13th INTERNATIONAL BIENNIAL CONFERENCE

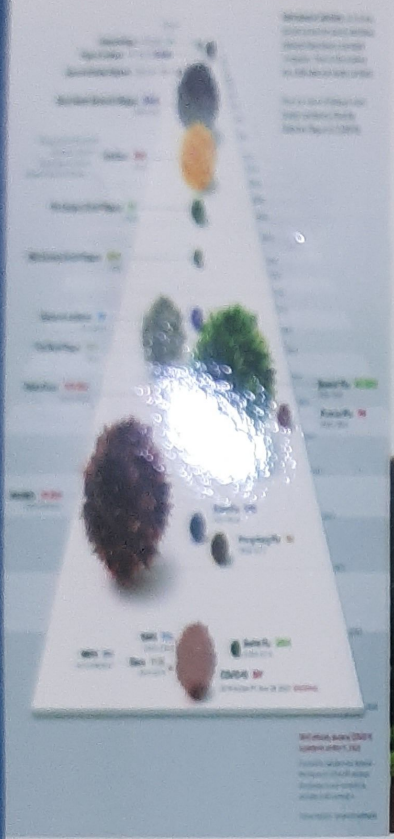
PAKISTAN SOCIETY FOR MICROBIOLOGY

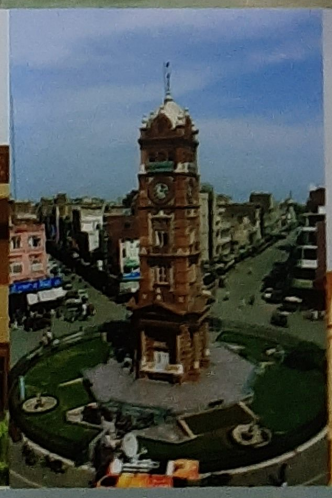
TIBC-PSM-2021

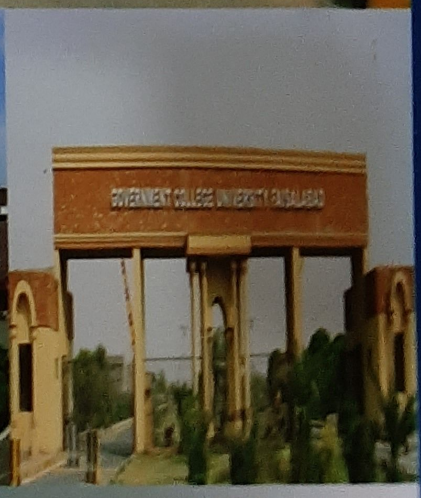
THEME

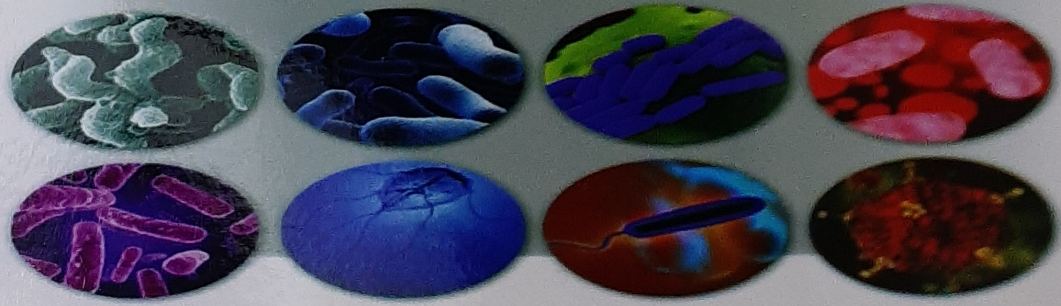
Pandemics-Epidemics: Public Health Challenges for Microbiologists

ABSTRACT BOOK

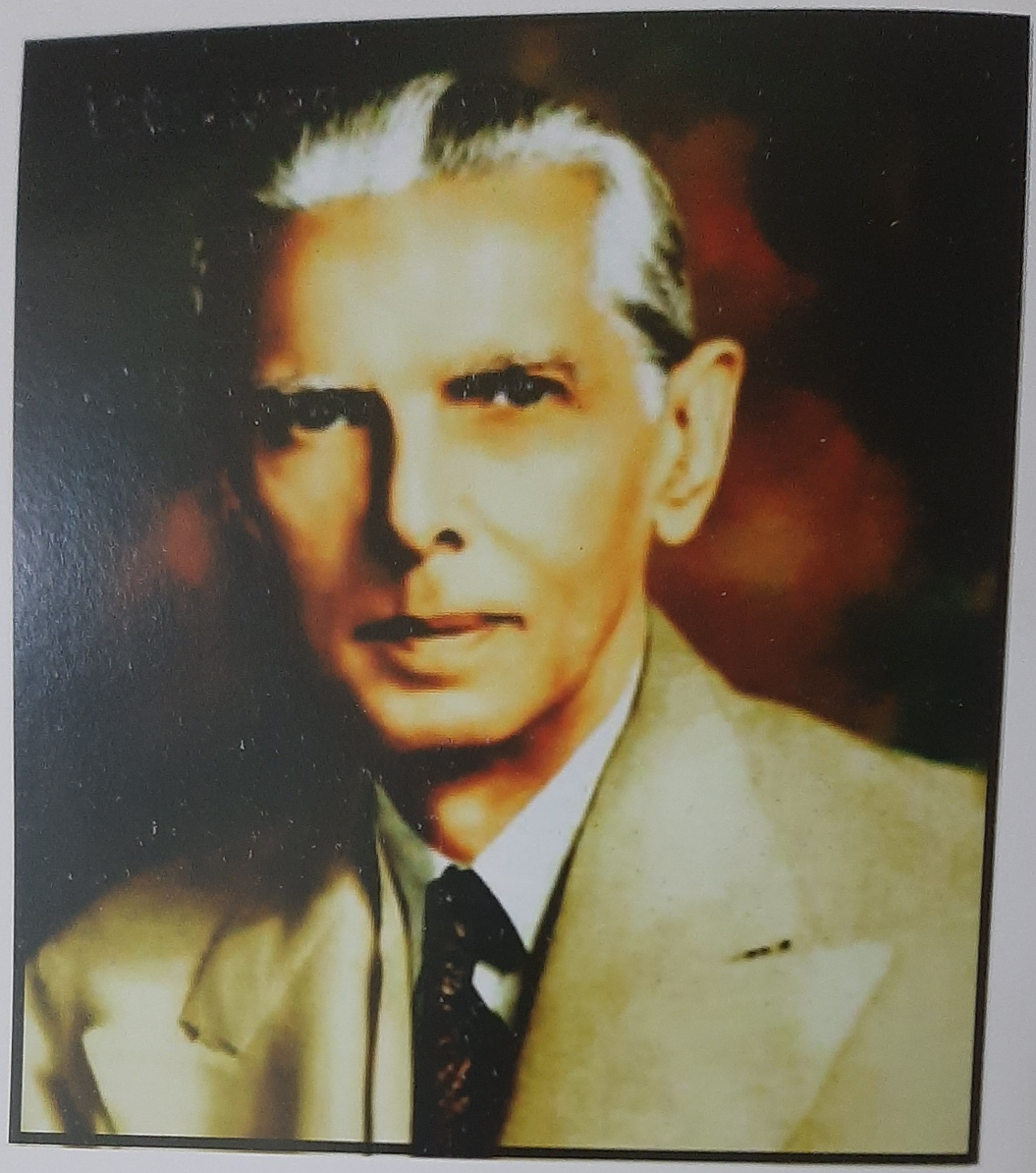








Father of the Nation



My young friends, I look forward to you as the real makers of Pakistan, do not be exploited and do not be misled. Create amongst yourselves complete unity and solidarity. Set an example of what youth can do. Your main occupation should be in fairness to yourself, to your parents, in fairness to the State, to devote your attention to your studies. If you fritter away your energies now, you will always regret.

Quaid-e-Azam Muhammad Ali Jinah
(Islamic College, Peshawar - 12th April, 1948)

TABLE OF CONTENTS

Message from Prof. Dr. Shahid Kamal

(Vice Chancellor, GCUF/ Patron-in-Chief)

It is a great privilege and an honour for me, as a Patron-in-Chief, to welcome you all to the 13th International Biennial Conference PSM (TIBC-PSM-21) organized by Department of Microbiology, Government College University Faisalabad (GCUF). Primarily, I would like to extend my appreciation to the department for ever increasing development and contribution as an important international player in the field of Microbiology. The theme of the conference *"Pandemics-Epidemics: Public Health Challenges for Microbiologists"* is appropriate to the current scenario of emerging and re-emerging infectious diseases like COVID-19 and Dengue around the globe. I am sure this event will not only add to this profile but also help in further advancement in all areas of the discipline of Microbiology especially in diagnostics, therapeutics and prevention.

Government College University Faisalabad is a dynamic seat of learning aspiring to provide highly motivating and conducive academic environments for quality education, research and professional development. The university is striving for excellence by living up to the international standards of education. The GCUF is the only general university in central Punjab catering the needs of more than 20 million populations and covering about three to five districts. In the recent years, the university has achieved phenomenal success through significant contribution in cutting-edge research which is reflected through the meteoric rise of the institution in HEC ranking. It's my pleasure to share that this year GCUF is ranked at 1st position in Punjab, 2nd in Pakistan, among 601-800 in Times Higher Education (THE) World University Ranking and among 201-250 in Times Higher Education (THE) Emerging Economies University Ranking.

We offer innovative syllabi encompassing the latest trends and approaches in the fields of higher education. Our curricula inculcate creativity, objectivity and critical thinking, impart values of commitment and singleness of purpose to learning and professionalism and promote confidence, adaptability and high level communication skills in students to ensure success of our graduates in this

highly competitive and challenging global world order. We are developing inter-disciplinary approach and social cohesion. The University administration and its teaching faculty is actively engaged in the challenging task of developing well-equipped labs to produce quality research in multiple disciplines including biological/ life sciences and in establishing liaisons with market and industry to ensure job opportunities for skilled graduates and young professionals. Furthermore, University also encourages its faculty to participate in conferences and to win scientific projects both nationally and internationally.

Keeping in view the national and international health challenges and current pandemics, the role of Microbiologists has become pivotal for the well-being of the society. We have a well-established Microbiology department and it is very promising that the department has taken the initiative in organizing the 13th International Biennial Conference in collaboration with Pakistan Society for Microbiology. I hope this event will provide a platform for sharing and learning the scientific notions in vast discipline of Microbiology. I congratulate the whole team for organizing such a wonderful scientific event and bringing a diverse national and international scientific community together.

My best wishes are with the organizers, international & national distinguished speakers, delegates and participants. I wish this event a great success.

President Welcomes TIBC-PSM 2021 Delegates

On behalf of all members of organizing committee, I would like to extend a very warm welcome to all the national and International delegates to the **13th *International Biennial Conference of Pakistan Society for Microbiology (TIBC-PSM-2021)*** hosted by the Department of Microbiology at Government College University – Faisalabad. Unfortunately, due to the ongoing pandemic and recent discovery of a highly mutated Omicron Variant of COVID-19 which poses a new threat to both vaccinated and unvaccinated population, a number of countries have put travel and other restrictions to control the spread of this new highly transmissible variant. A number of our international Delegates may not be able to join us physically but they will attend the conference online. New emerging and reemerging viral diseases have threatened humanity throughout history. The novel coronavirus 2 responsible for the COVID-19 pandemic engulfed the entire world in less than 6 months, with high mortality in the elderly and those with associated comorbidities. The pandemic severely disrupted the world economy by lockdowns, which was controlled by self-distancing, wearing masks, travel restrictions and avoiding gatherings and Vaccination. The theme of this year's conference ***"Pandemics-Epidemics: Public Health Challenges for Microbiologists".*** has been specially chosen to highlight the current emerging viral diseases, epidemics and pandemics as well as increasing emergence of drug resistance in human pathogens. Antibiotic resistance is a major global health concern, as our ability to treat infectious disease is challenged by multi-drug resistant bacteria.

The Scientific program of the Conference consist of plenary lectures, symposia, round table discussions and poster presentations with special Sessions on **COVID-19 Delta ,Omicron Variant Infections and Vaccines ", *Biosafety and Bio Risk Management, Academia-Industry Linkage as well as Pre-conference Hands-on Training Workshops on Meta genomics , Viral Diagnostics , Probiotics for Food Safety and Health Antimicrobial Resistance: A Public Health Challenge" will be held during the this 3 day mega event*** with participation of more than 500 delegates from Colleges , University teachers ,their young scholars , R&D institutions , industry representatives will help in developing collaborations and recommendations which will be mutually beneficial and will go a long way in providing incentives and encouragements for promoting Microbiological Sciences in Pakistan . **.** This Conference aims to bring together basic, applied and regulatory Bio scientists, health care providers and policymakers to address issues relating to medical sciences, stem cell therapy, genomics, proteomics, rapid spread of infectious diseases and applications of Molecular Biotechnology / Microbiology for the production of better antibiotics, vaccines, immuno-therapeutics, diagnostic reagents and green pesticides to improve the health of food animals, crops as well as the quality of human life. COVID -19 Pandemic has reemphasized the importance of teaching and training in all fields of Microbiology, Immunology and Molecular Biology, need to establish and enrich Microbiological Diagnostic Facilities for rapid detection of causative viruses and their variants through conventional methods, genomic sequencing and disease surveillance and global data sharing for containing the pandemics and saving human lives.

As always, I would like to congratulate microbiologist's community of Pakistan, specially Microbiology Alumni of Karachi University for their contributions in the last 60 years of excellence in teaching and research. Thanks to the vision and foresight of our teacher late **Prof. Dr. Ahmed Ali Anwer** who convinced the Karachi University management regarding the importance and broad applications of Microbiology in Health, Food, Pharma and Diagnostic Products Industry, to establish the very first Department of Microbiology in Pakistan as early as 1957. More than 50 years ago, at a meeting much smaller than this one, a group of microbiologists founded **Pakistan Society for Microbiology** at University of Karachi to promote the new discipline of science

under the patronage of late **Prof. Dr. Anwer** and **Prof. Dr. Essa Abdullh** - Founder President of PSM. Today, we are the oldest and largest society of Life Sciences in Pakistan, with more than 3000 members representing all provinces of Pakistan, working in close collaboration with American Society for Microbiology. Studies carried out to investigate the activities of microbes and their relationship with man, animals and plants resulted in major breakthroughs in healthcare sector. Efforts are now on all over the world to reduce incidence and find solutions to control dreadful diseases specially COVID -19, AIDS, Hepatitis, XDR and MDR TB and Typhoid. Microbiologists have a great responsibility to reduce massive crop destruction due to microbial plant diseases, improving animal health and productivity, soil fertility, reducing post-harvest spoilage of other farm products. Finally, with molecular keys within a researcher's reach, useful information can be gleaned. Today our motherland indeed is still a jungle in which microorganisms run rampant. Dysentery, Typhoid, Dengue, Severe Measles and flu are endemic. HIV and TB cases are on the increase. Polio is not yet eradicated. With all the recent technological developments, it is, within the realms of Microbiology to at least lower the incidence of unwelcome vermin in our food, water and Air. Recent information regarding human microbiome has been one of the most cutting-edge advances in biomedical research. We now know that every human being provides home to 10–100 trillion symbiotic micro-organisms, including bacteria, viruses, and archaea. The collective genomes of these micro-organisms form what is known as the human microbiome which impacts our physiological functions in many ways, such as contributing to metabolic functions, protecting against pathogens, and interacting with immune system. Research is now very much focused upon investigating how exactly its interactions within the body contribute to health and disease. Once we get an understanding of these interactions, we can start developing drugs to investigate the potential of microbiome modulating therapies for the treatment of disease. Microbiota population in our tissues can become imbalanced when there are more 'bad', pathogenic bacteria present than 'good' bacteria, resulting in illnesses, offering immense potential for microbiome therapeutics. Presently, a large proportion of novel microbiome modulators are being investigated for infectious diseases, gastrointestinal (GI) disorders metabolic disorders, dermatology, oncology, and neurological disorders.

On behalf of Pakistan Society for Microbiology , I would like to thank **Prof. Dr. Shahid Kamal** – Vice Chancellor – Government College University -Faisalabad for hosting TIBC-PSM 2021 as well as unflinching support and cooperation received from **Prof. Dr. Hidayat Rasool** - Chairman LOC and Head of Department of Microbiology and members of his dynamic team specially **Dr Usman Qamar Dr. .Mohsin Khursheed , Dr. Bilal , Saqlain ,** Heads of different Sub- Committees for their very enthusiastic participation in making excellent arrangements to welcome TIBC-PSM-2021 delegates at GC University- Faisalabad. I would also like to record my appreciation for our collaborating institutions - Women University Sawabi , NIBGE , NIAB , UAF and all members of Central Organizing Committee who have worked day and night under the patronage of Prof.Dr. Farhan Essa Abdullah – Patron in Chief- PSM and all members of Pakistan Society for Microbiology and ASM , specially Prof.Dr. Tanveer Abbass – Chair KU Microbiology Dr. Sadaf Akbar , Dr.Sadia Khalil Dr. Yasir Raza , Dr. Saeed Khan and many others for their continuous support , help and encouragement which enabled us to undertake the challenge of organizing the present and past 12 International Conferences in various cities of Pakistan including Bhurban and Malam Jabba , monthly seminars and for regularly organizing training courses to update recent advances . I am quite confident that the hard work that we all have put in for TIBC-PSM – 2021, will provide a scientifically stimulating environment to help the participants to develop implementable recommendations. The interactive discussions of experts and young scholars will go a long way in communicating recent technological advances and applicability of Microbiology in every walk of life. I wish great success to the conference and once again extend a very warm welcome to all delegates to Faisalabad – Manchester City of Pakistan.

Prof. Dr. Shahana Urooj Kazmi
Vice Chancellor – Women University Sawabi

Chairperson – TIBC-PSM-2021
President – Pakistan Society for Microbiology / F- ASM Country Ambassador

Message from Prof. Dr. Farhat Jabeen

(Dean Faculty of Life Sciences, GCUF/ Patron)

It is a great pleasure for me to welcome you to the "13th International Biennial Conference PSM" (TIBE-PSM-21) organized by the Department of Microbiology, Government College University Faisalabad, Punjab, Pakistan. I am very happy to host and welcome all the distinguished national & international speakers, delegates, faculty members, research scholars and students to share their knowledge, experience and to explore better ways of educating our future leaders. It is our mission to develop professionals with a global perspective, who are willing to take an active role in the progress of science. This conference is a wonderful initiative by the Department of Microbiology, Government College University Faisalabad and Pakistan Society for Microbiology by providing an excellent opportunity for students to travel and learn from their counterparts from all over the country, allowing for a more potent exchange of knowledge. I must congratulate Prof. Dr. Muhammad Hidayat Rasool, Chairperson, Department of Microbiology and his dynamic team for organizing this event and bringing together a diverse Microbiology community together.

The research areas in Microbiology offers promising prospects, and this event will provide a platform to share the scientific knowledge and ideas. The conference will include plenary talks from distinguished international and national guest speakers, scientific oral and poster presentations as well as pre-conference workshops. I hope you will have the three most productive days of motivating and interesting discussions. I sincerely wish that this conference will be a great success not only as a chance to share knowledge and experience in higher education but as a beginning of a long and fruitful cooperation and friendship among fellow educators devoted to the most meaningful and worthwhile task of teaching and training the youth, who will shape our future. We are looking forward to your active participation in this spectacular event.

I hope that you will enjoy your stay in Faisalabad, the Manchester of Pakistan. I wish this conference a great success.

Message from Prof. Dr. Muhammad Hidayat Rasool

(Chairman, Department of Microbiology, GCUF/ Principal Organizer)

It's a great pride and privilege for me to welcome distinguished international & national scientists/ microbiologists, renowned academicians, researchers, faculty members, alumni, industrial partners and students to the department of Microbiology, Government College University Faisalabad (GCUF), Punjab, Pakistan to participate in 13th International Biennial Conference PSM (TIBC-PSM-21) scheduled from 22-24 December 2021. The department of Microbiology is striving for excellence in exploring the invisibles since 2011. It has an excellent history of a decade with deep roots in microbiology, bacteriology, virology, immunology, and its applied branches. I am honored to serve as Chairperson of a dynamic group of faculty and to oversee exciting new changes in the department since its establishment to date. At present, it is housed in New Campus of GCUF with state-of-the-art laboratory facilities and among one of the leading departments of the university.

In the last decade, department has continued to achieve new heights in education and research excellence. Our research groups consistently publish on both fundamental and applied microbiology research in leading scientific journals. Another testimony to research excellence is the ability of our Principal Investigators to win large competitive research grants from national and international funding agencies. Students, academicians, researchers, and representatives from industry are always welcome to contact for support, guidance, and collaboration.

Today, the pace of discovery is accelerating like never before. Revolutions are underway in our ability to see, analyze in culture, analysis and information technologies that are completely transforming how we approach our discipline. These new tools are revealing rich new insights with dizzying speed. The microbes, however, continue to do what they've done for billions of years – grow, interact, evolve, and shape the world around them and we will continue to study them, to focus particularly on the bacteria and viruses that threaten human health and on strategies to combat them, and to harness microbial power to improve life where possible.

Come and see the excitement of our students and researchers who are engaged day and night to face the challenges of 21st century. As we have a moral and scientific duty to contribute towards a better understanding of infectious diseases, their mechanisms, treatments and prevention through meaningful research and educational initiatives. I am confident that we will achieve these goals with the dynamic, collegial, and collaborative colleagues in our midst. We are excited to look forward to you all during December, 2021 in Faisalabad, the Manchester of Pakistan.

Welcome to our world; it is a wonderful place to be. I wish this conference a great success.

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Introducing Faisalabad-Manchester City of Pakistan

The Host City

Faisalabad (Lyallpur District until 1979), the Manchester of Pakistan is one of the first planned and systematic cities in the sub-continent envisaged by the British Government, after the name of Sir James Lyall. Lyallpur was the first name ascribed to this fertile land of Sandal Bar. The unique bond present between the sister countries Pakistan and Saudi Arabia paved the way to rename the city after the name of our friend, the King of Saudi Arabia, King Faisal (Late). Initially a part of the Jhang District, it gained the status of a separate district in 1904. The city is catering to the needs of more than six million people. Agriculture and industry remain the hallmark of Faisalabad with a powerful and ever-developing thrust in these sectors. Faisalabad International Airport, Faisalabad Dry Port and Industrial Zone linked with the Motorway are facilitating this hub of industrial activities.

The historical Clock-Tower with Eight Bazars designed after Union Jack presents a magnificent example of town planning located in the province of Punjab to the west of Lahore, the provincial capital. With the proper infrastructure and road linkages, Faisalabad serves as an example as a district, as a division, and as a city for the developing world. The Faisalabad International cricket and hockey stadiums host international matches. The city has produced great leaders in the fields of politics, education, health, sports, agriculture, economy, bureaucracy, and industry.

The Host University

Government College University Faisalabad (GCUF) has emerged as a leading center of learning and research in a short time. GCUF is situated at the Jhang Road not far from the historical Clock Tower. The main campus is spread on 37.5 acres and a state-of-the-art new campus on an area of 200 acres about 5 kilometers away from the Main Campus at Jhang Road. The University with its dynamic faculty and innovative syllabi has become a catalyst of intellectual, social, and industrial change. It caters to the diverse needs of society by imparting education in almost all the major fields of learning. The University has more than 100 years of history of existence to its credit. Its journey started as a primary school in 1897 in the present building of Government College for Women, Karkhana Bazar, Faisalabad. It was promoted to High School and Intermediate College in 1905 and 1924, respectively. It was elevated to the degree level in 1933 and postgraduate disciplines were introduced in 1963. The long journey that started with the humble beginning reached its climax when it was granted the status of University in October 2002. The GCUF has a long history of excellence and distinction as an institution. Renowned scholars and eminent personalities have served this institution in various capacities at various stages of its history. It has produced outstanding personnel who have earned great fame not only for themselves but also for the nation.

Currently, it is ranked at 251-300 in Times, Higher Education Asia Ranking 2021, and 5th position among all Pakistan Universities. The GC University has more than 50 departments offering about 177 different degree programs at BS, MA/MSc, MPhil, and PhD levels. It has more than 600 faculty members out of which 395 are PhDs and 222 are MPhil. More than 25000 students are currently enrolled on-campus in addition to sub-campuses and approximately 400 Government and private colleges affiliated with GCUF. The GC University Faisalabad is the largest and only public sector university (General category) in central Punjab catering to the needs of millions of people.

The Host Department

Department of Microbiology in GCUF is striving for excellence in exploring the invisible since its establishment in 2011. Initially, it was housed in Basic Sciences Block Old Campus and later on shifted to Liaqat Block 2nd floor New Campus in 2017. The academic programs at an undergraduate and postgraduate level including BS, MPhil, and PhD in Microbiology are being offered in the department which is duly approved by university statutory bodies and HEC, Islamabad. At present department have 17 highly qualified and competent Faculty Members and approximately 400 enrolled students with an overall student-teacher ratio of 1:23. Department of Microbiology has purpose-built undergraduate and state of the art postgraduate research labs equipped with basic as well as advanced instrumentation for practical work of undergraduate and research work of postgraduate students.

Since its establishment, Department has set high standards in academics & research in the field of Microbiology. It has produced a number of graduates and postgraduates in the vast discipline of Microbiology well equipped with the theoretical knowledge and practical skills in Microbiology. These are the true ambassadors of the Department of Microbiology who are serving the nation throughout the country as well as internationally. Department has various research groups working in their domains and faculty members have won many research grants of approximately 30 million in total from different funding agencies Like Higher Education Commission (HEC) Pakistan, Pakistan Science Foundation (PSF), Pakistan Health Research Council (PHRC) and Health Security Partner, USA. Department of Microbiology has liaisons with leading teaching & research institutions and industries of the country for internship of undergraduate and research collaboration of postgraduate students. The department has organized many scientific events and our students have also won several laurels in extra-curricular and co-curricular activities.

SCIENTIFIC PROGRAM

Wednesday, December 22, 2021 (Day 01)
Quaid-e-Azam Auditorium, Main Hall, New Campus, GCUF

Inaugural Ceremony (09:20 am To 11:00 am)		
09:20 am	09:30 am	Arrival and Seating of Honorable Guests & Chief Guest
09:30 am	09:40 am	Recitation of Holy Quran & Naat-e-Rasool Maqbool (SAW)
09:40 am	09:50 am	Welcome note by **Prof. Dr. Muhammad Hidayat Rasool**, Chief organizer/ Chairman, Department of Microbiology, GCUF
09:50 am	10:00 am	Address by **Prof. Dr. Shahana Urooj Kazmi**, Chairperson Central PSM-ASM/ VC, WUS, KPK
10:00 am	10:30 am	Address by **Prof. Dr. Atta-ur-Rahman** (Nishan-e-Imtiaz) Chief Guest/ Chairman, Prime Minister's Task Force on Science and Technology
10:30 am	10:40 am	Address by **Prof. Dr. Shahid Kamal**, Patron-in-Chief/ VC, GCUF
10:40 am	10:50 am	Vote of Thanks by **Prof. Dr. Farhat Jabeen**, Patron/ Dean Faculty of Life Sciences, GCUF
10:50 am	11:00 am	Distribution of Souvenirs
Inauguration of Poster Display & Scientific Exhibition (11:00 am To 11:15 am)		
Tea Break (11:15 am To 11:30 am)		
Parallel Technical Session A1 Quaid-e-Azam Auditorium Main Hall (11:30 am To 1:30 pm) **Theme:** Antimicrobial Resistance, Antimicrobial Agents, Nano Antibiotics **Session Chair:** Prof. Dr. Farhan Essa Abdullah, CEO, ELDC Karachi **Session Co-Chair:** Dr. Muneera Baloch, University of Karachi, Sindh **Moderator:** Ms. Maryam Akram		
Keynote speaker: 11:30 am - 11:50 am		**Contribution as Microbiologist in Training and Capacity Building for COVID-19 Diagnostics and Research in Current Pandemic: Sharing Two Years Personal Experience** Dr. Essa Abdullah Memorial Lecture, *Prof. Dr. Saeed Khan, Dow University of Health Sciences, ASM country Ambassador*
11:50 am	12:00 pm	**Whooping cough caused by Bordetella pertussis and *Bordetella para pertussis* among patients of all age groups in Khyber Pakhtunkhwa province of Pakistan** *Muhammad Ali Syed, The University of Haripur, Haripur, KPK*
12:00 pm	12:10 pm	**Molecular epidemiology of tuberculosis and *RPOB* gene mutations in *Mycobacterium tuberculosis* isolated from patients in Marden Medical Complex, Marden, Pakistan** *Muzamil Shah, Abdul Wali Khan University Mardan, KPK*
12:10 pm	12:20 pm	**Molecular detection of extensively drug-resistant *Salmonella* Typhi and carbapenem-resistant pathogens in pediatric septicemia patients in Pakistan - a public health concern**. *Atifa Ambreen, Government College University Faisalabad, Punjab*
12:20 pm	12:30 pm	**Emergence of diverse genotypes of *S. aureus* harboring Panton-Valentine Leukocidin (*PVL*) and Accessory Gene Regulator (*Agr*)** *Nauman Javed, University of the Punjab, Lahore, Punjab*
12:30 pm	12:40 pm	**New Delhi Metallo-lactamase *Escherichia coli* belonging to Sequence Type 131 in tertiary care hospitals of Southern Punjab, Pakistan** *Faiza Sarwar, Government College University Faisalabad, Punjab*
12:40 pm	12:50 pm	**Antibiotic resistance modulation of enteric *E. coli* isolated from houbara bustard bird** *Afshan Muneer, Cholistan University of Veterinary & Animal Sciences Bahawalpur, Punjab*
12:50 pm	01:00 pm	**Determination of antibiotic resistant genes in *Bifidobacterium* species isolated from commercial fermented foods.** *Muhammad Amir Javaid, University of Agriculture Faisalabad, Punjab*
01:00 pm	01:10 pm	***Pseudomonas aeruginosa*: Extended Spectrum β- Lactamase & amp; Metallo β-Lactamase producer a Potential Global Threat in the Near Future** *Farkhnda Afaque, Jinnah University For Women, Karachi, Sindh*
01:10 pm	01:20 pm	**Antifungal susceptibility profile of invasive *Candida glabrata* isolates (2009-2020) from a tertiary care hospital laboratory in Pakistan.**

		Saba Memon, Aga Khan University, Karachi, Sindh
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session A2 Quaid-e-Azam Auditorium Hall II (11:30 am To 1:30 pm) **Theme:** Clinical Microbiology and Infectious Diseases **Session Chair:** Prof. Dr. Zafar Ali Chaudhary (TI) VC, FMU, Faisalabad **Session Co-Chair:** Dr. Shah Jahan, UHS, Lahore **Moderator:** Ms. Fatima Noreen		
Keynote speaker: 11:30 am -11:50 am		**Quality high through put techniques for diagnosis for COVID-19 infection** *Dr. Kamran Shaukat, United Kingdom*
11:50 am	12:00 pm	**Ursolic Acid and its Amide Derivatives Disrupts Clinical Acinetobacter Baumannii isolates** *Ayaz Ahmad, University of Karachi, Sindh*
12:00 pm	12:10 pm	**Optimization of major toxins production potential of *Clostridium perfringens* type B under various physico-chemical conditions.** *M. Khubaib Sattar, University of Veterinary & Animal Sciences, Lahore, Punjab*
12:10 pm	12:20 pm	**Identification of *Mycoplasma hominis* pathogens in semen using polymerase chain reaction and "flow-through" hybridization technology** *Rubina Ghani, Sohail University, Karachi*
12:20 pm	12:30 pm	**Association between Hepatitis C virus infection and type-2 diabetes mellitus.** *Tahira Qamash, Abdul Wali Khan University Mardan, KPK*
12:30 pm	12:40 pm	**Molecular Characterization of Toxigenic Aspergillus flavus Isolated from Sick Broiler Lungs and Risk Factors Analysis** *Saba Sana, University of Veterinary and Animal Sciences Lahore, Punjab*
12:40 pm	12:50 pm	**Prevalence of Streptococcal Super antigen genes** *Hina Musa, University of Karachi, Sindh*
12:50 pm	01:00 pm	**Immunohistochemical based detection of *H. pylori* in gastric biopsy patients.** *Abdullah Riaz, Government College University Faisalabad, Punjab*
01:00 pm	01:10 pm	**Methylation modification of the STAT1 gene in HCV induced hepatocellular carcinoma** *Umaira Zakir, Ziauddin University, Karachi, Sindh*
01:10 pm	01:20 pm	**Environmental and health impact of antibiotic residues in food chains** *Aqsa Shahid, Government College University Faisalabad, Punjab*
01:20pm	01:30 pm	**Mutational Analysis of Full Genome Sequence of SARS-CoV2 isolated from Pashtun Pakistani Patient** *Ome Kalsoom Afridi, Women University Swabi, KPK*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session A3 STC Hall (11:30 pm To 01:30 pm) **Theme:** Agricultural Biotechnology and Environmental Microbiology **Session Chair:** Dr. Sajid-ur-Rahman, DG, AARI, Faisalabad **Session Co-Chair:** Dr. Mudasser Habib, NIAB, Faisalabad **Moderator:** Ms. Ayesha Maham		
Keynote speaker 11:30 am -11:50 am		**Enhanced Solubilization and Purification of 3ABC Non-structural Protein of Foot-and-Mouth Disease Virus from Bacterial Inclusion Bodies** *Dr. Mudasser Habib, Nuclear Institute for Agriculture & Biology, Faisalabad, Punjab*
11:50 am	12:00 pm	**Improving microbial population and rice production through integrated use of organic and mineral nutrient sources** *Amanat Ali, Nuclear Institute of Agriculture, Tando Jam, Sindh*
12:00 pm	12:10 pm	**Saccharothrix Algeriensis NRRL B-24137 Potentiates Chemical Fungicide Carbendazim in Treating Fusarium Oxysporum f.sp. Vasinfectum-Induced Cotton Wilt** *Rizwan Asif, Qarshi University, Lahore, Punjab*
12:10 pm	12:20 pm	**Evaluation of Plant Growth Promoting Traits of Cadmium Contaminated bacteria for its use in Phytoremediation** *Zahra Kalim, Government College University Faisalabad, Punjab*
12:20 pm	12:30 pm	**Methyl-Jasmonate is a regulator to induce immunity in tomato during individual and**

		combined Pseudomonas syringae and NaCl stress *Hamid Manzoor, Bahauddin Zakariya University, Multan, Punjab*
12:30 pm	12:40 pm	**Distribution of phosphate solubilizing bacteria near seashore of Karachi, Sindh** *Qanita Ismail, FAUUST Karachi, Sindh*
12:40 pm	12:50 pm	**Three layered strategies to obtain keratinase production: immobilization, co-culture of bacterial strains and use of waste product as a substrate.** *Sana Khalique, University of Karachi, Sindh*
12:50 pm	01:00 pm	**Evaluation of metal resistance potential of bacteria isolated from rhizosphere of marine halophytes** *Drousham Nasir, FAUSST, Karachi, Sindh*
01:00 pm	01:10 pm	**Effect of Feeding Tanniferous Plants on Immune Response against Peste des Petits Ruminants (PPR) Vaccine in Goats** *Rida Fatima, Nuclear Institute for Agriculture & Biology, Faisalabad, Punjab*
01:10 pm	01:20 pm	**Combined application of nanoparticle and selected bacterial strains on the growth promotion of wheat plant.** *Muhammad Arif Ali, Bahauddin Zakariya University, Multan, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Visit of Scientific Research Posters in corridors of Quaid-e-Azam Auditorium Main Hall (01:30 pm To 02:00 pm)		
Lunch and Prayer Break (02:00 pm To 02:30 pm)		
Parallel Technical Session A4 Quaid-e-Azam Auditorium Main Hall (02:30 pm To 04:30 pm) **Theme:** Bioinformatics and Proteomics **Session Chair:** Dr. Shahid Mansoor (TI), Director NIBGE, Faisalabad **Session Co-Chair:** Dr. Amir Mehmood, GCUF **Moderator:** Mr. Muhammad Qasim		
Keynote speaker 02:30 pm - 02:50 pm		**Viral disease in plants, animals and human: characterization and control strategy** *Dr. Shahid Mansoor, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Punjab*
02:50 pm	03:00 pm	**Advanced Genomics Epidemiology and Bioinformatics in Medical Microbiology** *Afsheen Arif, KIBGE, University of Karachi, Sindh*
03:00 pm	03:10 pm	**The prospects of CRISPR-Cas system for the amelioration of antibiotic resistance** *Maria Rasool, Government College University Faisalabad, Punjab*
03:10 pm	03:20 pm	**Pan Based Genomic Analysis of *Campylobacter jejuni*** *Talyha Khalid, Jinnah University for Women, Karachi, Sindh*
03:20 pm	03:30 pm	**Novel role of ABC (ATP Binding Cassette) efflux pump in providing isopentenol tolerance in *E. coli*** *Asad Ali Shah, Government College University Faisalabad, Punjab*
03:30 pm	03:40 pm	**Molecular cloning and characterization of a thermostable esterase produced by Anoxybacillus spp.,** *Ayesha Mohiud Din, Virtual University, Lahore, Punjab*
03:40 pm	03:50 pm	**Molecular detection of *Acinetobacter baumannii* from fish meat available in local market.** *Samiyah Tasleem, FUUAST, Karachi, Sindh*
03:50 pm	04:00 pm	**Sequencing of 16s rRNA gene for molecular characterization of Bacillus cereus in street vended foods.** *Asadullah Marri, Sindh Agriculture University, Tando Jam, Sindh*
04:00 pm	04:10 pm	**Detection of *Pseudomonas aeruginosa* and their Virulence Genes Isolated from Burn Wound Infection and environmental samples** *Rakhshanda Erum, University of Karachi, Sindh*
04:10 pm	04:20 pm	**Aberrant STAT1 methylation as a non-invasive biomarker in hepatocellular carcinoma** *Rizwan Khan, Ziauddin University, Karachi*
04:20pm	04:30pm	**Engineering and computational analyses of glycerol dehydratase enzymes with improved resistance to inactivation for industrial production**

		Abdul Nasir, Ajou University, Republic of Korea
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session A5 Quaid-e-Azam Auditorium Hall II (02:30 pm To 04:30 pm) **Theme:** Covid-19 Situation in Pakistan **Session Chair:** Prof. Dr. Salvatore Rubino, Italy **Session Co-Chair:** Prof. Saeed Khan, DUHS, Karachi **Moderator:** Ms. Kaneez Fizza		
Keynote speaker 02:30 pm - 02:50 pm		**Circulation of SARS COV-2 variant of concern (VOC) traced by Whole Genome Sequencing, the case of Sardinia Italy** *Prof. Dr. Salvatore Rubino, Italy*
Keynote speaker 02:50 pm - 03:10 pm		**Living the experience of COVID-19 pandemic in one of the most locked country in the world** *Prof. Abdul Jabbar, Melbourne Australia*
03:10 pm	03:20 pm	**Understanding the Clinical characteristics of COVID-19 and Genome sequences of SARS-CoV-2 in Local Patients** *Shah Jahan, University of Health Sciences, Lahore, Punjab*
03:20 pm	03:30 pm	**Comparative genomic analysis of the structural proteins of SARS-COV-2 strains prevalent in Pakistan.** *Arooj Shafiq, Salim Habib University, Karachi, Sindh*
03:30 pm	03:40 pm	**Anti-SARS-COV-2 potential of ethanolic and methanolic extracts of *Allium sativum*, *Zingiber officinale*, *Eucalyptus globulus* and *Cinnamomum verum*** *Aleena Khan, University of Veterinary & Animal Sciences, Lahore, Punjab*
03:40 pm	03:50 pm	**Impact of COVID-19 pandemic on children with transfusion dependent blood disorders.** *Usmani Faiza, Children Hospital, Karachi*
03:50 pm	04:00 pm	**Impact of pandemics (COVID-19) on Global Health and challenges for Microbiologists** *Mudassar Mohiuddin, Islamia University of Bahawalpur, Punjab*
04:10 pm	04:20 pm	**Age and gender specific study of COVID-19 positive cases: A tertiary care hospital experience.** *Fatima Ahmed, Children Hospital Karachi, Sindh*
04:20 pm	04:30 pm	**Transmission dynamics of SARS-CoV-2 in Karachi, Sindh** *Sheikh Kausar, Aga Khan University, Karachi, Sindh*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session A6 STC Hall (02:30 pm To 04:30 pm) **Theme:** Nanotechnology, Probiotics and Bacteriophages **Session Chair:** Prof. Dr. Farhat Jabeen, Dean Life Sciences GCUF **Session Co-Chair:** Dr. Muhammad Tariq, NIBGE, Faisalabad **Moderator:** Ms. Urwat-ul-Isha		
Keynote speaker 02:30 pm - 02:50 pm		**Antibacterial potential of Probiotic bacteria and their applications** *Dr. Muhammad Tariq, National Institute for Biotechnology & Genetic Engineering, Faisalabad, Punjab*
02:50 pm	03:00 pm	**Therapeutic potential of *Lactobacillus* post-biotics and its competitive activity against Salmonella and *E. coli* in poultry birds** *Iqra Mumtaz, University of Agriculture Faisalabad, Punjab*
03:00 pm	03:10 pm	**Probiotics protect intestinal microbial dysbiosis and subsequent aero-gastric infections of *S. aureus* and *P. aeruginosa*** *Affhan Shoaib, Salim Habib University, Karachi, Sindh*
03:10 pm	03:20 pm	**Ecofriendly photosynthesized zirconium oxide nanoparticles as antibiofilm agent against MDR *Acinetobacter baumannii.*** *Sumreen Hayat, Government College University Faisalabad, Punjab*
03:20 pm	03:30 pm	**Silver nanoparticles: synthesis and characterization by using glucans extracted from *Pleurotus ostreatus*** *Haleema Sadia, BUITEMS, Quetta, Baluchistan*
03:30 pm	03:40 pm	**Comparative effect of organic acids and aqueous extract of garlic and ginger on survival of**

		***Campylobacter jejuni* on chicken meat** *Saira Gul, University of Veterinary & Animal Sciences, Lahore, Punjab*
03:40 pm	03:50 pm	**Controlling of lepidopterous pests (honeycomb and white marked tussock moths) by bacillus strains isolated from the local fields of Kohat.** *Syeda Fatima Gilani, Kohat University of Science and Technology, Mardan, KPK*
03:50 pm	04:00 pm	**Essential oils composition, antioxidant, antibacterial, and ɑ- glucosidase activity of Scutellaria edelbergii Rech. F.** *Muddaser Shah, Abdul Wali Khan University Mardan, KPK*
04:00 pm	04:10 pm	**Combined treatment of probiotics with therapeutic doses of ultraviolet radiations for immunity induction in immunocompromised patients following chemotherapy** *Qurat ul Ain, Government College University Faisalabad, Punjab*
04:10 pm	04:20 pm	**Assessment of cholesterol reducing potential of Commensal bacterial species isolated from human milk** *Habib-ur-Rahman, University of Agriculture Faisalabad, Punjab*
04:20pm	04:30pm	**Laboratory Formulated Cost Effective Plant Extract Media Combinations and Commercially Available Dehydrated Medium for Aspergillus niger; a Comparative Growth Assay** *Sadiha Saleem, Jinnah University For Women, Karachi, Sindh*
Concluding Remarks by Session Chair and Distribution of Certificates		
Tea (04:30 pm To 05:00 pm)		
Thursday, December 23, 2021 (Day 02)		
Parallel Technical Session B1 Quaid-e-Azam Auditorium Main Hall (09:00 am To 11:00 am) **Theme:** Antimicrobial Resistance, Antimicrobial Agents, Nano Antibiotics **Session Chair:** Dr. Syed Abdul Aziz, University of Ottawa, Canada **Session Co-Chair:** Dr. Afreenish Amir, NIH Islamabad **Moderator:** Ms. Memoona Sultan		
Keynote speaker 09:00 am - 09:20 am		**Analysis of SARS-CoV-2 and factors predicting next spillover of its more contagious variant** *Dr. Syed Abdul Aziz, University of Ottawa, Canada*
09:20 am	09:30 am	**Pakistan AMR National Action Plan: Development & Implementation** *Afreenish Amir, National Institute of Health, Islamabad*
09:30 am	09:40 am	**Comparative drug susceptibility of colistin resistant *Escherichia coli* isolated from milk in district Marden**. *Khadija Altaf, Abdul Wali Khan University Mardan, KPK*
09:40 am	09:50 am	**Molecular Epidemiology of Extensively-Drug Resistant *Acinetobacter baumannii* ST 2 Co-Harboring *blaNDM* and *blaOXA* From Clinical Origin** *Mahtab Ahmad, Government College University Faisalabad, Punjab*
09:50 am	10:00 am	**Prevalence and risk factors for second line drug resistance in drug resistant tuberculosis patients in Pakistan: a retrospective cohort study** *Irfan Ullah, Pakistan Council for Science and Technology, Islamabad*
10:00 am	10:10 am	**Occurrence of Extended Spectrum β-lactamase *E. coli* in cattle and buffaloes of Islamabad, Pakistan** *Hamid Irshad, National Agriculture Research Council, Islamabad*
10:10 am	10:20 am	**Prevalence and Antimicrobial susceptibity pattern of Carbapenem resistance from Pediatric bloodstream infections** *Ali Hassan, Government College University Faisalabad, Punjab*
10:20 am	10:30 am	**Molecular detection of multidrug resistant *pseudomonas aeruginosa* from raw meat samples** *Muhammad Qasim, Government College University Faisalabad, Punjab*
10:30 am	10:40 am	**Quinolones Derivatives: Active against MDR Bacteria associated with Urinary Tract Infection** *Amtul Sami, Women University Swabi, Swabi, KPK*
10:40 am	10:50 am	**Isolation of *Vibrio cholerae* from clinical and drinking water samples during Cholera**

		Outbreak in Khairpur Sindh Pakistan. *Amjad Ali Mughal, Shah Abdul Latif University, Khairpur, Sindh*
10:50am	11:00am	**Synergy of Allium cepa zinc oxide Nanoparticle on Antibiotics Against Biofilm Forming Multi drug Resistant Uropathogens** *Saba Sana, University of Veterinary and Animal Sciences, Lahore, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B2 Quaid-e-Azam Auditorium Hall II (09:00 am To 11:00 am) **Theme:** Clinical Microbiology and Infectious Diseases **Session Chair:** Prof. Dr. Habib Bokhari, VC, KUM **Session Co-Chair:** Prof. Dr. Aqeel Ahmed, HSU, Karachi **Moderator:** Ms. Atiqa		
Keynote speaker 09:00 am - 09:20 am		Pitfalls in global response to infectious diseases and its impact on global health & economy-preparedness is key word to remember *Prof. Dr. Habib Ali Bokhari, Kohsar University Murree, Punjab*
09:20 am	09:30 am	**Activity of plant essential oils against antibiotic resistant Enterococcus faecalis isolated from diarrheic children** *Tehrim, University of Veterinary & Animal Sciences, Lahore, Punjab*
09:30 am	09:40 am	**Prevalence and antimicrobial susceptibity pattern of bacteria from pediatric blood culture** *Aina Farooq, Government College University Faisalabad, Punjab*
09:40 am	09:50 am	**Evaluation of Phospholipase and Proteinase Activity of *Candida spp*., Isolated from Patients with Surgical Site Infection** *Rakhshanda Erum, University of Karachi, Sindh*
09:50 am	10:00 am	**Antibiogram Profile and Evaluation of Biofilm Formation by Bacterial Clinical Isolates** *Faryal Anjum, University of Karachi, Sindh*
10:00 am	10:10 am	**Prevalence and antimicrobial susceptibility pattern of Enterobacteriaceae isolated from hospitals wastewater.** *Muhammad Rizwan, Government College University Faisalabad, Punjab*
10:10 am	10:20 am	**Molecular epidemiology and characterization of Hepatitis delta virus from different areas of Khyber Pakhtunkhwa, Pakistan** *Izhar ul Haq The University of Haripur, Haripur, KPK*
10:20 am	10:30 am	**Case-control Sero-epidemiological study on Hepatitis C in Cancer Patients from Lahore** *Aneeqa Haleem, Government College University Faisalabad, Punjab*
10:30 am	10:40 am	**Isolation and Molecular Typing of Multidrug Resistant Bacteria from Antibiotics-Containing Waste and Their Potential for Environmental Applications** *Inam Ali Larik, Shah Abdul Latif University, Khairpur, Sindh*
10:40 am	10:50 am	**Occurrence of Multi-drug Resistant *Mycobacterium tuberculosis* from Patients of Pulmonary Tuberculosis in Faisalabad** *Muhammad Mujahid, Government College University Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B3 STC Hall (09:00 am To 11:00 am) **Theme:** Immunology, Virology and Vaccinology **Session Chair:** Prof. Dr. Sajjad-ur-Rahman, Director IOM, UAF **Session Co-Chair:** Dr. Ghulam Fatima, Civil Hospital, Karachi **Moderator:** Ms. Sadia Saleem		
Keynote speaker 09:00 am - 09:20 am		**Molecular characterization of SARS-CoV-2 Spike gene from suspected patients** *Prof. Dr. Sajjad-ur-Rahman, University of Agriculture Faisalabad, Punjab*
09:20 am	09:30 am	**Characterization and complete genome sequence of 229E-related Coronavirus from dromedary camels of Pakistan** *Ali Zoaib, Islamia University of Bahawalpur, Punjab*
09:30 am	09:40 am	**The COVID-19 outbreak: a novel SARS-COV2 virus infection prevalence rate at a Government Tertiary Care Hospital of Karachi Pakistan** *Ghulam Fatima, Civil Hospital Karachi*

09:40 am	09:50 am	**Spectrum of protozoal and bacterial infection in hospitalized patient with acute watery diarrhea Karachi-Pakistan** *Nain Tara, WUS, Swabi*
09:50 am	10:00 am	**Diagnostic potential of liver specific microRNAs and cytokine IL-33 as non-invasive biomarkers of hepatitis** *Muhammad Imran Arshad, University of Agriculture Faisalabad, Punjab*
10:00 am	10:10 am	**Development of diagnostic test to determine the incidence of Mycoplasmosis in bovines in Karachi** *Syed Khurram Fareed, University of Karachi, Sindh*
10:10 am	10:20 am	**Seroepidemiological Study of Crimean Congo Hemorrhagic Fever in District Mardan** *Muhammad Ali, Abdul Wali Khan University, Mardan, KPK*
10:20 am	10:30 am	**Outer membrane vesicles from Gram-negative bacteria and their interactions with host cells.** *Zia ur Rehman, Kohat University of Science and Technology, Kohat, KPK*
10:30 am	10:40 am	**Inhibition of viral PL-Pro (Papain-like proteases) protein and cytokine release Syndrome using coumarin derivatives through *in-silico* & *in-vitro* approaches Filling two needs with one deed…!** *Hira Noor, University of Karachi, Sindh*
10:40 am	10:50 am	**A serological survey of severe fever with thrombocytopenia syndrome virus (SFTSV) and Crimean Congo hemorrhagic fever virus (CCHFV) from Faisalabad, Pakistan.** *Muhammad Saqib, Islamia University of Bahawalpur, Punjab*
10:50am	11:00am	**Genetic analysis of env gene (gp120) of human immunodeficiency virus type -1 (HIV-1) prevailing among HIV positive population in Pakistan** *Salma Zahid, The University of Haripur, KPK*
Concluding Remarks by Session Chair and Distribution of Certificates		
Tea Break (11:00 am To 11:30 am)		
Parallel Technical Session B4 Quaid-e-Azam Auditorium Main Hall (11:30 am To 01:30 pm) **Theme:** Biosafety and Public Health **Session Chair:** Dr. Paul D. Brown, Jamaica **Session Co-Chair:** Dr. Farzana Rashid, LCWU, Lahore **Moderator:** Ms. Maryam Ramzan		
Keynote speaker 11:30 am - 11:50 am		**Small interfering RNAs targeting *agrA* and *sarA* attenuate pathogenesis of *Staphylococcus aureus*** *Dr. Paul Dean Brown, Jamaica*
Keynote speaker 11:50 am -12:10 pm		**Prevalence of pathogenic free-living amoeba in diverse environmental resources across Pakistan and its impact on public health in future** *Prof. Abdul Matin, University of Baltistan, Skardu, Gilgit-Baltistan*
12:10 pm	12:20 pm	**Knowledge and Attitude Towards Vaccination and its Impact on Different Variants of SARS-COV-2 Among General Public of Pakistan: A Cross Sectional Study** *Asad Ali, Provincial Public Health Reference Laboratory, Lahore, Punjab*
12:20 pm	12:30 pm	**Assessment of biosafety implementation in clinical diagnostic laboratories in Pakistan in relevance to the COVID-19 pandemic** *Mamoona Sattar, Donghua University, China*
12:30 pm	12:40 pm	**Mask using practices during current pandemic** *Hafsa Khalid, PCSIR, Karachi, Sindh*
12:40 pm	12:50 pm	**Microbial Pollution and its Impacts on Community residing near Sewerage Drains of Lahore, Pakistan.** *Farzana Rashid, Lahore College for Women University, Lahore, Punjab*
12:50 pm	01:00 pm	**Knowledge and Attitudes towards stethoscope hygiene and bacterial contamination.** *Khalid Ahmed, Aga Khan University, Karachi, Sindh*
01:00 pm	01:10 pm	**Sero-epidemiology of Hepatitis C in healthy population of district Chiniot** *Hadeesa Raza Malik, Government College University Faisalabad, Punjab*

01:10 pm	01:20 pm	**COVID-19 vaccines** *Syeda Ghufrana, Jinnah University For Women, Karachi, Sindh*
01:20 pm	01:30 pm	**Prevalence of needle stick injury and nursing practices regarding safe injection and sharp disposal working in critical care of two tertiary care hospitals** *Shah Zeb, Saidhu Medical College, Swat, KPK*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B5 Quaid-e-Azam Auditorium Hall II (11:30 am To 1:30 pm) **Theme:** Industrial and Pharmaceutical Microbiology **Session Chair:** Dr. Ahsan Akhtar, Managing Director Aptly Pharmaceutical, Faisalabad **Session Co-Chair:** Dr. Malik Hassan Mahmood, GCUF, Faisalabad **Moderator:** Mr. Mubashir Raza		
11:30 am	11:50 am	**Antibiotic stewardship in regional context, challenges and opportunities** *Dr. Malik Hassan Mahmood, Government College University Faisalabad, Punjab*
11:50 am	12:00 pm	**Response Surface Methodology for Enhanced CMCase Production by B. Licheniformis TLW-3** *Tabassum Kiran, FUUAST, Karachi, Sindh*
12:00 pm	12:10 pm	**Bioevaluation of antioxidative and antimicrobial efficacy of indigenously produced varieties of Moringa oleifera leaves** *Razia Noreen, Government College University Faisalabad, Punjab*
12:10 pm	12:20 pm	**Biodegradation of Aflatoxin through environment friendly bacteria** *Ali Akbar, University of Baluchistan, Quetta, Baluchistan*
12:20 pm	12:30 pm	**Improving Microbial Population and Rice Production through Integrated Use of Organic and Mineral Nutrient Sources** *Amanat Ali, Nuclear Institute of Agriculture, Tando Jam, Sindh*
12:30 pm	12:40 pm	**Biodegradation and Decolorization of Azo dye RB-221 by novel strain Pannonibacter phragmitetus-IMI-1C isolated from TWW of Faisalabad.** *Fatima Mujahid, Government College University Faisalabad, Punjab*
12:40 pm	12:50 pm	**Entrepreneurship in STEM: Opportunities and Challenges** *Bushra Jamil, BJ Micro Lab, Rawalpindi, Punjab*
12:50 pm	01:00 pm	**Assessment of Biodegradation by *Bacillus subtilis* in combinations with Biosurfactant and extracellular Lipase**. *Rabeea Laeeq, University of Agriculture Faisalabad, Punjab*
01:00 pm	01:10 pm	**Analysis and Evaluation of Iron Chelating and Anti-Cancer Activities of Extract from *Streptomyces* spp.**, *Imran Shah, National University of Medical Sciences, Islamabad*
01:10 pm	01:20 pm	**Evolution of indigenously developed rapid identification of medically important Gram positive group of cocci by urea & arginine** *Mehak, Jinnah University For Women, Karachi, Sindh*
01:20 pm	01:30 pm	**Determination of anti-hyperglycemic potential of phyto-alkaloid in High Fat diet-Streptozotocin Administered Rats** *Shumaila Mehdi, Government College University Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B6 STC Hall (11:30 am To 1:30 pm) **Theme:** One Health and Zoonotic Diseases **Session Chair:** Dr. Abdul Shakoor Chaudhry, UK **Session Co-Chair:** Prof. Dr. Aftab Ahmad Anjum, UVAS, Lahore **Moderator:** Ms. Amina Mehmood		
Keynote speaker 11:30 am - 11:50 am		**Chronic Inflammation: A major cause of chronic diseases** *Prof. Dr. Aqeel Ahmed, Salim Habib University, Karachi, Sindh*
Keynote speaker 11:50 am - 12:10 pm		**Biomass and toxins optimization of *Clostridium perfringens* toxinotype D under dynamic parameters** *Aftab Ahmad Anjum, University of Veterinary & Animal Sciences, Lahore, Punjab*

12:10 pm	12:20 pm	**Evaluation of seroprevalence and associated risk factors of Toxoplasmosis in sheep and goats in District Jhang-Pakistan** *Muhammad Shafique, Government College University Faisalabad, Punjab*
12:20 pm	12:30 pm	**Characterization of staphylococci from selected fish species of local fish farms** *Muhammad Ali Syed, The University of Haripur, Haripur, KPK*
12:30 pm	12:40 pm	**Prevalence of *mcr-1* gene in *E. coli* isolated from the poultry fecal samples** *Iqra Bashir, Government College University Faisalabad, Punjab*
12:50 pm	01:00 pm	**Detection of mycotoxigenic fungi and mycotoxins in poultry feed from poultry farms in Baluchistan** *Ghulam Ishaq Khan, University of Baluchistan, Quetta, Baluchistan*
01:00 pm	01:10 pm	**Comparative drug susceptibility of colistin resistant *Escherichia coli* isolated from milk in district Mardan** *Khadija Altaf, Abdul Wali Khan University Mardan, KPK*
1:10 pm	01:20 pm	**Effect of exogenous protease on growth performance, microbial count and meat quality of broiler reared on fish meal-based diet** *Najam-Us-Sahar, University of Agriculture Faisalabad, Punjab*
01:20 pm	01:30 pm	**Microbial assessment of poultry meat for the presence of targeted bacteria and antibiotic resistance profile of *Salmonella spp.*** *Sadaf Tagar, Mehran University of Engineering and Technology, Jamshoro, Sindh*
01:30 pm	01:40 pm	**Epidemiological statistics of FMD virus serotypes A, O and Asia-1 in Punjab, Pakistan during 2014-2019.** *R. Rafique, University of Agriculture Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Scientific Research Posters at Quaid-e-Azam Auditorium Main Hall (01:30 pm To 02:00 pm)		
Lunch and Prayer Break (02:00 pm To 02:30 pm)		
Parallel Technical Session B7 Quaid-e-Azam Auditorium Main Hall (02:30 pm To 04:30 pm) **Theme:** Nanotechnology, Probiotics and Bacteriophages **Session Chair:** Prof. Dr. Shahana Urooj Kazmi, VC, WUS **Session Co-Chair:** Dr. Bushra Jamil, BJ Lab Islamabad **Moderator:** Ms. Uswa Siddique		
Keynote speaker 02:30 pm - 02:50 pm		**Vaccines in the control of COVID-19 - emerging and re-emerging diseases** *Prof. Dr. Shahana Urooj Kazmi, VC, WUS, Swabi*
02:50 pm	03:00 pm	**Isolation and Molecular Characterization of *Pseudomonas aeruginosa* Bacteriophages from Sewage water** *Abu Bakar Siddique, Government College University Faisalabad, Punjab*
03:00 pm	03:10 pm	**Potent Quinoline Inhibitors against Causative agents of gonorrhea and chancroid** *Aisha Khalil, ICCBS, University of Karachi, Sindh*
03:10 pm	03:20 pm	**Green synthesis, characterization and biological evaluation of silver nanoparticles using extract of Euphorbia Serpens Kunth** *Niaz Ali Khan, Kohat University of Science and Technology, Kohat, KPK*
03:20 pm	03:30 pm	**Synergistic effect of *Lavandula angustifolia* l oil on the antimicrobial activity of gentamicin against methicillin resistant *Staphylococcus aureus*** *Summra Ahmed, University of Karachi, Sindh*
03:30 pm	03:40 pm	**Silver Nanoparticles Inhibit Biofilm Formation and EPS Production of Multi-Drug Resistant *Klebsiella pneumoniae.*** *M. Hussnain Siddique, Government College University Faisalabad, Punjab*
03:40 pm	03:50 pm	**Antibacterial activity of Cuminum cyminum against Multidrug-Resistant Bacteria** *Zainab Yaseen, Government College University Faisalabad, Punjab*
03:50 pm	04:00 pm	**Molecular Characterization and Antifungal susceptibility of Ochratoxin A Producing Fungi isolated from Poultry Feed to Plant Essential oils** *Gul Naz, Government College University Faisalabad, Punjab*
04:00 pm	04:10 pm	**Immunomodulatory role of Enterococcus faecium strain LCM08 in mice**

		Muneera Naz Baloch, University of Karachi, Sindh
04:10 pm	04:20 pm	**Antifungal potential of Chitinase extracted from *Bacillus subtilis* under In-Vitro condition** *Maheen Shafiq, University of Agriculture Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B8 Quaid-e-Azam Auditorium Hall II (02:30 pm To 04:30 pm) **Theme:** Agricultural Biotechnology and Environmental Microbiology **Session Chair:** Prof. Dr. Mujadad Ur Rehman, AUST, Abbottabad **Session Co-Chair:** Dr. Ali Akbar UOB, Quetta **Moderator:** Ms. Zainab Shabir		
Keynote speaker 02:30 pm - 02:50 pm		**Implications of agricultural biotechnology for plant improvement in a variable climate** *Prof. Dr. Muhammad Iqbal, Government College University Faisalabad, Punjab*
02:50 pm	03:00 pm	**Control of disease caused by *Phytophthora capsici* in pepper plant using the soil-borne *Bacillus spp.*, isolated from Kohat Khyber Pakhtunkhwa** *Mutahira Subhan, Kohat University of Science and Technology, Kohat, KPK*
03:00 pm	03:10 pm	**Immuno-modulatory Effect of Dietary Probiotic Supplementation on Peste des Petitis Ruminants Vaccine in Goats** *Ayesha Gill, Nuclear Institute for Agriculture & Biology, Faisalabad, Punjab*
03:10 pm	03:20 pm	**Fungal Diversity in different microenvironment of dust** *Saqib Iqbal, Kohat University of Science and Technology, KPK*
03:20 pm	03:30 pm	**Investigating the impact of combined application of *Bravibacterium spp.*, and nanoparticle on the alleviation of chromium stress in Brassica plants** *Muhammad Hassan Akhtar, Government College University Faisalabad, Punjab*
03:30 pm	03:40 pm	**Plant based products as microbiological media: A double green approach** *Sadia Khalil, FAUUST, Karachi, Sindh*
03:40 pm	03:50 pm	**Optimization of condition for Methanogenic Bacteria in Marai Village for Biogas Production in Portable Anaerobic Digester** *Niaz Muhammad, Kohat University of Science and Technology, KPK*
03:50 pm	04:00 pm	**Bioremediatory potential of PGPR to alleviate acid stress on the growth of *Triticum sativum*** *Ambreen Ahmed, University of the Punjab, Lahore, Punjab*
04:00 pm	04:10 pm	**Isolation, Screening and Biochemical Characterization of Plant Growth Promoting *Rhizobacteria* from chilli eminent growing areas of Sindh** *Qurban Ali Panhwar, Nuclear Institute of Agriculture, Tando Jam, Sindh*
04:20 pm	04:20 pm	**Insecticidal activity of *Bacillus* strains isolated from the local fields of Kohat against the pests belonging to order *Lepidoptera*** *Syeda Fatima Gilani, Kohat University of Science and Technology, KPK*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session B9 STC Hall (02:30 pm To 04:30 pm) **Theme:** Antimicrobial Resistance, Antimicrobial Agents, Nano Antibiotics **Session Chair:** Prof. Dr. Abdul Matin, UOB, Skardu **Session Co-Chair:** Dr. Hazir Rehman, AWKUM, Mardan **Moderator:** Ms. Khadija Tufail		
Keynote speaker 02:30 pm - 02:50 pm		**Microbes for Food and Health** *Dr. Abdul Shakoor Chaudhry, UK*
02:50 pm	03:00 pm	**Molecular characterization of extensively drug-resistant *Salmonella* Typhi clinical isolates from Lahore, Pakistan** *Mohsin Khurshid, Government College University Faisalabad, Punjab*
03:00 pm	03:10 pm	**Characterization of bacterial pathogens from commercially available ready to eat salads and vegetables used in salads sold in Hyderabad, Sindh, Pakistan** *Aijaz Hussain Soomro, Sindh Agriculture University, Tando Jam, Sindh*
03:10 pm	03:20 pm	**Isolation of MDR *Campylobacter spp.*, in commercial poultry and *tet* (o) mediated gene resistance against tetracycline.** *Usman Waheed, University of Veterinary and Animal Sciences, Jhang*

03:20 pm	03:30 pm	**Prevalence of *QnrA* and *QnrB* resistance genes in *Klebsiella pneumoniae* comparing with *Lactobacillus.*** *Alishbah Roobi, University of Agriculture Faisalabad, Punjab*
03:30 pm	03:40 pm	**Isolation and identification of Staphylococcus aureus from commercially important fishes of Karachi local fish markets and its antibiotics sensitivity** *Syeda Sadaf Akber, Hashmanis Group of Hospitals, Karachi*
03:40 pm	03:50 pm	**Prevalence and antimicrobial sensitivity pattern of ESBL producing Gram negative bacteria** *Abdul Wahab, Government College University Faisalabad, Punjab*
03:50 pm	04:00 pm	**Life Saving Antibiotics and Bacterial Resistance** *Hinz Zaidi, University of Karachi, Sindh*
04:00 pm	04:10 pm	**Endocrine role of gut microbiota and its therapeutic implications for neurological and psychiatric disorders** *Aniqa Arshad, Government College University Faisalabad, Punjab*
04:10 pm	04:20 pm	**Prevalence and antimicrobial susceptibity paradigm of bacteria isolated from Urinary Tract Infections** *Naila Ziafat, Government College University Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Tea (04:30 pm To 05:00 pm)		
Friday, December 24, 2021 (Day 03)		
Parallel Technical Session C1 Quaid-e-Azam Auditorium Main Hall (08:30 am To 10:30 am) **Theme:** Clinical Microbiology and Infectious Diseases **Session Chair:** Dr. Omar Bagasra, USA **Session Co-Chair:** Dr. Bushra Uzair, IIU, Islamabad **Moderator:** Mr. Zeeshan		
Keynote speaker 08:30 am - 08:50 am		**Infectivity of Human Olfactory Neurons to SARS-CoV-2: A Link to Anosmia** *Dr. Omar Bagasra, USA*
08:50 am	09:00 am	**Development of topically applied films for curing burn patients** *Bushra Jamil, BJ Micro lab, Rawalpindi, Punjab*
09:00 am	09:10 am	**Detection of *Helicobacter pylori* Through Microscopy and PCR in Gastric Biopsies** *Tahir Ullah, Kohat University of Science and Technology, KPK*
09:10 am	09:20 am	**The molecular basis of extensively drug resistant *Salmonella* Typhi isolates from pediatric septicemia patients** *Iqra Latif, Government College University Faisalabad, Punjab*
09:20 am	09:30 am	**Molecular Characterization of *E. coli* Associated Neonatal Sepsis in Pakistan** *Amna Mumtaz, COMSATS, Islamabad*
09:30 am	09:40 am	**Genetic diversity of *Staphylococcus aureus* strains from a tertiary care hospital in Rawalpindi, Pakistan** *Muhammad Ali Syed, The University of Haripur, Haripur, KPK*
09:40 am	09:50 am	**Transfusion Transmissible Infections among multi transfused Beta-thalassemia patients** *Muhammad Mudassar, Government College University Faisalabad, Punjab*
09:50 am	10:00 am	**Occurrence of *Ampc* beta-lactamase producing *Pseudomonas aeruginosa* isolated from skin infection** *Hira Naeem, Government College University Faisalabad, Punjab*
10:00 am	10:10 am	***Sirt2* Gene Expression in Gastric Cancer Patients of Peshawar** *Ali Raza Shah, Kohat University of Science and Technology, KPK*
10:10 am	10:20 am	**Occurrence and antibiotics sensitivity profiling of bacteria from operation theatres of DHQ Hospital, Narowal Punjab Pakistan** *Muhammad Atique Arshad, Government College University Faisalabad, Punjab*
10:20 am	10:30am	**Evaluation of antibacterial activity of *Nigella sativa* against Multidrug Resistant Bacteria** *Arooba Khalid, Government College University Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session C2 Quaid-e-Azam Auditorium Hall II (08:30 am To 10:30 am)		

Theme: Nanotechnology, Probiotics and Bacteriophages **Session Chair:** Dr. Arslan Zaidi, NIBGE **Session Co-Chair:** Dr. Inam Ali Larik, SALU, Khairpur **Moderator:** Ms. Rida Zafar		
Keynote speaker 08:30 am - 08:50 am		**National Probiotics Lab: Strategy for regulation, development & commercialization of probiotics in Pakistan** *Dr. Arsalan Zaidi, National Institute for Biotechnology and Genetic Engineering Faisalabad, Punjab*
08:50 am	09:00 am	**Green Synthesized Functionalized Microspheres for Targeted Delivery of Essential Oil Entrapped TiO2 nanoparticles against MDR *S. aureus*** *Bushra Uzair, International Islamic University, Islamabad*
09:00 am	09:10 am	**Moxifloxacin HCl loaded biodegradable chitosan nanoparticles for potential antibacterial and wound healing efficacy** *Akhtar Rasula, Government College University Faisalabad, Punjab*
09:10 am	09:20 am	**Antibacterial activity of tio2 nanoparticles and their effect in mice** *Farzana Rashid, Lahore College for Women University, Lahore, Punjab*
09:20 am	09:30 am	**Antibacterial potential of plants and algal extracts against clinically important bacteria and fungi** *Maham Jameel Siddiqui, FUUAST, Karachi, Sindh*
09:30 am	09:40 am	**Resistance modulation through nanoparticle coupled antibiotics against enteric *S. aureus* of Houbara bustard bird** *Amjad Islam, Cholistan University of Veterinary & Animal Sciences Bahawalpur*
09:40 am	09:50 am	**Role of probiotics in the reduction of Ochratoxin A form poultry feed** *Kinza Kiran, Government College University Faisalabad, Punjab*
09:50 am	10:00 am	**Antibacterial activity of silver nanoparticles against clinical isolates of *Klebsiella pneumoniae*** *Tabinda Khurshid, Government College University Faisalabad, Punjab*
10:00 am	10:10 am	**Antimicrobial activity of some medicinal plant extracts against bacteria causing diarrhea** *Barkat Ali, Dadabhoy Institute of Higher Education, Karachi, Sindh*
10:10 am	10:20 am	**Occurrence of hypervirulent Klebsiella pneumoniae in clinical settings and lytic potential of bacteriophages against the isolates** *Moeed Ahmad, Government College University, Faisalabad, Punjab*
Concluding Remarks by Session Chair and Distribution of Certificates		
Parallel Technical Session C3 STC Hall (08:30 am To 10:30 am) **Theme:** Industrial and Pharmaceutical Microbiology **Session Chair:** Prof. Dr. Shazia Tabassum Hakim, USA **Session Co-Chair:** Dr. Sadia, FUUAST, Karachi **Moderator:** Ms. Aimen Khalid		
Keynote speaker 08:30 am - 08:50 am		**COVID or Dikos Ntsaaígíí (the 'cough' that kills) and Indigenous Populations Globally** *Dr. Shazia Tabassum Hakim, USA*
08:50 am	09:00 am	**Evaluation of growth promotion potential of chromium tolerant PGPR using Zea mays L. in chromium contaminated soil** *Iman Fatima, University of the Punjab, Lahore, Punjab*
09:00 am	09:10 am	**Isolation of Bio surfactant producing bacteria from different fuel contaminated sites of Sindh** *Ubaidullah khan, FUUAST, Karachi, Sindh*
09:10 am	09:20 am	**Studying diversification of microbes in soil around industrial zones of Makori, Gurguri, Nashpa, district Karak, Khyber Pakhtunkhwa** *Naeem Usman, Kohat University of Science and Technology, Kohat, KPK*
09:20 am	09:30 am	**Assessment of Microbe as Potential Bioplastic Producing Entrant** *Fasiha Saeed, Jinnah University For Women, Karachi, Sindh*
09:30 am	09:40 am	**Bioremediation potential of wastewater in Karachi, Pakistan** *Hafsa Shaikh, Salim Habib University, Karachi, Sindh*
09:40 am	09:50 am	**Bacterial isolates and their antimicrobial susceptibility profile of superficial and deep-seated**

		Skin and Soft Tissue infections *Rao Abid, Sindh Institute of Urology & Transplantation, Karachi, Sindh*
09:50 am	10:00 am	**Preparation & Characterization of *E. coli* DH5α bacterial ghosts and their evaluation as a Drug Delivery Vehicle** *Sadia Masood, National University of Science & Technology, Islamabad*
10:00 am	10:10 am	**Ferulic acid production by *Lactobacillus acidophilus* through fermentation** *Lubna Soomro, Sindh Agriculture University, Tando Jam, Sindh*
10:10 am	10:20 am	**Bioremediation of hydrocarbons in wastewater by soil isolates** *Hafiza Shehla, Salim Habib University, Karachi, Sindh*
Concluding Remarks by Session Chair and Distribution of Certificates		
Scientific Research Posters at Quaid-e-Azam Auditorium Main Hall (10:30 pm To 11:00 pm)		
Closing Ceremony (11:00 am To 12:30 pm)		
11:00 am	11:10 am	Arrival and Seating of Honorable Guests & Chief Guest
11:10 am	11:20 am	Recitation of Holy Quran & Naat-e-Rasool Maqbool (SAW)
11:20 am	11:30 am	Remarks of the participants about the Conference
11:30 am	11:40 am	Conference Brief and Recommendations by **Prof. Dr. Muhammad Hidayat Rasool**, Chief Organizer/ Chairman, Department of Microbiology, GCUF
11:40 am	11:50 am	Address by **Prof. Dr. Shahana Urooj Kazmi,** Chairperson Central PSM-ASM/ VC, WUS KPK
11:50 am	12:00 pm	Address by **Prof. Dr. Iqrar Ahmed Khan**, Sitara-e-Imtiaz, Guest of Honor VC, UAF
12:00 pm	12:10 pm	Address by **Prof. Dr. Shahid Mahmood Baig,** (SI), Chief Guest/Chairman, Pakistan Science Foundation, Islamabad
12:10 pm	12:20 pm	Concluding Remarks by **Prof. Dr. Shahid Kamal**, Patron-in-Chief/ VC, GCUF
12:20 pm	12:30 pm	Distribution of Souvenirs
Lunch (12:30 pm To 1:00 pm)		

Workshops

Prof. Dr. Iqrar Ahmad Khan (S.I)
Vice Chancellor
University of Agriculture, Faisalabad

Cordially invites you
at the

Workshop on Laboratory Scale Screening of Multidrug Resistant Pathogens

at the Occasion of

13th Biennal Conference of Pakistan Society for Microbiology at GCUF

Convener:

Prof. Dr Sajjad ur Rahman
Director,
Institute of Microbiology, UAF

Focal Person:

Dr Mashkoor Mohsin,
Associate Professor,
Institute of Microbiology, UAF

Venue:

Monday; Decmber 20,2021
AMR research lab BSL2/3 Buildings UAF
Time: 9:30 AM to 2:00 PM

Contact:

mashkoormohsin@uaf.edu.pk
+92-300-9659046

Program Breakup

Time	Events
09:30-09:45 AM	Opening Ceremony
9:45-10:15 AM	Tea / Group Photo
10:30-11:30 AM	• Biochemical identification of bacteria using API-20E kit • Selective identification of critically important antimicrobials using antibiotic supplemented media
11:30-12:30 AM	• Antibiotic susceptibility testing using Kirby Bauer method • MIC using E-test strips
12:30-1:30 PM	PCR detection of antibiotic resistance genes
01:30- 02:00 PM	Closing Ceremony

Organized by: Institute of Microbiology,University of Agriculture ,Faisalabad

PSM-NIBGE Hands-on Training on Molecular Viral Diagnostics

at

National Institute for Biotechnology and Genetic Engineering (NIBGE)

on

Tuesday December 21, 2021

Resource persons
Dr. Imran Amin
Dr. Shahid Mansoor, SI
Dr. Waqas Rafiq
Mr. Muhammad Jawad Akbar Awan

Session 1		
Time	**Title**	**Speaker**
9:00- 9:40	An overview of current knowledge of virology	Dr. Shahid Mansoor SI
9:40- 10:20	Viral diagnostics and use of PCR and real-time quantitative PCR	Dr. Imran Amin
10:20-10:45	**Tea break**	
10:45- 1:30	DNA extraction and conventional PCR for viral diagnostics	Dr. Imran Amin Dr. Waqas Rafiq Mr. Muhammad Jawad
1:30-2:00	**Lunch/Prayer break**	
Session 2		
2.00-3:15	RNA extraction and real-time quantitative PCR for viral diagnostics (Ist part)	Dr. Imran Amin Dr. Waqas Rafiq Mr. Muhammad Jawad
3:15-3:30	**Tea break**	
3:30-5:00	RNA extraction and real-time quantitative PCR for viral diagnostics (2nd part)	Dr. Imran Amin Dr. Waqas Rafiq Mr. Muhammad Jawad

Who can apply?

1. MPhil/PhD Students with biotechnology/microbiology/genomics background
2. No. of students per course: 10-25
3. Course Fee: Rs. 3500.00

Venue: NIBGE Faisalabad

PSM-NIBGE Hands-on Workshop on Microbial Metagenomics and Bioimaging

at

National Institute for Biotechnology and Genetic Engineering (NIBGE)

on 21 December, 2021

Resource persons

Dr. Asma Imran
Mr. M. Farooq
Mr. Javed Iqbal
Mr. Ahmad

Session 1		
Time	**Title**	**Speaker**
9:00- 9:30	Metagenomics and bioimaging	Dr. Asma Imran
9:30- 10:00	Confocal Microscopy	Dr. Asma Imran
10:20-10:20	**Tea break**	
10:30- 1:30	DNA extraction from soil/microbes Microbial genomics Data interpretation and analysis	Mr. M. Farooq Mr. Ahmad Mr. Shoaib
1:30-2:00	**Lunch/Prayer break**	
Session 2		
2.00-3:15	Transmission Electron Microscopy	Mr. Javed Iqbal
3:15-3:30	**Tea break**	
3:30-5:00	Transmission Electron Microscopy	Mr. Javed Iqbal

Who can apply?

1. MPhil/PhD Students with biotechnology/microbiology/genomics background
2. No. of students per course: 10-25
3. Course Fee: Rs. 3500.00
4. Venue: NIBGE Faisalabad

ASM-PSM WORKSHOP AT GOVERNMENT COLLEGE UNIVERSITY FAISALABAD

ASM-PSM workshop " ONE HEALTH APPROACH - DISEASE SURVEILLANCE AND CONTROL IN PAKISTAN"

Workshop Facilitator/ Organizer: Fatima Aziz ASM Young Ambassador (Aga Khan University- Karachi)

Speakers:

Ms. Fatima Aziz (ASM Young Ambassador/ Aga Khan University)
Dr. Zahida Fatima, (FAO/ PARC– Islamabad)
Dr. Ghulam Fatima (Civil Hospital Karachi
Dr. Usman Qamar (Govt College University Faisalabad)
Dr. Shahana Urooj Kazmi (WUS - KPK)

Session Details	Speaker/ Facilitator
Ms. Fatima Aziz	Emergency preparedness & biorisk management.
Dr. Zahida Fatima	ONE HEALTH: A Perspective for Pakistan
Dr. Ghulam Fatima	Nosocomial infections: Epidemiology, prevention and control
Dr. Usman Qamar	Advancing the One Health response to Antimicrobial Resistance
Dr. Shahana urooj Kazmi	Concluding Remarks

Who can apply?

The students with One Health, Biosafety, Infectious Diseases/ Zoonotic diseases Microbiology, Public Health
Preferably: M.Phil./ PhD students and professionals from Biomedical Laboratories / veterinarian
Number of participants: 25-30
Duration: 3-4hours
Venue: NIBGE Faisalabad

Session Description:

The One Health (OH) concept recognizes that the health of humans is connected to the health of animals and the environment. One Health is a day-to-day and very practical concept and forging a strong links between human and animal health ecosystem, the environment and public policy. This workshop aims to emphasize on **One Health concept in Pakistan.** This workshop will also bring together a significant number of diverse researchers to disseminate information about Zoonoses, pathogen diversity, antibiotic challenges, pandemic preparedness in emerging infectious diseases & biorisk assessment.

Learning objective:

Implementing One Health strategies for disease surveillance, response, preparedness, prevention and control activities in the current and future pandemic preparedness.

Outcome of the workshop:

Upon completion of this workshop, the participants will be able to:

1. Recognize and understand the concept of One Health and zoonotic potential of presented diseases
2. Control of emerging and re-emerging infectious diseases
3. Measures to tackle antimicrobial resistance
4. Biorisk Management under One health umbrella

ASM-PSM WORKSHOP " BIOSAFETY AND BIOSECURITY"

Workshop Facilitator/ Organizer:
Dr. Nain Tara Bukhari
HOD & Assistant Professor, Department of Clinical Laboratory Sciences, Women University Swabi, KPK, Pakistan
Dr. Amtul Sami
HOD & Assistant Professor, Department of Microbiology & Molecular Biotechnology, Women University Swabi, KPK, Pakistan

Objective:
Epidemics of disease like corona virus might pose substantial tasks to international safety committees by failing national economies, and automatically effect on international employment. Biosafety is complementary to biosecurity, when working with potentially infectious microorganisms and other biological hazards and refers to the implementation of laboratory practices and procedures, specific construction features of laboratory facilities, safety equipment, and appropriate occupational health programs**.** This workshop will focus to converge the safety of diverse researchers and provide information about the safety practice and measures in order to handle the pathogenic organism to reduce the biorisk.

Dr. Nain Tara Bukhari HOD & Assistant Professor,
Dr. Amtul Sami HOD & Assistant Professor,
Dr. Muhammad Usman Qamar, Assistant Professor GCUF
Number of participants: 30-40
Workshop Duration: 3hours

Session Description:
Dr Nain Tara Bukhari "Role of Biosafety in Hospital Acquired Infection"
Lecture with hands on learning which include: Hand Washing Techniques, PPEs, Donning & Doffing, Spill Kit preparation

Dr. Amtul Sami "Sterilization and Good Microbiological Practices"
Lectures and spill management procedure

Dr. Muhammad Usman Qamar "Laboratoey Acquired Infections"
Lecture and SOP for needle stick injury procedure.

Learning objective:
Main objectives of this workshop are to highlight on Biosafety and Biosecurity in medical practices during laboratory working and personal hygiene in pandemic situation**.**

Outcome of the workshop:
After completion of this workshop, the participant will be able to:
1. Understand the concept of personal hygiene and safety practices during working in microbiological lab.
2. Spill managements after working in pathogenic laboratory
3. Measures to confrontation of antimicrobial pathogenicity.

4. Biosafety and biosecurity practices.

WORKSHOP ON RESEARCH ETHICS AND INTEGRITY

Facilitators:

Professor Dr. Syed A. Aziz (Ph.D.)
Dr. Sadaf Ahmed (Ph.D.)
Shamoon Noushad (M.Phil, CRCP)

The argument for the use of animals in research, and in particular in research for human conditions, tends to be a practical one. Although there are some suffering and harm to the animals, the benefits to people and animals outweigh the disadvantages: the ends justify the means. While there are still useful aspects of this work's justification, it is quite different from research on animals in which the goal is to benefit humans. In comparison, Human research is conducted with or about people, or their data or tissue. Therefore, human participation in the study is to be understood broadly to include human beings' involvement.

Outline

How To Deal with Specimen, Animals & Humans Within Boundaries of Ethics????

Benefits as a Result of Animal Research

The Legal Obligations

What are the Issues with Animal Research?

Basic animal rights

The 3Rs

Animal (Scientific Procedures) Act

Laboratory Animal "Program

A Historical Perspective on the Animal Welfare Regulations

Case Study

Human subjects

Evolution of Research Ethics Guidelines

Principles of Research Ethics

Vulnerable Populations

Foundations of Ethics in Research

Ethical Codes, Standards and Regulations

Pillars of Protection for Human Subjects Research

Case Study

WORKSHOP ON ACADEMIC RESEARCH WRITING

The workshop bridges the research, writing and publishing stages of a project, emphasizing research output and developing a publication strategy. The workshop will tackle issues including publication timeframes, rejection rates, optimizing your submissions, and the research strategies and skills you need to help your research blossom into a beautiful publication (while reaching the best audience for your work.)

Duration	4 hours
Target Audience:	This workshop is open to all researchers at all levels and will be particularly useful for those beginning their research careers.
Outline	Where & How to publish, Referencing, Plagiarism, How to execute a paper, Journal submission Ethics, Impact factor; myths & facts ,Peer review, how reviewers access manuscript.
Outcomes	Participants will be able to structure good manuscripts and to make it as revealing as possible Builds a better sense of how each section of a document operates as an individual informative unit and as part of a larger logical whole.

Training in how to respond to editors and reviewers who read your work.

Improved overall writing skills with immediate and individual feedback.

Benefits for the Individual, Learn and gain knowledge first hand from the researchers, Open forum for discussion – easy learning.

Through research-based learning, students can develop the intellectual skills of critical analysis and also valuable transferable skills such as group work, time and resource management and data handling.

WORKSHOP ON HOW TO WRITE A RESEARCH PROPOSAL

This workshop will try to appraise the techniques in writing a grant proposal. This exercise will bridge the gap between a request for a grand to obtain the research fund. The workshop will tackle the issues in formulating a good proposal for successful funding.

Duration	4 hours
Target Audience:	This workshop is open to all researchers at all levels and will be particularly useful for those beginning their research careers.
Outline	How to write the proposal, Referencing, how to the grant, where to submit, Ethics, Peer review, Outcomes Participants will be able to formulate a good grant proposal and to make it as revealing as possible Builds a better sense of how each section of a document operates as an individual informative unit and as part of a larger logical whole.

Training in how to respond to reviewers., Benefits for the Individual, Learn and gain knowledge firsthand from the researchers., Open forum for discussion – easy learning., Through research-based learning, students can develop the intellectual skills of critical analysis and also valuable transferable skills such as group work, time and resource management and data handling.

Glimpses of 12th International Biennial Conference of Pakistan Society for Microbiology


Glimpses of 12th International Biennial Conference of Pakistan Society for Microbiology
GENERATION OF MICROBIAL THERAPEUTICS
February 20-22, 2019


PSM-Covid-19 Awareness Session 2020-2021
Novel Corona Virus Epidemic
Pandemic : Impact on Global Health,
Mobility and Economy
CONSUMERS ASSOCIATION OF PAKISTAN

GLIMPSES OF PSM- WUS COVID19 SCREENING AND VACCINATION CAMP AT WOMEN UNIVERSITY SWABI

Organizing Committee Meetings TIBC-PSM 2021 at GCUF, NIBGE, NAIB, WUS, etc

KEYNOTE SPEAKERS

Abstracts of Keynote Speakers

Vaccines and Vaccination in the Control of New and Emerging Viral Disease Epidemics/ Pandemics

Prof. Dr. Shahana Urooj Kazmi, VC, Women University Swabi, KPK

New emerging and reemerging viral diseases have threatened humanity throughout history. A combination of different factors including environmental changes, increased urbanization, modernization favoring mobility of people, altered human behaviors and lack of public health facilities have played a key role in the emergence and uncontrolled spread of animal viruses –a threat to human health. The novel coronavirus 2 (SARS-CoV-2) responsible for the coronavirus disease COVID-19 pandemic engulfed the entire world in less than 6 months, with high mortality in the elderly and those with associated comorbidities. The pandemic severely disrupted the world economy by lockdowns, which was controlled by self-distancing, wearing masks, travel restrictions and avoiding gatherings. Now with high morbidity and millions of deaths, addition of vaccine(s) to existing countermeasures is the best hope for pandemic control. The unprecedented scale and rapidity of dissemination of recent emerging infectious diseases pose new challenges for vaccine developers, regulators and global health authorities. Vaccine manufacturing and distribution are complex and challenging. While speed is essential, clinical development to emergency use authorization, vaccine safety and surveillance of virus variants are also critical. Access to vaccines and vaccination needs to be prioritized in low- and middle-income countries. The combination of these factors ensures success of efforts to bring the current and any future emerging infectious disease pandemics to a close. For over 200 years, vaccines have protected us against diseases that threaten lives and prohibit our development, bringing us closer to a world free from many diseases. Investment and new research is enabling groundbreaking approaches to vaccine development, which changed the science of immunization forever, bringing us closer still to a healthier future. Vaccination has greatly reduced the burden of infectious diseases, but vaccine safety is important which gets more public attention than vaccination effectiveness, but independent experts have shown that vaccines are far safer than therapeutic medicines. Today, vaccines have an excellent safety record and most “vaccine scares” have been shown to be false alarms, some countries have recorded serious fall in vaccination coverage, causing re-emergence of pertussis and measles. Vaccines not only protect the immunized, but can also reduce disease among unimmunized individuals in the community through “indirect effects” or “herd protection. The decline of disease incidence is greater than the proportion of individuals immunized because vaccination reduces the spread of an infectious agent by reducing the amount and/or duration of pathogen shedding. Vaccines are the cornerstone of the management of infectious disease outbreaks and are the surest means to defuse pandemic and epidemic risk. The faster a vaccine is deployed, the faster an outbreak can be controlled. Standard vaccine development cycle is not suited to the needs of explosive pandemics. New vaccine platform technologies have shortened the cycle and made it possible for multiple vaccines to be more rapidly developed, tested and produced. Two COVID-19 vaccines were developed using mRNA technology (Pfizer–BioNTech and Moderna, both showing safety and high efficacy, approved by FDA for emergency use authorization. While innovative and encouraging for other Emerging Infectious Diseases, it is too early to say that mRNA vaccines represent a universal vaccine approach that could be broadly applied to other EIDs While COVID-19 mRNA vaccines are a useful proof of concept, gathering lessons from their large-scale deployment and effectiveness studies still requires more work and time. Despite advances in diagnostics, therapeutics and vaccines, world travel and increased global interdependence have added layers of complexity to containing these infectious diseases. As human societies grow in size and complexity, an endless variety of opportunities are created for infectious agents to emerge into the unfilled ecologic niches we continue to create, constant vulnerability of populations to emerging and reemerging pathogens and their respective risks, rapidly evolve into devastating outbreaks and pandemics. The understanding of emerging infectious diseases has evolved over the past two decades. A look back at the SARS-CoV outbreak in 2002 recorded few deaths and infections its high mortality and transmissibility caused significant global disruption The epidemic ended as work on vaccines was initiated. Traditionally research and development for vaccines takes between 5 and 10 years to develop a vaccine for an infectious agent. This approach is not well suited for the needs imposed by the emergence of a new pathogen during an epidemic. What made the COVID-19 pandemic remarkable is that the whole research and development pipeline, from the first SARS-CoV-2 viral sequenced to interim analyses of vaccine efficacy trials, was accomplished in just under 300 days. Realizing the shortcomings in vaccine development during public health emergencies – an NGO – CEPI contributed to timely vaccine development capabilities in anticipation of epidemics. CEPI initially focused on diseases of WHO priority

pathogens for EIDs—Middle East respiratory syndrome (MERS), Lassa fever, Nipah, Rift Valley fever (RVF) and chikungunya etc based on sequencing to clinical trials in weeks rather than months or years, such as mRNA and DNA technology, platforms that were useful when COVID-19 was declared a global health emergency in January 2020, and a pandemic in March 2020. Recombinant proteins vary greatly in design for the same pathogen (subunit, virus-like particles) and are often formulated with adjuvants but have longer development times. Virus-like particle-based vaccines used for hepatitis B and human papillomavirus are safe, highly immunogenic, efficacious and easy to manufacture in large quantity. The technology is also easily transferable. Whole inactivated pathogens (SARS-CoV-2, polio, cholera) or live attenuated vaccines (SARS-CoV-2, polio, chikungunya) are unique to each pathogen. Depending on the pathogen, these vaccines also may require biosafety level 3 manufacturing (at least for COVID-19 and polio), which may limit the possibility of technology transfer for increasing the global manufacturing capacity. Other vaccines are based on recombinant vector platforms, subdivided into nonreplicating vectors (adenovirus 5 (Ad5), Ad26, highly attenuated vectors and live attenuated vectors such as the measles-based vector or the vesicular stomatitis virus (VSV) vector. Either each vector is designed with specific inserts for the pathogen targeted, or the same vector can be designed with different inserts for the same disease. WHO prequalified, making VSV an attractive 'platform' for COVID-19 and perhaps for other EID vaccines although the −70 °C ultracold chain storage requirement still presents a big challenge. The lessons learned dealing with COVID-19 pandemic need to be applied for the development of future vaccines against emerging infectious diseases and novel pandemic pathogens. The permanent threat of emerging human pathogens calls for vigilance, surveillance and preparedness for vaccine development and deployment, all activities to be conducted flawlessly between epidemiologists, Microbiologists and other scientists, developers, human and veterinary health authorities, regulators and funders. Global health stakeholders have already learned the strategy to develop vaccines but they still need to have knowledge about making and using them with due regard to equity and access. Randomized controlled trials might underestimate the protective effect of vaccines at the population level. This would occur if the COVID-19 vaccine, in addition to conferring direct protection to individuals, reduces transmission of COVID-19 between individuals, providing protection to unvaccinated individuals and enhanced protection of vaccinated individuals in contact with vaccinated individuals. Vaccine-induced herd protection, which might be crucial to the public health value of a vaccine, will be missed when trials are individually randomized and analyses fail to take account of the geographical distribution of individuals in the population. SARS-CoV-2 is evolving, with new lineages being reported all over the world. Other variants of concern have been described in the UK, Brazil, South Africa with enhance lethality and transmissibility, having increased affinity for the human ACE2 receptor. The B.1.351 variant with nine spike alterations; it rapidly emerged in South Africa during the second half of 2020 and was found to evade neutralizing antibodies elicited by infection and vaccination with previously circulating lineages. For many people who have already been infected with SARS-CoV-2 globally with some level of immunity, new variants such as, Omicron pose a significant reinfection risk, although vaccine-induced cell-mediated immune responses might mitigate this risk. We do not know how variants will evolve under vaccine-induced immune pressure during the vaccination or whether choices that alter the schedule or dose may effect virus evolution. Whether current vaccines will be effective against UK and Brazil variants requires surveillance in both humans and animals. With the recent discovery of Omicron Variant of COVID-19, the global community is now focused to determine the protective potential of current vaccines against Omicron as well as its transmissibility and virulence potential – through sharing of genomic sequencing data, surveillance studies and vaccination status. In order to continue fighting the current and future pandemics, we need to collaborate and develop a global network of pathogen sequencing, surveillance and diagnostic infrastructure to help us detect disease threats. Vaccination is the best tool to protect our community against this devastating virus, we therefore urge all eligible to get vaccinated as well as get a Booster in addition to strictly following SOPs, health and safety measures including masking, social distancing and hand washing.

Contribution as Microbiologist in Training and Capacity Building for COVID-19 Diagnostics and Research in Current Pandemic: Sharing Two Years Personal Experience

Dr. Essa Abdullah Memorial Lecture,

Prof. Dr. Saeed Khan, DUHS, ASM Country Ambassador

The World Health Assembly have placed specific responsibilities on WHO Member States for building and strengthening national capacities for the surveillance, detection, assessment, early notification and response to disease outbreaks and other emergencies of potential public health concern. Laboratories and Microbiologist are obviously playing a critical role in this surveillance and response process. In this framework, monitoring and evaluation of laboratory capacity require a standardized approach and methodology. Therefore, in the beginning of the pandemic, I along with my students developed a in-house method to investigate SARS-CoV-2. We prepared and validated our own Kit. For this WHO and Health Department, Sindh government selected 8 different health care facilities in Sindh to facilitate and provide training to their staff to follow the SOPS regarding response to COVID and good laboratory practice. For variant detection we prepared our own in-house PCR kit for detection of different variants of COVID-19. The amplified product was then sequenced by Sanger sequencing and results were analyzed using Chromas and Mega 7. Some of the mutations studied for variant assessment were D614G, N501Y, 69/70 deletion, E484K, and P681H. For verification of B.1.1.7, the results were further established using SARS-CoV-2 Variant B.1.1.7 Identification Kit by Real-time PCR Melting Curve Analysis. Between July and November, the GSD Nova Type III SARS-CoV-2 Kit was used for the qualitative detection of mutations associated with the Beta, Gamma, Epsilon, Kappa, and Delta variants. Strengthening program resulted that laboratoris does not have any written SOPs for test that were performed nor for the rules and regulation followed by the lab. The staff did not wear PPE due to unavailability, there was no waste management system, and no facility for PCR in lab. There is lack of shortage of technicians, no quality assurance process was followed. After the training the developed SOPS are in initial implementation phase which required serious effort from the laboratory leadership. The staff are now wearing some PPE however there is shortage of PPE in the laboratory. Emergency response, biohazard signs, donning/doffing, spill SOPS are pasted on walls of required areas. For variant detection our data between March and June, identified that Alpha variant was circulating in different areas of Sindh. Samples that were analyzed between July and November among which we have identified Delta variant that was more prevalent. For validation of results, samples were confirmed by Sanger sequencing, which remains the gold standard for genomic surveillance in clinical research. The overall result of Laboratory Strengthening Program shows that beside limited resources the implementation of basic rules and regulation has significantly improved the practices of laboratory workers. Behavioral change was observed at every lab in second visit. They were more conscious about the risk assessment before proceedings to their work in laboratory. After training we observed significant change in awareness level, practices, and attitude of the employees. The identification of the Alpha variant in Sindh during the period of March-June corresponds to its rapid transmission across the globe within those few short months. Our results also show the prevalence of other variants within the population, accounting for more than half the samples that were analyzed. Between July and November, the Delta variant was the more dominant one among all samples that were examined during this period. With the emergence of these variants, it is important to strengthen quarantine programs and improvise better lockdown measures to control virus transmission effectively. Furthermore, by detecting the presence of these variants, we can assess the relationship between COVID-19 vaccine efficacy against these mutations. This

data also represents the need to check for variants, other than the ones reported, in the country as this remains the crucial time for their detection.

Quality High Through Put Techniques for Diagnosis for Covid-19 Infection

Dr. Kamran Shaukat, UK

A novel strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) disease (COVID-19) has been recently identified as an infectious disease affecting the respiratory system of humans. This disease is caused by SARS-CoV-2 that was identified in Chinese patients having severe pneumonia and flu-like symptoms. COVID-19 is a contagious disease that spreads rapidly *via* droplet particles arising through sneezing and coughing action of an infected person. World health organization declared COVID-19 outbreak a public health emergency of international concern on 30 of January 2020. Without question, COVID-19 is the most intensively studied infectious disease in history with >125,000 PubMed citations from January 2020 to April 2021. This contrasts with influenza, about 8,000 citations, and tuberculosis, 10,000 citations in the same time period. Since COVID 19 pandemic, several diagnostic approaches have been re-adapted, and also improved from the previous detections of SARS coronavirus. The best strategy to handle this situation seems to rely on a triad of detection methods: (i) highly sensitive and specific techniques as the gold standard method, (ii) easier and faster point of care tests accessible for large population screening, and (iii) serology assays to complement the direct detection and to use for surveillance. The diagnosis of positive cases is necessary to ensure prompt care to affected people and also to curb further spread of infection in the population. In this presentation, we will cover the techniques and tests currently available to produce quality high throughput results to combat the rapid transmission of virus.

Enhanced Solubilization and Purification of 3ABC Non-structural Protein of Foot-and-Mouth Disease Virus from Bacterial Inclusion Bodies

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Nonstructural 3ABC protein of foot and mouth disease virus (FMDV) is used to differentiate vaccinated from naturally infected animals. It is a polyprotein which is cleaved into membrane associated 3A protein, three copies of 3B and 3Cpro mediated by virally encoded 3C protease. The expression of this protein in E. coli results into the formation of inclusion bodies which require solubilization in high concentration of chaotropes and extensive refolding process prior to purification of the native protein. Protein aggregation during refolding leads to the poor recovery of protein in functional form. Alternatively, Mild solubilization methods have been proposed to recover the native and soluble protein from inclusion bodies present in E. coli. In this study, 3ABC protein was expressed predominantly as inclusion bodies using E. coli host and solubilized in mild non-ionic detergent followed by purification through Ni-NTA chromatography. The protein recovery using this solubilization method, showed higher yield as compared previously described solubilization methods for 3ABC protein. This method also favored higher stability of the 3ABC recombinant protein stored at different temperatures. The reactivity of the proteins was analyzed by western blotting and ELISA which showed their ability to use them as antigen for the development of immunoassays. In conclusion, this study demonstrates an efficient and high yielding purification method of protein without refolding process than previously described methods involving renaturation steps.

Circulation of SARS Cov-2 variant of concern (VOC) traced by Whole Genome Sequencing, the case of Sardinia Italy

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The World Health Organization (WHO) declared over 223 million coronavirus disease (COVID-19) confirmed cases, including more than 4,6 million deaths worldwide from the beginning of the pandemic. As seen for other viruses, the SARS-CoV-2 spread follows a fluctuating pattern displaying spikes and drops, indicating an increase or a reduction in the number of new infections. Two SARS-CoV-2 waves hit Sardinia (Italy) in 2020: the first occurred early in the year and asecond after the summer; while writing this paper, a third wave of DELTA variant, began in June 2021, is still ongoing. Our report investigates the pattern and the epidemiological context for the COVID-19 recorded cases in north Sardinia, explicitly focusing on the second surge, which started from the second week of August 2020 until the end of March 2021. Based on available data from the local public health laboratory and regional sources, we describe the distribution of the newly infected cases and define the impact of the SARS-CoV-2 variants of concern. Interestingly, we defined viral mutations that aid in vaccine evasion and breakthroughs and mutations that may limit the epidemiological transmission and spread of SARS-CoV-2. Our data will expand the understanding of the mechanisms that allowed the ALPHA and DELTA variants entry in Sardinia and help health authorities to predict the future waves of infections.

Antibacterial potential of Probiotic bacteria and their applications

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (WHO). The antibacterial potential of probiotic bacteria from different origins in Pakistan was explored. The molecular analysis based on 16s rRNA gene revealed the dominance of *Lactobacillus* in different sources with 5 other genera including *Enterococcus*, *Weissella*, *Streptococcus*, *Leuconostoc* and *Bacillus*. Shortlisting was done for their survival at *in vitro* GI conditions and antibacterial activity against human pathogens. Some probiotic strains were subjected active compound identification. Pilot scale studies are underway to use some probiotic Bacillus for control of hospital-borne pathogens by developing probiotic sanitizer. We also investigated the effects of Aloe vera gel (AvG) and multi-layered microencapsulation on the survival of *Lactobacillus* species in cottage cheese during 28 days of refrigerated storage. The results suggest that cottage cheese fortified with Aloe vera gel filled alginate-chitosan beads loaded with either *L. rhamnosus* or *L. plantarum* strains, can prevent pathogen invasion, maintain functional qualities, and deliver more probiotics to the human gut. *Lactobacillus*

rhamnosus GG (ATCC 531030) is a proved and widely used probiotic strain but it cannot metabolize lactose and degrade milk proteins. We made *L. rhamnosus* GG lactose positive and proteolytically active by conjugation with the dairy *Lactococcus lactis* subsp. *cremoris* NCDO 712 strain carrying the conjugative plasmid (pLP712) encoding lactose operon and the proteinase gene *(prtP)*. In contrast to its parental strain LGG, the ability of LAB49 to metabolize lactose and degrade casein enabled strong and fast growth in milk.

Analysis of SARS-CoV-2 and Factors Predicting Next Spillover of its More Contagious Variant.

Dr. Abdul Aziz, University of Ottawa Canada

At the beginning of 2020, the world has started experiencing the epidemic of a novel coronavirus; by the mid of March 2020, it has been declared a pandemic. The disease has been named COVID-19, and the virus labelled as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on the type of infection it is causing. Coronaviruses are not new to us, there are15 different coronaviruses known to us. In the last 20 years, this is the fourth coronavirus pandemic, and SARS-CoV-2 seems to be the deadliest among all with the ability to continue producing more contagious variants. In this review, we attempted to give an outline of the SARS-CoV-2 about its origin, transmission, tropism, zoonotic, vaccines and the factors that are contributing to its contagious and virulent nature. We also sought to predict future pandemics and commented on the impact of COVID-19 disease during pregnancy. There is evidence that the novel coronavirus can penetrate CNS and have an impact on the brain by cytokine storm or by rouge autoimmune effect. Accumulating evidence also indicates that the pandemic might have a massive impact on mental health particularly to those who are predisposed to COVID-19. Though a couple of approved vaccines are being in use, in the future, we may see some more vaccines and their effect around the globe. Our biggest concern is the constant mutation of SARS-CoV-2, recently a variant referred to as SARS-CoV-2 VUI 202012/01 (Variant Under the investigation, the year 2020, month 12, variant 01), has been identified through viral genomic sequencing in the United Kingdom (UK). It is defined by multiple spike protein mutations present as well as mutations in other genomic regions. As the COVID-19 pandemic continues, a new, highly transmissible form of SARS-CoV-2 has emerged, named as strain B.1.1.7, which was originally identified in the UK and includes the spike protein variant N501Y. Similarly, another more contagious lineage B.1.1.248, informally termed as the Brazil variant has 17 amino acid changes. In this review, our aim is to reveal the factors which can impede the ability of this highly lethal virus to reproduce into more contagious variants.

Pitfalls in Global Response to Infectious Diseases and its Impact on Global Health & Economy-Preparedness is Key Word to Remember

Habib Bokhari

Vice Chancellor, Kohsar University Murree

The significant burden of diseases so far has been estimated to result in >50 million total annual deaths worldwide and infectious diseases alone result in approximately half of them. The spectrum of emerging new diseases as well as re-emerging old diseases is on rise as their infectious agents evolve, adapt and spread at enormous speed in response to changing ecosystems, behavior and moving population patterns. The inequities of health status and disease burden as seen by the current ongoing pandemic reflect the fact that high income countries (HICs) with

the world's best health care infrastructure are finding it extremely difficult to cope with the ongoing onslaught of COVID-19 and what to say about low & middle income countries (LMICs) mainly due to wider gaps between have and have nots. The world is currently under siege of coronavirus outbreak with initial epicenter in Wuhan city, the critical challenge is how to respond to such catastrophic pandemic and develop rapid & cost-effective methodologies for extensive testing globally, sharing data, and developing early warning systems for better preparedness by coordinated efforts and keeping an eye not only on what is happening around the globe but also dealing with any other associated unforeseen challenges for effective timely response by preparing & engaging skilled workforce.

Molecular Characterization of Sars-Cov-2 Spike Gene from Suspected Patients

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Throughout history, infectious diseases with pandemic potential have often developed and spread. Humanity has previously been impacted by significant epidemics and pandemics, including the plague, cholera, influenza, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV). The world is now experiencing a new coronavirus epidemic dubbed as SARS-CoV-2. In this study, a total number of 50 nasopharyngeal swabs were collected from suspected patients in the sterile tube containing the virus transport medium (VTM). Prior to the screening and confirmation, RNA was extracted. Then, the samples were screened and confirmed by E gene, N gene, and RdRP gene using RT-qPCR by observing the CT-Values. The extracted RNA was converted to cDNA to amplify the partial Spike gene region including the receptor binding domain (RBD). Conventional PCR was setup and amplification were carried out under optimal conditions and with particular primers to obtain the desired Spike gene having product size of 719 bp. The amplified product was run through Gel electrophoresis to separate and visualize the product based on their molecular weight to get the desired amplicon size of the S gene. The product was visualized using Gel documentation system. Amplified product of Spike gene was purified from the gel. The purified DNA was sent to ZOKEYO Internationals, China for sequencing. The Clustal W technique was used to align all the sequences using Lasergene's EditSeq and MegAlign tools. Aligned sequences were submitted to the NCBI and accession numbers were obtained. During this study, E484k, N501Y and D614G mutations were observed. Due to the point mutation, the amino acid was changed leading to enhanced transmission, binding affinity to hACE2 receptor, and reduced sensitivity to antibody neutralization. These mutations constitute a public health problem around the world.

Small interfering RNAs targeting *agrA* and *sarA* attenuate pathogenesis of *Staphylococcus aureus*

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The use of small interfering RNA (siRNA) gene silencing is a promising therapeutic option as it does not impose selective pressure on bacteria that is often associated with the development of resistance. The study assessed the

effect of siRNA targeted to *sarA* and *agrA* in *S. aureus* and the relationship between the transcriptional response, biofilm formation and pathogenicity. siRNAs designed against *agrA* and *sarA* were electroporated into methicillin-resistant and methicillin-susceptible *S. aureus* strains. mRNA levels, growth kinetics, biofilm formation and minimal inhibitory concentration were measured. Efficacy of siRNA in bacteria was assessed using survival assays in a *C. elegans* model. Differences in gene expression before and after siRNA treatment were anaysed using the paired t-test, while the log rank test was used to assess the significance of any difference among survival rates of nematodes. Biofilm formation decreased significantly in siRNA treated strains and growth rates of siRNA treated strains were significantly higher compared to untreated strains. We observed significant decreases in the transcriptional response in siRNA treated strains, with concomitant significant increases in the lifespan of *C. elegans* worms exposed to siRNA-treated versus untreated strains. siRNA targeted to *agrA* and *sarA* lowered mRNA transcription and pathogenicity of *S. aureus.*

Revalence of Pathogenic Free-Living Amoeba in Diverse Environmental Resources Across Pakistan and Its Impact On Public Health in Future

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Free living amoebae (FLA) are opportunistic protozoan pathogens and therefore play a predatory role and help to control microbial communities in the ecosystem. In contrast, the ability of pathogenic FLA (*Acanthamoeba*, *Balamuthia* and *Naegleria*) to produce central nervous system infections in human especially is also a growing concern worldwide. Here, we evaluated prevalence of pathogenic FLA from environmental resources like air, soil and water across Pakistan. One hundred and twenty-one various water, 78 soil and 30 air samples were examined. FLA was identified by morphological characteristics of their cysts on non-nutrient agar plates seeded with *E. coli*. Additionally, the PCR was performed with genus-specific primers followed by direct sequencing of the PCR product for molecular identification. Overall, FLA was recovered from ~52 % of the examined samples. *Acanthamoeba* was found in 38 and 8 % and *Naegleria* in 18 and 5% of water and soil samples respectively while *Balamuthia* was not recovered from any medium. Interestingly *Acanthamoeba* was recovered on 30°C while two *Naegleria* species were successfully isolated and cultured on both 30 and 42°C. This is the first report demonstrating inclusive survey for pathogenic FLA from various environmental sources across Pakistan, which suggests FLA could be a potential threat to public health to which the population is exposed.

Antibiotic Stewardship in Regional Context, Challenges and Opportunities

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Antibiotic stewardship program is direly needed to combat global threat of antibiotic resistance. The study aimed to determine knowledge, perspectives and practices of attending physicians regarding various aspects of antibiotic stewardship program including antibiotic stewardship roles, rational use of antibiotics, antibiotic resistance, prescribing practices and factors associated with these practices. In this qualitative study, a total of 19 semi-

structured, in-depth interviews with doctors of three tertiary care public sector hospitals in Multan and Dera Ghazi Khan were conducted. The convenient sampling method was adopted to collect the data. Sample size was collected using saturation point criterion. Thematic analysis approach was used to deduce findings. The analysis of data yielded 04 major segments, 12 sub segments with 24 categories. The themes included, (i) perception about antibiotic use and antibiotic stewardship, (ii) antibiotic prescription practice with rational use and antibiotic resistance, (iii) strategies adopted by hospital management to ensure quality and safe distribution of antibiotics, (iv) implementation of antibiotic stewardship program: barriers, suggestion and future benefits. Physicians had misconceptions about the use of antibiotics. The perception regarding antibiotic stewardship programs has been found poor. Moreover, very few activities related to antibiotic stewardship program. The participants gave many suggestions for successful implementation of antibiotic stewardship program in order to overcome the challenge of antibiotic resistance, including development of guidelines for the use of antibiotics, strict legislation regarding use of antibiotics, active participation of healthcare professionals and awareness program among the general public about the use of antibiotics. This study concluded that improvement in the knowledge of doctors regarding antibiotic stewardship program is needed. It also highlighted the need for development of antibiogram of hospital and lack of rules for the safe use of antibiotics are the key elements promoting irrational utility of antibiotics and development of antibiotic resistance.

Chronic Inflammation: A Major Cause of Chronic Diseases

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Inflammation is a protective immune response against various agents, and plays a vital role in heal-ing the injuries. Inflammation may be acute or chronic. Chronic inflammation may have a negative impact on different tissues and organs. The symptoms in chronic inflammation symptoms are gen-erally vague that are usually overlooked. Chronic inflammation is an indication of some underlying problems which needs to be identified to protect the body from different damages that may lead to tissue death and internal scarring. Fur-thermore, old age, obesity, unhealthy diet, smoking, stress, sleep problem may contribute in increas-ing the risk of chronic inflammation. Some long-term diseases like asthma, peptic ulcer, tuberculo-sis, rheumatoid arthritis, peritonitis, Crohn's disease, sinusitis, active hepatitis etc., are also associ-ated with chronic inflammation. The hospitalised COVID-19 patients exhibit pneumonia, and pulmonary edema, also ARDS, which is a causative syndrome of death in 70% of the fatal cases, in which aggressive inflammatory re-sponses occur. Hospitalised patients with severe infection show high levels of IL-2, IL-7, IL-10, and many others cytokines in serum, suggesting that severe COVID-19 is dictated as a cytokine release syndrome, which is a disorder induced by cytokine storms. Among the elevated levels of inflamma-tory mediators, the blood levels of IL-6 are noticeably higher in non-survivors compared to sur-vivors. IL-6 is a key cytokine in the acute-phase inflammatory response that stimulates CRP and fibrinogen production in the liver, release of white blood cells and platelets from bone marrow, and activation of endothelium and hemostasis. CRP is a sensitive marker of systemic inflammation produced by the liver. CRP is a nonspecific acute-phase reactant that has traditionally been used to detect acute infection and inflammation. So the best practices would be to monitor health status by checking inflammatory markers such as IL-6, CRP, ESR etc., and taking appropriate medication, if needed, and consuming healthy diet (rich in antioxidants), containing fruits, nuts, vegetable, eggs etc., with at least mild exercise; and avoid-ing unhealthy lifestyle, consuming junk (toxic) food, alcohol, stop smoking and lazy life style

Implications of agricultural biotechnology for plant improvement in a variable climate

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The climate changes at global level are not only affecting plant productivity, but also threatening the ever-increasing human population that is projected to be a ten billion in the year 2050. Crop improvement is a continuous process that started since the advent of agriculture. However, current agricultural biotechnology is focused on producing genetically engineered plants (transgenic plants) to make superior and environment friendly plants as well as improving microbial inoculants to be used to control plant pests, as fertilizer supplements, and to aid in atmospheric nitrogen fixation. Similarly, plants have been modified for better yield and nutritional profile, herbicide tolerance, pest-resistance, and to produce plantibodies including edible vaccines. Plants especially trees could be modified for reduced lignin content to be used in a paper, and bioethanol industries. Further, the uses of new and innovative techniques are expected to improve plant productivity under abiotic stresses to ensure food security.

Microbes for Food and Health

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Abundant quantities of microbes such as bacteria, fungi, protozoa, and viruses occur naturally in various ecosystems. Some of these microbes play a significant role in the promotion of food supply whereas others could be beneficial or determinantal for the health of almost all biological systems. For example, specific microbes can facilitate nitrogen fixation in the soil to enrich leguminous plants to produce foods of good nutritional value. Some microbes are known to break highly fibrous or waste materials to synthesise valuable products. This is particularly relevant to anaerobic microbes that naturally exist in ruminant animals such as buffalo, camel, cattle, goats, and sheep. These animals can utilise lignocellulosic materials with the help of billions of rumen microbes to yield energy and microbial protein to satisfy their nutrient needs. In fact, these food producing animals perform an excellent work to biologically convert indigestible fibrous materials into nutrient rich foods such as meat and milk. Similar anaerobic microbial activities take place in the hind gut of almost all animals and human beings, but this happens on a much smaller scale. In addition, desirable microbes such as lactobacilli, can fight against

incoming pathogens to maintain gut integrity, motility, and health. Indeed, microbes such as yeast can enhance the quality and shelf life of baked or fermented food products. Conversely, microbes are known to produce poisons, toxins and allergies resulting in multiple diseases and fatalities of animals, plants, and human beings. Thus, an understanding of a chosen microbial ecosystem is essential to either harness their beneficial features or to develop protection strategies against their potential hazards in different situations. This paper will examine the opportunities and obstacles that are involved in studying the positive or negative aspects of some selected microbes by using only a few relevant examples of selected animal species.

Infectivity of Human Olfactory Neurons to SARS-CoV-2: A Link to Anosmia

Omar Bagasra, Pratima Pandey, Jessica R. Sanamandra, Jarrett M. Houston, Ewen McLean, Helmut Albrecht

We sought to determine whether SARS-CoV-2 infections are associated with anosmia and if this virus infects other neuronal cells. We utilized male and female olfactory neuronal cell lines as well other olfactory cells lines to determine the viral targets. Methods: We utilized four undifferentiated and two partially differentiated human developing neuronal cell lines. Infectivity was confirmed by RT-qPCR, immunofluorescence assay (IFA) probing with anti-SARS-CoV-2 antibody, evaluation of cytopathic effects and neurite formation. Since both olfactory cell lines were terminally differentiated, we induced partial differentiation of all cell lines with retinoic acid (RA) to determine whether differentiation was a factor in viral permissiveness. The expression of serine protease, transmembrane serine protease 2 (TMPRSS2), and angiotensin-converting enzyme II (ACE2) receptors were examined by RT-qPCR and IFA to determine the mechanism of viral entry. Results: Four-to-five days after exposure both olfactory cell lines exhibited morphological evidence of infection; IFA analyses indicated that ~30% of the neurons were SARS-CoV-2 positive. At two weeks, 70–80% were positive for SARS-CoV-2 antigens. The partially differentiated (CRL-2266 and CRL-2267) and undifferentiated cell lines (CRL 2142 CRL 2149, CRL-127 and CDL-2271) were essentially non-permissive. After RA treatment only CRL-127 exhibited slight permissiveness (RT-qPCR). The TMPRSS2 receptor showed high expression in olfactory neurons but low expression in RA treated CRL-127. ACE2 expression by RT exhibited high expression in olfactory neurons whereas other cell lines showed low expression including RA-treated cell lines. ACE2 expression slightly increased in CRL-127 post RA-treatment. Conclusions: Our studies confirm neurotropism of SARS-CoV-2 to olfactory neurons with viral entry likely mediated by TMPRSS2/ACE2. Other neuronal cell lines were non-permissive. Our results established that the nerve cells were infected regardless of male or female origin and strengthened the reported association of COVID-19 with loss of smell in infected individuals.

National Probiotics Lab: Strategy for regulation, development & commercialization of probiotics in Pakistan

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National reference laboratories are at the pinnacle of scientific services in modern societies. They have pivotal roles in the diagnosis, surveillance and analysis of public health data and play a ‘behind the scenes supportive role

in national food regulatory systems. The development of the PSDP driven National Probiotics Lab (NPL) project at NIBGE is an important step in governmental efforts to address the health and safety challenges posed by the influx of functional foods into the national market. This address gives an overview of the various research initiatives that NPL has undertaken to date, related to advances in the study of indigenous probiotic reservoirs (human and non-human), technological aspects of probiotic product development and commercial pilot ventures hand in glove with the local food industry. In addition, a special focus shall be placed on the regulatory aspects of nationally marketed supplements and functional foods to shed light on the vexing question of the proof of claims. An outlook on probiotic research and how we can capitalize on this rapidly evolving field will also be deliberated upon.

COVID or Dikos Ntsaaígíí (the 'cough' that kills) and Indigenous Populations Globally

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In technologically advanced countries like Canada and Brazil and in the US, Indigenous people been dying at disparate rates to the general population to the COVID-19. Conversely, there was one noteworthy exception i.e. indigenous Australians. They experienced almost six times less Covid-19 related disparities. Why? Because they were provided with flexible grant funding in the beginning of pandemic for their 110 remote communities, allowing local Indigenous health agencies and medicine men/ women to tackle the problem locally with appropriate culturally aware retort. As per United Nations' 2009 report, there are between 370 to 500 million Indigenous Peoples globally, in over 90 countries, representing approximately 5,000 different cultures and roughly 7,000 languages, who make up approximately 5-6 percent of the total global population. The legacy of inequality and exclusion has made these indigenous communities more vulnerable to the impacts of climate change and natural hazards, including disease outbreaks for example COVID-19. Majority of these communities often have minimal access to clean water, soap, personal protective equipment (PPE), enough food and public sanitation. Local medical services are underfunded for many. Hospitals and clinics do not have capacity to meet the high demand for diagnosis like COVID-19 testing and treatment in the general population, leave off the indigenous communities located farther away, who often experience stigma or discrimination. One major cause behind this situation is un-availability of local data. Many Indigenous Peoples across the globe lack access to data disaggregated by Indigenous connection or documentation like nation, tribe, and ethnicity. Even in the US, disaggregated data were not available for COVID19 infection rates among Indigenous Peoples at the start of the pandemic. Additionally, misclassification or lack of classification on death certificates also leads to unavailable or underreported COVID-19 mortality data. As a result, Indigenous Peoples lack the data to track the size, spread, and distribution of cases and fatalities for Indigenous nations and populations for prevention, surveillance, mitigation, and evaluation purposes. Overall there is a dire need for development, maintenance, storage and access to local indigenous data in order to develop better strategies to deal with the situations like this COVID-19 in future.

ABSTRACT FOR ORAL PRESENTATIONS

Whooping Cough Caused by Bordetella Pertussis and *Bordetella Para Pertussis* Among Patients of All Age Groups in Khyber Pakhtunkhwa Province of Pakistan

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Pertussis is a highly infectious respiratory tract infectious disease affecting people of all age groups. Pertussis resurgence has been reported from different countries of the world. The study was aimed to isolate and identify *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii* strains from typical and asymptomatic pertussis patients with unrecognized persistent cough. A cross sectional study was carried out in Khyber Pakhtunkhwa, Pakistan. Different hospitals and local areas were approached for sample collection. Participants of the study were typical, atypical or suspected cases of whooping cough. One hundred thirty-six samples were collected by cough plate method and by nasopharyngeal swabs (in case of infants). For culture, charcoal agar supplemented with 10% horse blood was used. Initial screening was carried by microscopy and growth patterns on selective medium and biochemical tests such as oxidase and urease tests. Molecular identification was performed by using primers for insertion sequences *IS481*, *IS1001*, *h1001* found in *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella holmesii* respectively whereas PCR for promoter region sequence of *Bordetella pertussis* was also performed for further confirmation of *B. pertussis*. Of 136 samples, 19 (13.9%) were found to be *Bordetella* by PCR for insertion sequence *IS481*, *IS1001*, *ptxP* and *h1001*. From 19 PCR positive isolates, 8 (42.1%) were found to be *B. parapertussis* while 11 (57.8%) as *Bordetella. pertussis*. Of 11 *Bordetella. pertussis* isolates, 5 (26.3%) were found negative for *ptxP* promotor region DNA sequence and were suspected as *Bordetella holmesii.* However, these isolates were not confirmed as *Bordetella holmesii* by PCR. It might possible that these isolates of *Bordetella pertussis* have mutant pertussis toxin promoter region or this sequence is absent. In the present study, both *Bordetella pertussis* and *Bordetella parapertussis* were recovered from typical as well as atypical cases. All age groups were found to be affected with the disease in this region. Change in the pathogen etiology and waning of vaccine induced immunity may be the major reasons behind resurgence of the disease.

Molecular Epidemiology of Tuberculosis and *rpoB* Gene Mutations in Mycobacterium Tuberculosis Isolated from Patients In Mardan Medical Complex, Mardan, Pakistan

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Tuberculosis remains in the top 10 killer diseases in the world. Good prognosis of tuberculosis is always linked to its early detection and thus helps in preventing the outbreaks. The current study was designed to assess the burden of TB disease among suspects who visited Mardan Medical complex, Mardan, and to check the frequency of *rpo*B gene mutation in *Mycobacterium tuberculosis* that confers resistance to rifampin. A total of 791 samples were processed (147 samples were analyzed through fluorescent microscopy, while the remaining 644 samples were tested via TB-PCR (GeneXpert). Five probes were used to amplify the complementary sites in the entire resistance hotspot region (81 bps) of the beta subunit of *M. tuberculosis* RNA polymerase. Results of the study revealed that 30 out of 147 patients were tested positive for *M. tuberculosis* on microscopy while the GeneXpert detected *M. tuberculosis* in 176 out of 644 tested samples. Among the GeneXpert positive samples, only 10 (5.31%) were identified to have a mutation in the *rpo*B gene. These mutants are resistant to one of the most potent antibiotics, rifampin, used for the treatment of Tuberculosis. Resistance to the last line drug is an alarming issue. It takes years and costs huge amount of money to make new drugs available in the market. Prompt and effective containment strategies at community level are the need of the day to control increasing morbidity and mortality due to Tuberculosis.

Molecular Detection of Extensively Drug-Resistant *salmonella* typhi and Carbapenem-Resistant Pathogens in Pediatric Septicemia Patients in Pakistan - A Public Health Concern.

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To determine the prevalence of multidrug (MDR) and extensively drug-resistant (XDR) pathogens from pediatric blood samples in total, 4543 children's blood samples were processed in the BacT/ALERT system. Confirmation

of the isolates and MIC was determined in VITEK® 2 system. Molecular identification of blaIMP, blaVIM and blaOXA-48 was done by PCR. Of 4543 blood cultures, 458 (10%) were positive for bacterial growth and Salmonella Typhi (415; 90%) remained the primary pathogens. Antibiogram revealed 208 (50.1%) and 137 (33%) were MDR and XDR S. Typhi, respectively. Klebsiella pneumoniae displayed 46% resistance to imipenem. One hundred twelve (81.7%) XDR Typhi were positive for blaCTXM, whereas 14 (66.6%) blaVIM were found in carbapenem-resistant bacteria.

Emergence of Diverse Genotypes of *S. aureus* Harboring Panton-Valentine Leukocidin (*pvl*) and Accessory Gene Regulator (*agr*)

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Staphylococcus aureus is an important human pathogen which can transmit in both hospital and the community. Methicillin resistant *S. aureus* (MRSA) inclines to be resistant towards several antibiotics. Methicillin resistance is conversed by the procurement of the mecA gene, present in mobile genetic element called staphylococcal cassette chromosome mec, SCC-mec. Additionally, Panton Valentine Leucocidin (pvl) is a gene which encodes a powerful cytotoxin which is sturdily allied with CA MRSA strains. Life frightening infections are chiefly related with CA MRSA that are more virulent because of presence of pvl gene. It is encoded by two genes lukS(PV) and lukF(PV). Agr gene is a quoram sensing gene and produce biofilm. The main purpose was to assess and analyze the molecular characterization of mecA, pvl and agr genes among clinical isolates of MRSA. Moreover, isolates of MSSA were included as control. The samples were isolated from clinical patients and normal healthy individuals. For this, 50 clinical samples were collected labeled as (S1-S50) and 30 samples from healthy individuals (H1-H30) were collected all of them were S. aureus. Almost all the strains were non-motile except S30 and H12. All samples were cultured on blood agar medium. All strains were gram positive cocci. Those strains were characterized morphologically by biochemical testing. The biochemical characterization showed that all the strains were DNase, Coagulase and catalase tests were positive while most of the strains were negative for oxidase test except S21, S37 and H11 and also negative for VP test. Results were positive for starch hydrolysis (66%), gelatin hydrolysis (68%), nitrate reduction (76%), MR (70%), citrate (85%) and urease tests (80%). and they were aerobic in nature having ability to form biofilm. The antibiotic susceptibility profiling was done for strains by Kirby-Bauer disk diffusion method. Twelve antibiotics were used. Vancomycin, chloramphenicol and Linezolid showed more susceptible towards isolated strains while penicillin and oxacillin were showed highest resistance. For genotypic analysis, DNA was extracted by GeneJET genomic DNA purification kit. The isolated DNA was then subjected to the PCR amplification for the presence of mecA, pvl and agr genes. PCR results indicated that the MRSA was 86% positive for pvl while 47% of MSSA were pvl positive while 12 % for agr gene and 62% positive for MSSA. On conclusion, this study revealed that the high percentage of the pvl and mecA genes are associated with the diseased population of MRSA while the healthy individuals also carry mecA and pvl gene in high amount 42% and 47% respectively, which is actually alarming. Our study showed antibiotic sensitivity level for various antibiotics. The isolated strains can further be studied for screening of other significant virulence factors for effective control of those superbugs.

New Delhi Metallo-lactamase *Escherichia coli* Belonging to Sequence Type 131 in Tertiary Care Hospitals of Southern Punjab, Pakistan

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New Delhi Metallo-lactamase belongs to B1 superfamily of Metallo-β-lactamase and is rapidly disseminated globally. blaNDM producing pathogens confer resistance against a wide range of antibiotics including carbapenems. In present study, 215 CREC isolates were obtained from 4091 E. coli isolates in clinical samples (respiratory tract sample; tracheal secretions, sputum; blood, tips, urine, catheters, fluids, wound swabs, and pus) from five hospitals of southern Punjab, Pakistan. Antimicrobial susceptibility testing and MICs of isolates were performed followed by blaNDM typing and multi-locus sequence typing (MLST) to characterize CREC strains. blaNDM-1 blaNDM-5 blaNDM-7 blaNDM-4 variants were identified in 30(52.6%), 18(31.6%), 7(12.3%), 2(3.5%) isolates respectively. The ESBL genes such as blaCTX-M (CTX-M1, CTX-M15) and blaTEM were also found whereas 16S methylases; rmtB and armA were found in 69 (63.3%) and 55 (50.5%) CREC isolates, respectively. MLST of blaNDM carrying E. coli revealed eight STs with ST131 belonging to 21 (37.8%) was the most prevalent sequence type followed by ST3329 11 (19.3%), ST2279 8 (14%), ST8051 7 (12.3%), ST88 4 (7%), ST6293 3 (5.3%), ST209 2 (3.5%) and ST3059 1 (1.8%) respectively. E. coli strains harboring blaNDM-1 and blaNDM-5 genes belonged to ST131, blaNDM-1 belonged to ST3329, blaNDM-5 belonged to ST2279, blaNDM-7 to ST8051, blaNDM-1 to ST88, blaNDM-1 to ST6923, blaNDM-4 to ST209, and blaNDM-1 to ST3059 respectively. Sequence type 131 was predominant ST of blaNDM positive strains. Large-scale surveillance studies coupled with active infection control policies are suggested on urgent basis to avoid an epidemic in future.

Antibiotic Resistance Modulation of Enteric *E. coli* Isolated from Houbara Bustard Bird

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Escherichia coli (E. coli) is major among gram negative bacteria that appears as commensal as well as pathogenic bacteria in wider range of hosts. Houbara bustard bird is among rare migratory birds whose health parameters with especial context to bacterial resistance are necessarily be monitored time to time. In current study, enteric E. coli of hourbara bustrard birds from Houbara Foundation International Bahawalpur were targeted for their

prevalence, antibiogram, and response to antibiotic coupled nanoparticles. Total of n=72 fecal samples using purposive sampling technique of statistics were aseptically collected from cloaca. Following guidelines of Burgey's manual of determinative bacteriology, E. coli were identified while antibiotic susceptibility was performed according to clinical and laboratory and standard institittue. MgO and Sodium Alginate (Gel) nanoparticles were prepared by chemical methods and tested against E. coli for antibiotic efficacy. Probability as well as non-probability statistical tools using SPSS version 22 of statistical software at 5% probability were applied on obtained data. Current study found, 68.06% (49/72) of fecal samples positive for E. coli. Response of these bacteria against fusidic acid, vancomycin, and cefoxitin showed 40%, 50%, 40%, and 40% resistance, respectively. It was also noted from the study that 50% of isolates were found intermediate susceptible against levofloxacin. The highest to lowest minimum inhibitory concentration were as followed cefoxitin coated with gel and MgO nanoparticles (GMC), tylosin coated gel nanoparticle (GT), ampicillin coated with gel and MgO nanoparticles (GMA), Cefoxitin coated MgO nanoparticle (MC), gel nanoparticle alone (G), ampicillin coated gel nanoparticle (GA), and gel tylosin coated gel and MgO nanoparticle (GMT). Microscopic examination of these isolates for confirmation of antibacterial effect was validate in the form of excessive filamentation. The study thus concluded emerging antibiotic resistance in E. coli while nanoparticles coupled antibiotics as an effective tool to counter resistance.

Determination of Antibiotic Resistant Genes in *Bifidobacterium* Species Isolated from Commercial Fermented Foods

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Antibiotic resistance is a challenging and emerging problem for the community. Pathogen become resistant against different antibiotic groups due to excessive and misuse of antibiotics. Bacterial species attain antibiotic resistance genes through several mechanisms to escape from immune system and antibiotics. Specific genes of bacteria show resistant against antibiotics. Genome sequencing provides the evidence about the source and origin of these genes. Bifidobacterium is used as probiotics and available in commercial fermented foods. Current study is designed to determine the antibiotic resistant genes in Bifidobacterium. Bifidobacterium was isolated from commercial fermented foods and identified by using selective growth media. Identification of isolated bacterium was carried out by different biochemical test. Antibiotic sensitivity assay was performed to evaluate the sensitivity of bacteria against antibiotics. gDNA was isolated and amplified by using PCR, gel electrophoresis. 16s rRNA gene sequencing was used to analyze the DNA expression of antibiotic resistant genes in Bifidobacterium. These resistant genes were compared with the lab strain of Lactobacillus which contains the highly resistant genes. The frequency of AR genes of Bifidobacterium was non-significant as compared to the lab strain of Lactobacillus. The bacterial genera of normal microbiota Bifidobacterium was found to have antibiotic resistance genes but according to graphical data DNA expression values was non-significant so these bacteria cannot transfer the resistant genes.

Pseudomonas aeruginosa: Extended Spectrum β- Lactamase & amp; Metallo β-Lactamase producer a Potential Global Threat in the Near Future

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The main objective of the study was to look for the MDR; as drug resistance has been increasing tremendously and also analyzing the association between the virulent determinants and the antibiotic resistance in Pseudomonas aeruginosa. Microbial resistance is always been a threat in the treatment of such MDRs having versatile drug resistance mechanism making them resistant to most of the higher drugs which make the options limited for the treatment and in selecting antimicrobial therapy. This prospective study was conducted related to drug resistance in P. aeruginosa. For this purpose, random samples of the clinical isolates were collected having the sample size 50 isolates. All the clinical isolates were first subjected to a series of conventional tests to aid the identification of the required bacterium by performing Gram staining, growth on MacConkey and Cetrimide agar as well as by performing biochemical tests that included TSI and citrate. After that the isolates were examined for the presence of the virulence factors by different phenotypic methods including growth on Egg Yolk Agar, Blood Agar, DNase Agar, and Trypticase Soy Broth. The antimicrobial susceptibility was identified by Kirby Bauer method. The isolates were 100%, 90%, 40% positive for the phospholipase, DNase and biofilm formation respectively. 90% of the isolates showed α- hemolysis whereas the rest of them were γ-hemolytic. The findings of antimicrobial susceptibility showed that 70% of the isolates were positive for ESBL production while only 30% were MBL producers. The study speculated that some of the virulent factors may contribute to the antibiotic resistance so these can be given the more consideration and the researchers have to look forward in developing the methodologies to detect these determinants efficiently.

Antifungal Susceptibility Profile of Invasive *Candida glabrata* Isolates (2009-2020) from a Tertiary Care Hospital Laboratory in Pakistan

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Invasive infections with *Candida glabrata* are a global concern due to poor clinical outcomes and propensity to acquire resistance to commonly used antifungal agents. Monitoring emerging resistance and trends in *Candida glabrata*, an important agent of candidemia in Pakistan, is critical for patient management. Therefore, this study evaluated antifungal resistance and minimum inhibitory concentrations distribution in invasive *C. glabrata* isolates from Pakistan. This is a cross-sectional and descriptive study was conducted from Jan 2009-Mar 2020 at a clinical laboratory Aga Khan in Pakistan. Antifungal susceptibility of invasive *C. glabrata* isolates against azoles, echinocandin and amphotericin B were performed using colorimetric broth microdilution and interpreted using CLSI criteria. Demographics, clinical history and outcome were studied. Chi-square test was used to demonstrate association between antifungal resistance andclinical characteristics of the patients. We identified 277 patients

with invasive *C. glabrata* infection. Forty-eightisolates (18.4%) were resistant to fluconazole (MIC≥64 µg/ml). One isolate was resistant to amphotericin (MIC=2µg/mL); one isolate each was resistant to anidulafungin(MIC=1µg/mL) and micafungin (MIC=0.5 µg/mL). MIC_{90} for fluconazole was64 µg/mL and other triazoles 2 µg/mL, caspofungin 0.12 µg/mL, anidulafungin 0.06 µg/mL, micafungin 0.03 µg/mL and amphotericin 0.5 µg/mL respectively.FluconazoleMIC≥64 µg/mL(resistance), caspofungin MIC>0.06 µg/mL and amphotericin MIC>0.25µg/mL (above MIC_{50}) weresignificantly associated with patient being alive at the time of reporting, no use of healthcare devices, nor infection with other fungi.Fluconazole resistance was significantly associated with prior antifungal use by the patient. Surveillance data of antifungal resistance among common *Candida* species should be monitored closely for identification of resistant strains.

Quality High Through Put Techniques for Diagnosis for Covid-19 Infection

Dr. Kamran Shaukat, UK

A novel strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) disease (COVID-19) has been recently identified as an infectious disease affecting the respiratory system of humans. This disease is caused by SARS-CoV-2 that was identified in Chinese patients having severe pneumonia and flu-like symptoms. COVID-19 is a contagious disease that spreads rapidly *via* droplet particles arising through sneezing and coughing action of an infected person. World health organization declared COVID-19 outbreak a public health emergency of international concern on 30 of January 2020. Without question, COVID-19 is the most intensively studied infectious disease in history with >125,000 PubMed citations from January 2020 to April 2021. This contrasts with influenza, about 8,000 citations, and tuberculosis, 10,000 citations in the same time period. Since COVID 19 pandemic, several diagnostic approaches have been re-adapted, and also improved from the previous detections of SARS coronavirus. The best strategy to handle this situation seems to rely on a triad of detection methods: (i) highly sensitive and specific techniques as the gold standard method, (ii) easier and faster point of care tests accessible for large population screening, and (iii) serology assays to complement the direct detection and to use for surveillance. The diagnosis of positive cases is necessary to ensure prompt care to affected people and also to curb further spread of infection in the population. In this presentation, we will cover the techniques and tests currently available to produce quality high throughput results to combat the rapid transmission of virus.

Ursolic Acid and its Amide Derivatives Disrupts Clinical *Acinetobacter Baumannii* Isolates

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Hospital acquired infections due to antimicrobial resistant pathogens has emerged globally with increased morbidity and mortality. A group of ESKAPE pathogens holds a key importance in hospital acquired infections. *Acinetobacter baumannii* is one of the members of ESKAPE pathogens, its spreading widely and acquired multiple drug resistance (MDR) even with the last resort drug colistin at rapid phase which posed an aptitude of problem in term of its treatment or management. World Health Organization (WHO) categorized *A. baumannii* among the list of pathogens for which new pharmacophore required on urgent basis. Compounds from medicinal

plants and their derivative might act as potential drug candidates for MDR pathogens. So, in this study Ursolic acid (UA) and its synthetic amide derivatives were screened against standard (ATCC: 19606) and clinical isolates of A. baumannii strains. In the first phase of this study clinical isolates were collected and identified as A. baumannii strains phenotypically as well as genotypically. Then the ursolic acid and its derivatives were screened for antimicrobial, biofilm inhibiting and eradicating potential. Out of tested compounds amide derivative of UA (KSUA-2,4) was found to possess better antimicrobial concentration at 77.87µg/ml against colistin resistant A. baumannii strains (Colistin MIC > 100µg/ml). Compound KSUA-2,4 significantly inhibited or eradicated >60% biofilm formation of tested standard and clinical isolates at MIC. Microscopic analysis further confirms the biofilm inhibition and eradication potential of this compounds. Atomic Force Microscope analysis (AFM) further confirms the antimicrobial properties KSUA-2,4 and suggesting that the antimicrobial action might be due the the membrane leakage. Considering this evidence, microbial membrane potential was determined by using FACS analysis which confirm the loss of membrane potential after compound treatment. Gene expression analysis further explained that this compound inhibits biofilm formation by reducing the gene expressionof *bap* (biofilm gene) and *abaR* (quorum sensing). So, urasolic acid amide derivative KSUA-2,4 might be used to tackle *Acinetobacter baumannii* related nosocomial infections and further evaluated as a drug candidate.

Optimization of Major Toxins Production Potential of *Clostridium perfringens* type B under Various Physico-chemical Conditions

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Clostridium perfringens type B is responsible for causing dysentery in newborn lambs. The present study was conducted to evaluate physico-chemical conditions for maximum production of major toxins of C. perfringens type B. A total of (n=35) samples were collected from diarrheic lambs suspected for C. perfringens and cultured on perfringens agar. Anaerobic growth positive cultures were characterized as C. perfringens based on biochemical profile. These isolates were confirmed on molecular basis by amplification of 16S rRNA gene followed by sequencing. Nucleotide sequences were submitted to NCBI GenBank® and accession number retrived were MW867097.1, MW867098.1, MW867099.1, MW867100.1 and MW867101.1. Toxin type of isolates were confirmed by amplification of alpha, beta and epsilon toxin genes by using toxin gene specific primers. Confirmed isolates (n=05) were subjected to varied physical (temperature and pH) and chemical conditions (culture media for clostridial growth). It was observed that highest hemolytic units of alpha toxin were produced at 37°C (11.03±0.16HU/mL) in acidic pH (16.72±0.15HU/mL) by using Robertson Cooked Meat Medium (21.45±0.53HU/mL). Moreover, highest cytotoxic units of beta toxin were produced at 37°C (09.32±0.19CU/mL) in acidic pH (14.63±0.28CU/mL) by using Robertson Cooked Meat Medium (18.65±0.34CU/mL). Furthermore, highest hemolytic units of epsilon toxin were produced at 37°C

(08.52±0.29HU/mL) in acidic pH (11.96±0.45HU/mL) by using Robertson Cooked Meat Medium (16.57±0.19HU/mL). Similar pattern of ELISA percentages was recorded for alpha, beta and epsilon toxins of C. perfringens. It was concluded that optimized conditions might be used to produce maximum toxins for manufacturing cost-effective vaccine of lamb dysentery.

Identification of *Mycoplasma hominis* Pathogens in Semen using Polymerase Chain Reaction and "Flow-Through" Hybridization Technology

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Infertility is a disorder of the reproductive system affecting both male and female populations and is defined as the inability to achieve pregnancy after 12 months of unprotected sexual intercourse. The association remains controversial due to the variable prevalence, sample sizes, and different methods used to diagnose genital mycoplasma infection. The aim of this study was to access in the cause of infertility due *Mycoplasma hominis* as well as diagnosis and common pathogens, those causing infertility like Chlamydia trachomatis, and Neisseria gonorrhoeae Material and Methods: Semen samples were obtained by masturbation into sterile containers after sexual abstinence of 72 hours and the concentrations of sperm as well as sperm motility were determined within one hour of collection processed and remaining semen sample was used for extracting DNA and was freezed to perform PCR assay. The amplicons are subsequently hybridized to pathogen-specific capturing probes via "Flow-through" hybridization. During our study we came across with the STI pathogens present in our population and the reason for infertility was the main cause. *Mycoplasma hominis,* Chlamydia trachomatis and Neisseria gonorrhoeae were detected in their wife's were screened. The main route for the transfer of STI pathogens were the men special those who visited commercial sex workers or hotel-based sex workers as they were working in other cities and the complained for infertility. Screening for bacterial STI pathogens, like *Mycoplasma hominis,* Chlamydia trachomatis and *Neisseria gonorrhoeae* is strongly recommended because these pathogens can cause serious reproductive complications.

Association between Hepatitis-C Virus Infection and Type-2 Diabetes Mellitus

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Hepatitis-C is one of the most alarming infectious diseases in the world infecting 2-3% of the total world's population. Hepatitis-C directly damages liver and liver associated tissues such as pancreas. Thus Hepatitis-C virus can be considered a risk factor for the development of type-2 diabetes mellitus. The aim of this study was to determine any possible association between Hepatitis-C infection and type 2 diabetes mellitus. A systematic research survey was conducted on Google Scholars using the terms; "Hepatitis-C and diabetes mellitus". A total

of 17 research articles were retrieved that were published between 2000-2019. According to the researches published globally, Hepatitis-C virus (HCV) infection has been considered highly associated with type-2 diabetes mellitus (T2DM). T2DM was found in advanced stages of HCV infection such as; chronic infection and cirrhosis. Highest association in terms of co-morbidity has been reported from South Korea in 2008 with 43% HCV infected patients were diabetic. Similarly, another research published from Algeria in 2010, reported 39% of co-prevalence of HCV and T2DM in tested population, while several other research studies from Pakistan in 2007, 2013, 2017 and 2020 reported co-morbidity of HCV and T2DM in 10% -26% of the total tested population. It can be concluded that there is a strong association between HCV and T2DM. HCV is considered as a "silent killer". Approximately 10% of Pakistani population is HCV carrier which implies a drastic increase in T2DM cases in coming years.

Occurrence of Hypervirulent Klebsiella pneumoniae in Clinical Settings and Lytic Potential of Bacteriophages against The Isolates

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Antibiotic resistance is a major health hazard around the globe. The hypervirulent Klebsiella pneumoniae (hvKp) is associated with "HAIs (Hospital acquired infections)" and well as "CAIs (community acquired infections)". As there is a lack of new Antibiotics against multi-drug resistance "MDR (multi-drug resistance)" pathogens, the phage therapy may provide alternative approach to confer antibiotic resistance. This study was aimed to estimate the occurrence of hvKp and characterize the bacteriophage against the hvKp prevalent in clinical settings, which may be used as an alternate to antibiotics. Different clinical samples were collected to isolate K. pneumoniae and assessment of MDR was done as per CLSI guidelines 2020. The bacteriophage was isolated from hospital waste and double agar overlay method was used for phage purification and propagation. Spot test and one-step curve was performed to determine the host-phage interactions. For the evaluation of phage stability to environmental conditions the phage was incubated at various ranges of temperature, pH and chloroform. Total 50 clinical samples were collected and 22 (44%) were confirmed as K. pneumoniae. Among 22 confirmed K. pneumoniae total 11 (50%) isolates were detected as hvKp. Total 14 (64%) isolates were detected as MDR and 5 (35%) were among hvKp phenotype. Maximum resistance was observed against ampicillin (86%) followed by ceftriaxone (81%) which was highest among cephalosporins. The isolated bacteriophage showed broad host range, short latent period and stability. Overall, 16 isolates (85%) of K. pneumoniae were susceptible to phage infection among those 12 were MDR (75%) whereas, all 5 (100 %) hvKp were susceptible to phage infection. One step growth analysis revealed burst size of 190 phages / host bacterial cell with short latent period of 24 minutes. Taking together, a significant prevalence of hvKp was estimated in clinical settings and the isolated bacteriophage showed significant lytic activity as it inhibits the growth of all hvKp strains. Phage therapy may be exploited and used as potential alternative therapeutic approach against infections caused by this resistant pathogen.

Prevalence of Streptococcal Superantigen genes

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Streptococcus pyogenes can cause a wide range of disease from superficial infections of throat and skin, such as pharyngitis and impetigo, to serious and life threatening invasive infections, including necrotizing fasciitis, bacteremia, and Streptococcal toxic shock syndrome. Among numerous virulence factors produced by *S. pyogenes*, M-proteins and superantigens are mainly associated with *S. pyogenes* infections. Superantigens (SAgs) are highly potent T cells mitogens which share the ability to over-stimulate immune response by interfering with host adaptive immunity. *S. pyogenes* strains express 11 different but structurally related SPEs/ SAgs mostly encoded on prophages. *SpeA, SpeC, SpeH, SpeI, SpeK, SpeL, SpeM,* and Streptococcal Superantigen *SSA* are present on prophages while SpeG, SpeJ and smeZ (streptococcal mitogenic exoprotein) are encoded on the chromosome. Prophages are involved in horizontal gene transfer of bacterial DNA during the cell lysis, and lysogenic conversion by temperate phages resulted in conversion of nontoxigenic strain to pathogenic strain contributing enhanced pathogenesis. The aim of this study is to determine the prevelance of superantigen genes in invasive and non-invasive *S. pyogenes* strains and to screen their possible transfer to *Group G Streptococci* (GGS) and *Group C Streptococci* (GCS). The data suggests that more than 50% strains harbored more than one superantigen. Even few strains carried eight SAgs genes, suggesting that highly virulent strains are prevalent in the environment, and capable of transmitting these virulent genes to other strains as well as to non-pathogenic streptococci. Hence, the data of this study would lead to better knowledge about the prevelance and transfer of these superantigen genes in our population and assist us in better understanding of Streptococcal epidemiology and pathogenesis.

Immunohistochemical Based Detection of *Helicobacter pylori* in Gastric Biopsy Patients

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The *Helicobacter pylori* has ancestral relation with family of Proteobacteria, order Campylobacterales, family Helicobacteraceae. It is first most important cause of gastric carcinoma along with peptic ulcer and dyspepsia carcinoma. H. pylori also responsible for mucosa associated with lymphoid tissue (MALT)and Known Hodgkin Lymphoma. It is assessed that patients having H. pylori are at 30% to 40% possibility of evolving gastric ulcer and at 10% to 20% possibility of developing distal gastric cancer. It causes chronic inflammation and significantly increases the risk of duodenal, gastric ulcer and cancer. It is second leading cause of cancer related deaths in the world. Many stains are used for diagnosis of H. pylori. now a days Immunohistochemistry (IHC) is used for detection of H. pylori from gastric biopsies. In current study a total of 210 gastric biopsy specimens were collected from the patient with the history of gastritis from Chughtai Lab in 10% NBF. All tissue size was measured and gross examined these sample were processed in an automated tissue processor Tissue-Tek VIP VI (Japan). After processing, embedding of tissues was done in paraffin wax at Tissue-Tek TEC. 2- 3 µm sections were prepared using rotary microtome Leica 201 USA. A comparison was carried out between H&E and IHC.

Helicobacter pylori was detected in 88 cases out of 200 while 09 samples were either poorly preserved or autolyzed that's why further processing and diagnosis was not possible. One case was diagnosed as poorly differentiated adenocarcinoma; sample was collected from a female patient of 56 years. Out of 88 positive cases 66 (75%) were initially screened as positive for H. pylori by H&E staining due to the presence of mild or moderate colonization while remaining 25% cases were negative by H&E staining but with suspicion of neutrophils and lymphoid aggregates. All the 88 cases were further confirmed by IHC. Our findings confirmed that IHC is batter reliable and more accurate method in the diagnosis of H. pylori and it should be considered as the gold standard.

Methylation Modification of the STAT1 Gene in HCV Induced Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is a multistage carcinogenetic process, which includes the development of genetic as well as epigenetic modification in different genes. Hyper-methylation of promoter region of various tumour suppressor genes, including RASSF1a, GSTP1, p16, and APC cause transcriptional silencing of these genes, that is the reason of clinical outcome of HCC. STAT 1 gene is part of signalling cascade of JAK/STAT, plays important role in interferon (IFN) signalling, dysregulation in signalling has been implicated in tumour formation. Study shows that increased in methylation of promotor region of STAT1 were seen in cervical cancer, gastric cancer and ovarian clear cell carcinoma. However, no study has been reported to find the association between STAT1 promotor methylation and expression of gene in HCV induced HCC peripheral blood samples compared with controls. To evaluate the methylation status of promotor region of STAT1 gene and mRNA expression in peripheral blood of HCV induced HCC patients compared to healthy individuals. We examined the methylation status of the STAT1 gene by methylation specific PCR and then confirmed the methylation mediated silencing of STAT1 by real time PCR and in silico analysis. The result of present study illustrated that aberrant DNA methylation in STAT1 was detected in 14 of 22 (63.3%) cases. In contrast 22 (88%) healthy controls show no methylation signal ($p=0.01$). Subsequent real time PCR proved that methylation of STAT1 diminished expression of STAT1 mRNA. STAT-1 aberrant methylation results in transcriptionally silencing of gene and may be a potential biomarker for HCC diagnosis.

Environmental and Health Impact of Antibiotic Residues in Food Chains

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The presence of antibiotic residues in the food chain has gained much attention in the past few decades due to their increasing role in the role of antibiotic resistance as well as hypersensitivity disorders, mutagenic potential, carcinogenicity, suppression of bone marrow, and disruption of intestinal microflora. The misuse of antimicrobial agents for the treatment of various infections in humans as well as in the veterinary settings for growth promotion despite the fact their use is discouraging in the product consumed by humans i.e., milk, meat, and eggs. Furthermore, the accumulation of antibiotic residues into seawater adversely affects the aquatic environment. The situation will be more complex soon due to the production of food animals at the industrial scale which will result in the increased use of antimicrobial agents. The presence of antibiotic residues in the food chain is double endangerment due to their direct toxicity to the humans and emergence of antimicrobial resistance strains because of selection pressure which can ultimately lead to treatment failure. Here, we have discussed the factors that contribute to the accumulation of antibiotic residues in the food chain and natural ecosystem and their impact on human health.

Enhanced Solubilization and Purification of 3ABC Non-structural Protein of Foot-and-Mouth Disease Virus from Bacterial Inclusion Bodies

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Nonstructural 3ABC protein of foot and mouth disease virus (FMDV) is used to differentiate vaccinated from naturally infected animals. It is a polyprotein which is cleaved into membrane associated 3A protein, three copies of 3B and 3Cpro mediated by virally encoded 3C protease. The expression of this protein in E. coli results into the formation of inclusion bodies which require solubilization in high concentration of chaotropes and extensive refolding process prior to purification of the native protein. Protein aggregation during refolding leads to the poor recovery of protein in functional form. Alternatively, Mild solubilization methods have been proposed to recover the native and soluble protein from inclusion bodies present in E. coli. In this study, 3ABC protein was expressed predominantly as inclusion bodies using E. coli host and solubilized in mild non-ionic detergent followed by purification through Ni-NTA chromatography. The protein recovery using this solubilization method, showed higher yield as compared previously described solubilization methods for 3ABC protein. This method also favored higher stability of the 3ABC recombinant protein stored at different temperatures. The reactivity of the proteins was analyzed by western blotting and ELISA which showed their ability to use them as antigen for the

development of immunoassays. In conclusion, this study demonstrates an efficient and high yielding purification method of protein without refolding process than previously described methods involving renaturation steps.

Improving Microbial Population and Rice Production through Integrated Use of Organic and Mineral Nutrient Sources

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Application of organic matter to soil improves soil physicochemical properties and provides a suitable environment for PGPR and indigenous microbes to enhance their growth promotion activities in addition to supplying nutrients to growing plants. A field study was conducted at Experimental Farm of Nuclear Institute of Agriculture (NIA), Tandojam to assess the microbial dynamics under integration of organic and inorganic nutrient sources for enhancing rice growth and production. Most widely grown rice variety, NIA-Shandar was used as a test genotype. Two organic sources viz poultry manure (PM) and farmyard manure (FYM) each at the level of 10 t ha^{-1} were used along with half (60 and 45 kg of N and P_2O_5 ha^{-1}) and full levels (120 and 90 kg of N and P_2O_5 ha^{-1}) of mineral fertilizers in addition to zinc at the level of 10 kg ha^{-1}. The experiment was laid in randomized complete block design (RCBD) with three replications. The maximum general microbes (38×10^8cfu g^{-1} soil), N_2 fixing (19×10^7CFU g^{-1} soil), P solubilizing (45×10^7 CFU g^{-1} soil) and Zn solubilizing (13×10^7 CFU g^{-1} soil) microbial population were recorded in treatment where PM was integrated with half level of mineral fertilizer. Similarly, maximum plant height (106 cm), number of tillers (16) per hill, number of panicles (26) per plant, 1000-grain weight (26.01 g) and grain yield (6.92 t ha^{-1}) were also observed in poultry manure integrated with half dose of mineral fertilizer. Thus, poultry manure (10 t ha^{-1}) application along with half dose of the recommended (N and P_2O_5) chemical fertilizers can potentially improve microbial population and rice growth and yield.

Saccharothrix Algeriensis NRRL B-24137 Potentiates Chemical Fungicide Carbendazim in Treating Fusarium Oxysporum f.sp. Vasinfectum-Induced Cotton Wilt Disease

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Cotton (Gossypium hirsutum) wilt is one of the destructive disease caused by Fusarium oxysporum f. sp. vasinfectum and lead to 100% yield loss under favorable conditions. This study aims to estimate the potential of biological control agents Saccharothrix algeriensis NRRL B-24137 (SA) and chemical fungicides against cotton wilt pathogen under in-vitro and in-vivo conditions. The in-vitro study revealed that carbendazim showed maximum mycelia growth inhibition with a mean of 91% over control, which was further validated in glasshouse assay. In-vitro dual culture test of biocontrol agents with F. oxysporum determined that SA had a potential to inhibit mycelia growth by 68% compared to control. Further in glasshouse assay, the combination of the SA and carbendazim (10 µg/mL) showed a significant ($p < 0.05$) disease control. Moreover, results demonstrated that carbendazim and SA remarkably decreased the disease development up to 83% and subsequently, significant improvement was observed in the plant growth parameters (plant length, root length, and plant weight) compared to untreated plants. Conclusively, exploration and utilization of bioagent for fungal diseases in cotton may provide

a better line with maximum efficacy and with lesser adverse effects, which will pave a way towards better consequences in fungal treatments

Evaluation of Plant Growth Promoting Traits of Cadmium Contaminated bacteria for its use in Phytoremediation

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Cadmium (Cd) toxicity can have negative effects on plants, animals, and human beings. It has been reported to cause neurodegenerative disorders, breast cancer, diabetes, and prostate cancer. High concentration of Cd can also alter the physiological and biological function of plants by disturbing their photosynthetic activity, plant water status and membrane stability. The use of plant growth promoting rhizobacteria (PGPR) has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth. In this study metal tolerant bacteria were used to study their potential as plant growth promoting bacteria. Different traits like indole acetic acid (IAA), siderophore, 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), hydrogen cyanide (HCN), phosphate solubilization, catalase (CAT) production for studied for selected rhizobacteria. It was observed that these bacteria (n=10) were gram-positive, cadmium resistant and phosphate solubilizers whereas only seven isolates produced IAA and siderophore. Only four bacterial isolates were found positive for ACC-deaminase activity. It can be assumed that these isolates may have the potential to alleviate deleterious effects of Cd stress by improving the plant's growth directly or indirectly. However further research would be needed to fully elucidate their effect on plant growth in field conditions.

Methylejasmonate dependent defense signaling to restrict *P. aeruginosa,* and salinity induce debrides in tomato

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Plants are subjected to various environmental stresses simultaneously when they are grown in open field. These stresses may be abiotic, biotic either separate or both at a same time. Pathogen infection and salinity are two major stressors that restrict plant growth and development worldwide, but their interactive effect is still unknown. Methyl-Jasmonate (MeJA) is an important regulator of stress response in plants. Despite the plethora of scientific literature on the mitigating effects of MeJA under individual stress, its role in inducing defence against combined

pathogen and salinity stress; is still unexplored. We investigated the physiological and biochemical mechanisms of tomato plants mitigated by foliar application of MeJA when plants were exposed to sequential or individual action of *Pseudomonas syringae* pv. *tomato* and salt stress. NaCl-induced damages promoted the growth of pathogen and exaggerated disease severity in tomato plants. The decreased performance of salt-stressed plants under pathogen stress might be attributed to salinity-induced growth inhibition, decreased photosynthetic pigments, and destruction to photosystem II machinery. This combined interaction also provoked different interactions between antioxidant mechanisms and stress signaling by increasing lipid peroxidation and accumulation of H_2O_2. However, MeJA significantly enhanced resistance against *Pst* infection and tolerance against salinity stress by modulating levels of lipid peroxidation and H_2O_2, increasing activities of antioxidant enzymes, and accumulating compatible osmolytes such as proline and sugars. The ameliorating effect of MeJA in turn improved photosystem II machinery and photosynthetic pigments sequentially increasing the growth of tomato plants. The results of the present study suggest the vital defensive role of MeJA during combined (a)biotic stresses in tomato plants.

Distribution of Phosphate Solubilizing Bacteria Near Seashore of Karachi, Sindh

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Phosphorus is a macronutrient required for the proper functioning of plants. phosphorus plays a vital role in every aspect of plant growth and development; deficiencies can reduce plant growth and development. Phosphate solubilizing microbes (PSMs) are capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble phosphorus form. The goal of this study to check the qualitative and quantitative distribution of phosphate solubilizing bacteria across the karahi seashore. The phosphate solubilization ability of phosphate solubilizing microbes (PSMs) is detected by using conventional method such as selective media Pikovskaya's agar, are utilized both for qualitative and quantitative estimation of phosphate solubilization. Molecular tools and techniques also used for the detection of PSM such as polymerase chain reaction (PCR). As a result of this study by conventional method, 5 out of 75 cultures were showed clear zone formation while other isolates showed growth but not zone on this media. Positive cultures have the potential to be used as plant growth promoter under saline soils.

Three Layered Strategies to Obtain Keratinase Production: Immobilization, Co-culture of Bacterial Strains and Use of Waste Product as a Substrate

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Keratinases are extra-cellular proteases capable of transforming keratinous waste into the nitrogen enriched compost. Keratinases can be employed in a number of industrial applications such as leather de-hairing, manufacturing of bio-fertilizers for plant growth promotion, production of digestible animal feed of high nutritive

value from poultry waste etc. Use of a microbial consortium and its immobilization onto a solid support matrix greatly improves the enzyme production rate as compared to free cells. In this study, two keratinolytic strains namely *Bacillus licheniformis* MW45 and *Bacillus paralicheniformis* MW48 isolated from poultry soil were analysed for the keratinase enzyme production separately as well as in a combination in two different media. These strains were also investigated for enzyme production after immobilization on to different carriers in both the media. A total of six solid matrices were used including corncob, sodium alginate, agar-agar blocks, gelatin cubes, polyacrylamide gel blocks, and native chicken feathers. Incubation conditions were 45°C for 72 h on shaking. Afterward, Keratinase activity was assayed, and data was statistically analysed. Results revealed that keratinase production was enhanced when the producers were immobilized as consortium. Corn cob appeared as the most effective matrix as the enzyme units produced by immobilized cells were higher than those obtained from free movable cells. This study, therefore, explores the possible biotechnological applications of corncob for bacterial cell immobilization for the subsequent production of an industrially important enzyme.

Evaluation of Metal Resistance Potential of Bacteria Isolated from Rhizosphere of Marine Halophytes

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Halophytes are well studied for their salt tolerance. However, these saline habitats are getting contaminated due to various anthropogenic activities like urban waste, agricultural runoff, mining, industrial waste that are rich in toxic metals and metalloids. Increased concentration of heavy metals in soil effects the yields of crops and symbiosis which adversely affects the growth of plants. Halophytes by virtue of their tolerance to salinity also show high tolerance to heavy metals. To envisage this study samples from rhizoshpere of marine halophytes present at Clifton Beach, Karachi, Sindh were collected for isolation and distribution of metal resistance. Samples were processed for root endophytes, rhizospheric and rhizoplanic in the presence of Cu, Co, Ni, Zn, K and Pb. Isolation carried out under stress conditions in the presence of metals. Results showed that 46 isolates possess the ability to grow in the presence of heavy metals. These isolates were further tested on higher concentrations of heavy metals to get the maximum heavy metal resistance capability. Isolates with maximum heavy metal resistance for further growth promotion traits like phosphate solublization, bio surfactant production and Indol acetic acid production. Results revealed that these isolates possess the ability to be worked as plant growth promoting bacteria under stress condition although further confirmation from pot experiments is needed.

Effect of Feeding Tanniferous Plants on Immune Response against Peste des Petits Ruminants (PPR) Vaccine in Goats

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Peste Des Petits Ruminants (PPR) is an acute, viral, transmissible disease, which is caused by Peste des petits ruminants virus (PPRV) in small ruminants. PPR was first recordedin West Africa during 2nd world war. Live attenuated PPR vaccine (Nigeria 75/1) is being used for preventation and control of the disease. This study was designed to monitor the effect of feed supplementation on immune system, growth rate, body weight gain and feed conversion ratio (FCR). Seven experimental groups wereformedas G1 to G7. The experiment has three controls i.e. negative control for feed supplements and vaccine (G1), vaccine control group (G2), feed control group (G3). There were four treatment groups i.e. basal feed group (G4), feed supplemented with *Acacia ampliceps* 30% (G5), feed supplemented with *Acacia nilotica* 30% (G6) and feed with combination of the *Acacia ampliceps* 15% + *Acacia nilotica* 15% (G7). Treatment groups were vaccinated with PPR vaccine (Nigeria 75/1) to monitor the effect of feed supplementation on their immune system and health status. It was found that maximum body weight was gained in G4 i.e. 6.37 kg during the study period of two months. Moreover, maximum growth rate was recorded in G4 i.e. 112g/day, which is statistically significant than other groups ($P \leq 0.05$). Results of FCR showed that G4 and G6 hasbetter feed conversion efficiency as compare to other supplemented groups. It was also found thatG6 has highest antibody titresagainst PPRV as compare to other groups. It might be due to herbal contents (tannins and flavonoids) of*Acacia nilotica* which are immuno-stimulants. Whereas, G4 which was fed on basal feed without supplement has lowest antibody titres as compared to other groups. So, it was concluded that the feed with*Acacia nilotica* supplementhas immunostimulatory effect as well as it also hasbetter performanceas compare to other experimentalfeed supplements.

Optimization of Zinc and Phosphorus Application Rates for Maize Crop Inalkaline Calcareous Soils

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Nutrient management includes biological approaches which are important because of their compatibility with environment and economically viable for greater nutrient availability to obtain better for plant growth. Among the nutrients zinc (Zn) is one of the most important micronutrients, it is relatively immobile and unavailable for plant uptake in alkaline calcareous soil. In contrast, Zn toxicity is also a problem for plant growth. A novel biological approach based on arbuscular mycorrhizal fungi (AMF) inoculation is proposed to overcome Zn deficiency or alleviate the Zn toxicity to improve plant growth and yield in alkaline calcareous soils. The objective of the present study was to evaluate the effects of AMF inoculation on the growth, physiological processes, biochemical properties, and yield attributes of maize (*Zea may*s L.) under variable (both deficient and toxic Zn levels) Zn levels in alkaline calcareous soils. An experiment was conducted to optimize the phosphorus (P) and Zn levels with mycorrhizal colonization for maize growth attributes in pot-culture study under greenhouse conditions. Results showed that AMF colonization consistently increased the stomatal conductance, photosynthetic- and transpiration rates, chlorophyll content and total soluble protein contents, superoxide dismutase, catalase, and peroxidase activities of maize under both Zn deficient and toxic conditions. Improved physiological processes and biochemical properties as exerted by AMF inoculation, significantly increased the plant height, stem girth, cob weight, 1000-grain weight, and harvest index of maize under both Zn deficient and toxic conditions.

Advanced Genomics Epidemiology and Bioinformatics in Medical Microbiology

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The talk will overview the role of new technologies like next generation sequencing, microarray and bioinformatics and their potential in the paradigm shift of the conventional diagnostics of microbiology. In the last decade it took months and lot of money to sequence a bacterial genome but now it is a matter of hours and cost had reduced ten folds' times, giving a possibility to be utilized in routine diagnostics. Another recent technology WGS (Whole genome sequencing) has been proven significant in epidemiology, by establishing origin and transfer of infectious agent globally in several breakouts. As we are witnessing the pivot of world due to COVID-19 pandemic, that has changed many traditional and conventional norms. Another emerging technology nanopore sequencing technology provide portable, flexible, and inexpensive alternatives to NGS. Outbreak.info is a project from Scripps Research labs, California, USA to unify COVID-19 and SARS-CoV-2 epidemiology and genomic data, published research, and other resources. It also makes it easy to track global trends in COVID-19 cases and deaths. CDC has launched Advanced molecular detection program (AMD) with a COVID 19 epidemiology toolkit. genomics to epidemiologic investigations and public health response to SARS-CoV-2. It will be embraced by existing IRIDA (Integrated Rapid Infectious Disease Analysis Platform) and some other new and efficient databases and software that can enhance the diagnostics of infectious diseases like ARG-ANNOT: (Antibiotic Resistance Gene-ANNOTation) is a new bioinformatic tool that was created to detect existing and putative new antibiotic resistance (AR) genes in bacterial genomes., antibacTR: a computational pipeline designed to aid researchers in the selection of potential drug targets, one of the initial steps in antibacterial-drug discovery. MorphoCol, a new ontology-based tool for the standardised, consistent and machine-interpretable description of the morphology of colonies formed by human pathogenic bacteria. CLIMB: Cloud Infrastructure for Microbial Bioinformatics (CLIMB) facility, a shared computing infrastructure that has been designed from the ground up to provide an environment where microbiologists can share and reuse methods and data and PhyResSE; a simple-to-use web service. Delineating both lineage and resistance, it provides state-of-the-art methodology to life scientists and physicians untrained in bioinformatics. It combines elaborate data processing and quality control, as befits human diagnostics, with a treasure trove of validated resistance data collected from well-characterized samples in-house and worldwide. In the coming decade, it is likely that genomics and metagenomics will play major roles in routine medical microbiology.

The Prospects of CRISPR-Cas System for The Amelioration of Antibiotic Resistance

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Exposure to antibiotics gives rise to huge selective pressure that commences the spread and emergence of antibiotic resistance in both pathogenic and commensal bacteria. Developing novel and new antibacterial to confront antibiotic-resistant infections is the foremost priority in the field of medicine. The slow procedure of formulating new antibacterial makes this strategy implausible for confronting rapidly emerging antibiotic-resistant pathogens. Since the success rate for approval and the formulation of new and novel antibiotics is very low, therefore, alternative potential strategies i.e., the formulation of antibacterial peptides, anti-virulence compounds, nucleic acid-based antibacterial approaches, bacteriocins, and phage therapies must be explored to subsist the resistant infections caused by superbugs. An adaptable and potent approach is to ensure the execution of a revolutionary strategy; "the type II CRISPR-Cas system", which is based on a bacterial adaptive system aimed to confront the foreign genetic material invasion like, genetic mobile elements and also phages. CRISPR-based studies have gained interesting attention because the "CRISPR beauty" lies therein that it may easily attack and kill both antibiotic-resistant and antibiotic-sensitive pathogens. Moreover, this revolutionary CRISPR approach only targets pathogens while protecting the commensal ones within the microbiome. So, the future of the CRISPR craze predominantly lies in managing the 'microbial community composition' rather than using a conventional antibiotic broad-spectrum approach

Pan Based Genomic Analysis of *Campylobacter jejuni*

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Bacterial pan-genomic research provides a systematic view of genes found in strains. It provides a controlled view of bacterial genomics. Also, it offers detailed views of genetic diversity and adaptation among closely related strains, such as replication, gene loss, and horizontal gene transfer mechanism. Campylobacter jejuni is linked to a variety of food-borne illnesses around the world. Campylobacter jejuni has recently shown increased resistance to antibiotics such as macrolides (erythromycin), fluoroquinolones, and tetracycline. Campylobacter jejuni genomic analysis was conducted using the pan-genome analysis pipeline platform (PGAP). This study is aimed to do pan-genomics analysis and phylogenetic analysis to find functional genes involved in pathogenicity in the core, shared, and unique genomes. The complete genome sequence and annotation files of 114 strains of Campylobacter jejuni (Refseq) were taken from National Center for Biotechnology Information (NCBI). Among these strains, only 35 strains were selected to performed PGAP analysis. Results showed that in 35 strains of Campylobacter jejuni, we had found whole 2765 clusters, division of these clusters in the core, shared, and unique genome was 1,267, 876, and 622, respectively. Many single nucleotide polymorphisms (SNPs) were also present in the strains of Campylobacter jejuni. The phylogenetic analysis also discloses that the high rate of recombination acquired the Campylobacter jejuni strains' evolution can promptly produce novel phenotype possibly due to horizontal gene transfer (HGT) showed variability and diversity in allelic genes. Further research in molecular biology, sequencing, and pan-genomic analysis of evolving strains can understand the virulence factors of the Campylobacter jejuni and the pattern they produce.

Novel Role of ABC (ATP Binding Cassette) Efflux Pump in Providing Isopentenol Tolerance in *E. coli*

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Isopentenol is among many compounds being considered as candidates for advanced biofuels. However, *E. coli* has very low tolerance to isopentenol, limiting the cost-effective production of this biofuel. Cellular export systems, such as efflux pumps are one of the most frequently employed strategies which can provide a direct mechanism for reducing biofuel toxicity. To identify novel efflux pumps which can improve isopentenol tolerance, we attempted to exploit some well know ATP binding cassette (ABC) drug transporters in E. coli. Our results showed that a Periplasmic Membrane Fusion Protein from ABC confers two-fold increase in the isopentenol tolerance of E. coli against isopentenol as compared to the control. The Single knock-out mutant of Periplasmic Membrane Fusion Protein made E. coli hypersensitive to isopentenol.

Molecular Cloning and Characterization of a Thermostable Esterase Produced by Anoxybacillus spp.,

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A novel thermophilic, Gram-positive and spore forming bacterium considered as moderately thermophile was characterized. The strain was positive for both casein, catalase test and negative on the basis of gelatine hydrolysis test. The gene encoding esterase from thermophilic *Anoxybacillus sp* was cloned and sequenced. The recombinant protein was expressed in *Escherichia coli* BL21 under the control of strong IPTG inducible promoter. The gene coded for 383 amino acids and written as PET-Est. The catalytic pocket consists of triad of three residues serine, aspartic acid and histidine as explained by amino-acid sequence alignment. This specific site is required for esterase activity. PET-Est was active in a pH range (5-9) and in temperature range (45-80^{o}C) and has optimum temperature of 60^{o}C and optimum pH of 8. The enzyme shows thermostability and did not reduce activity after 30

min of incubation at 60°C. The docking results described that ethyl acetate fitted in binding cleft of esterase model. Blast results showed that *Anoxybacillus* esterase have almost 80 % identity to other species of same genus. Thus recombinant enzyme is potential candidate for industrial application due to distinct properties of thermostability.

Molecular Detection of *Acinetobacter baumannii* from Fish Meat Available in Local Market

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Acinetobacter is a genus of gram-negative bacteria belonging to a class of gammaproteobacteria. Due to intake of food contaminated with *Acinetobacter spp*, it causes many health problems. The serious health issues associated with the *Acinetobacter spp* are pulmonary diseases, hospital acquired infections, lungs infection, inflammation of meninges, gastro problems like diarrhea and bacteremia is also caused by this pathogen. 20 to 60% mortality is associated with the diseases caused by this pathogen. This disease is transmitted by the contact of one person to another person, contamination of food and water and contaminated equipment's in hospitals. The aim of our study the isolation of *Acinetobacter baumannii* from fishes available in different markets of city area of Karachi, Pakistan. *Acinetobacter baumannii* was identified by the use of microscopy, traditional biochemical identification and final verification was done by molecular techniques. From February to June, 2018, total 100 fish meat were taken from different markets in Karachi. Out of 100 fish meat samples collected from different market in Karachi 18 samples were confirmed as *Acinetobacter baumannii* by using microscopic and traditional biochemical testing techniques. These 18 samples were further processed for molecular confirmation by amplification of 16S rRNA gene. Out of 18 sample confirmed by microscopy and biochemical testing 12 samples were positive for *Acinetobacter baumannii* by PCR. Our study concludes that *Acinetobacter baumannii* is prevalent in fish meat available in different market of Karachi. These contaminated fishes are consumed by human and it is a great public health concern. Our study also conclude that fishes should not only be examined for food-borne diseases but should also be examined for *Acinetobacter baumannii* because it is a major public health concern.

Sequencing of 16s rRNA Gene for Molecular Characterization of *Bacillus cereus* in Street Vended Foods

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The 16S rRNA gene sequencing is a key procedure in molecular characterization of bacterial pathogens. Since poor food safety practices in street food vending harbor toxin producing microbial agents in street foods such as *Bacillus cereus* therefore in this study, six common types street vended foods (SVFs) i.e. Biryani, Cholay chat, Dheleem, Pakorey, Salad and Cane juice were examined for molecular characterization of *Bacillus cereus* using 16s rRNA gene sequencing. The result revealed that out of 108 SVFs (18 each street food), 35 (27.77%) samples were found contaminated with *Bacillus cereus*. The morphological, cultural and biochemical characterization confirmed the presence of *Bacillus cereus* in street food samples. Moreover, the DNA from bacterial isolates were recovered, genomic DNA were amplified via polymerase chain reaction (PCR) using specific bacterial 16S rRNA gene and for this universal primer (16F- ACGCGTCGACAGAGTTTGATCCTGGCT and 16R-

GGACTACCAGGGTATCTAAT) were used to amplify 16S rRNA gene. Finally, 16s rRNA gene sequencing further confirmed the presence of *Bacillus cereus* in SVFs. It was concluded from the findings that both phenotypic and genotypic characterization showed the occurrence of *Bacillus cereus* in all six SVFs therefore it is suggested that food safety assuring authorities should impart their crucial role in regulating strict implementation of Good handling and manufacturing practices by street food vendors in order to prevent consumers from enterotoxin related food poisoning.

Detection of *Pseudomonas aeruginosa* and their Virulence Genes Isolated from Burn Wound Infection and Environmental samples

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P. aeruginosa is ubiquitous organism, particularly common in hospital environments and causes serious wound infection in burn patients. Exotoxin A (ETA) is the major and most lethal virulent factor produced by P. aeruginosa. The aim of this study was to determine the presence of P. aeruginosa in burn wound infections and hospital environment and also determine the prevalence of virulence genes of P. aeruginosa in order to highlight factors contribute to increased morbidity and mortality in burn patients. 150 pus swabs collected from patients with burn wound infections, admitted in different hospitals of Karachi and 80 samples collected from hospital environment were identified by standard biochemical tests. Identification was confirmed by Polymerase chain reaction (PCR) using 16s rDNA gene. Virulence gene of P. aeruginosa was detected by amplifying a 396-bp region of the Exotoxin A (ETA) structural gene sequence using primer pair ETAl and ETA2 by PCR. The sensitivity and specificity of the putative genus-specific and species-specific PCR assays were 100%. Among the 64 P. aeruginosa strains isolated from 150 burn wound samples 53 (91.37%) were positive for ETA gene while in 11 P. aeruginosa isolated from 80 environmental samples 9 (81.81%) were positive for ETA gene. This study indicates that PCR is rapid method for identification of *P. aeruginosa* Isolated from burn wound infection and environmental samples. ETA gene contributed to the overall virulence of P. aeruginosa and may play an important role in the spread of infection by delayed wound healing.

Aberrant *STAT1* methylation as a non-invasive biomarker in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common types of cancer in the world and a reason behind different oncogenes activation and tumor suppressor genes inactivation. Hyper-methylation of tumor suppressor genes including *RASSF1a, GSTP1, p16, and APC* cause gene silencing as well as tumor cell invasion. *STAT 1*

gene is a part of signaling cascade of JAK/STAT and any dysregulation in signaling has been implicated in tumor formation. The current investigation focus on the methylation role of STAT1 gene as a non-invasive biomarker in the progression and diagnosis of hepatocellular carcinoma. STAT1 gene methylation status in 46 HCV induced hepatocellular carcinoma patients and 40 non-HCC controls were examined by methylation specific PCR. STAT1 gene expression was examined by real time PCR and further validated by various bioinformatics tools. *STAT1* methylation in HCV-induced HCC (67.4%) was significantly higher compared to the non-HCC controls ($p<0.01$). However, mRNA expression of STAT1 gene in methylated groups was significantly lower compared to unmethylated groups ($p<0.05$). Furthermore, insilco analysis of STAT1 validated our results and shown expression of *STAT1* mRNA was lower in liver cancer with the median 24.3 ($p= 0.085$). After using peripheral blood samples, we observed that *STAT1* silencing caused by aberrant methylation could be used as potential non-invasive biomarker for the diagnosis of HCV induced hepatocellular carcinoma.

Circulation of SARS Cov-2 Variant of Concern (Voc) Traced by Whole Genome Sequencing, The Case of Sardinia Italy

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The World Health Organization (WHO) declared over 223 million coronavirus disease (COVID-19) confirmed cases, including more than 4,6 million deaths worldwide from the beginning of the pandemic. As seen for other viruses, the SARS-CoV-2 spread follows a fluctuating pattern displaying spikes and drops, indicating an increase or a reduction in the number of new infections. Two SARS-CoV-2 waves hit Sardinia (Italy) in 2020: the first occurred early in the year and asecond after the summer; while writing this paper, a third wave of DELTA variant, began in June 2021, is still ongoing. Our report investigates the pattern and the epidemiological context for the COVID-19 recorded cases in north Sardinia, explicitly focusing on the second surge, which started from the second week of August 2020 until the end of March 2021. Based on available data from the local public health laboratory and regional sources, we describe the distribution of the newly infected cases and define the impact of the SARS-CoV-2 variants of concern. Interestingly, we defined viral mutations that aid in vaccine evasion and breakthroughs and mutations that may limit the epidemiological transmission and spread of SARS-CoV-2. Our data will expand the understanding of the mechanisms that allowed the ALPHA and DELTA variants entry in Sardinia and help health authorities to predict the future waves of infections.

Understanding the Clinical Characteristics of COVID-19 and Genome Sequences of SARS-CoV-2 in Local Patients

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SARS-CoV-2 is a causative agent for COVI-19 disease, initially reported from Wuhan, China. Pakistan has one of the world's largest populations, of over 200 million people, and is experiencing a severe infection caused by SARS-CoV-2 beginning in March 2021. Infected Patients experienced mild to severe symptoms, resulting in several fatalities due to a weak understanding of its pathogenesis, which is the same even to date. A cross-sectional study has been designed on four hundred and fifty-two symptomatic, mild-to-moderate, and severe/critical patients to understand the epidemiology and clinical characteristics of COVID- 19 local patients with their comorbidities and response to treatment. The mean age of studied patients was (58±14.42) years, and the overall male to female ratio was 61.7 to 38.2%, respectively. 27.3% of the patients had a history of exposure, 11.9% travel history, while for 60% of patients, the source of infection was unknown. The most prevalent signs and symptoms in ICU patients were dry coughs, myalgias, shortness of breath, gastrointestinal discomfort, and abnormal Chest X-ray ($p<0.001$), along with the high percentage of hypertension ($p=0.007$) and COPD ($p=0.029$) as leading comorbidities. Complete Blood Counts indicators were significantly increased in severe patients, while the Coagulation Profile and D-dimer values were significantly higher in mild-to-moderate (non-ICU) patients ($p < 0.001$). Serum Creatinine (1.22 umole L-1; $p = 0.016$) and LDH (619 umol L-1; $p < 0.001$) indicators were significantly high in non-ICU patients while, raised values of Total Bilirubin (0.91 umol L-1; $p = 0.054$), CRP (84.68 mg L-1; $p = 0.001$) and Ferritin (996.81 mg L-1; $p < 0.001$) were found in ICU patients. Drug Dexamethasone was the leading prescribed and administrated medicine to the COVID-19 patients, followed by Remdesivir, Meropenem, Heparin, and Tocilizumab, respectively. A characteristic pattern of Ground glass opacities (GGO), consolidation, and interlobular septal thickening were prominent in severely infected patients. Till now, very few SARS-CoV-2 genomes collected in Pakistan are reported on public databases. To understand the circulating variants in Lahore and surrounding areas with a combined population of 11.1 million, 102 samples were sequenced, covering one week from April 2021. The samples were randomly chosen from 2 hospitals with a diagnostic polymerase chain reaction (PCR) cutoff value of fewer than 25 cycles. Analysis of the lineages shows that B.1.1.7 (first identified in the UK, Alpha variant) dominates, accounting for 97.9% (97/99) of cases, with B.1.351 (first identified in South Africa, Beta variant) accounting for 2.0% (2/99) of cases. No other lineages were observed. In-depth analysis of the B.1.1.7 lineages indicates multiple separate introductions and subsequent establishment within the region. Eight samples were identical to European genomes (7 UK, 1 Switzerland), indicating recent transmission. Genomes of other samples show evidence that these have evolved, indicating sustained transmission over some time either within Pakistan or other countries with low-density genome sequencing. Vaccines remain effective against B.1.1.7. However, the low level of B.1.351, against which some vaccines are less effective, demonstrates the requirement for continued prospective genomic surveillance. These findings could be used for future research, control, and prevention of SARS-CoV-2 infected patients.

Anti-SARS-Cov-2 Potential of Ethanolic and Methanolic Extracts of Allium Sativum, Zingiber Officinale, Eucalyptus Globulus and Cinnamomum Verum

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This study was conducted to evaluate the anti-SARS-CoV-2 activity of ethanolic and methanolic extracts of *Allium sativum, Zingiber officinale, Cinnamomum verum* and *Eucalyptus globulus*. The *A. sativum, Z. officinale, C. verum* and *E. globulus* were identified and further subjected to ethanolic and methanolic extraction by employing Soxhlet apparatus. The cytotoxicity activity of each extract was evaluated using Vero cell line by MTT assay. Antiviral activity of each extract was determined using different dilutions of each extract. Ethanolic extract of *A. sativum* showed antiviral activity at lower concentrations (0.3-0.04mg/mL) followed by *C. verum* (0.17mg/mL) and *E. globulus* (0.67mg/mL), respectively. Methanolic extract of *E. globulus* presented antiviral potential at lowest concentration i.e., 0.90 mg/mL followed by *Z. officinale* (0.74mg/mL), *A. sativum* (0.43mg/mL) and *C. verum* (0.36mg/mL). The MTT Assay revealed that these concentrations of ethanolic and methanolic extracts of *A. sativum, Z. officinale, C. verum* and *E. globulus* were non-toxic and considered safe to use (Cell survival percentages more than 50%). It was concluded that these extracts may be alternative treatment for COVID-19.

Comparative Genomic Analysis of the Structural Proteins of SARS-cov-2 Strains Prevalent in Pakistan

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The ongoing pandemic caused by SARS-CoV-2 still poses a threat for human health despite the available vaccines as we speak. While scientific research has answered a lot of questions regarding both the disease and the agent, there still seems to be a sense of mystery regarding the novel coronavirus and its strains. Similarly, the mutant of SARS- CoV-2 are increasingly being reported in various parts of the world. To our knowledge, a comparative genomic analysis of the strain predominant in Pakistan is largely missing. We aim to perform an in-depth comparative sequence and structural analysis of SARS-CoV-2 structural proteins including Surface Glycoprotein (S), Nucleocapsid Phosphoprotein (N), Membrane Glycoprotein (M) and Envelope Protein (E) between the commonly reported varieties in Pakistan with the original Wuhan COVID-19 virus. For comparison of the viral strains, we focused on structural proteins of SARS-CoV-2. Genomic sequences were retrieved from

NCBI database. Genetic variations were identified based upon Multiple sequence alignment, while structural analysis was used to investigate their implications. Furthermore, bioinformatics tools, such as DynaMut2, mCSM, CUPSAT, MAESTROweb and SDM were used to determine effect of mutations on protein's structural stability. We represent genetic variance of SARS-COV-2 structural proteins in Pakistani population relative to the original Wuhan-Hu-1 reference. So far 250 genomic sequences reported from Pakistan. These data serve as a reference to consult the presence of a particular mutation in SARS-COV-2 structural protein in Pakistani population relative to the original Chinese variety. Variations in spike gene and other structural proteins give us the extent of viral evolution under the selection pressures of vaccine and global spread. Particularly in case of spike protein, D614G is reaching fixation around the world. However, we have not found D614G to be accompanied by other three mutations that Groves et al (2021) reported: C241T is located in the 5'UTR region; there is a silent mutation, C3037T; and C14408T results in the P323L amino acid change in the RNA-dependent RNA polymerase. The variations observed may render spike protein resistant to current vaccines. Structural and functional consequences of the observed mutations on these proteins stability provide an in-depth understanding of the SARS-COV-2 pathogenesis, structure-function relationships, and development of modern therapeutic approaches. We propose that multiple antigens (such as membrane M, envelope E and nucleocapsid N proteins) should be targeted simultaneously for vaccine development. The polyclonal response in such cases can deter the emergence of escape strains. Broad analysis of Pakistani variety with mutant UK, South African, Brazilian and Indian strains of SARS-CoV-2 is underway to be published in the future.

The COVID-19 Outbreak: A Novel SARS-COV2 Virus Infection Prevalence Rate at a Government Tertiary Care Hospital of Karachi Pakistan

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WHO declared novel coronavirus known as 2019-nCoV) as epidemic and public health emergency on 30 January 2020 in the 21 st century, has emerged all over China and spread globally, a highly contagious and pandemic of corona virus. The key objective of the study is to calculate the proportion of this pandemic infection in a government based tertiary care hospital setting and finds contributory risk factors and prevalence of crona virus infection. The complex etiology of the total 14983 Nasopharyngeal swabs were screened through Real Time PCR, to determine the cutoff values to be used for screening the potential carriers along with demographic data. initially 14.8 % cases were reported positive which raises to 31.6% in early may indicating highest peak reached 42.2% in mid may than no of positive case declined till early June 2020. Young population between age bracket of 20-39 were affected most (12 – 20%). 24.8% male were showing positive results. Almost 30% patients showed Respiratory symptoms of Flue and Fever and while 27% patients showed other symptoms. Peak season for respiratory symptoms ranges from mid -May to mid- June, 2020. Current study data highlights the predisposing factors of Covid19.

Impact of COVID-19 Pandemic on Children with Transfusion Dependent Blood Disorders

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SARS-CoV-2 is reported to transfer person to person, it is not known till today that COVID-19 virus is transmitted through blood transfusion. In Pakistan COVID-19 pandemic is ongoing and government of Pakistan imposed a lockdown at 31-March 2021. Due to such restrictive measures for slowing the spread of COVID-19 virus marked visible impact in treatment and follow-up of patients with blood disorders including thalassemia, hemophilia, aplastic anemia and leukemia. The main concern of patients with blood disorders during pandemic was shortage of blood products. This issue was also highlighted through Social media as, "Due to the shortage of blood, one bag was being transfused to two children suffering from thalassemia", alarming situation was faced at the moment when the blood level of children with thalassemia had dropped to 2.5 gm/dl which should ideally be above 10 gm/dl. Blood level of children's treated with hydroxyurea was not decreased dramatically. This study was conducted in Children Hospital Karachi. Total 398 multiple transfusion patients with blood disorders were studied. All 398 patients were transfused with 2900 PRCs product during March 2020 to Feb 2021. During follow-ups each patient which is reported with fever has been observed carefully and screened for COVID-19 and others test related to acute febrile infections. Only 1 out of 398 patients have been reported with positive COVID-19. After investigation it has been proved that the infection has been transmitted through contact after contact tracing. There was no evidence of transmission of COVID-19 through donated blood has been proved.

Impact of Pandemics (COVID-19) on Global Health and Challenges for Microbiologists

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Pandemic, a Greek word PAN meaning 'all' and DEMOS 'people'. Infectious disease spreading rapidly can cause pandemic. The history of mankind has some major pandemics including plague, cholera, flu, SARS and currently COVID-19. These pandemics were having variable morbidity and mortality rate and had affected people throughout the world. Some studies have highlighted tourism and globalization as the major reason for pandemics in the last century. In spite of these progressions of the modern medical era, the human novel coronavirus disease (COVID-19) initially identified in Wuhan, China in December 2019, is the worst pandemic, the world is going through till date. It has badly hit the world economy and also induced behavioral changes in people all over the globe. Outbreak response measures like quarantine and restricted movement of people significantly impacted social and economic growth. The coronavirus has four genera, *Alpha*, *Beta*, *Gamma*, and *Delta*; together making subfamily *Coronavirinae* and the family *Coronaviridae*. Despite all advancements and medicines discovered, the

Coronavirus disease-19 (COVID-19) has highest morbidity and mortality rates in a short period of time. Latest statistics of COVID-19 have reached over 2.3 billion people infected and 4.5 million lost their lives in a fight against this pandemic and the situation is still not under control. The world is currently facing its fourth wave and the causative agent typically has flu-like symptoms with fever and dry cough. The virus however has affinity for all major organs of the body leading to various complications with old age and immune compromised people at greater risk. Vaccination is under way with different efficacy rates for different vaccines underlining an urgent need to incorporate latest variants into new COVID vaccines, which are now undergoing research and clinical trials. Treatment discovery is still underway and supportive care and oxygen supplementation therapy is the only way to save the lives of infected people. The situation is still challenging for Microbiologists, with coronavirus being RNA virus, undergoing frequent variations. The virus mutates itself when transferred from one person to another. Moreover, the vaccines available are effective against COVID-19 virus for short duration and the individuals again have chances of getting infected with corona virus. The field of microbiology has significant role in diagnosing, treating and preventing the disease. Clinical microbiologists should continue to promote diagnostic stewardship and its importance during this pandemic phase. The effective management against the disease is based on the knowledge shared by the microbiologists. The discovery of vaccine against COVID-19 is main achievement and success of microbiologists. However, during this critical phase lot of Microbiologists have lost their lives and are true heroes who sacrificed themselves for saving humanity. The situation continues to remain as such, throughout this investigative process, starting from the collection and transport of specimens to the amplification and confirmation of viral RNA and the validation of clinical sensitivity and specificity. Microbiology endorses critical research requirements, like; Standardization of RT-PCR protocols, improvement in amplification techniques, sequencing of viral RNA including its mutations. Improvement in techniques and modification of the existent assays for measuring the viral infection rate in wastewater and environmental surveillance at the community level. Use of CRISPR technique for point-of-care applications and subsequent validation procedures.

Age and Gender Specific Study of COVID-19 Positive Cases: A Tertiary Care Hospital Experience

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In Pakistan, the first case of COVID-19 was reported on Feb. 26th, 2020 that marked the initiation of the first wave of COVID-19 in Pakistan that lasted till September before the beginning of the second wave from Oct. 2020. During the first wave, 9,712 people screened by Real Time PCR (RTPCR) at Genetic Lab of Children's Hospital Karachi, Research Institute for Genetics, Blood and Bone Marrow Transplant. Individuals from age 20-80 years old were studied and categorized on the different age groups and gender. Out of the 9,712 people, 1988 (20.3%) people reported positive for COVID-19. The positivity ratio out of 1981, 1021 (53.93%), 784 (34.16 %) and 68 (11.89) belonged to the age group 20-40, 40-60 and 60-80 years old respectively. On the basis of gender, 1478 (74.34%) out 1988 of the positive cases were men and 503 (25.7%) out of 1988 were women. Among these positive men 809 (54.75%), 503(34.05%) and 164(11.11) belong to the age of 20-40, 40-60 and 60-80 years old respectively. Among these positive women 258(51.40%), 171(34.27%) and 71(14.31%) belong to the age of 20-40,40-6 and 60-80 respectively. This data suggests that the prevalence rate of COVID-19 positivity is the highest among the age group 20-40 years old, whereas it was the lowest in the age group 61-80 years old and On the basis

of gender, men were infected by COVID-19 at a significantly higher rate than women. We did this age and gender specific study on COVID- 19 positive cases so that we can target that population to enhance precautions measures

Transmission dynamics of SARS-CoV-2 in Karachi, Sindh

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The trajectory of SARS-CoV-2 infections has been of concern in populous, low middle income countries such as Pakistan. We describe the rise and fall of COVID-19 cases in Pakistan with a particular focus on the metropolis, Karachi. This is a retrospective cross-sectional analysis of SARS-CoV-2 polymerase chain reaction tests conducted at The Aga Khan University Hospital. Geospatial mapping of countrywide data was done with particular emphasis on Karachi and Sindh province. Thirty-three percent of the 54,017 cases (mean age 39 ± 17 y), tested were positive for SARS-CoV-2 with the first case in end February and a peaked in mid June followed by a subsequent decline. In all ages except those ≤ 15 years, significantly more males were tested than females. Within each gender, approximately one third of all cases tested were positive. Early in the epidemic, COVID-19 was mostly associated with travelers. Subsequently, local transmission through towns in Karachi was observed. Highest SARS-CoV-2 test positivity per population density was found in a town with a lower socio-economic status whilst the greatest numbers of tests were conducted in a higher income setting. Initial slow SARS-CoV-2 transmission was likely associated with lockdowns and subsequent spread with re-opening, leading to peak transmission followed by selective lockdowns and decline in cases. Reducing the gender bias in testing together with a more rational testing would be key to manage continual virus transmission in the population.

Antibacterial potential of Probiotic bacteria and their applications

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (WHO). The antibacterial potential of probiotic bacteria from different origins in Pakistan was explored. The molecular analysis based on 16s rRNA gene revealed the dominance of *Lactobacillus* in different sources with 5 other genera including *Enterococcus, Weissella, Streptococcus, Leuconostoc* and *Bacillus*. Shortlisting was done for their survival at *in vitro* GI conditions and antibacterial activity against human pathogens. Some probiotic strains were subjected active compound identification. Pilot scale studies are underway to use some probiotic Bacillus for control of hospital-borne pathogens by developing probiotic sanitizer. We also investigated the effects of Aloe vera gel (AvG) and multi-layered microencapsulation on the survival of *Lactobacillus* species in

cottage cheese during 28 days of refrigerated storage. The results suggest that cottage cheese fortified with Aloe vera gel filled alginate-chitosan beads loaded with either *L. rhamnosus* or *L. plantarum* strains, can prevent pathogen invasion, maintain functional qualities, and deliver more probiotics to the human gut. *Lactobacillus rhamnosus* GG (ATCC 531030) is a proved and widely used probiotic strain but it cannot metabolize lactose and degrade milk proteins. We made *L. rhamnosus* GG lactose positive and proteolytically active by conjugation with the dairy *Lactococcus lactis* subsp. *cremoris* NCDO 712 strain carrying the conjugative plasmid (pLP712) encoding lactose operon and the proteinase gene *(prtP)*. In contrast to its parental strain LGG, the ability of LAB49 to metabolize lactose and degrade casein enabled strong and fast growth in milk.

Therapeutic Potential of *Lactobacillus* Post-biotics and its Competitive Activity against *Salmonella* and *E. coli* in poultry birds

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The ability of post-biotic as a replacement to antibiotic has been characterized as a potential approach to prevent gastrointestinal infection of *Salmonella* and *Escherichia coli* in poultry. Postbiotic have been recognized to enhance growth performance and immunity in birds. Postbiotics are secreted metabolites from bacteria when administered in sufficient amounts give health benefits to the host. The study was designed to screen Lipase, Amylase, and Protease enzymes from *Lactobacillus*. *Lactobacillus* was isolated from poultry droppings of healthy birds after identification, cell-free supernatant was extracted. The supernatant was characterized for evaluation of Lipase, Amylase, and Protease enzyme by the formation of a clear zone on Olive-oil, Starch, and Skim milk media, respectively. The study was comprised of 5 experimental groups for 3 weeks. At the end of treatment, body weight gain, feed conversion ratio, feed efficiency, were measured and histopathology for intestinal samples was performed. Results from the experiment showed that the group supplemented with postbiotic had a higher weight gain of 146.3g and the lowest feed conversion ratio of 2.34. Histopathology results revealed postbiotics supplementation increased the surface area for absorption of nutrients in the intestine by increasing villi length and depth of crypts.

Probiotics Protect Intestinal Microbial Dysbiosis and Subsequent Aero-gastric Infections of *S. aureus* and *P. aeruginosa*

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Studies on gut microbiome and the balance in this microbial ecosystem has been of great interest in past decade. Human digestive tract has immense assortment of microorganisms and the balance in this ecological niche is dependent on several factors including gastric acidity and motility etc. Gut microbial disturbances or dysbiosis may cause an assortment of illnesses or anomalous physiological states. Infections caused by pathogens and /or exposure to antibiotics can change gastro-intestinal microbial ecosystem and create the opportunity for

opportunists to over grow and translocate to extra-intestinal niches. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are well known opportunists and nosocomial pathogens. They appear to have the opportunity and the ability to promote intestinal and respiratory infections. They are largely the cause of morbidity and mortality in both hospital and community settings. These pathogens remain the important cause of pulmonary infections in case of Cystic fibrosis patients with a worldwide prevalence. Although antibiotics are still an effective means of treating bacterial infections, the alarming rise of multi drug resistant bacteria has urged to seek for the new therapeutic approaches. Thus, there is a need for the development of potent antimicrobials for the effective treatment of infections. Currently *Lactobacillus* and *Bifidobacterium* species are pulling in incredible enthusiasm as health supplements due to expanded familiarity of the beneficial roles in health and nutrition. There is evidence that maintaining balanced intestinal microbial ecosystem can antagonize the access of respiratory and enteric pathogens. Probiotic bacteria play role in keeping the gut microbial ecosystem stable by re-establishing normal microbiota and can possibly repress pathogen's colonization via modulation of immune response.

Ecofriendly Photosynthesized Zirconium Oxide Nanoparticles as Antibiofilm Agent against MDR *Acinetobacter baumannii*

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The worldwide increase of multi-drug resistance has directed the researchers to focus on ecofriendly ways of nanoparticles synthesis with effective antivirulence properties. Here, we report the antibiofilm and quorum quenching potential of zirconium oxide nanoparticles (ZrO2 NPs) synthesized from aqueous ginger extract against multi drug resistant (MDR) *Acinetobacter baumannii*. The results indicated that ZrO2 NPs were of tetragonal shape with average diameter of 16 nm. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for *A. baumannii* were 15.6 µg/ml and 31.2 µg/ml respectively as revealed by broth microdilution assay. Exposure of bacterial cells to ZrO2 NPs resulted in reactive oxygen species (ROS) generation which in turn led to cellular membrane disruption as observed by an increase in leakage of cellular contents such as proteins, sugars and DNA. The antibiofilm activity was evaluated by microtiter plate assay and the results revealed that the percentage inhibition of biofilm was found to be 14.3-80.6 %. ZrO2 NPs also obstructed the chemical composition of biofilms matrix by reducing the proteins and carbohydrate contents. These findings suggested the invitro efficacy of phytosynthesized ZrO2 NPs as antibiofilm agent that can be exploited in the development of alternative therapeutic options against MDR Acinetobacter baumanii.

Silver Nano-particles: Synthesis and Characterization by using Glucans Extracted from *Pleurotus ostreatus*

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The extensive use of antibiotics has led to drug-resistant bacteria, which is a major public health issue worldwide. Silver nano-particles have been recognized as efficient broad-spectrum antimicrobial agent. It uses mushroom glucans as reducing and capping agent to reduce its toxicity. In the present work, we performed the characterization of glucans and glucan– AgNPs. The crude glucans were extracted by hot water then fractionated and purified by anion exchange chromatography (DEAE-cellulose 52); the elution of glucans was monitored by phenol sulfuric acid assay. The major fractions were further purified with gel filtration chromatography (Sephadex-G200) then lyophilized. Green synthesis of silver nano-particles was performed by glucans from selected Pleurotus spps. Extracted glucans and synthesized glucan–AgNPs were confirmed by UV–Vis spectra at 190–250 nm and 380–420 nm, respectively. Size of glucan–AgNPs (100.1 nm) was determined by dynamic light scattering. The glucans were characterized by FT-IR, SEM and XRD while glucan–AgNPs were characterized by FT-IR, SEM–EDS, TEM and XRD. Based on FT-IR spectra, the functional groups associated with the glucans and glucan–AgNPs were determined. SEM analysis revealed that the structure of glucan was spongy with asymmetrical particle size while SEM–EDS/TEM analysis revealed the glucan–AgNPs were spherical with size range of 15–45 nm for Pleurotus ostreatus. XRD spectra of glucan showed diffraction peaks at 2θ=5.9°, 10.1° and 19.6°. By SEM–EDS/TEM analysis, the silver nano-particles against Braggs refection planes of (111), (200), (220) and (311) in X-ray diffraction, showed diffraction at 2θ=38.2°, 44.5°, 64.7° and 77.5° in pattern.

Comparative Effect of Organic Acids and Aqueous Extract of Garlic and Ginger on Survival of *Campylobacter jejuni* on Chicken Meat

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The present study dealt with the comparison of antibacterial potential of aqueous extracts of garlic, ginger and organic acids (acetic acid and lactic acid) against the survival of *Campylobacter jejuni* on chicken meat. Antibacterial activity of each aqueous extract and organic acid was determined against five isolates of *C. jejuni* by Agar well diffusion assay. To investigate the Minimum Inhibitory Concentration (MIC) of the aqueous extracts, broth microdilution method was employed. Highest zone of inhibition was recorded against garlic (22±2.00mm) followed by ginger (20.3±1.5mm), lactic acid (15.3±0.5mm) and acetic acid (13.6±0.5mm). The MIC of garlic and ginger was recorded as 35 and 25 uL/mL, respectively. To evaluate the antibacterial potential of the extracts and organic acids on food model, log reduction assay was performed. Aqueous extract of garlic showed the best results (3.31±0.39 log CFU/g) at 25˚C followed by ginger (2.36±0.13 log CFU/g) at 4˚C, lactic acid (2.22±0.02 log CFU/g) and acetic acid (1.36±0.09 log CFU/g) at 25˚C after 100 minutes of contact, respectively. In conclusion, organic acids, garlic and ginger have strong anti-*Campylobacter* activity and can be used safely in processing of chicken meat to reduce the *Campylobacter* load.

Controlling of Lepidopterous Pests (Honeycomb and White Marked Tussock Moths) By *Bacillus* Strains Isolated from The Local Fields of Kohat

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Agriculture is the most important sector for the economy of Pakistan. The pest attack is one of the most damaging factors in the field of plant pathology. The most common pest, white marked tussock moths, attacking the shade as well as ornamental plants and caused 80% damage to plants canopy. On the other hand, higher gross loss has also been reported in managing honeybees and their wax caused by wax moths that weakened the stored comb and colonies of bees. However, chemical pesticides have widely been used for the protection of these crops and combs, but the wide use of these chemicals from the last three decades has increased the number of environmental problems. Thus, the use of natural enemies of pests, such as viruses, bacteria, and fungi, can be an alternative for the pest control. Therefore, the objectives of the present study were to isolate novel insecticidal strains of Bacillus spp. to control the plant's insects belonging to the order Lepidoptera. Around 50 soil samples were collected from the local fields of District Kohat and cultured through serial dilutions using pour plate technique. The bacteria isolated from the soil samples were then characterized and identified using their colonial, morphological Gram staining and biochemical characteristics. The bacteria were identified as B. subtilis FD3, B. cereus FD6 and B. wiedmannii TD10. The strains were then subjected to the centrifugation for extracting metabolites. Finally, the

strains and their metabolites were applied to the larvae of Orgyia leucostigma and Galleria mellonella. It was observed that out of the three strains, the *B. subtilis* was the most toxic than *B. cereus* and *B. wiedmannii.* The larval survival was 99% without exposure to *B. subtilis* FD3 strain and decreased to 80% when exposed to *B. subtilis* FD3 strain after 48 hours. Moreover, *B. cereus* FD6 showed 50% mortality rate after 48 hours. Furthermore, the metabolites of *B. wiedmannii* TD10 showed 60% activity against the larvae of *Orgyia leucostigma* and 40% activity against *Galleria mellonella*. From this study, it may be concluded that *B. subtilis* FD3 strain showed significant toxicity against *Orgyia leucostigma* and *Galleria mellonella* and hence may be considered as a putative biocontrol agent.

Essential Oils Composition, Antioxidant, Antibacterial, and ɑ- Glucosidase Activity of *Scutellaria edelbergii* Rech. F.

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The current study was aimed to investigate essential oils (Eos) profile obtained through n-hexane from *Scutellaria edelbergii* via GC-MS analysis using the reported approach. Among the 46 important constituents with (97.79%), the main essential oils detected in *S. edelbergii* were Linoleic acid ethyl ester (19.67%), Ethyl oleate (18.45%), and Linolenic acid, methyl ester (11.67%). As the mentioned EOs have a key role to cure various complications, therefore, examined for various *in-vitro* antioxidant, antibacterial, and ɑ- glucosidase assays. Antioxidant activity of the Eos of *S. edelbergii* has been carried out with DPPH and ABTS by applying standard methods. The DPPH assay revealed a significant antioxidant capacity with value IC_{50} = 70 µg/mL and 105 for ABTS in comparison with standard ascorbic acid IC_{50} with 32 and 29 (µg/mL) respectively for DPPH and ABTS. The essential oils were also screened for their antibacterial bacterial significance using the standard method in comparison with the standard. An appreciable zone of inhibitions (ZOI) was noted in mm against *S. aureus* and E.*coli* with ZOI (17.33±0.3), and (16.11±0.3) respectively. Moreover, it has been also observed that EOs exhibited (98.95%) inhibition against enzymatic ɑ- glucosidase assay in comparison with standard acarbose (58.52%) at 0.5 µg/mL.

Combined Treatment of Probiotics with Therapeutic Doses of Ultraviolet Radiations for Immunity Induction in Immunocompromised Patients following Chemotherapy

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Chemotherapy is the frequent cause of a weaken immune system in patients taking anticancer treatments. The symptoms of this weaken immune system are susceptibility to get frequent infections, and these illnesses may become severe hardly able to cure. The gut microflora has been reported to modulate immunity, metabolic, psychological and cognitive mechanisms, and can be upregulated using probiotics such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*. Chemotherapy adversely affect populations of gut microbiota by inducing acute dysbiosis and alteration in physiological and psychological function. Probiotics may help boost immunity by inhibiting the growth of certain microbes therein. Probiotics enhance the natural defence system of the body by modulating the immune cells especially immunoglobulin A (IgA)-producing cells, T-lymphocytes and natural killer cells. Meanwhile, a transcriptional factor nuclear factor erythroid-2-related factor 2 (Nrf2) activates 100s of genes via metabolism regulating sirtuin proteins. Nrf2 can be activated through UVA rays, exercise, fasting, and various phytochemicals/nutraceuticals. Nrf2 activates cytoprotective, antioxidant, and anti-inflammatory genes to induce innate immunity and associated genes for immunocompromised system. Following chemotherapies, the huge mortalities are due to compromised and weak immunity subjected to impaired immune cell profiles and gut microbiota. It is assumed that therapeutic UVA exposure may help induce immunity along with Gut microbial profiles by inducing Nrf2 expression. This potential targeted treatment strategy can lead to immune related signaling pathways which can lessen the mortalities post chemotherapy.

Assessment of Cholesterol Reducing Potential of Commensal Bacterial Species Isolated from Human Milk

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Probiotic are living non-pathogenic microorganisms which on ingested in adequate amount are beneficial to human health. Probiotics mainly drive from two genera Lactobacillus and Bifidobacterium. Probiotics can be isolated from various sources but human milk offer enriched LAB flora which develop beneficial effects on human neonatal health. The objective of this study was to isolate commensal bacterial spp. from human milk and to characterize their probiotic potential particularly the cholesterol reducing capability. A total of 30 commensal bacterial strains was isolated and molecular identification was done. Molecular identification showed the diversity of commensal bacteria including including *Streptococcus epidermidis* (73%) *Staphylococcus hominis* (10%), *Staphylococcus hominis subspp novobiosepticus* (3%), *Bacillus safensis* (3%), *Streptococcus lacterius* (3%), *propionibacterium avidum* (3%) and *Streptococcus parasinguinis* (3%). All strains showed bile resistance at high concentrations i.e 0.3 %, 0.6% and 1.2% (w/v) and also exhibited significant growth at 4.2 and 5.7 pH. These

strains were observed as safe determined through hemolysis assay and antibiotic sensitivity assay. None of the strain showed antibacterial activity against the tested pathogens. A total of 22 strains showed positive BSH activity whereas the tested five strains showed cholesterol reducing potential. In the light of this study, it is revealed that human milk related commensal bacterial spp. has the probiotic potential and it is first time reported the probiotic potential of commensal bacterial spp. from human milk.

Analysis of SARS-CoV-2 and Factors Predicting Next Spillover of its More Contagious Variant

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At the beginning of 2020, the world has started experiencing the epidemic of a novel coronavirus; by the mid of March 2020, it has been declared a pandemic. The disease has been named COVID-19, and the virus labelled as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on the type of infection it is causing. Coronaviruses are not new to us, there are15 different coronaviruses known to us. In the last 20 years, this is the fourth coronavirus pandemic, and SARS-CoV-2 seems to be the deadliest among all with the ability to continue producing more contagious variants. In this review, we attempted to give an outline of the SARS-CoV-2 about its origin, transmission, tropism, zoonotic, vaccines and the factors that are contributing to its contagious and virulent nature. We also sought to predict future pandemics and commented on the impact of COVID-19 disease during pregnancy. There is evidence that the novel coronavirus can penetrate CNS and have an impact on the brain by cytokine storm or by rouge autoimmune effect. Accumulating evidence also indicates that the pandemic might have a massive impact on mental health particularly to those who are predisposed to COVID-19. Though a couple of approved vaccines are being in use, in the future, we may see some more vaccines and their effect around the globe. Our biggest concern is the constant mutation of SARS-CoV-2, recently a variant referred to as SARS-CoV-2 VUI 202012/01 (Variant Under the investigation, the year 2020, month 12, variant 01), has been identified through viral genomic sequencing in the United Kingdom (UK). It is defined by multiple spike protein mutations present as well as mutations in other genomic regions. As the COVID-19 pandemic continues, a new, highly transmissible form of SARS-CoV-2 has emerged, named as strain B.1.1.7, which was originally identified in the UK and includes the spike protein variant N501Y. Similarly, another more contagious lineage B.1.1.248, informally termed as the Brazil variant has 17 amino acid changes. In this review, our aim is to reveal the factors which can impede the ability of this highly lethal virus to reproduce into more contagious variants.

Pakistan AMR National Action Plan: Development & Implementation

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Pakistan joined in the international endorsement of AMR Global Action Plan at the 68th session of the World Health Assembly (2015). This commitment led to the development of a National AMR Strategic Framework for Containment of Antimicrobial Resistance (2016) and an operational AMR National Action Plan (2017). AMR

working group (NIH, CDC, WHO/EMRO) developed Pakistan AMR surveillance system (PASS) in 2018. We aim to discuss the activities and progress of AMR and IPC as per five key strategic objectives in NAP. GLASS initiated in 2017 with 5 pilot sentinel sites and now in 2021, there are 45 sites enrolled with PASS. Capacity building of the sites for quality data generation has been conducted, including LQMS, microbiology and WHONET trainings. National AMR Data portal established. AMR e-Module developed with ASM. Antimicrobial consumption and stewardship established in country since 2020. IPC activities strengthened through development of national guidelines on IPC, followed by implementation strategy. Progress in five strategic objectives of NAP AMR showed the success of AMR activities in the country. JEE score showed improvement since 2016. We still face challenges in form of dearth of microbiologist across the country which effects the quality testing. The laboratories have limited resources including quality control strains and diagnostic reagents. Lack of coordination between few key stakeholders is a considerable challenge. In LMICs, there are multiple stakeholders who invest in AMR and helped to establish the capacity of the country. Awareness campaigns started at national and provincial level brought a huge change in public perception on AMR. Engagement of Federal and Provincial Governments for legislation and implementation on AMR is building up.

Comparative Drug Susceptibility of Colistin Resistant *Escherichia coli* Isolated from Milk in District Marden.

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E. coli are prevalent in different environments and can cause various disorders i.e diarrhea, edema, and inflammation of serosa membrane, septicemia as well as mastitis. E. coli showed resistance to different antibiotics which represents a threat to humans and animals. Here we reported resistance of E. coli to colistin which is a last option of treatment. From local areas of Mardan milk samples were collected. According to standard guidelines of Clinical Laboratory Standard Institute bacteria were isolated. Out of positive isolates some showed multi drug resistivity. Vancomycin showed maximum resistance while minimum by Chloramphenicol. On other hand maximum susceptibility was found to Trimethoprim sulfamethoxazole and Cefipime. Colistin resistance genes were detected via Polymerase Chain Reaction. UDP was the most prevalent gene detected in isolates. It is concluded from this study that colistin resistance genes are dominant in milk and can be transferred to others via animal product i.e. milk and meat.

Molecular Epidemiology of Extensively-Drug Resistant *Acinetobacter baumannii* ST 2 Co-Harboring *blaNDM* and *blaOXA* From Clinical Origin

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The therapeutic management of carbapenem-resistant *Acinetobacter baumannii* (CR-AB) represents a serious challenge to the public health sector because these pathogens are resistant to a wide range of antibiotics, resulting in limited treatment options. The present study was planned to investigate the clonal spread of CR-AB in a clinical setting. A total of 174 *A. baumannii* clinical isolates were collected from a tertiary care hospital in Lahore, Pakistan. The isolates were confirmed by VITEK 2 compact system and molecular identification of *rec*A and *bla*OXA-51. Antimicrobial profile and the screening of carbapenem-resistant genes were carried out using VITEK 2 system and PCR, respectively. The molecular typing of the isolates was performed according to the Pasteur scheme. Of the 174 *A. baumannii* isolates collected, the majority were isolated from sputum samples (46.5%) and in the intensive care unit (ICU, 75%). Among these, 113/174 (64.9%) were identified as CR-AB, and 49.5% and 24.7% harbored *bla*OXA-23 and *bla*NDM-1, respectively. A total of 11 (9.7%) isolates co-harbored *bla*OXA-51, *bla*NDM-1, and *bla*OXA-23. Interestingly, 46.9% of the CR-AB belonged to sequence type 2 (ST2; CC1), whereas 15.9% belonged to ST1 (CC1). All of the CR-AB isolates showed extensive resistance to clinically relevant antibiotics, except colistin. The study concluded CR-AB ST2 clone harboring *bla*OXA-23 and *bla*NDM-1 are widely distributed in Pakistan's clinical settings, which could result in increased mortality. Strict compliance with the National Action Plan on Antimicrobial Resistance is necessary to reduce the impacts of these strains.

Prevalence and Risk Factors for Second Line Drug Resistance in Drug Resistant Tuberculosis Patients in Pakistan: A Retrospective Cohort Study

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The emergence of extensively drug resistant tuberculosis (XDR-TB) is increasing globally due to delayed or indiscriminate use of second line drugs (SLD) in multidrug resistant tuberculosis (MDR-TB) patients. The purpose of the present study was to determine resistance to second line anti-tuberculosis drugs and risk factors for SLD among drug resistant (DR-TB) patients. A retrospective cohort study was conducted from January 1, 2011, to June 31 in which all patients (857) with confirmed DR-TB (at the start of SLD treatment) presenting to Programmatic Management of Drug resistant TB unit in Khyber Pakhtunkhwa, Pakistan, 2014 were included. Drug susceptibility testing was done for first- and second-line drugs and risk factors for resistance to SLD were determined. Out of total 857 patients, almost half of the patients (n=387, 45.2%) showed resistance to at least one second line drugs. The prevalence of resistance to any injectable second line drugs was 4.3%, any oral second line drugs was 43.4% and XDR-TB patients were 3.8%. Multivariable analysis revealed that the strongest predictor for resistance to SLD was previous use of SLD (OR=2.72, 95% C.I. 1.69-4.36) and positive sputum smear results in the current TB episode (OR=1.75, 95% C.I. 1.13-2.71). Patients with previous use of SLD is a risk factor for resistance to second line drugs including XDR-TB. The occurrence of XDR-TB cases in Pakistan highlights the need to strengthen Pakistan's TB program with increased attention to rapid diagnosis of MDR-TB, improved nosocomial infection control policies and regulated treatment.

Occurrence of Extended Spectrum β-lactamase *E. coli* in Cattle and Buffaloes of Islamabad, Pakistan

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Extended spectrum β lactamase E. coli (ESBL E. coli) have been isolated from food animals and humans indicating an emerging veterinary public health issue resulting in high morbidity, mortality and treatment cost. In addition, ESBL E. coli are also responsible for development of further AMR as the antibiotics of last resort such

as carbapenems have to be given to affected patients which may results in Carbapenem-Resistant Enterobacteriaceae (CRE). ESBL E. coli is a highly diverse and ubiquitous group of microbes that can be easily transmitted between humans, animals and environment therefore, it may be considered as representative of AMR problem. A study was carried out with an objective to find the evidence of occurrence of ESBL E. coli in cattle and buffaloes slaughtered in a slaughterhouse of Islamabad, Pakistan. In total 150 recto-anal mucosal swab samples (RAMS) were collected from cattle (n= 79) and buffaloes (n= 71) slaughtered in a slaughter houses of Islamabad. The samples were enriched in buffered water for 24 hours at 37°C. Enriched samples were inoculated onto MacConkey agar (MAC-CEF) containing cefotaxime (0.8gm/L). A lactose positive colony per sample was selected and confirmed as E. coli by colony morphology and biochemical analysis. ESBL E. coli were confirmed using double disk synergism and double disk diffusion test following CLSI guidelines. The overall occurrence of ESBL E. coli was 44% (66/150). The occurrence of ESBL E. coli was higher in buffaloes 56.3% (40/71) compared to cattle 32.9% (26/79). Antibiotic sensitivity profile for each phenotypically confirmed ESBL E. coli isolate was evaluated against 18 different antibiotics. All the ESBL E. coli isolates were resistant to Ampicillin, Penicillin, Teicoplanin, Lincomycin, Cephradine and Neomycin. The results of the study indicated the occurrence of ESBL E. coli in health cattle and buffaloes slaughtered in a slaughterhouse of Islamabad, Pakistan therefore they may act source of transmission of ESBL E. coli to humans.

Prevalence and Antimicrobial Susceptibity Pattern of Carbapenem Resistance from Pediatric Bloodstream Infections

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Pediatric bloodstream infections (PBSIs) have constantly been recognized as the most abundant type of nosocomial infections comprise over 28% of the infections rates with substantial morbidity and mortality among pediatric populations globally. The rapidly emerging antimicrobial resistance (AMR) against highly effective drugs is now considered a serious threat to public health in developing countries. Alarmingly, Carbapenem-resistant Gram-negative bacilli, especially the carbapenemase enzymes producing strains of *Klebsiella* species and Escherichia *coli*, are considered as the emerging cause of nosocomial bloodstream infection in pediatric patients. Therefore, this study aimed to evaluate the frequency of pathogens causing PBSI and determined the drug susceptibility paradigm of clinical isolates of CRE from public and private hospitals of Lahore, Islamabad, Faisalabad. About 120 CRE isolates were procured from the blood cultures that were first identified phenotypically based on culture characteristics, colony morphology, Gram staining, and biochemical tests. The Kirby-Bauer disc Diffusion method were used to profile the antimicrobial susceptibility paradigm of the CRE isolates. The minimum inhibitory concentration (MIC) of the CRE isolates was evaluated against imipenem and meropenem by the broth microdilution assay. Out of 120 CRE isolates, about 67% (80/120) were *Klebsiella species*, 21% (25/120) were *Escherichia coli*, 9% (11/120) were *Enterobacter species*, and 1% was *Citrobacter species*. In the present study, 100% isolates showed resistance to gentamycin, carbapenems such as meropenem, imipenem, ertapenem, ceftriaxone, penicillin's, amoxycillin/clavulanic Acid, Ampicillin, cefotaxime, 99.2% to co-trimoxazole, 98.3% to Ciprofloxacin, Tazobactam, gentamycin, 97.5% to Tobramycin and about 0.5% to colistin and polymyxin B.

Molecular Detection of Multidrug Resistant Pseudomonas Aeruginosa from Raw Meat Samples

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Multidrug resistant (MDR) *Pseudomonas aeruginosa* is prominent nosocomial pathogen conferring resistance to a variety of antimicrobial agents. Due to the increasing rate of drug resistance in *P. aeruginosa* it is considered as severe threat to the public health worldwide. Recently emergence of MDR *P. aeruginosa* in food chain further worsens the health situation. In the present study, a total of 100 meat samples were collected (50 each from chicken and mutton) from different butcher shops and supermarkets. Isolation and molecular detection of MDR *P. aeruginosa* was done on *Pseudomonas* cetrimide agar and polymerase chain reaction. The antimicrobial susceptibility profile was detected by Kirby Bauer method. The study outcome revealed 24% prevalence of *P. aeruginosa* in meat samples and chicken samples were found more contaminated with the presence of *P. aeruginosa* 14/50 (28%) in contrast to mutton samples 10/50 (20%). Correspondingly, detection of *P. aeruginosa* contamination was more noticeable in fresh meat type 16/50 (32%) than frozen 8/50 (16%). The antibiotic susceptibility showed highest resistance against amoxicillin/clavulanic acid, ceftriaxone and colistin (100%) followed by aztreonam and ticracillin (95.83%), ciprofloxacin (91.6%) and meropenem (87.5%) while highest susceptibility was recorded for ceftazidime (41.67%) followed by imipenem (33.3%) and cefepime (25%). Among total 24 isolates, 22 (91.66%) were detected MDR isolates. Double disk synergy test detected 09 (40.90%) isolates as ESBL phenotypically. Among the 22 MDR isolates of *P. aeruginosa*, 06 were positive for *bla*CTXM and 03 for *bla*TEM genes. None of the isolates was found positive for *bla*NDM and *bla*OXA genes. Rational use of antibiotics, proper sanitary practices at slaughter houses and meat shops is required to curtail the presence of MDR pathogens from food chain.

Quinolones Derivatives: Active against MDR Bacteria associated with Urinary Tract Infection

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Multidrug resistance (MDR) is a phenomenon in which the infectious agents develop resistance against more than one class of antimicrobial drugs. MDR is a serious health issue all over the world. Antibiotics resistance has reached to an alarming level with the emergence of resistance against every new drug, which is globally affecting the lives of millions of people. Urinary tract infections (UTI) are common infectious diseases affecting150 million

people all over the world each year. Micro-dilution method was employed to measure the minimum inhibitory concentrations (MICs) of the active constituents by MTT assay. About (14) out of 2000 compounds related to quinolones derivatives were found to be highly active against MDR UTI strains. Various mechanistic and morphological studies were also conducted with selected potent reproducible inhibitors of MDR to evaluate their therapeutic potential. These studies include membrane potential study, fluorescent microscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), study of generation of reactive oxygen species (ROS), hemolytic activity and *ex-vivo* analysis. They provided key understanding about the mechanism of action of the newly identified antimicrobial agents. Reactive oxygen species (ROS) play a key role in the destruction of cellular morphology, and finally lead to cell death. After that, the compounds were also examined through *ex vivo* studies and hemolytic assay in order to evaluate their activity in the presence of human blood.

Isolation of Vibrio cholerae from Clinical and Drinking Water Samples During Cholera Outbreak in Khairpur Sindh Pakistan

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Cholera is a diarrheal disease that is caused by the bacterium *Vibrio cholerae* that transmits through contaminated water, food, oral-fecal route and spread through poor sanitation system. This study aims to investigate the reports of clinical and drinking water samples during an epidemic in Pakistan. This study was conducted in District Khairpur including 660 samples (360 from clinical and 300 from drinking water) from 2014-2016. All samples were enriched in Alkaline peptone water for 6 hours and then streaked on TCBS agar, incubated at 35ºC for 24 hours. The next day standard microbiological, biochemical, serological techniques were used for the identification of *V. cholerae* and further identification was performed using PCR. Out of 360 clinical samples, 76(21.11%) were positive for *V. cholerae*. The Species-specific ompW gene was amplified and shown at the correct size (588 bp) through agarose gel electrophoresis. The serotyping revealed that the isolates belonged to serogroup Inaba, O1. cholerae O1 strains shown typical El Tor phenotype similar to *V. cholerae* El Tor strain N16961 (PBR VP+) used as the reference strain in this study. All age groups were affected where the highest onset was seen in among those aged 19 years and above. The culture of drinking water (n=300), observed negative on TCBS agar.. As far as our knowledge, this is the first time when the presence of *V. cholerae* El Tor peak has been reported for causing cholera outbreaks in Khairpur Sindh, Pakistan. However, these findings can be used for further investigations and recognizing control measures.

Pitfalls in Global Response to Infectious Diseases and its Impact on Global Health & Economy-Preparedness is Key Word to Remember

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The significant burden of diseases so far has been estimated to result in >50 million total annual deaths worldwide and infectious diseases alone result in approximately half of them. The spectrum of emerging new diseases as well as re-emerging old diseases is on rise as their infectious agents evolve, adapt and spread at enormous speed in response to changing ecosystems, behavior and moving population patterns. The inequities of health status and disease burden as seen by the current ongoing pandemic reflect the fact that high income countries (HICs) with the world's best health care infrastructure are finding it extremely difficult to cope with the ongoing onslaught of COVID-19 and what to say about low & middle income countries (LMICs) mainly due to wider gaps between have and have nots. The world is currently under siege of coronavirus outbreak with initial epicenter in Wuhan city, the critical challenge is how to respond to such catastrophic pandemic and develop rapid & cost-effective methodologies for extensive testing globally, sharing data, and developing early warning systems for better preparedness by coordinated efforts and keeping an eye not only on what is happening around the globe but also dealing with any other associated unforeseen challenges for effective timely response by preparing & engaging skilled workforce.

Activity of Plant Essential Oils against Antibiotic Resistant *Enterococcus faecalis* Isolated from Diarrheic Children

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Activity of Plant Essential oils and their fractions was determined against characterized isolates of antibiotic resistant *Enterococcus faecalis* recovered from diarrheic children. Isolates were confirmed by PCR targeting 16S rRNA gene amplification followed by nucleotide sequencing and accession numbers retrieved were MW349990.1, MW349859.1, MW332122.1, MW356805.1, MW349975.1, MW349988.1, MW356790.1, MW356244.1, MW341593.1 and MW332549.1. These isolates were screened for antibiotic susceptibility to a wide range of antibiotic groups and mean zone of inhibition (ZOI) of all antibiotics were recorded as resistant. Antibacterial activity of plant essential oils (n=05) was checked against three antibiotic resistant isolates of *E. faecalis*. Three plant essential oils having higher ZOI including *Cinnamomum verum, Syzygium aromaticum* and *Nigella sativa* were used against resistant *E. faecalis* isolates to determine minimum inhibitory concentration (MIC) and lowest effective MIC observed was of *S. aromaticum* (11.39±3.94 mg ml^{-1}). *S. aromaticum* n-hexane plus chloroform fraction displayed higher mean ZOI (16.67±2.51 mm) while the lowest MIC was of n-hexane oil fraction. Based upon GC/MS analysis, the most effective fatty acid was Eugenic acid which is present in higher proportion in both fractions. These fractions of essential oils proved safe for the treatment of antibiotics resistant diarrheic cases of children caused by *E. faecalis*.

Prevalence and antimicrobial susceptibity pattern of bacteria from pediatric blood culture

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Bloodstream infections are major cause of morbidity and mortality among children of pediatrics age group. Bacterial septicemia is leading cause of mortality among all other mortalities that happens due to nosocomial infections. Source of most common infectious cases has been reported in recent past from Operation Theatre (OT) and other sensitive units like ICU's. Hospital's contaminated environment plays a crucial role in the transmission of HAI's. This study was conducted to test the prevalence and antimicrobial sensitivity of blood culture developing multidrug resistance. Gram negative as well as gram positive bacteria were collected from hospitals of various pediatric age groups. Specimens were analyzed by using microbiological procedures and phenotypically identified by performing different biochemical methods. Antimicrobial-susceptibility testing was performed by using Kirby Bauer Disk diffusion assay according to the CLSI guidelines. According to the outcomes of study 150 (50%) samples exhibited growth upon cultures from which majority of isolates were *Klebsiella pneumoniae* (12.0%), *Pseudomonas aeruginosa* (5.3%), *Acinetobacter baumannii* (5.3%), *Salmonella typhi* (40.0%) and Coagulase negative staphylococcus (37.3%), respectively. The antimicrobial susceptibility profiling showed that 44% of isolates were MDRs and 21.3% XDRs respectively. Gram negative isolates showed maximum resistance against Ampicillin (76.1%), Chloramphenicol (73.01%), in *Salmonella typhi*, Cefepime (54%), Piperacillin-Tazobactem (40.9%) in *Pseudomonas aeruginosa*, Meropenem (71.8%) in *Acinetobacter baumannii*, Ceftriaxone (58.7%), Amikacin (56.7%) and Trimethoprim-sulfamethoxazole (74.6%) in *Klebsiella pneumoniae*. It was concluded that Gram negative bacteria were found to be one of the main reasons for resistance to multiple antibiotics.

Evaluation of Phospholipase and Proteinase Activity of *Candida spp*., Isolated from Patients with Surgical Site Infection

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Surgical Site Infections (SSIs) are classified as the major challenge of surgery. Candida albicans can cause severe life-threatening infections thus it represents a serious public health challenge, with increasing medical and social-economical importance. Phospholipase and proteinase are two putative virulence factors of Candida spp that have been shown to directly contribute to pathogenicity. The aim of this study was to determine the prevalence of

candida spp in surgical wound and evaluate the relative level of the proteinase and phospholipase exo-enzymes produced by candida spp. Pus swabs (180) were collected from patients having surgical site infections admitted at different Hospitals of Karachi. Samples were inoculated on Sabouraud's Dextrose Agar for the isolation of candida spp which were subsequently identified by Germ tube test, Chromogenic (Biggy) Agar, Corn meal Agar, Rice Agar and by Carbohydrate assimilation. Cultures of candida spp were tested for phospholipase and proteinase activities by plate method. Fifty-two (29%) Candida isolates were obtained from 180 pus samples of which the most frequent was *Candida albicans* (42%) followed by *C. glabrata* (27%), *C. Krusei* (18%), *C. tropicalis* (8%) and *C. parapsilosis* (5%). Enzymatic activity was more pronounced in *Candida albicans* with 88% phospholipase and 93% proteinase. Incontrast, non *C. albicans* species demonstrated only 27% and 69% phospholipase and proteinase activity respectively. The results indicate that *C. albicans* isolated from Surgical Site Infections showed significant extracellular proteinase and phospholipase activity as compared to the percentage of non-*C. albicans.* We believe that production of both phospholipase and proteinase enzymes could be an important virulence factor so we can use these enzymes as virulent marker for diagnosis and management of severe infections.

Antibiogram Profile and Evaluation of Biofilm Formation by Bacterial Clinical Isolates

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A variety of bacteria can shift between planktonic forms (single cells) and establishing communities in the form of biofilms, growing on solid surfaces or rooted in a matrix of extracellular polymeric substance (exopolysaccharides). Biofilm formation by bacterial pathogens can lower vulnerability to antibiotic treatments and may result in the development of chronic infections; thus, biofilm formation can add to important virulence factors. Currently much attention is needed towards understanding the ecology of biofilms and its inhibitors. Biofilms have been identified to be accountable for several human disease and about 80% of bacterial infections in the body. The goal of this study is to collect clinical bacterial isolates from hospitals, to determine their potential of biofilm formation. Total 300 (150 gram-negative and 150 gram-positive) clinical samples were collected from Memon Medical Institute (MMI) Hospital in Karachi, Pakistan. Among those samples 156 (95 gram-positive and 61 gram-negative) were able to form biofilm. Antibiotic resistance/sensitivity of collected isolates was done to determine the relation between antibiotics resistance of the organisms and potential of biofilm formation. Bacterial cultures were isolated from different human samples (pus, blood, HVS, urine, and wound) and identified. The identified cultures were then screened as biofilm former by help of Congo Red Agar (CRA) method. Screened organisms were sub-cultured and their qualitative analysis was done by measuring the absorbance through Trypticase Soya Broth (TSB) method in 96 well microtiter plate. Relationship between the antibiotic resistance and biofilm forming property of the isolated organisms was determined. Results indicated that the higher resistance linked to the increased potential of biofilm formation by the clinical pathogens.

Prevalence and Antimicrobial Susceptibility Pattern of Enterobacteriaceae Isolated from Hospitals Wastewater

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Hospital wastewater (HWW) is bearing in mind the main root to proliferate multidrug-resistant among the Enterobacteriaceae family. In developing countries, mostly untreated hospital wastewater (UHWW) is directly exposed to the environment without any treatment. A total of 100 samples of HWW and community wastewater were collected aseptically. Samples were sub-cultured through basic, selective and UTI Chrome agar. Bacteria were identified based on cultural characteristic and different biochemical tests. Antimicrobial susceptibility testing was performed as per CLSI 2016 guidelines. The molecular characterization of the CTX-M was done bt PCR to present its association with multidrug- resistant. Among the total 165 isolates, the predominant bacteria were E. coli (48.1%) followed by K. pneumonia (25.3%), Shigella species (8.0%) and P. aeruginosa (12.7%) from hospital wastewater while E. coli (30.3%) followed by K. pneumonia (32.6%), Shigella species (20.9%) and P. aeruginosa (11.6%) were isolated from community wastewater. A total of 50 bacteria were found as CTX-M producers, E. coli (n=26) was prominent bacteria noted having CTX-M followed by K. pneumonia (n=12), Shigella species (n=5), P. aeruginosa (n=5) and S. marcescens (n=2). Amongst the isolates from HWW, E. coli was resistant to cefepime and ceftriaxone (100%), tigecycline (94.73%), ciprofloxacin (73.68%) and ampicillin-sulbactam (71.05%) while K. pneumonia was resistant to tigecycline (100%), meropenem (95.0%). Finally, the AST of CTX-M producers and Non-CTX-M producers was also compared to find the relation of CTX-M to multidrug-resistant. Tigecycline was observed as a predominant resistant (≥96%) antibiotic followed by meropenem (≥40%) against all isolated CTX-M producers. The conclusion represented the existence of MDR Enterobacteriaceae in untreated hospital wastewater. These bacteria had a chance to travel to the inlet of community wastewater without any wastewater treatment. All efforts revealed to realize the requisite that hospital wastewater should be treated with standard treatment plants before exposing to community wastewater.

Molecular Epidemiology and Characterization of Hepatitis Delta Virus from Different Areas of Khyber Pakhtunkhwa, Pakistan

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Hepatitis Delta virus is an unusual, single-stranded RNA virus that is dependent on hepatitis B surface antigen. HDV transmit a disease to patients that are earlier infected by HBV (hepatitis B virus). It propagates only in the presence of HBV because as sub satellite virus, it is dependent on HBV. Since HDV is partial RNA virus which requires the support of HBV to proliferate in host. HDV causes co-infection or super infection along with harsh complication as compared to only HBV infection. The aim of the study was to determine the prevalence of hepatitis D virus in positive samples of hepatitis B virus. Total two hundred positive samples of hepatitis B virus were collected from different areas of KPK. Out of two hundred cases, only eighty samples were found RT-PCR positive for HBV. Furthermore, ELISA (Enzyme Linked Immunosorbent Assay) were used to screen eighty positive for HDV, fourteen samples were found HDV positive. Nested PCR techniques was used for to confirm the positive HDV samples. Result of nested PCR shows that only 14.3% samples were found HDV positive and 85.7% samples were found negative. Overall data of

our study shows that the prevalence of HDV infection is usually higher in males than female and also investigate that the prevalence of hepatitis D virus infection is still remain high in Peshawar division.

Case-control Sero-epidemiological Study on Hepatitis C in Cancer Patients from Lahore

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Present case control study was designed to evaluate the association of HCV infection and cancer in district Lahore and Shaukat Khanum Hospital Lahore. 100 cases (cancer positive) were taken from oncology department SKH. 100 controls (cancer negative) were selected from healthy volunteers from different regions of Lahore. Controls were matched on age, gender, rural/urban areas, blood transfusion, surgery and body piercings/tattooing. Age based sero-prevalence of HCV in cases was 35% in above 55 years, 26% in 26-40 years, 25.6% in 41-55 years and 6% in 10-25 years. In controls it was 12.5% in more than 55 years, 27% in 41-55 years, 11% in 26-40 years and 4% in 10-25 years. It was 36% in females and 18% in males in cases. In controls it was 12.5% in males and 13% in females. It was 46% in rural areas 17% in urban areas in the cases. In controls, it was 13% in urban areas and 12.5% in rural areas. It was 37.2% in group with history of transfusion and 14% in group with history of not transfused in cases. In controls it was 20% with history of transfusion and 12% in group with history of not transfused. Surgery based sero-prevalence in cases was 25% and was 23% in non-operated. In controls, it was 18.75% with the history of surgery and was 12% in non-operated. It was 26% with history of body piercings and tattooing and 23% with negative history in the cases. In controls it was 33% with positive body piercings and tattooing history and was 8% with negative history of tattooing. The 24% was overall sero-prevalence of HCV in cases whereas in controls it was 14%. It was concluded that cancer is a significant risk factor for HCV infection. Cancer patients are 1.84 times more prone to develop HCV than healthy individuals. Urban/rural status, blood transfusion, body piercings and tattooing are those factors which were found to be positively associated with HCV. However, gender, age and surgery had no association with HCV. Proper screening of HCV at mass level in cancer infected patients should be started.

Isolation and Molecular Typing of Multidrug Resistant Bacteria from Antibiotics-Containing Waste and Their Potential for Environmental Applications

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The waste from majority of hospitals generated is not disposed-off properly, whereas multinational pharmaceutical companies (MNCs) and very few use third party to manage the system. Here 22 bacterial strains of MDR and XDR isolates from environmental samples including ESBL and MLSB strains show promising potential for application in the environmental clean-up of antibiotics. Antibiotics are chemical substances to kill microbes they are commonly used for the treatment of microbial infections. Extreme antibiotics discharged into the environment from medical waste, industrial and sewage effluents may lead to increased persistent antimicrobial resistance (AMR) in non-target microorganisms creating superbugs, thereby posing great threatening concern to the society. With the aim to isolate multidrug-resistant (MDR) bacteria and their subsequent application for environmental cleanup of antibiotics pollution from the environment, main objectives of present study were to generate local data on environmental pollution caused by antibiotics usage in Khairpur, Sukkur and Karachi cities of Sindh. Another objective was to determine the local impact of antibiotic pollution on natural microbiome causing dissemination of MDR among the environmental bacteria. Finally, to determine the application potential of selected MDR bacteria for environmental cleanup of antibiotics, in order to decontaminate the polluted sites. In present study, first two objectives were covered. For achieving this, local data regarding antibiotic usage and antibiotics-containing waste management were collected through survey using pre-designed questionnaire, followed by collection of waste samples from hospitals and industries using standard protocols. All the samples were processed for the isolation and screening of multidrug-resistant (MDR) or extended drug-resistant (XDR) bacteria, followed by identification of the isolates using digital (Micro-scan walkaway system) and manual antibiogram studies were performed. The survey study revealed that Karachi being a metropolitan city and industrial zone produces significant amount of antibiotics-containing wastes followed by Hyderabad and Khairpur, however, the awareness regarding antibiotic resistance and management strategies were more violated in small cities like Khairpur. The most prevalent method for treating antibiotics-containing waste was incineration followed by landfilling in Karachi and Hyderabad, while incineration was commonly practiced in Khairpur. Total twenty-two (22) different MDR/XDR bacterial strains were selectively isolated from environmental samples on antibiotics-containing media. The bacterial isolate *S. aureus* PWW2, showed extensive resistance to macrolides, lincosamides and Streptogramin B group (MLSB) and Methcillin (MRSA), while the isolates *Acinetobacter baumani* LMW2 and *E. coli* LMW3 were extended-spectrum beta-lactamases (ESBL) producers.

Occurrence of Multidrug Resistant *Mycobacterium tuberculosis* from Patients of Pulmonary Tuberculosis in Faisalabad

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Tuberculosis (TB) is an irresistible disease brought about by a bacterium called Mycobacterium tuberculosis, which influences the lungs the majority of all. Multidrug-resistant tuberculosis (MDR-TB) demonstrates protection to isoniazid and rifampicin the two of which are the first-line anti-TB drugs. This is one of the basic wellbeing concerns everywhere throughout the world and MDR-TB speaks to a noteworthy danger to control of the disease overall due to its high mortality and constrained treatment. Methodology: The present study intended to find out the spread of MDR-TB in Faisalabad metropolis. A total of 500 sputum samples were collected from suspected patients at tertiary care hospital, Faisalabad. All the information regarding patients and their previous history of infection was collected through a predesigned questionnaire. After collection all the sputum samples

were directly screened for acid fast bacilli through Z-N and Fluorescent staining. Molecular confirmation of MDR-TB done by Gene-Xpert MTB/RIF assay. Results: Out of 500 samples 262(52.4%) were male and 268(47.6%) were females among those 419 were new patients and 81 were previously treated patients. ZN staining reveled 97 (19.4%) positive cases and mostly (n=33; 34.02%) were found in 21-40 years. However, 114 (22.8%) MTB cases were identified by Gene-Xpert and among these, 08 (7.01%) were MDR-TB. Out of 08 cases 5(62.5%) were females and 03 (37.5%) were male patients. Conclusion: There was high prevalence of MTB (22.8%) and MDR-TB (1.6%) in clinical setting of Faisalabad. Therefore, it is the need of the hour to conduct surveillance study to overcome this problem.

Molecular characterization of SARS-CoV-2 Spike gene from suspected patients

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Throughout history, infectious diseases with pandemic potential have often developed and spread. Humanity has previously been impacted by significant epidemics and pandemics, including the plague, cholera, influenza, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV). The world is now experiencing a new coronavirus epidemic dubbed as SARS-CoV-2. In this study, a total number of 50 nasopharyngeal swabs were collected from suspected patients in the sterile tube containing the virus transport medium (VTM). Prior to the screening and confirmation, RNA was extracted. Then, the samples were screened and confirmed by E gene, N gene, and RdRP gene using RT-qPCR by observing the CT-Values. The extracted RNA was converted to cDNA to amplify the partial Spike gene region including the receptor binding domain (RBD). Conventional PCR was setup and amplification were carried out under optimal conditions and with particular primers to obtain the desired Spike gene having product size of 719 bp. The amplified product was run through Gel electrophoresis to separate and visualize the product based on their molecular weight to get the desired amplicon size of the S gene. The product was visualized using Gel documentation system. Amplified product of Spike gene was purified from the gel. The purified DNA was sent to ZOKEYO Internationals, China for sequencing. The Clustal W technique was used to align all the sequences using Lasergene's EditSeq and MegAlign tools. Aligned sequences were submitted to the NCBI and accession numbers were obtained. During this study, E484k, N501Y and D614G mutations were observed. Due to the point mutation, the amino acid was changed leading to enhanced transmission, binding affinity to hACE2 receptor, and reduced sensitivity to antibody neutralization. These mutations constitute a public health problem around the world.

Characterization and Complete Genome Sequence of 229e-Related Coronavirus from Dromedary Camels of Pakistan

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Currently, seven coronaviruses (CoVs) have been reported to infect humans. Out of theses, four are globally endemic and may cause mild to moderate respiratory disease. Little is known about the evolutionary origin of human coronaviruses. Bats serve as ecological reservoir for several CoVs, however, recent reports of the presence of MERS, HKU-23 and 229E-related CoVs in dromedary camels highlights their important role as intermediate host in zoonotic transmission cycle of CoVs. Present epidemiological study was performed on 812 nasal swab samples collected from Pakistani dromedary camels between 2015 and 2018. Samples were screened for the presence of novel CoVs by conventional polymerase chain reaction (PCR) method. Seven samples were detected positive by PCR and sequences obtained following Sanger sequencing were edited using Geneious R11. NCBI BLAST searches revealed that all the seven samples were dromedary associated 229E-related CoVs. Interestingly, all the seven camels were from the similar heard and was suffering from respiratory diseases. One positive sample (J22/Pakistan/2016) was further selected for whole genome sequencing. The genome characteristics of J22/Pakistan/2016 virus was typical of other dromedary-associated 229E viruses isolated from Middle East and Kenya. The J22/Pakistan/2016 was 27380 bp long with 38.4% of GC content. The J22/Pakistan/2016 shared 99.91%-99.74% nucleotide identity at genomic level with Middle Eastern strains and 98.51% from Kenyan strain. The virus differed from alpaca coronavirus by 0.64% and almost 7.92-8.2% by HCoV-229E. Phylogenetic analysis revealed that alpaca, dromedary and human-associated 229E CoVs clustered together sharing a common ancestor with bat-related 229E. Specific host and geographic associated differences were also observed from dromedary and bat-related 229E CoVs in the nucleocapsid genes. J22/Pakistan/2016 was having patterns similar to Arabian CoVs further supporting the dromedary-associated 229E viruses from Middle East and Pakistan might have evolved from another pool of viruses as compared to Kenyan CoVs. Genetic analysis of dromedary-associated 229E from Pakistan further supports the hypothesis that dromedary-associated 229E may have diverged from bats. Camels live in close association with humans in camel rearing countries and the presence of both Alpha and Betacoronaviruses along with other important viruses emphasizes the need for continuous surveillance of viruses in these animals.

The Covid-19 Outbreak: A Novel Sars-Cov2 Virus Infection Prevalence Rate at A Government Tertiary Care Hospital of Karachi Pakistan

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In the history of Corona RNA viruses (COV), the two important zoonotic COV are SARS and MERS that caused outbreak in china and Middle East in 2002 and 2012. In late December 2019 a novel corona virus outbreak occurred in the province of Wuhan, China from a sea food market of the city hubei. In the split of time this novel COV focused by worldwide and WHO declared it as an outbreak a "public health emergency" In Pakistan the first two cases of the virus reported on 26 February 2020, and by 17 June, each district in Pakistan had recorded at least one confirmed case of COVID-19. According to the studies, the first viral spread was through animal to human because of virus origin from sea food market but human to human droplets transmission increased rapidly and associated with pneumonia and pneumonia like symptoms (4). The virus spreads primarily via nose and mouth secretions through droplets produced by coughing, sneezing, and talking. The phylogenetical analysis of the nCoV2 showed 79% similarity towards the SARS-CoV, 50% towards MERS and 98.7% towards the bat corona virus strain BtCoV/4991 in accordance of the GenBank. Sea food market wuhan investigation found that Wuhan Corona virus originated from bats that might exposed to the contaminated things into the wuhan market. (4) This novel corona virus affects the upper and the lower respiratory tract. Like other respiratory viruses, this infection may take a mild course with few or no symptoms. In the reported death cases, the time from symptom onset to death has ranged from two to eight weeks (8). The study objective was to monitor the rate of this pandemic infection in a government based tertiary care hospital setting. This descriptive study was conducted in out-patient and in-patient departments at Dr. Ruth K.M. Pfau Civil Hospital, Karachi, the largest tertiary care government hospital in the province of Sindh, from the 3 months' period of 27 March to June2020. The first Government hospital at Karachi, which started the COVID-19 testing in this pandemic situation along with few private sectors. Patient having pneumonia like symptoms and other respiratory symptoms, fever, cough, flu was screened for the SARS cov2 too because of this pandemic situation. The Out Patient Department samples were collected in a designated triage area while samples of admitted Patient were collected by a trained staff with the all provided bio safety facilities of proper Personal protective equipments. The total 14983 Nasal or Oropharyangeal swab samples collected in a commercially provided viral transport media (VTM) in these 3 months of time period. After collection, the samples were sent to the designated laboratory for COVID-19 testing. Patient demographic details including age, gender, address, and contact exposure or travel history were collected and the symptoms and other clinical signs were noted. The SARS-COV2 RNA was extracted from the 1ml of VTM samples through QiA ampR viral RNA mini kit provided by Qiagen Germany. The Qia ampR Viral RNA Sample kit followed by different steps of Nucleic acid lysis solution, spin column transfers, wash and elution buffers then get viral nucleic acid elute into a separate tube to further perform RT-PCR detection/amplification. The multiplex Real time reverse transcription PCR system were performed according to Maccura Chinese based amplification kit containing specific primers and fluorescent probes targeting Open reading frame ORF1ab /Nucleopcapsid protein N/and Envelop Protein E gene sequence of SARS –CoV-2. The viral RNA/nucleic acid are detected by monitoring the fluorescence intensity in real time. Novel corona virus (nCOv) is nowadays a pandemic worldwide causing low to moderate and severe respiratory illness. The nCOV identified on basis of genomic analysis as in the group of beta corona virus that is a 7th member of this corona virus family The pandemic is the biggest public health challenge, the world has faced in this recent era. The preliminary evolutionary measures and molecular epidemiological analysis provided the basic tool to identify this unknown viral cause. Along with virus identification the molecular phylogenetic study can help in vaccine and drug development against the disease (10).The primary action is to control the spread of infection, having close attentions and monitoring the viral spread continuously by public health and government authorities for providing the better response and action(4) As far as no specific antiviral treatment or vaccine unavailability for this novel corona virus as per the public health authorities World health organization main focused to the frequent hand

washing, quarantine and proper isolation with use of all bio safety measures is the only preventive way to protect this COVID-19 infection . Results will be presented.

Diagnostic Potential of Liver Specific Micrornas and Cytokine IL-33 As Non-Invasive Biomarkers of Hepatitis

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Liver specific microRNAs (miR) and Interleukin-33 (IL-33) are proposed as biomarkers of hepatitis, we deciphered level of liver specific microRNAs (miR) and IL-33 in HCV and HBV affected human patients. The sera samples of HCV (n=225) and HBV (n=80) human patients and healthy control (HC; n=50) were collected for qPCR based relative quantification of liver specific miR-122, miR-192 and miR-29. Quantification of serum IL-33 in chronic HCV patients (n=214), HBV (240) and in HC (n=50) was carried out by Human IL-33 ELISA Kit. Hospital data of studied human patients showed an increase in AST/ALT levels with presence of increased level of HCV viral load or HBV sero-positivity (HBsAg) by ELISA. The liver specific miR-122, miR 192 and miR-29 were raised in the sera of acute and chronically HCV infected human patients but not in hepatocellular carcinoma (HCC) patients. The HCV affected patients aged between 31-80 years with higher prevalence in males (35%) than in females (23%). The predisposing factors for chronic hepatitis B were blood transfusion (OR=2.55), injection drug abuse (OR=5.56), surgery (OR=2.4) and dental operations (OR=2.35). The level of miR-122 and miR-192 in the sera were increased in chronic HBV patients and miR-122, miR-129 and miR-192 were increased in the sera of acute HBV patients. The soluble level of IL-33 in sera and ALT/AST were significantly increased in chronic HCV patients as compared to HC. IL-33 level in HBV and HCV human patient's sera was significantly raised compared to HC. A positive correlation was found between serum IL-33 level in hepatic subjects and their AST/ALT levels indicating potential of IL-33 as a diagnostic marker of liver injury. In conclusion, the serum level of IL-33 and liver specific miR-122, miR-129 and miR-192 were over-expressed in HCV/HBV human patients evidencing its diagnostic and biomarker potential in liver diseases.

Development of Diagnostic Test to Determine the Incidence of Mycoplasosis in Bovines in Karachi.

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Respiratory syndrome has emerged as a health hazard affecting cattle and buffaloes. This syndrome is an acute and frequently fatal respiratory tract infection. The study concludes that *Mycoplasma*/ *Acholeplasma* are main agent responsible for respiratory issues. Dot-ELISA against *M. bovis* and *A. laidlawii* were developed. It is useful for the detection of antibodies against respective species of *Mycoplasma* and *Acholeplasma* for routine diagnosis. However, it could be useful tool for diagnosticians directly in the livestock farm. So the development of Dot-ELISA kit is required that would be valuable for veterinary doctors and farmers to identify the *Mycoplasmosis* in livestock farms. Likewise, Rapid serum agglutination (RSA) was also successfully developed in Microbiology

laboratory but its sensitivity and specificity was found less as compare to Dot-ELISA. It is also cheap, rapid and valuable in the field diagnosis. Results were obtained within few minutes from suspected and non-suspected cases of Mycoplasmosis. The highest incidence and sero-prevalence was reported in well saturated livestock, mixed population of animals (buffaloes, cattle and goats) and hygienically poor farms. On the basis of Dot-ELISA, in the buffaloes was very high to *Mycoplasma* infection in Surjani Town (18.4% *M. bovis* antibodies) and in Nagori Colony (49.4% *A. laidlawii* antibodies). Likewise, early infection detection through RSA was determined in Surjani Town (7.9% antibodies against *M. bovis*) while 40% buffaloes were exposed with *A. laidlawii* in Madina Society. The prevalence of *Mycoplasmosis* is high in Karachi. Antibodies were detected even from those asymptotic buffaloes. It is fact, the cultural technique is gold standard but many of mycoplasmas/ acholeplasmas may not grow due to many reasons or it needs more expertise, medium supplements and time required to grow. However, sero-prevalence by using Dot-ELISA and RSA were revealed 48.8% and 33.2% respectively

Seroepidemiological Study of Crimean Congo Hemorrhagic Fever in District Mardan

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Crimean Congo Hemorrhagic Fever (CCHF) is a viral zoonotic tick-borne disease mainly caused by CCHF virus and massively circulating in domestic and wild animals. The virus is transmitted to livestock population via bite of Hyloma ticks and to human through direct contact with blood of verimic animals or body fluid. In the present study, seroprevalence of IgG antibodies to CCHF in human and livestock's population and risk factors associated with CCHF among both populations were determined. A total 55 households/ Farms were visited in district Mardan in which 400 serum samples from livestock, and 70 serum samples from farmers were collected. All farmers were extensively interviewed to identify major risk factors. All the serum samples were tested on Indirect Elisa (ID Screen) to determine the IgG antibodies in both Populations. A total 15.25% (n= 61/400) prevalence was recorded in livestock population. Highest ratio was detected in buffalo (20%) followed to Goat (16.6%) and Cow (12%). While no positive sample were detected in farmers (n=0/70). Tehsil Rustum was highly exposed to CCHF having 17% prevalence. Lowest rate was detected in Tehsil Mardan (13.3%). Among the major risk factors of CCHF, it was observed that Hyloma ticks being circulating in all households (91%) is on the top. All individual was in closed contact with livestock. Most of the participants (92.8%) were unaware of CCHF and its transmission which increased risk of CCHF. On the basis of above facts, it is concluded that there is a high ratio of CCHF in livestock population, although these cases are present sporadically. Adoption of proper preventive measures and awareness campaign are need, otherwise it may cause severe mortality in livestock's which will directly affect country economy.

Outer Membrane Vesicles from Gram-Negative Bacteria and Their Interactions with Host Cells

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Bacteria in general and Gram-negative bacteria in particular continuously shed outer membrane vesicles (OMVs) to their extracellular environment. OMVs are lipid membrane bound, non-replicative, spherical structures often decorated with proteins and LPS which are in analogous to the bacterial outer membrane from which they are released. In the recent years, OMVs have gained considerable attention due to the facts that they have various cellular, biochemical and pathological roles. Helicobacter pylori (H. pylori) is a gastric pathogen infecting half of the world's populations. Although, the majority are asymptomatic, in some individuals H. pylori infections can progress into peptic ulcers and in some into gastric cancers. Like other Gram-negative bacteria, H. pylori continuously sheds OMVs and has been extensively studied previously. Despite of so much interests, very little is known about the extra- and intracellular trafficking of OMVs. In most, if not all, of the cases OMVs need to be internalized into the cells. OMVs can use multiple internalization pathways including but not limited to; clathrin mediated endocytosis, caveolin mediated endocytosis, Macropinocytosis and phagocytosis. Similarly, in order to induce biological response, OMVs need to avoid lysosomal digestion and escape from endosome. We have recently observed that, prior to internalization, OMVs are attached to the cell surface filopodia. Various mechanisms are utilized by filopodia to bring OMVs to the cell surface, where internalization takes place. These include filopodia retraction and surfing of OMVs on the surface of filopodia. Moreover, with the help of various imaging tools including electron microscopy, confocal microscopy and live cells microscopy we have deciphered the cellular trafficking of OMVs. We are very hopeful that our current findings will help us to understand the cellular trafficking of OMVs subsequently helping us to understand this mode of virulence transmission by the bacteria.

Inhibition of Viral pl-pro (papain-like proteases) Protein and Cytokine Release Syndrome using Coumarin Derivatives through Insilico & Invitro Approaches: Filling Two Needs with One Deed

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SARS-CoV-2 infection recently emerged as a pandemic affecting millions of people worldwide. Cytokine storm or cytokine release syndrome is the deadly consequence of the fight between immune system and the virus. Interestingly the its not alone virus titer that leads to critical condition but the storm of cytokines that includes mainly TNF-α, IL-1β, GM-CSF, IL-6, IL-18, IL-2 and IFN-γ. In proposed study we identified natural compounds derivative that can inhibit the viral protein and cytokine storm simultaneously using in-silico and in-vitro approaches. Docking was performed to check the antiviral potential against PL-pro viral protein. Extracellular and intracellular oxidative burst of reactive oxygen species was inhibited by colorimetric and chemiluminescence assay. Intracellular nitric oxide inhibition was checked on RAWJ774.2 cells. Cytokines namely TNF-α, IL-1β, GM-CSF, IL-6, IL-2 and IFN-γ were quantified by ELISA. Alamar blue assay was used for accessing the

proliferation of the cells. Cytotoxicity of the compound was checked on normal human fibroblast cell line by MTT 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl tetrazolium bromide) assay. Our compound has shown inhibition of intracellular and extracellular species and iNOS (IC50 10.22±4.8). It significantly decreased the level of pro inflammatory cytokines TNF-α (IC50 0.22±0.09), IL-1β (IC50 1.61±0.19), GM-CSF (IC50 4.1±1.2), IL-6 (IC50 2.2±0.29), IL-2 (IC50 1.058± 0.08) and IFN-γ (IC50 .058± 0.08). It was non-cytotoxic on normal human cells but showed inhibitory effects against leukemic cells presenting hypercytokemic microenvironment. Our compound belonging to coumarin derivative HN9359 has showed a great potential to suppress the cytokine release syndrome and the viral protein simultaneously and can be categorized as an antiviral and anti-inflammatory lead.

A Serological Survey of Severe Fever with Thrombocytopenia Syndrome Virus (sftsv) and Crimean Congo Hemorrhagic Fever Virus (cchfv) from Faisalabad, Pakistan.

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Hemorrhagic septicemia is highly acute, fatal and septicemic bacterial disease mainly affecting water buffaloes and cattle, caused by *Pasteurella multocida* with much higher incidence in animals younger than 2 years. The disease is clinically manifested by high fever, respiratory distress, salivation, nasal discharge, tongue protrusion, hot and painful swelling in throat region and edema in brisket and occasionally gastrointestinal discomfort. The successful prophylaxis regime includes the adjuvant-based vaccination of the animals. The present study was, therefore, been planned to prepare and evaluate the comparative efficacy of 2 formulations of chitin derived chitosan adjuvanted hemorrhagic septicemia (HS) vaccines and comparing its efficacy against oil based HS vaccine. Chitosan adjuvanted vaccine was prepared by diluting 0.2% (W/V) chitosan in 25 mM sodium acetate solution (pH 5.0) and adding suspension culture of *Pasteurella multocida* at 1:2 and 1:4 chitosan and similarly 1:2 mineral oil vaccine was prepared. Adult male rabbits (n=60) were randomly be divided into 4 groups (1 to 4) of 15 each, having equal weight and age. Rabbits in group 1 were kept as control and did not receive any vaccine. Group 2 were vaccinated with 1:2 Chitosan adjuvanted vaccine and similarly group 3 and 4 were given 1:4 chitosan adjuvanted and 1:2 oil adjuvanted vaccine respectively. Sera were collected on weekly interval post vaccination for seven weeks and titrated for Antibody titers through indirect hemagglutination (IHA) test. Challenge/Protection test was given after 4 weeks of vaccination in 5 rabbits from each group and results were subjected to statistical analyses. The results depicted significantly higher IHA titers in group 4 followed by group 3 and 2 respectively. But these titers did not sustain longer and after 4 weeks, there was a decline in titers of all vaccinated groups at a different rate. After 4 weeks, IHA titers of group 3 were significantly higher than others, followed by group 4 and 2 respectively. Therefore, it can be concluded that initially oil adjuvanted vaccine imparted highest protection against HS, but later on after 4 weeks, protection was maximum with 1:4 chitosan adjuvanted vaccine.

Small Interfering RNAs Targeting *agrA* and *sarA* Attenuate Pathogenesis of *Staphylococcus aureus*

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The use of small interfering RNA (siRNA) gene silencing is a promising therapeutic option as it does not impose selective pressure on bacteria that is often associated with the development of resistance. The study assessed the effect of siRNA targeted to *sarA* and *agrA* in *S. aureus* and the relationship between the transcriptional response, biofilm formation and pathogenicity. siRNAs designed against *agrA* and *sarA* were electroporated into methicillin-resistant and methicillin-susceptible *S. aureus* strains. mRNA levels, growth kinetics, biofilm formation and minimal inhibitory concentration were measured. Efficacy of siRNA in bacteria was assessed using survival assays in a *C. elegans* model. Differences in gene expression before and after siRNA treatment were anaysed using the paired t-test, while the log rank test was used to assess the significance of any difference among survival rates of nematodes. Biofilm formation decreased significantly in siRNA treated strains and growth rates of siRNA treated strains were significantly higher compared to untreated strains. We observed significant decreases in the transcriptional response in siRNA treated strains, with concomitant significant increases in the lifespan of *C. elegans* worms exposed to siRNA-treated versus untreated strains. siRNA targeted to *agrA* and *sarA* lowered mRNA transcription and pathogenicity of *S. aureus.*

Prevalence of Pathogenic Free-Living Amoeba in Diverse Environmental Resources Across Pakistan and Its Impact On Public Health in Future

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Free living amoebae (FLA) are opportunistic protozoan pathogens and therefore play a predatory role and help to control microbial communities in the ecosystem. In contrast, the ability of pathogenic FLA (*Acanthamoeba*, *Balamuthia* and *Naegleria*) to produce central nervous system infections in human especially is also a growing concern worldwide. Here, we evaluated prevalence of pathogenic FLA from environmental resources like air, soil and water across Pakistan. One hundred and twenty-one various water, 78 soil and 30 air samples were examined. FLA was identified by morphological characteristics of their cysts on non-nutrient agar plates seeded with *E. coli*. Additionally, the PCR was performed with genus-specific primers followed by direct sequencing of the PCR product for molecular identification. Overall, FLA was recovered from ~52 % of the examined samples. *Acanthamoeba* was found in 38 and 8 % and *Naegleria* in 18 and 5% of water and soil samples respectively while *Balamuthia* was not recovered from any medium. Interestingly *Acanthamoeba* was recovered on 30°C while two *Naegleria* species were successfully isolated and cultured on both 30 and 42°C. This is the first report demonstrating inclusive survey for pathogenic FLA from various environmental sources across Pakistan, which suggests FLA could be a potential threat to public health to which the population is exposed.

Knowledge and Attitude Towards Vaccination and its Impact on Different Variants of SARS-CoV-2 Among General Public of Pakistan: A Cross Sectional Study

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Globally, a COVID vaccination campaign is ongoing stop the spread of this deadly viral infection. Perceptions and attitude of the people towards the vaccines and their knowledge about the pandemic is largely affected by the rumors circulating in the public. This study aimed to assess the knowledge, attitude and perceptions of the general public towards vaccination. This study was based on a self-devised e-survey form and followed a convenient study design. The survey was divided into 3 sections: demographic information, knowledge towards Covid-19 and vaccines, and attitude towards vaccination. The survey was distributed in general public via electronic links through social platforms in 4000 individuals following Checklist for Reporting Results of Internet ESurveys (CHERRIES) guidelines. Knowledge was measured by 17 questions. Similarly, attitude was measured by 6 questions, on a 3 points Likert's scale. Statistical Package for Social Sciences (SPSS) version 26 was used for statistical analysis. Chi-square was used to determine association between various variables. Multiple regression analysis was performed for variables showing significant differences. A total of 3010 responses were received in which 53.39% (n=1607) were female and 46.25% (n=1392) were male and their age ranged from 16-71 years. The healthcare workers constituted 31.53% (n=949). Most of the participants were employed (75.75%). Students constituted 18.74% (n=564) and majority had graduate level of education 65.32% (n=1966). Majority (73.85%, n=2223) were fully vaccinated while (6.31%, n=190) were unvaccinated. Majority were vaccinated with Sinovac 43.74% (n=734) followed by Sinopharm (33.79%, n=567). The infectivity rate was 18.74%. A small proportion (5%) of the individuals were affected by the virus after their second shot of the vaccine. The mean knowledge and attitude score was 8.12 ± 2.83 SD (out of 17) and 3.90 ± 1.50 SD (out of 12), respectively; with an overall correct rate of 47.91% for knowledge. 46.26% of the participants were knowledgeable and 65.12% were having positive attitude. The mean knowledge score was higher in participants with high education level (8.91 vs 7.86), urban living type (8.19 vs 7.96), and fully vaccinated (8.4 vs 7.75 and 6.35) participants. The participant's priority source of knowledge was social media 70.8% (n=1188), followed by government services 21% (n=352). Majority (62.63%, n=1051) had wrong knowledge that they will get positive PCR result upon vaccine. Positive attitude about SOPs observation was recorded in 86.11% of the participants. Only (23.48%, n=394) believed that covid vaccines are effective against all variants. Concern was shown by 64.42%, n=1081 participants for knowing the variant if they are infected. The female participants were statistically more knowledgeable than males with a p value of <0.0001. The vaccination rate is good. Positive attitude is vey low in general public about the COIVD vaccination. The overall knowledge about COVID and its vaccination is also very low and more effective campaign is required to effectively eliminate the negative attitude towards COVID vaccination drive.

Assessment of Biosafety Implementation in Clinical Diagnostic Laboratories in Pakistan in Relevance to The Covid-19 Pandemic

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Laboratory diagnostics capacity is crucial for optimal national response to a public health emergency like the COVID-19 pandemic. Preventing laboratory acquired infections and the loss of critically required human

resources, especially in emergencies like the COVID-19 pandemic, requires laboratories to have a good biorisk management system in place. This study aimed to evaluate laboratories' biosafety and biosecurity situation in Pakistan during the COVID-19 pandemic. In this cross-sectional study, a self-rated anonymous questionnaire was distributed to laboratory professionals (LPs) working in clinical diagnostic laboratories, including laboratories performing PCR-based COVID-19 diagnostic testing, in Punjab, Sindh, Khyber Pakhtunkhwa (KP), and Gilgit-Baltistan (GB) Provinces and Islamabad during March 2020 – April 2020. The questionnaire assessed knowledge and perceptions of LPs, resource availability, and commitment by top management in these laboratories. 58.6% of LPs performing COVID-19 testing reported that their laboratories did not perform a biorisk assessment before starting COVID-19 testing in their facility. Only 31% of LPs were aware that COVID-19 testing could be performed at the BSL-2 level as per the WHO interim biosafety guidelines. A sufficiently high percentage of LPs didn't feel confident in their ability to handle the COVID-19 samples (32.8%), spills (43.1%), or other accidents (32.8%). These findings demonstrate the need for an effective biosafety program implementation, proper training, and the establishment of competency assessment methods. These findings also suggested that identifying and addressing gaps in existing biorisk management systems through sustainable interventions and preparing LPs for surge capacity is very important to better address health emergencies.

Mask Using Practices During Current Pandemic

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The Novel Coronavirus (covid-19) continues to be a major health burden all over the world with astonishing reports of fatalities. The burden on healthcare systems is tremendous, as Covid-19 is spreading via respiratory droplets, A medical face mask with an adequate microbial barrier can also be effective in reducing the emission of infective agents from the nose and mouth of an asymptomatic carrier or a patient with clinical symptoms. Many different types of masks are available, numerous people complain that wearing masks causes them difficulty in breathing and causes rashes, acne, allergies, and other problems. A correctional study was designed and data were collected via an online survey to highlight people's perspectives on this whole matter. A total of 240 participants responded to the questionnaire. Male and Female responders were about 32% and 68% respectively. About 77 % of people considered that they like to wear masks during the current pandemic. 27.2% responded that they re-use surgical masks. 93.5% of these responders agreed that wearing masks is effective against current Covid-19. 71% responded that it was difficult for them to wear a mask for a long time. 59.5% preferred face fitted mask. 31.1% reported that mask usage causes them acne, 11.5% reported that they get redness on the face,8.7% reported pigmentation on their skin, 55.7% felt suffocated, 29.5% face itchiness, 7.2% face swelling. 30.6% agreed on mask makes it hard for them to communicate. When they were asked where they buy face masks, about 75.7% purchased from Pharmacy, 7% from general stores, 8.1% from local shops and about 3.8% mentioned street vendors. 92.4% of responders who use masks feel protected by the use of masks. About 83.2% were agreed that wearing a mask is still necessary after vaccination. 82.7% agreed on masks should be tested in the laboratory. On

the basis of this study, it has been concluded that most of the population were agreed that wearing a mask is an effective way to help in reducing Covid-19 transmission without causing any major side effects, provided that, masks are clean and used correctly.

Microbial Pollution and Its Impacts On Community Residing Near Sewerage Drains of Lahore, Pakistan

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Microorganisms are present across all ecosystems however their concentration varies as per the conditions of different sites. Huge microbial communities are located in the regions offering favorable atmosphere for their long-term sustainable growth, such as uncovered sewerage drains which hold heaps of solid waste in them. Owing to the increased proportion of these biological pollutants, water and air quality is highly compromised in such areas. This study was carried out along the Cantt drain, Sattu Katla drain, Lower Chotta Ravi drain, Upper Chotta Ravi drain and Shalimar Escape Channel drain of Lahore. Samples of water and air collected from these drains and nearby dwellings were assessed to check microbial load in them. A questionnaire survey was carried out to evaluate the impacts on life quality of people living near open drains. The quality of drinking water supplied in those areas was also analyzed according to the standards of National Environmental Quality Standards (NEQS). 55% drinking water samples were contaminated with feacal coliforms. Bacterial species isolated from water and air samples were identified through phenotypic and biochemical characterization. Among identified species, more were gram negative and belong to class Enterobacteriaceae. According to the results, identified bacterial species were E. coli, Shigella, S. aureus, K. pneumoniae, P. aeruginosa, P. mirabilis and Salmonella. This study provided a look into the life quality of public residing near open drains of Lahore District and also explained the quality of drinking water supplied in those areas.

Knowledge and Attitudes Towards Stethoscope Hygiene and Bacterial Contamination

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To compare the knowledge, attitudes, and practices related to stethoscope hygiene in the medical students, residents, and identify bacterial colonizers on the stethoscopes It was a cross-sectional study conducted from the year 2015 to 2016 included medical students, residents and attending physicians at Aga Khan University Hospital, Karachi. knowledge, attitudes, and practices about stethoscope hygiene were acquired by a questionnaire. Sterile swabs were collected from diaphragm and earpieces of each stethoscope and sent for bacterial identification. The cumulative responses on items of knowledge were categorized into positive; “have knowledge” and negative; no knowledge. Attitudes were characterized into positive with consideration of responses into ‘important’ merged

with 'minimally important' and negative attitudes were acquired by 'not important' responses Practices were considered by summing of individual responses. The final responses based on gender, designation and location of sample collection, questions on knowledge, attitude and practices were compared with the presence of organisms in the studied samples by Chi-Square test and the results were considered significant with p values <0.05. The comparison of the relationship in between knowledge and practices related to the stethoscope hygiene showed that 73(65%) participants had knowledge but did not have time to practice stethoscope cleanliness regularly (p<0.01) which may have resulted in bacterial contamination(p=0.06) Bacterial contamination was observed in stethoscopes of more than half of health care professionals especially in tertiary health care establishments.

Sero-Epidemiology of Hepatitis C in Healthy Population Of District Chiniot

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Approximately 180 million individuals are diseased with infection of Hepatitis C virus, although Pakistan has the maximum occurrence ratio of infection, an estimated 8.0 million of total Pakistani population is positive for HCV antibodies. However, in Pakistan exact epidemiological evidence is relatively inadequate. One of the severe health concerns in District Chiniot, Punjab, Pakistan is the occurrence of viral Hepatitis C infection. Therefore present study was conducted to evaluate the sero-prevalence of Hepatitis C virus in healthy population of District Chiniot. Overall 180 blood specimens were drawn from healthy residents of three Tehsils of district Chiniot i.e. Chiniot, Bhuwana and Lalian out of which 79 were female and 101 were male. Serum specimens were used to perform Enzyme linked Immunosorbant assay (ELISA) to spot anti HCV immunoglobulin. From current study, it was estimated that overall Sero-prevalence was 26.66% among tested population of district Chiniot Pakistan. It was revealed from present study that district and tehsil Chiniot had the highest sero-prevalence ratio (31.34%) which was higher than Tehsils Lalian and Bhuwana. Those with age of above 40 years were predisposed to HCV infection (33.33%). Similarly, transfusions of blood (42.30%) dental techniques (38.63%), surgical interventions (36%) body piercing (35.71) were found to be the significant risk factors for HCV transmission. So it is need of time to aware general public of district Chiniot Pakistan about such high prevalence of Hepatitis C infection, mode of HCV spread and precautionary measures for the better control of this fatal disease in area in near future.

COVID-19 Vaccines

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A recently-discovered SARS-CoV-2, caused pandemic of respiratory illness, first appeared in China, in December'2019. As the world is in the midst of COVID-19 pandemic, researchers compete to develop effective vaccines. On 18th, February'2021, seven different vaccines across three platforms have been mounted in

countries, i.e., Pfizer, Moderna, Sinovac, AstraZeneca, Gamaleya, Novavax, Johnson & Johnson and CoronaVac vaccines. U.S. FDA authorized Johnson & Johnson vaccine as 66% effective, in comparison to 95% Moderna and Pfizer. AstraZeneca vaccine showed 64% safety after only one standard dose. According to an article submitted to FDA, Moderna vaccine provides 80.2% protection after one dose, in contrast, 95.6% after the second (in aged 18 to 65, it's 86.4% in those over 65). The CoronaVac vaccine is effective differently according to different researches. Another, BBIBP-CorV, 79% effective after two doses, but hasn't been verified internationally. Outside China, It has been approved in Bahrain, Egypt, Jordan, Seychelles, and UAE. In UAE its first recorded efficacy is 86%. Health experts show concerns over Pakistan's decision to allow private companies to import vaccines. Healthcare workers are vaccinated on February'3rd after receiving 500,000 doses from Chinese company, setting-up over 500 inoculation centers. 53,000 healthcare workers have been vaccinated while 65 are now registering to receive the jab. China's Sinopharm vaccine was also brought on December'31st and a national vaccine committee was formed. There are three companies; AJM, Sindh Medical Stores Services and AJP, which are now allowed to import the jabs. These will purchase the Astrazeneca, CanSino and Sputnik V vaccines.

Prevalence of Needle Stick Injury and Nursing Practices Regarding Safe Injection and Sharp Disposal Working in Critical Care of Two Tertiary Care Hospitals

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This observational cross-sectional study design (Pilot) was conducted to assess the prevalence of needle stick injury and nursing practices regarding safe injection and sharp disposal in critical care units of two tertiary care hospitals from 1st July to 30th August, 2014. Data was collected using self-developed questionnaire about the prevalence of NSI and nurses' practices regarding safe injection and sharp disposal after thorough literature review, and then was given to the expert for review. Finally, the data was collected from the participants after verbal consent. The study result showing that about half of the nurses have no knowledge regarding disposal of sharp and it has been found that 47.8% re-cap the needle prior to disposal. While 32.6% reported needle prick injury. Inadequate knowledge among nurses about safe nursing practices and lack of using preventive measures from Needle stick injury were identified. Lack of reporting is also a factor identified in this study.

Antibiotic Stewardship in Regional Context, Challenges and Opportunities

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Antibiotic stewardship program is direly needed to combat global threat of antibiotic resistance. The study aimed to determine knowledge, perspectives and practices of attending physicians regarding various aspects of antibiotic stewardship program including antibiotic stewardship roles, rational use of antibiotics, antibiotic resistance, prescribing practices and factors associated with these practices. In this qualitative study, a total of 19 semi-structured, in-depth interviews with doctors of three tertiary care public sector hospitals in Multan and Dera Ghazi

Khan were conducted. The convenient sampling method was adopted to collect the data. Sample size was collected using saturation point criterion. Thematic analysis approach was used to deduce findings. The analysis of data yielded 04 major segments, 12 sub segments with 24 categories. The themes included, (i) perception about antibiotic use and antibiotic stewardship, (ii) antibiotic prescription practice with rational use and antibiotic resistance, (iii) strategies adopted by hospital management to ensure quality and safe distribution of antibiotics, (iv) implementation of antibiotic stewardship program: barriers, suggestion and future benefits. Physicians had misconceptions about the use of antibiotics. The perception regarding antibiotic stewardship programs has been found poor. Moreover, very few activities related to antibiotic stewardship program. The participants gave many suggestions for successful implementation of antibiotic stewardship program in order to overcome the challenge of antibiotic resistance, including development of guidelines for the use of antibiotics, strict legislation regarding use of antibiotics, active participation of healthcare professionals and awareness program among the general public about the use of antibiotics. This study concluded that improvement in the knowledge of doctors regarding antibiotic stewardship program is needed. It also highlighted the need for development of antibiogram of hospital and lack of rules for the safe use of antibiotics are the key elements promoting irrational utility of antibiotics and development of antibiotic resistance.

Response Surface Methodology for Enhanced CMCase Production by *B. Licheniformis* TLW-3.

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The demand of cellulase enzyme has been increased globally because of its diverse industrial applications. Cellulases are applied in various industrial setups like textile, agricultural, pharmaceutical, detergents, paper, wine and food industries. The most demanding application of cellulases is the production of biofuel from the agro industrial waste. The consortia of celluases (with different isozymes) work synergistically for the complete degradation of cellulosic biomass. There are various factors that can influence on enzyme production including temperature, pH, medium composition, incubation time, inoculation concentration, incubation condition etc. Classical method i.e one variable at a time (OVAT) has drawback that unable to find out the interactive effect of multiple factors on enzyme production. Response surface method (RSM) is the alternative method that is used to study the significance of multiple factors interaction on enzyme production. During the presented study central compost design (CCD) was constructed by using Minitab software (version17) with six factors (temperature (˚C), pH, incubation time (hour) and concentration of peptone, carboxymethyl cellulose (CMC) and yeast extract) that were previously optimized by OVAT. Total 53 experiments were designed and performed. Obtained results were

analyzed with the help of Minitab 17. Results were found significant and the model was fit with 0.11 lack of error which shows the fitness of the model. Contour and response surface plots showed good interactions among the factors.

Bioevaluation of Antioxidative and Antimicrobial Efficacy of Indigenously Produced Varieties of *Moringa Oleifera* Leaves

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Moringa oleifera leaves were used worldwide for the treatment of different diseases due to the of presence of bioactive compounds present in it. Indigenously produced Multan and Indian variety of *Moringa oleifera* leaves were used for ethanol and methanol extract preparation by ultra-sonication. Phytochemical analysis was done by High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared Spectrophotometer (FTIR) which showed presence of bioactive compounds responsible for antimicrobial and antioxidant activity. HPLC determine the presence of kaempherol in Multan variety while presence of salicylic acid and sinapic acid in Indian variety make it strong antimicrobial and antioxidant agent. FTIR analysis showed the presence of carbohydrates, proteins and amides in both varieties with varied. Antioxidative activity of the *Moringa oleifera* leaves (indigenously produced Multan and Indian variety) was checked by DPPH assay. Total Phenolic Content (TPC) was higher in Multan variety of *Moringa oleifera* leaves while Total Flavonoid Content (TFC) was higher in Indian variety. Antibacterial activity of the *Moringa oleifera* leaves was studied by agar well diffusion method. Four bacteria were used for this purpose. In both varieties of *Moringa oleifera* leaves Escherichia coli gave the highest activity than Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis but the Multan variety gave higher zone of inhibition than Indian variety of *Moringa oleifera* leaves. Presence of active compounds in both varieties of the *Moringa oleifera* leaves showed its potential for antioxidant and antimicrobial activity.

Biodegradation of Aflatoxin Through Environment Friendly Bacteria

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Aflatoxins are the secondary metabolites of fungal species usually produced in tough environmental conditions. The interruption of fatty acid formation resulting in Aflatoxins production. It is equally important in food and feed due to its health complication for animals and human beings. Biodegradation through environment friendly microbes are acceptable strategy for the control of mycotoxins. A study has been designed to evaluate the environment friendly bacterial species for the biodegradation of Aflatoxins. Specific double strength broth media (BHI, TSB, MRS and SDB) were mixed with standard Aflatoxins B1 to obtained 30 ppb final concertation. Different bacterial species (*Rhodococcus erythropolis*, *Bacillus lichenoformis*, *Bacillus subtilis*, *Lactobacillus Pantosis*, *Lactobacillus casei*, Actinomycetes spp., and Saccharomyces crevice) were added to the mixture

containing aflatoxins and incubated for 48-72 h. The concentration of the aflatoxins in the mixture were confirmed with Agra strip, Agra quant and HPLC at starting and end of incubation. Results showed that *Rhodococcus erythropolis*, *Bacillus lichenoformis* and *Bacillus subtilis* are able to degrade or alter the structure of aflatoxins to the undetectable level of the Agra strip (less than 4 ppb) and Agra Quant (less than 2 ppb), these results were confirmed by HPLC analysis which showed the reduction of aflatoxins to 0.57, 0.95 and 0.91 ppb by *Rhodococcus erythropolis*, *Bacillus lichenoformis* and *Bacillus* subtilis respectively. These results conforming the biodegradation of aflatoxins B1 used in the study. The organisms used in the study are environment friendly and is capable of having the potential to decrease the of aflatoxins to the acceptable consumption level. Uses of these microbes for biodegradation will have impact over the biocontrol strategies of the mycotoxins.

Improving Microbial Population and Rice Production Through Integrated Use of Organic and Mineral Nutrient Sources

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Rice is one of the major cereal crops in the world and it plays major role in the agricultural economy of Pakistan. Rice provides high nutritional food values that fulfill the dietary requirement of the humans. A field study was conducted at Nuclear Institute of Agriculture (NIA) Farm, Tandojam to assess the microbial dynamics under various organic sources for enhancing rice growth and yield. Most widely grown rice genotype, NIA-Shandar was used as a test genotype. Two organic sources viz poultry manure & FYM (each 10 t ha^{-1}) were used along with half (60 & 45 kg of N and P_2O_5 ha^{-1}) and full levels (120 & 90 kg of N and P_2O_5 ha^{-1}) of mineral fertilizers. The zinc was applied at the rate of 10 kg ha^{-1}. The experiment was laid in randomized complete block design (RCBD) with three replications. The maximum general microbes (38×10^8cfu g^{-1} soil), N_2 fixing (19×10^7CFU g^{-1} soil), P solubilizing (45×10^7 CFU g^{-1} soil) and Zn solubilizing (13×10^7 CFU g^{-1} soil) microbial population was recorded in poultry manure where half N & P_2O_5 fertilizer was applied. Similarly, the maximum plant height (106 cm), number of tillers (16), number of panicles (26), 1000-grain weight (26.01 g) and grain yield (6.92 t ha^{-1}) were also observed in poultry manure integrated with half fertilizer application. Thus, poultry manure (10 t ha^{-1}) application along with half level of chemical fertilizer can potentially improve microbial population and rice growth and yield.

Biodegradation and Decolorization of Azo dye RB-221 by Novel Strain *Pannonibacter phragmitetus*-IMI-1C Isolated from TWW of Faisalabad

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Unprecedented industrialization is leading the world towards the environmental crisis. Excessive water pollution is one of the major contributors to ecological crisis. Among all, in textile industries azo dyes are major contributors to water pollution, due to pervasive use. The reluctant and carcinogenic nature of azo dyes poses difficulties in degradation. The current research was designed to evaluate the bioremediation and degradation

potential of novel strain *Pannonibacter phragmitetus*-IMI-1C isolated from textile waste-water of Faisalabad. The *Pannonibacter phragmitetus*-IMI-1C a gram-negative facultative anaerobe, decolorized the reactive azo dye RB-221 at the concentration of 5g L^{-1} up to 96 % within 24-72 hrs. The dye decolorization assay was performed using OFAT (one factor at a time) approach, including temperature of 37 ^{o}C, pH range of 7-9, inoculum concentration of 50 µl and supplementation of 1% starch and peptone as carbon and nitrogen source accordingly. UV–Visible spectrophotometry and FT-IR spectral analysis confirmed the biodegradation of RB-221. Phylogenetic analyses based on 16S ribotyping revealed that the *Pannonibacter phragmitetus*-IMI-1C belong to genus *Pannonibacter*. Thus, the significant decolorization represented by the novel strain can be utilized for the biodegradation and decolorization azo dyes to reduce the pollution burden on environment.

Isolation of MDR *Campylobacter* Species in Commercial Poultry and Tet(O) Mediated Gene Resistance Against Tetracycline

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According to the CDC report, Campylobacter is on the top of foodborne causing pathogens followed by the salmonella, Yersinia and E. coli. Mainly two species *Campylobacter jejuni* and *Campylobacter coli* are frequently causing illness in humans. These are gram-negative, microaerophilic, non-spore-forming, and comma to curved shaped microorganisms. The common route of transmission is the faecal-oral route. The different kinds of antibiotics are used to cure the infection in humans, as well as these antibiotics, are used in the poultry industry as medicine along with feed additives. The unjustified use of antibiotics has caused resistance in Campylobacter against several antibiotics. The antibiotic resistance of campylobacter against tetracycline class of antibiotic at a molecular level was determined with specific primer sequences that target the specific resistant genes. For this purpose, the Campylobacter was isolated and identified from fecal samples of poultry birds on a selective media CCDA with selective supplements. Later on, it was confirmed microscopically and by biochemical tests. The spiral to curved shape of pink colour clolonies indicates the samples were Campylobacter positive. *C. coli* and *C. jejuni*, were confirmed by multiplex PCR. The campylobacter prevalence in the area of study was 70%. The antibiotic resistance of isolated campylobacter spp. was checked by Kerby Beurer disk diffusion method. It was found that 90% of isolates were highly resistant against tetracycline and for and doxycycline the percentage was 75%. Further, the resistant gene responsible for the development of resistance was targeted by using specific primer sequences in conventional PCR. The gene responsible for the resistance of isolates was *tet(O)* and 30% of the isolates were detected positive for the presence of antibiotic resistance gene in them. It was confirmed from this study that the campylobacter has developed a high level of resistance against tetracycline. This study concluded that the Campylobacter species in broiler chicken has developed resistance against Tetracycline due to the *tet(O)* gene . To control the prevalence of antibiotic resistant bacteria, there is a need of public health concern to make strategies to overcome the resistance pattern.

Entrepreneurship in STEM: Opportunities and Challenges

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Entrepreneurship is creating or setting up a business not only for the purpose of generating profit but also for providing job opportunities. Entrepreneurs are problem solvers and product developers. They use their financial asset for hiring a professional team and combine the innovation, knowledge and leadership leads to a successful startup or a business. To be a successful entrepreneur, one should be people-oriented and have perseverance. One must have the ability to take risk, believe in his/her potential and have the courage to move forward in all circumstances. Most importantly have the ability to take risk and can assess the risk. For STEM students, entrepreneurship is of specific importance and relevance as it helps them not just to be able to solve real life existing complications but to be able to recognize and identify emerging needs and glitches. It is becoming increasingly apparent that in today's world, there is a need as well as a desire for STEM and entrepreneurship to work together. In Pakistan entrepreneurship is slowly emerging yet we are far behind. It is high time that we should introduce modern teaching methods and inculcate entrepreneurial initiative in students. A course on entrepreneurship can be included in the syllabus and we must invite businessmen and policy makers to motivate young minds for entrepreneurship. This must be pitching competitions, opportunities to win seed funding and facilities of incubation centers. In Pakistan, there are many good public sector research institutes yet there is a void gap in private sector. Only few research institute are meant for research and development. BJ Micro Lab is one of them. It is SECP registered company and is working in academia to promote and facilitate research in STEM.

Assessment of Biodegradation by *Bacillus subtilis* in Combinations with Biosurfactant and Extracellular Lipase.

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Polyethylene is one of the major plastic pollutants infesting the land and marine environment. The excessive use of polyethylene everywhere and its recalcitrance to natural degradation has caused it to accumulate in the environment, becoming one of the major pollutants of ecosystem. Scientists use microorganisms with a few additional factors for biodegradation of polyethylene. This study is designed to use bacteria with enzymes to biodegrade polyethylene in laboratory conditions. *Bacillus subtilis* is chosen for this study and lipase enzyme were used in combination with the bacteria. Samples were collected from soils infested with plastic in different locations. After purification and identification of the *B. subtilis* strain MR21, extracellular lipase and biosurfactant were isolated from *B. subtilis* and used in different combinations with the bacterial isolate and incubated in culture medium with new and aged polyethylene (PE) films. Confirmation of biodegradation was evaluated by Scanning Electron Microscopy, Fourier Transform Infrared spectroscopy and gravimetric analysis for weight loss. The analyses showed that *B. subtilis* was able to biodegrade polyethylene best with the biosurfactant and caused an average 29% weight loss in aged polyethylene and 17% in new polyethylene. FTIR analysis showed formation of ketones, aldehydes, vinyl and other functional groups in the samples. SEM analysis showed surface

deterioration in polyethylene samples. The study showed that *Bacillus subtilis* caused significant PE biodegradation with biosurfactant, but lipase had no significant on the action of the bacteria in biodegradation.

Analysis and Evaluation of Iron Chelating and Anti-Cancer Activities of Extract from *Streptomyces* spp.,

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Streptomyces are very promising source of natural compounds; more than 6000 different bioactive compounds have been isolated from this genus. Iron homeostasis is very important for normal physiology, but in some specific conditions such as in iron overload this balance is disturbed and free iron is available in plasma, this iron overload is called hemochromatosis and has been connected with several complications like diabetes, cardiac failure, liver cirrhosis and cancer. This study was focused on extraction of the bioactive metabolites from *Streptomyces* sp. BD32, the ethyl acetate crude extract was assessed for their phytochemicals which revealed the presence of phenols, flavonoids, alkaloids, and tannins. After that, the chromatographic analysis of ethyl acetate extract using TLC and HPLC displayed different UV active metabolites which could be responsible for the given pharmacological activities. Moreover, GCMS analysis was carried out that unveiled the phenol and ester compounds as the main ingredients of the extract. The antioxidant potential of the extract was determined using DPPH assay, which exhibited potent IC50 of 0.034 mg/mL as compared to the positive control ascorbic acid 0.12 mg/mL. Furthermore, the iron chelation activity of the extract exhibited significant chelation potential by 250 and 125 µg/mL of the extracts, whereas 62.5 µg/mL displayed moderate chelation of the ferrous ion, similarly the cytotoxicity of the extract was evaluated using MTT assay with a range of extract concentrations, in which 51% cytotoxicity was exhibited by 350 µg/mL of the extract, whereas 65% of cells growth was inhibited by 700 µg/mL respectively. The current study demonstrates that the active compounds from microbes living in desert could be used for future potential therapy in the field of medicine.

Evolution of Indigenously Developed Rapid Identification of Medically Important Gram Positive Group of Cocci by Urea & Arginine

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The evolution of a Rapid identification system of gram positive group of cocci progress is a basic advantage of clinical purpose. The diagnosis of infections is conditional, to tremendous importance, on laboratory action and its estimation going on it can reduce the chances for bacteria to become resistant and enhance their mortality and morbidity data, and assay for diagnosing bacterial infections in the pathological laboratory and it is significant for the treatment of the disease and preventing the overuse of antibiotics. commercially available systems operating

these techniques for bacterial detection and identification have been proposed, the supervisor formulated the formulations for Gram-negative bacteria and she preferred us to work on formulating for Gram-positive bacteria as a portable role of her experiment lab. The rapid system was developed for the rapid identification of biochemically less active in comparisons with other groups of bacteria that covers 14 different bacterial species. This research was to use the known strains received from a particular pathology are well established human pathogens. The known strains were then being applied to the API strips. Strips were not contaminated. In these strips, sugar fermentation and enzyme profile examination showed the ability of the microbes to metabolize in urea, and arginine of different Strain of Staphylococcus, Streptococcus, after evaluation some strain gives 100% concordant results and ensures the possibility to be used in clinical diagnosis. It gives effect in 3-4 hours. Total 16 bacterial strains which were used in research, were collected from the pathological lab. All strains are known and isolated from various parts of the body. and cultures of 16 known strains and add into 16 saline tube, now take strips and inoculate the sugars into the strips and dry the sugars After drying, inoculate the culture. incubate at 37oC for 14-24 hours. After incubation check strips for colour change. Document the effects as colour revealed positive and negative results. I had performed this research in which I concluded 14 culture of gram-positive cocci involved 2 genera consist of staphylococcus and streptococcus and urea & arginine were used which gave the outcomes as all the species enormously fermented however some species do not ferment these sugars, whilst some of them give a negative result. If this rapid identification system of gram-positive cocci progress and its lower the risk of bacteria to become resistant and can easily be used in hospitals for clinical purposes.

Biomass and Toxins Optimization of *Clostridium perfringens* Toxinotype D under Dynamic Parameters

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Bacterial toxins play vital role in causing enterotoxaemia. To muddle through enterotoxaemia, its control and prevention is a huge challenge for farmers in Pakistan. Present study was designed to optimize the biomass and toxins production by indigenously characterized *Clostridium perfringens* type D isolates under various physicochemical parameters, which may provide a lead towards local vaccine production in Pakistan. Indigenous isolates were characterized based on 16S rRNA with accession numbers MW349974.1, MW341428.1 and MW332258.1. These isolates were identified as toxinotype D through toxin gene specific PCR toxinotyping. Optimization of biomass and toxins production was determined under the influence of temperature, time of incubation, glucose, vitamin and mineral mixture, tween-80, sodium chloride and sodium acetate. Higher biomass was produced after incubation for 48hours at 37°C (58.23±0.68, 59.10±0.10 and 57.37±0.30mg/mL). A higher biomass was produced (28.58±01.11, 27.58±0.24 and 29.84±0.16mg/mL) at 0.5% concentration of sodium acetate. A higher hemolytic units of alpha toxin were produced (4.53±0.04, 4.26±0.20 and 4.52±0.02HU/mL) at

37°C after 24 hours in RCM broth. Whereas, a higher hemolytic units of alpha toxin were produced (20.81±0.00, 20.41±0.36 and 19.67±01.33HU/mL) at 0.2% concentration of glucose. Higher hemolytic units related to epsilon toxin were produced (34.05±0.00, 33.37±0.59 and 33.09±0.97HU/mL) at 0.2% concentration of glucose. Under these conditions, maximum biomass and toxins units were observed; these conditions could be used to produce maximum biomass and toxin units for vaccine production at industrial scale.

Evaluation of Seroprevalence and Associated Risk Factors of Toxoplasmosis in Sheep and Goats in District Jhang-Pakistan

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Toxoplasmosis is a zoonotic infection caused by a pathogenic protozoan, *Toxoplasma gondii (T. gondii)* responsible for huge economic and health losses in developing countries. The current study was conducted to assess the seroprevalence of Toxoplasmosis and associated risk factors in sheep and goats in District Jhang, Punjab, Pakistan. Blood samples (n=400) were collected from both genders of goats (n=219) and sheep (n=181) in District Jhang. The seroprevalence of *T. gondii* was examined using Latex agglutination test. Additional data regarding hygienic conditions, water source, gender, breed, age of animal was also collected on a predesigned questionnaire. The overall seroprevalence of *T. gondii* was found 34.25% (137/400) in District Jhang. Higher seroprevalence was recorded in goats {36.52% (80/219)} as compared to sheep {31.49% (57/181)}, however, it was non-significant ($p>0.05$). Gender-wise seroprevalence was found 32.59% (44/135) and 35.09% (93/265) in male and female animals, respectively ($p>0.05$). Further, the association of Toxoplasmosis between different age groups was significantly higher in older animals having age >24 months 42.75% (62/145) than younger animals with age <12 months 26.60% (29/109) and 11-24 months 31.50% (46/146) ($p<0.05$). The seroprevalence was also higher 40.81% (80/196) in animals drinking water from outdoor water source than in animals drinking from indoor water source 27.94% (57/204) ($p<0.05$). Moreover, seroprevalence was significantly higher 43.11% (97/225) in animals kept in vicinity of cats than in absence of cats 22.85% (40/175) ($p<0.05$). However, reproductive status, breeds, flock size had non-significant impact on the prevalence of *T. gondii*. Thus, it is concluded that the presence of cats near animals, larger flock size, older age of animals, and poor hygienic conditions are main risk factors of Toxoplasmosis in sheep and goats and these could be a potential threat of infection for livestock industry and public health.

Characterization of *Staphylococci* from Selected Fish Species of Local Fish Farms

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Resistance to antibiotics is receiving a lot more attention from all over the world. These resistant strains may be transmitted from humans to fish which is of major concern. Detection, antibiotic sensitivity testing and molecular characterization of staphylococciin general and *S. aureus* in particular, from the intestinal tract of Rohu (Labeo *rohita*) and Silver fish (Hypophthalmichthys *molitrix*) from local fish farms of Haripur, was investigated in thus study. Bacteriological testing has been carried out on 100 intestinal fish samples comprising Rahu fish (n = 50) and Silver Carp (n = 50) of various weights, during the period from June 2021 to August 2021.Bacteria were grown on Manitol Salt Agar, a selective growth medium, after being processed with sterile equipments and then identified using microscopy and different biochemical tests.Fift two (52) samples were confirmed as staphylococci through biochemical tests and 42 samples showed fermentation on MSA while 57 were coagulase positive. PCR was used to confirm the presence of *S. aureus.nuc* and *mecA* were the two particular primers used. *nuc* was used to identify *Staphylococcus aureus* strains, and methicillin resistant bacteria were obtained using *mecA* gene from confirmed *S. aureus* isolates. *nuc* positivity was found in 5 isolates (9.80%), while *mecA* positivity was found in 3 isolates (5. 88%).Antibiotic sensitivity was determined using the CLSI scale. The resistance to *S. aureus* in bacterial isolates was evaluated using seven antibiotics, which shows variable results.

Prevalence of *mcr-1* gene in *E. coli* Isolated from the Poultry Fecal Samples

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Multidrug resistance is posing an alarming threat to population health globally and needs immediate surveillance. The AMR is rapidly spreading to all countries including Pakistan via horizontal gene transfer via some mobile genetic factors. The rising resistance due to *mcr* gene possessing has provoked the risk of being resistance to last resort antiniotic, colistin. The rising multiple drug resistance among poultry due to *Eneterobacteriaceae* is a major challenge to public health and it needs immediate surveillance to stop rising ESBL/Carbapenemase resistance. A total of 100 cloacal swabs were obtained from different poultry farms in the territory of Faisalabad, Pakistan. Antimicrobial susceptibility profiling was carried out using Kirby-Bauer Disk Diffusion test following CLSI guidelines 2016. Resistance pattern was determined by measuring zones of inhibition using graduated scale and then comparing their values with the guidelines provided by the CLSI. Molecular characterization of *mcr-1* gene was done approaching PCR assay using specific primers. The current study indicates cloacal swabs (n=100) were isolated from different poultry farms from poultry chicken. Out of 100 collected cloacal swabs 17 were positive for colistin specific *E. coli* and they all possess resistance to *mcr-1* (mobilized colistin resistant) gene. According

to the present study a total of 17 *mcr-1* resistant isolates of *E. coli* were isolated from cloacal swabs obtained from poultry droppings. Antimicrobial susceptibility profiling was done using Kirby-Bauer disk diffusion method and the resistance pattern was determined using CLSI guidelines 2016. Molecular detection of *mcr-1* gene was carried out using Polymerase chain reaction (PCR) using specific primers. According to the current study, a total of 17 out of 100 cloacal swabs were confirmed as *E. coli* and all were harboring plasmid mediated *mcr-1* gene. Out of those 17 isolates, 100% of the isolates (n=17) were resistant to colistin and tetracycline, Trimethoprim/sulfapethoxazole showed 82.35% resistant, individually. Cefepime, pipercacillin/tazobactum, amikacin and imipenum showed 100% sensitivity.

Detection of Mycotoxigenic Fungi and Mycotoxins in Poultry Feed from Poultry Farms in Baluchistan

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Mycotoxins are secondary toxic metabolites produced by fungi that occur naturally in agricultural commodities worldwide. Aflatoxins, Ochratoxin A, Fumonisins, Trichothecenes and Zearalenone are the most important mycotoxins types. Different species are responsible for the production of mycotoxins such as, Aspergillus, Fusarium, Penicillium and Claviceps genera. These mycotoxins can be carcinogenic, cytotoxic, mutagenic, teratogenic, neurotoxic, nephrotoxic, estrogenic, and immunosuppressant. A study was conducted for the presence of mycotoxigenic fungi and mycotoxins in poultry feed used by the poultry farmers of Balochistan. It was found that 40 out of the total 43 (93%) samples are contaminated with fumonisin tested by fumonisin detection Agra strip and ELISA tests. Whereas, the samples tested for aflatoxins showed that 29 out of 43 (66%) feed samples are contaminated with aflatoxin conformed by Agra strips and ELISA kits. The mycotoxins presence was also conformed with the help of thin layer chromatography compared with standard toxins. It was concluded that the majority of poultry feeds are contaminated with aflatoxin and fumonisin. The occurrence of these mycotoxins can be from the raw materials or form other sources during transportation and storage. This research was supported by the AIP-PARC-2017 project funded by USAID.

Comparative Drug Susceptibility of Colistin Resistant *Escherichia Coli* Isolated from Milk in District Mardan

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E. coli are prevalent in different environments and can cause various disorders i.e diarrhea, edema, and inflammation of serosa membrane, septicemia as well as mastitis. E. coli showed resistance to different antibiotics which represents a threat to humans and animals. Here we reported resistance of E. coli to colistin which is a last option of treatment. From local areas of Mardan milk samples were collected. According to standard guidelines of Clinical Laboratory Standard Institute bacteria were isolated. Out of positive isolates some showed multi drug resistivity. Vancomycin showed maximum resistance while minimum by Chloramphenicol. On other hand

maximum susceptibility was found to Trimethoprim sulfamethoxazole and Cefipime. Colistin resistance genes were detected via Polymerase Chain Reaction. UDP was the most prevalent gene detected in isolates. It is concluded from this study that colistin resistance genes are dominant in milk and can be transferred to others via animal product i.e. milk and meat.

Effect of Exogenous Protease On Growth Performance, Microbial Count and Meat Quality of Broiler Reared On Fish Meal-Based Diet

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This study determined the effect of protease supplementation in fish meal-based diets on growth, digestibility and meat quality of commercial broiler birds. Nine experimental diets were formulated using three levels i.e. 0, 25 and 50% of the protein source of fish meal, with or without alkaline protease (CIBENZA® DP100). Four hundred and thirty-two (432) day-old mixed sex broiler chicks were randomly divided into thirty-six experimental units of 12 chicks each. Feed consumption and body weight were measured weekly. On the last day of trial, two birds from each pen were picked up and processed for carcass parameters. Meat samples were analyzed for meat quality parameters includes pH, water holding capacity and cooking loss. Data collected were analyzed by analysis of variance technique under Completely Randomized Design in a factorial arrangement (3×2) (Diet × Protease) and mean values were compared using Tukey's test if value of $P<0.05$. Improved body weight gain and FCR were recorded in birds fed diet having protease enzyme in fish meal-based diet during day 1-42. Highest dressing percentage and thigh meat yield was observed in treatment groups replaced 25% of SBM with fish meal on protein equivalent basis with Enzyme as compared to 50% fish meal based diet with supplementation of enzyme. Relative organ weights heart weight showed significant *($P<0.05$)* difference. There was no significant difference among treatments regarding coliform count either supplemented with different levels of fish meal or with protease. No two-way interactions *($P>0.05$)* were found between protease and fish meal levels on water holding capacity, pH and cooking loss. An interaction *($P<0.05$)* between protease and fish meal levels on uric acid, serum albumin, cholesterol and triglycerides. Higher platelet count was measured for group formulated diet containing 50% fish meal. White blood count and hemoglobin levels were highest in treatment group having replaced 50% of SBM with fish meal on protein equivalent basis with enzyme. It can be concluded that the addition of protease in 25% fish meal based diet had improved growth performance, crude protein digestibility and carcass characteristics.

Microbial Assessment of Poultry Meat for the Presence of Targeted Bacteria and Antibiotic Resistance Profile of *Salmonella* spp.

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The broiler meat is a food that is widely consumed in many forms, but is also as major reservoir of many pathogenic bacterial species, such as Salmonella, Shigella, and E. coli. In this study, poultry meat samples were assessed for the presence of Salmonella, Shigella and E. coli. For this purpose, a total of 38 meat samples were collected from Hyderabad and Jamshoro districts of Pakistan. Meat samples were purchased from the poultry slaughtering facilities and were brought to the microbiology lab aseptically within one hour. Then by using sterilized cotton swab, the surface sampling of samples was done on approximately 1 sq. inch. The swabs were then diluted in 5 ml solution of 1 X peptone Buffered solution (PBS). After shaking the solution gently, 100 µl of diluted solution was spread thoroughly on Salmonella-Shigella agar plates and plates were incubated for 48 h and on completion of incubation period, the colonies of all three targeted bacteria were identified on the basis of morphological characteristics and were counted. The results revealed very poor quality of all meat samples. Except one sample, all the samples (97.36%) were positive for positive for Salmonella, Shigella and E. coli. The antibiotic resistance of Salmonella was also determining by using disc diffusion test after confirming it with triple-sugar-iron agar test and Urease test. All the isolates exhibit resistance to more than one antibiotic. Resistance to ampicillin, azithromycin, ceftazidime, gentamicin, cefotaxime, erythromycin, neomycin, streptomycin and sulphamethoxazole was 100%, 2.6%, 0%, 24.3%, 27%, 37%, 45%, 32.4% and 51.3%.

Epidemiological Statistics of FMD Virus Serotypes A, O and Asia-1 In Punjab, Pakistan During 2014-2019

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Foot and Mouth Disease is an endemic disease and three serotypes (A, O, Asia-1) are prevalent in Pakistan. The present study shows the frequency of Foot and Mouth Disease Virus (FMDV) serotypes (A, O, Asia-1) in Punjab Province of Pakistan by using antigenic Enzyme-Linked Immunosorbent Assay (ELISA). This study was conducted from 2014-2019 by using a total of 184 epithelial samples from field. Out of 184 samples, 106 samples showed positive results. Out of three serotypes, serotype „A" was the most prevalent (39.62%) followed by serotype „O" (33.96%) while the least prevalent was serotype Asia-1 (26.41%). It was marked that no other serotype of FMD reported during this study. This study also described the year wise prevalence of three prevailing FMD serotypes (A, O, Asia-1). During year 2014, the serotype „A" was more prevalent (95.83%**)** followed by serotype „Asia-1" (4.16%**)** while no case of serotype „O" was found. During year 2015, the prevalence trend was almost the same as in 2014. In this year, serotype „A" was 55.55% while serotype „Asia-1" 37.03% followed by the least prevalent serotype „O" as 7.40%. During year 2016, 19 positive cases were reported from which the maximum prevalence was serotype „O" and „Asia-1" (42.10%) followed by serotype „A" (15.78%) as least prevalent. During year 2017, the positive cases for FMD increased remarkably i.e. 29 in number. This year serotype „O" was highest in number with prevalence 75.86%, and serotype „Asia-1" 20.68% whereas only one case reported for serotype „A" with only 3.44% prevalence. During year 2018 and 2019, only 7 cases described with no evident case of serotype „A". During these two years 4 cases of serotype „O" were observed followed by 3 cases of serotype „Asia-1".

Key words: Prevalence, FMD Virus, Serotype A, O & Asia-1, ELISA, Geographical Distribution, National Control Strategy.

Isolation and Molecular Characterization of *Pseudomonas aeruginosa Bacteriophages* from Sewage Water

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Among the infected wound patients, the most commonly found pathogens is *Pseudomonas aeruginosa* and it play major role in morbidity and mortality. Multiple antibiotics are used for the treatment of pseudomonal infections but the resistance against *Pseudomonas aeruginosa* has been increased immensely. Antibiotic resistance reached at threatening level so for the treatment of such infections caused by *P. aeruginosa* need to be treated by an alternative therapy. For the alternative therapy of antibiotics bacteriophages are reflected to be a very good substitute. The main objective of the proposed study was to weigh the lytic potential and characterization of the bacteriophages isolated against the *P. aeruginosa.* Total (n=40) of sewage sample was collected from the different spots of the District Faisalabad, Pakistan. For the isolation of bacteriophages double agar overlay method was used and for the identification Transmission electron microscopy was carried out. Furthermore, for the characterization of the isolated bacteriophages different techniques were performed used including host range assay, killing assay, pulse field gel electrophoresis and SDS-PAGE. From 40 samples of sewage sample only 18 were found positive for bacteriophages against *P. aeruginosa.* To check the host range of the isolated bacteriophage total 25 isolates of *P. aeruginosa* was used and complete lysis was recorded against (21) isolates of *P. aeruginosa* in the case of phage with the designated name *7A* while least (03) were recorded for *P21* bacteriophage. All the bacteriophages showed high lytic activity against their host bacterium. Pulse field gel electrophoresis showed different size bands of bacteriophages DNA. Different proteins bands range between from 20 kDa to 155 kDa was determine by using the SDS-PAGE. It concluded that *P. aeruginosa* was the most leading pathogen among the infected wound patients and most of the isolates of *P. aeruginosa* were showed resistant to multiple antibiotics (MDR). Isolated bacteriophages presented good lytic activity against MDR *P. aeruginosa* isolates. In future bacteriophage therapy considers a good substitute for the treatment of such infections against the commonly used antibiotics.

Potent Quinoline Inhibitors against Causative Agents of Gonorrhea and Chancroid

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Gonorrohea, sexually transmitted disease, had a high morbidity. Due to the number of antibiotic-resistance stories, incidence of this disease has been rising rapidly. The organism of *Neisseria gonorrhoeae* showed an extra-ordinary ability to develop resistance against all antimicrobials introduced for its treatment. Gonorrohea is also known to facilitate the acquisition of HIV infections. Gonorrohea showed resistance to a number of antimicrobial agents, including ciprofloxacin, tetracycline, azithromycin, penicillin and extended spectrum cephalosporins (ESCs) globally. It has been recently classified as a "Priority 2" micro-organism in the World Health Organization (WHO). The increase resistance to azithromycin and the decreased susceptibility to ceftriaxone worldwide suggested that the currently introduced dual antimicrobial regimens might not be effective in long term-solutions. A combination of antimicrobials or the discovery of novel/new agents is the only solution of an integrated, global approach to gonorrohea control. In this concern our study focused to explore various classes of chemical compounds against susceptible and resistant strains of Gonorrohea and *Haemophilus ducreyi* by using Alamar Blue Assay. Some derivatives of 5-Nitroquinolin-8-ol were found to have significant activity against these organisms *in vitro.* The MICs of active compounds were also evaluated. The effects of non-toxic compounds were investigated by using Atomic Force Microscopy (AFM) and fluorescence microscopy. These compounds can be studied in future for drug development against sexually transmitted diseases.

Green Synthesis, Characterization and Biological Evaluation of Silver Nanoparticles Using Extract of *Euphorbia Serpens* Kunth

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The silver nanoparticles are synthesized via green synthesis approach using Euporbia serpens Kunth. The maximum 80% yield was obtained at 2:14 of 10% plant extract solution and 1Mm silver nitrate solution. Furthermore, the UV-visible spectroscopy and furrier transformer infra-red spectroscopy justified the reduction and stabilization of silver nanoparticles from its precursors. Two broad peaks with absorption maxima in rang 550 nm to 650 nm strongly suggested the flavonoid with aromatic benzene conjugated at C-2 and C-3. Ag-NPs Characteristic absorption peak was observed at 420 nm. The SEM and TEM analysis demonstrated the spherical shape of the synthesized nanoparticles with particle sizes ranging from 30 nm to 80 nm. FTIR transmission bands at 2920 cm-1, 1639 cm-1, 1410 cm-1, 3290 cm- 1and 1085 cm-1 were attributed to C-H, C=O, C-C, N-H and C-N functional groups respectively. XRD peaks could be attributed to (111), (200), (220) and (311) crystalline plane of the faced centered cube (FCC) crystalline structure of the metallic silver nanoparticles. The Ag-NPs showed good antibacterial activity against all the tested bacteria at each concentration. The particles were found to be more active against Escherichia coli with 20 ± 06 mm and Salmonella typhi with 18 ± 0.5 mm zone of inhibition in reference to standard antibiotic amoxicillin with 23 ± 0.3 mm and 20 ± 0.4 zone of inhibition respectively. All the tested concentration of silver nanoparticles showed comparable % RSA with the standard reference ascorbic acid in the range 60% to 75%. The percent motility at 3 hours post incubation showed quick response and most

Etramorium caespitum were found deceased or paralyzed. Similarly, the percent mortality showed a linear response at concentration and time.

Synergistic Effect of *Lavandula angustifolia* l Oil on the Antimicrobial Activity of Gentamicin against Methicillin Resistant *Staphylococcus aureus*

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Probiotics are beneficial bacteria that have a significant impact on nearly every medical profession. They are commonly utilized in functional foods and drinks to improve gastrointestinal working. Younger generations have a strong desire for health-related products which drives the probiotic industry. Lactic acid bacteria (LAB) are being studied for their positive effects because they improve the flavor and texture of fermented goods and prevent pathogenic microbes that causes food deterioration. These important bacteria are now being developed for their therapeutic potential in the form of oral or mucosal medicinal delivery vectors. It is widely understood that resident bacteria play an important role in human health maintenance and intestinal microbiota is critical in avoiding pathogen invasion and overgrowth. *Staphylococcus aureus* is a ubiquitously harmful microorganism that causes a wide range of ailment. There is growing evidence of *Staphylococcus aureus* colonization in the gastrointestinal system as well as the development of drug-resistant strains that pose a danger to antibiotic treatment. Therefore, an alternative therapy is in demand that may decolonize pathogens most of which are biofilm-forming bacteria that defy antibiotic treatment. For many years, medicinal plants have sparked interest owing to their varied biological and therapeutic properties. These are phytochemicals and proteins receiving special attention due to their bio- and cyto-compatibility. Lectins are carbohydrate binding proteins that are particular in their identification of sugar molecules. As lectins are omnipresent, interest has been generated in isolating them from various sources and evaluating their medicinal potential. Therefore, the goal of this study was to determine the anti-biofilm activity of banana proteins and bioactive metabolites generated by Lactic acid bacteria against Methicillin Resistant *Staphylococcus aureus* (MRSA) also several studies were performed in order to assess the synergistic impact of banana proteins with probiotics. Banana proteins has the ability to inhibit biofilm formation as indicated by results and the cell free supernatant from Lactococcus species also inhibited growth and development of biofilm. Furthermore, the probiotic cells itself demonstrated competitive suppression of pathogens and a decrease in biofilm formation while their synergistic effect was heightened. This study shows how probiotics and plant protein may be employed to treat MRSA biofilm in a variety of ways. Hence, probiotics as well as their components may aid in the defense against resistant forms of Staphylococcus aureus and combining them with plant proteins opens up new avenue for combating antimicrobial resistance.

Silver Nanoparticles Inhibit Biofilm Formation and EPS Production of Multi-Drug Resistant *Klebsiella pneumoniae.*

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Antibiotic resistance against present antibiotics is rising at an alarming rate with need for discovery of advanced methods to treat infections caused by resistant pathogens. Silver nanoparticles are known to exhibit satisfactory antibacterial and antibiofilm activity against different pathogens. Considering the importance, AgNPs were synthesized chemically and characterized by UV-Visible spectroscopy, scanning electron microscopy and X-ray diffraction. Antibacterial activity against MDR *K. pneumoniae* strains was evaluated by agar diffusion and broth microdilution assay. Cellular protein leakage was determined by Bradford assay. The effect of AgNPs on production on extracellular polymeric substances was evaluated. Biofilm formation was assessed by tube method qualitatively and quantitatively by microtiter plate assay. The cytotoxic potential of AgNPs on HeLa cell lines was also determined. AgNPs exhibited an MIC of 62.5 and 125 µg/ml, while MBC of 250 and 500 µg/ml. The production of extracellular polymeric substance decreased after AgNPs treatment while cellular protein leakage increased due to higher rates of cellular membrane disruption by AgNPs. The percentage biofilm inhibition was evaluated to be 64% for *K. pneumoniae* strain MF953600 and 86% for MF953599 at AgNPs concentration of 100 µg/ml. AgNPs were evaluated to be minimally cytotoxic and safe at concentrations of 15-120 µg/ml. The data evaluated by this study provided evidence of Ag NPs being safe antibacterial and antibiofilm compounds against MDR *K. pneumoniae.*

Antibacterial Activity of *Cuminum cyminum* against Multidrug-Resistant Bacteria

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Cuminum cyminum seeds extract and essential oil can be used to kill or inhibit the growth of multidrug-resistant bacteria. This study had been designed for the determination of the antibacterial activity of Cuminum cyminum seed extracts against MDR clinical isolates. Collections of the clinical isolates were done from different hospital settings in Faisalabad. Identification of isolates was done by different biochemical tests including Gram's staining, Catalase test, Oxidase test, Voges-Proskauer test, Methyl red test, Indole test, Urease test, and Citrate Utilization test. These isolates were confirmed as multidrug-resistant strains by checking their antibiotic susceptibility through Kirby-Bauer disc diffusion assay. The alcoholic and aqueous extracts of Cuminum cyminum were prepared at different concentrations. Agar well diffusion method was performed with different concentrations of alcoholic and aqueous extracts and essential oil of Cuminum cyminum and showed that these resistant microbes are sensitive Cuminum cyminum. MIC and MBC assay was also employed to check the antibacterial potential of cumin. The different MIC values were determined for all clinical multidrug-resistant isolates. The effect of Cuminum cyminum combined with antibiotic was determined by a two-dimensional checkerboard assay. FIC index was calculated and FIC value below or equal to 0.5 showed that they have a strong synergistic effect when employed after combination of both.

Molecular Characterization and Antifungal susceptibility of Ochratoxin; A Producing Fungi Isolated from Poultry Feed to Plant Essential oils

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Occurrence of Ochratoxin A producing fungi in poultry feed is common problem and a potential hazard to poultry. There is a need to control fungal growth to improve the quality of feed. Plant essential oils (Garlic, turmeric, Black seed, Eucalyptus, Clove, Cumin, Cardamum and Cinnamon oil) were evaluated for antifungal activity against ochratoxin A producing fungi isolated from poultry feed and feed ingredients (n=120). The fungi were characterized by macroscopic and microscopic characters followed by polymerase chain reaction. Ochratoxin A production was detected by thin layer chromatography and High-performance liquid chromatography. Antifungal potential of PEOs was evaluated by well diffusion assay and Minimum Inhibitory Concentrations (MIC) was determined by micro broth dilution method. A total of 1842 fungal isolates were recovered and *A. ochraceous, A. terreus,* and *A. parasiticus were* detected as ochratoxin A producing fungus. Among tested essential oils *S. aromaticum C. verum* and *E. cardamomum* showed antifungal activity against *A. ochraceous* (33.67 ± 0.57mm), *A. parasiticus* (24.00 ± 2.00mm) and *A. terreus* (54.67 ± 0.57mm). The least MIC was of *S. aromaticum C. verum* and *E. cardamomum*, the respective MICs were 0.52±0.22, 0.65±0.22 and 2.08±0.90µg /mL against *A. ochraceous*, 0.65±0.22, 1.30±0.45, and 2.6±0.90 µg /mL against *A. terreus* and 1.04±0.45, 1.30±0.5 and 2.08±0.90 µg /mL against *A. parasiticus*. It was concluded that essential oils have ability to inhibit Ochratoxin A contaminating fungi of poultry feed.

Immunomodulatory Role of Enterococcus faecium Strain LCM08 in Mice

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Lactic acid bacteria are the diversified population of Gram positive organisms, found in a large number of ecological niches. The most important attribute of Lactic acid bacteria is probiotic. Probiotics are effective in modulating the immune system of the host by competitively excluding the gut pathogens as well as stabilising the gut microbiota. The focus of this research was to investigate the immunomodulatory potential of *Enterococcus faecium* strain LCM08 under in vivo conditions. Since the strain survived in vitro conditions like acidic pH, bile salts, and digestive enzymes, it was administrated intragastrically to mice for 5 days and followed up for 20 days. The results indicated that probiotic test candidate significantly increased the T- cells count *i.e.* count raised from

8% to 44% whereas no change was recorded in control mice group. Moreover, IgA level was also elevated from 1.302µg/mL to 1.328µg/mL in the test group. Furthermore, mice remained healthy and active throughout the experiment. These findings suggested that the test strain is a promising probiotic candidate that could be applied in food products for health benefits after further in vivo assessments in human.

Antifungal Potential of Chitinase Extracted from *Bacillus Subtilis* Under In-Vitro Condition

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Fungal pathogens have been recognized as a worldwide threat to human health, decreasing crop production and causing infectious diseases in plants and animals. *Bacillus* species have been identified as biocontrol agents for harmful fungal pathogens by degrading their cell wall. It has been proved in controlling the fungal diseases by showing chitinase activity against fungal pathogens. In this study, presence of extracellular chitinase and antifungal potential of *Bacillus subtilis* was studied. The chitintolytic bacterium was isolated from rhizopheric soil and identified through standard microbiological techniques, molecular characterization by PCR and sequencing as a *Bacillus subtilis* strain MR21. Extracellular chitinase activity of *B. subtilis* strain MR21 was observed by using 3% of colloidal chitin prepared from shrimp shell. The antifungal activity of chitinase from *Bacillus subtilis* was determined against pathogenic fungal species of *Aspergillus niger* and *Rhizoctonia solani* through broth microdilution assay. *Aspergillus niger* showed 0.83±0.674 optical density against chitinase of *Bacillus subtilis* MR21 while *Rhizoctonia solani* showed 0.78±0.256. Our results revealed that *Aspergillus Niger* showed more inhibition as compared to *Rhizoctonia* solani. Therefore, chitinase from *B. subtilis* showed antagonistic activities against pathogenic fungi and used in the treatment of fungal infection.

Implications of Agricultural Biotechnology for Plant Improvement in A Variable Climate

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The climate changes at global level are not only affecting plant productivity, but also threatening the ever-increasing human population that is projected to be a ten billion in the year 2050. Crop improvement is a continuous process that started since the advent of agriculture. However, current agricultural biotechnology is focused on producing genetically engineered plants (transgenic plants) to make superior and environment friendly plants as well as improving microbial inoculants to be used to control plant pests, as fertilizer supplements, and to

aid in atmospheric nitrogen fixation. Similarly, plants have been modified for better yield and nutritional profile, herbicide tolerance, pest-resistance, and to produce plantibodies including edible vaccines. Plants especially trees could be modified for reduced lignin content to be used in a paper, and bioethanol industries. Further, the uses of new and innovative techniques are expected to improve plant productivity under abiotic stresses to ensure food security.

Control of Disease Caused by *Phytophthora capsici* in Pepper Plant using the Soil-Borne *bacillus spp.,* Isolated from Kohat Khyber Pakhtunkhwa

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Okra (*Abelmoschus esculentus*) locally known as bhindi is a member of family *Malvaceae*. It is rich source of vitamins, minerals, proteins, and carbohydrates. The okra crop is grown on 15500 hectares' area with total production of 117900 tons. Its yield decreases due to diseases caused by root-knot nematodes. *Meloidogyne Incognita* is the most destructive one which cause huge economic losses to okra production. Nematicides are generally used to control the nematodes however they have several adverse effects including toxicity, environmental contamination, and hazard. The PGPR have shown considerable potential as biological control agents. *Bacillus* species reduced population densities of the soybean cyst nematode in the greenhouse, micro plot, and field studies. Therefore, the present study was designed to determine the plant growth promoting traits and nematocidal potential of bacteria isolated from the fields of district Kohat for the management of root-knot nematodes (*Meloidogyne species*) in okra plant. Roots of infected okra plant and rhizospheric soil were collected from different locations in the District Kohat Khyber Pakhtunkhwa, Pakistan. The root-knot nematodes were isolated by using the Baermann funnel, and the root incubation methods and were identified based on their phenotypes. The bacteria were isolated from the rhizospheric soil by using serial dilution plating on LuriaBertani (LB) agar plates. *Bacillus subtilis* were identified based on morphological and biochemical characteristics. Plant growth promoting traits of *B. subtitles* were also investigated. Finally, the strains, their cell pellet and their metabolites were applied to the juvenile and eggs of *Meloidogyne incognita*. It was observed that *B. subtilis* has biocontrol potential against *Meloidogyne incognita.* The juvenile survival was 98% without exposure to *B. subtilis* and decreased to 70% when exposed to *B. subtilis* broth culture after 24 hours. Moreover, cell pellet and metabolites of *B. subtitles* showed 55% and 65% mortality rate against the juvenile of *M. incognita* respectively after 24 hours. Furthermore, the *B. subtilis* broth culture, their cell pellet and their metabolites showed 50%, 30% and 45% activity against the eggs hatching of *M. incognita*. The *B. subtilis* also showed plant growth promoting traits by zinc and phosphate solubilization, ammonia and indole acetic acid production. From this study, it may be concluded that *B. subtilis* showed nematocidal activity against *Meloidogyne incognita* and produced nematocidal metabolites. It also promotes plants growth by zinc and phosphate solubilization, ammonia and indole acetic acid production.

Immuno-modulatory Effect of Dietary Probiotic Supplementation on Peste des Petitis Ruminants Vaccine in Goats

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Probiotics are known to confer health benefits upon administration in animal diet. Probiotics have ability to modulate immune responses in animals. It improves the health status of animal by competing with pathogenic microbes and enhancing utilization of nutrients that induces positive effects on gut microflora. Two threatening issues to sustainable goat production are poor body conditioning and presence of highly contagious Peste des petits ruminant's disease. The aim of this study was to investigate the effects of *Scharomyces cerevisiae* supplementation on weight gain, growth performance, and feed conversion efficiency and to check its immuno-modulatory effects on PPR disease in goats. Twenty female goats having average weight 30kg were divided into five groups, four goats in each group. These experimental groups were designated as G1 through G5; the group G1 (no feed supplement, no vaccine) was control group for both feed supplemented (G4, G5) and vaccine treated (G3) groups, vaccine control group offered with basal feed (G2), feed control group with vaccine treatment (G3), vaccinated group offered with basal feed (G4) and a vaccinated group with basal feed supplemented with yeast (G5). Groups G3, G4 and G5 were vaccinated with PPR vaccine (strain Nigeria 75/1) on day 0 through *in ocular* (I/O) mode of vaccination. Competitive enzyme linked immuno sorbent assay (c-ELISA) was performed to detect the serum antibodies against PPR virus. After 60 days, there was higher weight gain, growth rate and better FCR in yeast supplemented group compared with all other groups ($P \leq 0.05$). Moreover, relatively better immune response against PPR vaccine was also observed in experimental animals. Group G5 (yeast supplemented group) showed highly significant results against negative controls in enhancing the anti-PPRV antibody titres in goats and 56% increase in weight gain as well in growth rate simultaneously and 15.32% better feed conversion efficiency. The G4 group (basal feed group) showed non-significant results with respect to antibody titres compare with vaccinated group but showed high growth rate and weight gain. It is concluded that probiotic (*Saccharomyces cerevisiae*) supplementation resulted in better goat performance as well as positive immuno-modulatory effect on PPR vaccine.

Fungal Diversity in Different Microenvironment of Dust

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Dust comprised of the things like animal hair, textile fibers, paper fibers, human skin cells, human hair, food particles, soil particles, pollen, or even insect follicles or parts. Fungi are prevalent in distribution in dust and are a serious threat to public health. The concentration of fungal spores in dust depends on some factors including moisture, nutrients and temperature. *Cladosporium spp, Penicillium spp, Cladosporium spp., Aureobasidium spp., yeasts,* and *Aspergillus niger* are the most commonly cultural fungi from dust. Dustborne fungi are serious threat to human's health as the cause many health hazards. The aim of this study was to investigate the fungal diversity in different dust microenvironment of Bannu. The area was selected because it is highly populated area of KPK and there is a lot of dust pollution in it. The study was carried out at the Department of Microbiology at Kohat University of Science and Technology. This study comprises of 10 dust samples collect from different site of Bannu. All the samples were processed using culture based method on SDA media for 48 hrs to 72 hrs for the appearance of fungal colonies and from those samples 7 isolates were selected for further identification. After morphology analysis of fungal colonies, the isolates were subjected to lactophenole cotton blue stain in order to study the microscopic examination. Spores and fungal hyphae were observed in each isolate under microscope. *Fusarium spp, Aspergillus spp, Paecilomyces spp, Penicillium spp, cladosporium spp, Rhizopus spp*. were identified on the basis of their colony morphology and spores study. After studying each sample, it was concluded that the most abundant *spp* in all the dust sample was *Aspergillus Spp. Aspergillus spp* produce spores which after inhaling case many diseases depending on the immunological status of the host in humans.

Investigating the Impact of Combined Application of *Bravibacterium Spp.,* and Nanoparticle on The Alleviation of Chromium Stress in Brassica Plants

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Among toxic metals, chromium is highly toxic with adverse effects on living organisms. For the removal of such toxic metals from the soil, microbe assisted phytoremediation is environment friendly approach. The pot experiment was conducted to study the alleviative role of *Bravibacterium sp*. and nanoparticle (ferric oxide NP) in *Brassica juncea* under Cr stress. For this, seeds with and without bacterial inoculum were grown in pots containing different chromium concentrations (0, 10, 20 and 40 mg/kg). At harvesting, *Bravibacterium sp*. exhibited positive effect on leaf area (21%), root length (6%), shoot length (7%), fresh weight (14%) and dry weight (17%) of *Brassica juncea* in the presence of chromium. Similary, nanoparticles also improved leaf area, fresh weight, dry weight shoot length and root length by 21%, 32%, 25%, 9% and 12% respectively. *Bravibacterium sp*. and NP application increased the photosynthetic pigments such as Chl. A, Chl. B and total Chl. were 7%, 5% and 6% increased respectively in case of PGPR. However, biochemical parameters such as phenolics, flavonids, amino acid, soluble sugar and soluble protein increased 21%, 75%, 11%, 49% and 70% respectively when PGPR & NP were applied. Antioxidant enzymes SOD, POD, CAT and MDA were also increased up to 15%, 85%, 25% and 33% respectively. This study concluded that the application of the PGPR and NP reduced the toxic effect of chromium and enhanced the growth parameters, antioxidant activities and biochemical attributes of the plants.

Plant Based Products as Microbiological Media: A Double Green Approach

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Plants are subjected to various environmental stresses simultaneously when they are grown in open field. These stresses may be abiotic, biotic either separate or both at a same time. Pathogen infection and salinity are two major stressors that restrict plant growth and development worldwide, but their interactive effect is still unknown. Methyl-Jasmonate (MeJA) is an important regulator of stress response in plants. Despite the plethora of scientific literature on the mitigating effects of MeJA under individual stress, its role in inducing defence against combined pathogen and salinity stress; is still unexplored. We investigated the physiological and biochemical mechanisms of tomato plants mitigated by foliar application of MeJA when plants were exposed to sequential or individual action of *Pseudomonas syringae* pv. *tomato* and salt stress. NaCl-induced damages promoted the growth of pathogen and exaggerated disease severity in tomato plants. The decreased performance of salt-stressed plants under pathogen stress might be attributed to salinity-induced growth inhibition, decreased photosynthetic pigments, and destruction to photosystem II machinery. This combined interaction also provoked different interactions between antioxidant mechanisms and stress signaling by increasing lipid peroxidation and accumulation of H_2O_2. However, MeJA significantly enhanced resistance against *Pst* infection and tolerance against salinity stress by modulating levels of lipid peroxidation and H_2O_2, increasing activities of antioxidant enzymes, and accumulating compatible osmolytes such as proline and sugars. The ameliorating effect of MeJA in turn improved photosystem II machinery and photosynthetic pigments sequentially increasing the growth of tomato plants. The results of the present study suggest the vital defensive role of MeJA during combined (a)biotic stresses in tomato plants.

Optimization of Condition for Methanogenic Bacteria in Marai Village for Biogas Production in Portable Anaerobic Digester

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Biogas is a pure, reliable and sustainable energy source, which can generated from organic waste by anaerobic digester. In this project, we have designed a specific portable anaerobic digester for domestic use in rural areas (Marai village) to facilitate the people and to protect the forest by using biogas. We have made 200-liter portable biogas digester (PBD). Because, open ground biogas plants, in most situations, require a large area, constant agitation, and a very stable atmosphere. Therefore, essential changes had done according to the environmental condition to maintain the methanogenic bacteria for biogas production in PBD in every type of environment. We have insulated the PBD up to 3 inches to maintain the temperature for methanogens in different environmental conditions. The PBD supported by a storage tank of big rubber tubes, where produced gas will be stored and used at required time. The PBD charged with manure in farm of slurry (175L) and culture of methanogenic bacteria

(3L). The PBD was incubated at Marai village of Kohat for 14 days and after incubation gas storage tank was fill with gas and It has been used for domestic purpose by using burner for cooking and other activities. Due to this prototype, we are convincing the people, to use PBD for biogas production instead of wood consequently it will protect the forest. In this study, the optimized conditions by insulation to methanogenic bacteria for the production of biogas are successful. The study includes that use of vegetable waste, kitchen waste and fruit waste is also a good source of biogas production through PBD for domestic use. This study concludes that use of PBD for biogas production from kitchen waste and manure and its use for cooking and heating in rural and urban areas will protect the forest on glob.

Bioremediatory Potential of PGPR to Alleviate Acid Stress on the Growth of *Triticum Sativum*

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Plants are continually exposed to a variety of abiotic and biotic stresses in their natural surroundings, which can have negative impact on their productivity. Acid rain is one of such factors now-a-days due to increasing air pollution, which not only affects environment but also affects growth and development of plants. Plant Growth-Promoting Bacteria (PGPB) can enhance plant growth and protect them against several biotic and abiotic stress by utilizing a wide variety of direct and indirect mechanisms. In the current work, impact of various concentrations of sulfuric and nitric acid on the growth and development of *Triticum aestivum* L. was assessed. Moreover, the effect of single and co-inoculation of selected PGPB strains i.e., S5a and S12 on the growth was evaluated. Various growth and biochemical parameters were recorded such as germination percentage, shoot length, root length, number of leaves, fresh weight, chlorophyll content and protein contents of treated plants which were compared with control plants. The results showed that plants treated with low concentration of acids showed increment in growth parameters as micronutrients become more available to plants in optimum environmental conditions and in this way small concentrations of acids enhanced the growth. On the other hand, plants treated with higher concentrations i.e., 0.5, 1 and 2N concentrations of sulfuric acids not only showed decline in growth and biochemical parameters but also showed foliar injuries and bending and yellowing of leaves. However, it was observed that bacterial cultures treated plants showed resistant towards abiotic stress created by acids. It was observed that PGPB were more susceptible to nitric acid as compared to sulfuric acids.

Isolation, Screening and Biochemical Characterization of Plant Growth Promoting Rhizobacteria from Chilli Eminent Growing Areas of Sindh

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A study was conducted to isolate plant growth promoting rhizobacteria (PGPR) from chilli eminent growing areas of Sindh. A total of 25 soil and chilli plant samples were collected for isolation, screening and identification of beneficial microbes. The most efficient PGPR identification was carried out through 16S rRNA sequencing technique. The general bacteria were more abundantly found in all locations as compared to nitrogen fixers and phosphate solubilizers. General bacteria were abundant in Maher Ghulam Mustafa-3, Kunri (8.89 log CFU g^{-1} soil), maximum nitrogen fixing bacterial population (7.88 log CFU g^{-1} soil) was in Khushal Das, Bacha Band and highest population (7.86 log cfu g^{-1} soil) of phosphate solubilizing bacteria was recorded in Mahmoodabad Farm. Twenty microbial isolates were screened based on their different colony morphological characteristics. Among them 16 isolates had ability of atmospheric N_2 fixation, 13 were able to produce biofilm on solid medium and 14 isolates had antagonistic ability against plant pathogen. The isolated microbes solubilized inorganic phosphate in the range 18.75-114.75%, NIA-KC-03 was the most efficient P-solubilizer (114.75%) and the highest IAA producer (35.30 mg L^{-1}). Sixteen (16) microbes were positive and 04 were negative in Gram reaction. The chilli plant rhizosphere was colonized by various genera of PGPR including *Alcaligenes, Stenotrophomonas, Aeromonas* and *Brevundimonas*. The best performing PGPR with their beneficial traits would be considered as potential bio-inoculants and could be used for sustainable chilli cultivation system.

Insecticidal Activity of *Bacillus Strains* Isolated from the Local Fields of Kohat Against the Pests Belonging to Order *Lepidoptera*

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Agriculture is the most important sector for the economy of Pakistan. The pest attack is one of the most damaging factors in the field of plant pathology. The most common pest, white marked tussock moths, attacking the shade as well as ornamental plants and caused 80% damage to plants canopy. On the other hand, higher gross loss has also been reported in managing honey bees and their wax caused by wax moths that weakened the stored comb and colonies of bees. However, chemical pesticides have widely been used for the protection of these crops, but the wide use of these chemicals from the last three decades has increased the number of environmental problems. Thus, the use of natural enemies of pests, such as viruses, bacteria, and fungi, can be an alternative for the pest control. Therefore, the objectives of the present study were to isolate novel insecticidal strains of Bacillus spp. to control the plant's insects belonging to the order Lepidoptera. Around 50 soil samples were collected from the local fields of District Kohat and cultured through serial dilutions using pour plate technique. The bacteria isolated from the soil samples were then characterized and identified using their colonial, morphological Gram staining and biochemical characteristics. The bacteria were identified as B. subtilis FD3, B. cereus FD6 and B. wiedmannii TD10. The strains were then subjected to the centrifugation for extracting metabolites. Finally, the strains and their metabolites were applied to the larvae of Orgyia leucostigma and Galleria mellonella. It was observed that out of the three strains, the B. subtilis FD3 was the most toxic than B. cereus FD6 and B. wiedmannii TD10. The larval survival was 99% without exposure to B. subtilis FD3 strain and decreased to 80% when exposed to B. subtilis FD3 strain after 48 hours. Moreover, B. cereus FD6 showed 50% mortality rate after 48 hours. Furthermore, the metabolites of B. wiedmannii TD10 showed 60% activity against the larvae of Orgyia leucostigma and 40% activity against Galleria mellonella. From this study, it may be concluded that B. subtilis FD3 strain showed significant toxicity against Orgyia leucostigma and Galleria mellonella and hence may be considered as a putative biocontrol agent

Microbes for Food and Health

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Abundant quantities of microbes such as bacteria, fungi, protozoa, and viruses occur naturally in various ecosystems. Some of these microbes play a significant role in the promotion of food supply whereas others could be beneficial or determinantal for the health of almost all biological systems. For example, specific microbes can facilitate nitrogen fixation in the soil to enrich leguminous plants to produce foods of good nutritional value. Some microbes are known to break highly fibrous or waste materials to synthesise valuable products. This is particularly relevant to anaerobic microbes that naturally exist in ruminant animals such as buffalo, camel, cattle, goats, and sheep. These animals can utilise lignocellulosic materials with the help of billions of rumen microbes to yield energy and microbial protein to satisfy their nutrient needs. In fact, these food producing animals perform an excellent work to biologically convert indigestible fibrous materials into nutrient rich foods such as meat and milk. Similar anaerobic microbial activities take place in the hind gut of almost all animals and human beings, but this happens on a much smaller scale. In addition, desirable microbes such as lactobacilli, can fight against incoming pathogens to maintain gut integrity, motility, and health. Indeed, microbes such as yeast can enhance the quality and shelf life of baked or fermented food products. Conversely, microbes are known to produce poisons, toxins and allergies resulting in multiple diseases and fatalities of animals, plants, and human beings. Thus, an understanding of a chosen microbial ecosystem is essential to either harness their beneficial features or to develop protection strategies against their potential hazards in different situations. This paper will examine the opportunities and obstacles that are involved in studying the positive or negative aspects of some selected microbes by using only a few relevant examples of selected animal species.

Molecular Characterization of Extensively Drug-Resistant *Salmonella* Typhi Clinical Isolates from Lahore, Pakistan

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During the past decades, various studies have reported the multidrug-resistant (MDR) phenotypes among the Salmonella enterica serovar Typhi (*S.* Typhi) isolates from African and Asian countries. The rise in the frequency of such MDR isolates with increased resistance to cephalosporins as well as fluoroquinolones and cephalosporins is frightening. The recent outbreaks from the Sindh province of Pakistan caused by the extensively drug-resistant (XDR) S. Typhi strains have highlighted the importance of infection control measures. For this study, a total of eighty-two typhoid cases were investigated that were obtained from the febrile children referred to the tertiary care hospitals in Lahore, Punjab, Pakistan during 2018. The standard microbiological procedures were used for the identification of S. Typhi followed by confirmation using polymerase chain reaction by specific primers. The

antimicrobial resistance testing was performed using the Kirby Baur method and minimum inhibitory concentrations were determined through broth microdilution assay. Further, the extended-spectrum beta-lactamases (ESBL) genes were confirmed by PCR among the S. Typhi strains. The 35/83 (43%) isolates were resistant to first-line drugs as well as cephalosporins and fluoroquinolones therefore considered as XDR. The blaTEM and blaCTX-M genes were mainly found among these XDR strains. Such a higher prevalence of these XDR S. Typhi strains raises the apprehension about transmission prevention among the community and proper infection management in clinical settings. The study also highlights the concerns regarding the decreasing antibiotic arsenal for effective management of typhoid fever in Pakistan.

Characterization of Bacterial Pathogens from Commercially Available Ready to Eat Salads and Vegetables Used in Salads Sold in Hyderabad, Sindh, Pakistan

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Vegetables used in salad are prepared at homes and in restaurants are consumed in raw form, the vegetables are handled and processed under unsafe conditions which favors the growth of pathogen. The main reason of an increasing outbreak of foodborne diseases through consuming ready to eat salads is the presence of pathogens. This study is therefore planned to isolate and characterize the pathogens present in ready to eat salads and vegetables used in salad. For this purpose, a total of 60 samples including 30 samples of different commercially available ready to eat (RTE) salads (vegetable salads, fruit salads, cream salads) and 30 different vegetables used in salad (cucumber, cabbage, lettuce) samples sold in Hyderabad, Sindh, Pakistan were purchased. Out of 60 samples analyzed, *Escherichia coli* was present in 27 samples (45%), *Klebsiella* were detected in 11 samples (18.3%), *Staphylococcus aureus* were detected in 9 samples (15%) *Streptococcus* were detected in 7 samples (11.6%) whereas *Salmonella* spp. was isolated from 5 samples (8.3%). The antibiotic sensitivity test of all the isolates was determined by Kirby Bauer-disk diffusion method with antibiotic containing discs on Mueller-Hinton Agar media. The results revealed that *Escherichia coli* were resistant to ampicillin, enrofloxacin, tetracycline, oxytetracycline and ciprofloxacin and sensitive to norfloxacin. *Klebsiella* were resistant to ampicillin, enrofloxacin, tetracycline, ciprofloxacin and oxytetracycline and norfloxacin. *Staphylococcus aureus* were resistant to ampicillin, norfloxacin tetracycline, oxytetracycline and enrofloxacin and sensitive to ciprofloxacin. *Streptococcus* were resistant to ampicillin, enrofloxacin, ciprofloxacin, oxytetracycline and tetracycline and sensitive to norfloxacin. *Salmonella* were resistant to ampicillin, oxytetracycline, tetracycline, ciprofloxacin and enrofloxacin and sensitive to norfloxacin.

Isolation of MDR *Campylobacter Spp*., in Commercial Poultry and *Tet* (O) Mediated Gene Resistance Against Tetracycline

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According to the CDC report, Campylobacter is on the top of foodborne causing pathogens followed by the salmonella, Yersinia and *E. coli*. Mainly two species *Campylobacter jejuni* and *Campylobacter coli* are frequently causing illness in humans. These are gram-negative, microaerophilic, non-spore-forming, and comma to curved shaped microorganisms. The common route of transmission is the faecal-oral route. The different kinds of antibiotics are used to cure the infection in humans, as well as these antibiotics, are used in the poultry industry as medicine along with feed additives. The unjustified use of antibiotics has caused resistance in Campylobacter against several antibiotics. The antibiotic resistance of campylobacter against tetracycline class of antibiotic at a molecular level was determined with specific primer sequences that target the specific resistant genes. For this purpose, the Campylobacter was isolated and identified from fecal samples of poultry birds on a selective media CCDA with selective supplements. Later on, it was confirmed microscopically and by biochemical tests. The spiral to curved shape of pink colour clolonies indicates the samples were Campylobacter positive. *C. coli* and *C. jejuni*, were confirmed by multiplex PCR. The campylobacter prevalence in the area of study was 70%. The antibiotic resistance of isolated campylobacter spp. was checked by Kerby Beurer disk diffusion method. It was found that 90% of isolates were highly resistant against tetracycline and for and doxycycline the percentage was 75%. Further, the resistant gene responsible for the development of resistance was targeted by using specific primer sequences in conventional PCR. The gene responsible for the resistance of isolates was *tet(O)* and 30% of the isolates were detected positive for the presence of antibiotic resistance gene in them. It was confirmed from this study that the campylobacter has developed a high level of resistance against tetracycline. This study concluded that the Campylobacter species in broiler chicken has developed resistance against Tetracycline due to the *tet(O)* gene. To control the prevalence of antibiotic resistant bacteria, there is a need of public health concern to make strategies to overcome the resistance pattern.

Prevalence of Qnr and Qnr B Resistance Genes in *Klebsiella pneumonia* Comparing with Lactobacillus

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Infections are the second leading cause of death worldwide after cancer. It becomes an alarming condition and challenge to treat chronic infectious conditions due to vast usage of antibiotics. These antibiotics became resistant to many classes of bacteria through acquiring resistance genes. Ciprofloxacin is a broad-spectrum antibiotic that acts as a bactericidal but has major issue of developing resistance. *Klebsiella pneumonia* acquired resistance genes, *Qnr A* and *Qnr B* against ciprofloxacin is a major crisis in the effectiveness of antibiotic. *Klebsiella pneumonia* is a highly pathogenic organism in which plasmid-mediated quinolone resistance through horizontal gene transfer is very common. Current study was design to analyze the prevalence of *Qnr A* and *Qnr B* genes availability in *Klebsiella pneumonia* and possible correlation with lactobacillus as possible contributor to transfer

of resistance genes through horizontal gene transfer to other bacterial species. The expression level of *Qnr* A and *Qnr* B genes were measure through qRT-PCR. Absolute quantification results revealed that the expression level of *Qnr A* were significantly expressed in *klebsiella* ($P < 0.01$). *Qnr B* ($P \leq 0.01$) was highly expressed in pathogenic strains in comparison to control samples. In pathogenic strains, both *Klebsiella pneumonia* and *lactobacillus* strains acquired resistance genes in comparison to control samples. Results revealed that lactobacillus might involve in the distribution of resistance genes to other bacterial population. The data was analyzed statistically by using one-way ANOVA and DMR.

Isolation and Identification of *Staphylococcus aureus* from Commercially Important Fishes of Karachi Local Fish Markets and Its Antibiotics Sensitivity

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Fish meat has an excellent nutritional value being rich in proteins, vitamins and unsaturated fatty acid; it is also one of the most important feed stuffs as it is the cheapest source of animal proteins. As it is highly perishable food item and the biological degradation is faster than vegetables. Therefore, it has to be handled, stored and marketed with extreme care in minimum possible time. Cross contamination with harmful agents through bad handling and unhygienic practices cause illness to the consumers. For this purpose, this study was designed to investigate the prevalence of human pathogens especially *S. aureus* among commercially important fishes locally marketed in Karachi. In this study a total of 180 fish samples of 9 species (20 samples of each) were examined for the incidence of *S. aureus* which were collected from local fish markets of Karachi. Out of 180 processed samples 94 were found positive for *S. aureus* and 33 were found positive for MRSA where the 53 samples were found negative for both. All the *S. aureus* isolates were then preceded for antibiotic susceptibleness towards commonly used antibiotics. The results show that 95 (52.7%) of isolates were found sensitive to Ampicillin- 10 µg (AM), 35 (19.4%) were found resistant and 50 (27.7%) were found intermediate respectively, 70 (38.8%) were found sensitive to Polymaxin-300 IU (POL), 55 (30.5%) were resistant and 55 (30.5%) were found intermediate. 50 (27.7%) of isolates were found sensitive to Chloramphenicol-30 µg (CL), 45 (25%) were found resistant and 85 (47.2%) were found intermediate, 35 (16.6%) of isolates were found sensitive towards Gentamicine-30 µg (GM), 80 (44.4%) showed resistance and 65 (36.1%) were found intermediate, 30 (16.6%) of the *S. aureus* isolates were found sensitive towards CO-Trimaxazol-25µg (CO-T), 88 (48.8%) were showed resistance activity and 70 (38.8%) were having intermediate activity. Similarly, 27 (15%) of the isolates were found sensitive to Ciprofloxacin 5µg (CIP), 90 (50%) were having resistance activity and 63 (35%) were found having intermediate activity respectively. The present investigation showed that the prevalence of human pathogens such as *S. aureus* and MRSA were found high which means all the possible sources of bacterial contamination like hygienically handling of fish, its proper transportation, and storage during the fishing and after being marketed must be addressed.

Prevalence and Antimicrobial Sensitivity Pattern of ESBL Producing Gram Negative Bacteria

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The quick upsurge in extended spectrum beta lactamases (ESBLs) cases among major Gram negative bacteria is raising danger and a worldwide topic of great concern. In particular, the increase in 3rd & 4th generation cephalosporins resistance is a very important issue. This study was undertaken to determine the prevalence and antimicrobial sensitivity of ESBL developing multidrug resistance Gram negative bacteria from various samples collected from hospital environment and various surgical instruments and fomites inside hospitals in district Jhang. All the specimens were examined using routine microbiological procedures. As per the guidelines of Clinical and Laboratory Standards Institute, isolated Gram negative bacteria were subjected to sensitivity tests against different antibiotics using Disc Diffusion method. Multi drug resistant specimens were considered for ESBL's production detection by Double Disc Synergy test (DDST). Out of 200 isolates, 109 (54.5%) were positive by DDST. Most of the ESBL producing isolates exhibited resistance towards drugs such as cefotaxime and ceftazidime. On the basis of overall outcomes of current study, it was concluded that increase in number of ESBL producing Gram Negative multi drug resistant bacteria is alarming in Pakistan, where we are already facing problems of higher level of antibiotics resistance. Therefore, detection of ESBL may be used as an important tool for the assessment and thus control of the spread of resistance.

Life Saving Antibiotics and Bacterial Resistance

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The discovery of antibiotic is considered to be one of the greatest achievements in the field of medicine. Antibiotic treatment not only enhanced clinical outcomes but also resulted in decrease morbidity and mortality in critical care patients. However, rapidly evolving bacterial resistance against antibiotics as a consequence of indiscreet use of antibiotics and lack of new drug molecule development is becoming a global threat. According to report published in the 'Proceedings of the National Academy of Sciences' in 2018, Pakistan was observed to be third highest consumer of antibiotics among the low and middle income countries. The present study is based on antimicrobial susceptibility testing (AST) and resistance determination against lifesaving antibiotics including cabapenems, cephalosporins, glycopeptides, monobactam, polymyxin and penicillin. The antibiotics were tested for efficacy against pathogens labeled under the WHO list of antibiotic- resistant. The disk diffusion method was employed as per CLSI guidelines. The highest susceptibility was observed towards polymyxin by Pseudomonas aeruginosa while the lowest towards ticarcillin in all tested Gram-negative bacteria. Resistance against carbapenems was low with imipenem being the most effective antibiotic against Staphylococcus aureus and Escherichia coli while meropenem proved to be the most active against Klebsiella pneumoniae. Cephalosporins and monobactam showed average results with comparatively lower susceptibility when compared to other antibiotics. 50% strains were multi-drug resistant. Comparison of results with previous studies showed important resistance trends over the years in Karachi. In Pakistan, the national antibiotic resistance surveillance program needs the impetus to control the injudicious use of antibiotics and increasing resistance.

Endocrine Role of Gut Microbiota and Its Therapeutic Implications for Neurological and Psychiatric Disorders

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Recent advances in microbiome research have increased the interest of scientists to study the interaction of gut-microflora with host cells because it affects behavior, metabolism, and the immune system. But the mechanism by which microorganisms modulate the functions of host cells is still unknown, the ongoing studies have been focused on deciphering the signaling pathways linking the microbiota and hormones as the studies have shown the explicit hormonal changes in the presence of a certain microbial species. Several studies reported that modulation in gut-brain microbial communication can cause various psychological disorders. Microbiota produces numerous hormones which interact with the hormones of the host cell; which plays role in the regulation of expression. The function of the gut-brain axis can be improved by several therapeutic strategies, such as FMT (fecal microbiota transplantation), prebiotics, and probiotics. Here we have summarized the endocrine role of gut microbiota and its implications in neurological and psychiatric disorders. The mechanism by which intestinal microbiota interact with the brain and causes neurological and psychiatric disorders are still unknown, therefore, further research is needed to explore the interaction between brain and intestinal microflora.

Prevalence and Antimicrobial Susceptibity Paradigm of Bacteria Isolated from Urinary Tract Infections

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UTIs are one of the commonest nosocomial infections and these are correlated with increased morbidity and mortality. Escherichia coli is most abundant aetiological agents usually found in digestive system while *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* and *Enterococcus* are also responsible for urinary tract infection. UTI predominantly spreads especially in developing countries causing substantial mortality and morbidity. Uropathogens contain specific virulence factors such as siderophore, toxins and adhesions that have ability to colonize and invade urinary tract. The empirical antibiotic therapy of UTI is usage of Fluoroquinolone, Aminopenicillin, Cefotaxime, ceftriaxone, Imipenem, meropenem and levofloxacin

etc. The purpose of this study was to evaluate the prevalence and antimicrobial susceptibility pattern of bacteria isolated from UTIs patients. Samples were collected and cultured on different culture growth media to analyze the bacterial growth. About more than 50% isolates showed positive bacterial growth and the most prevailing and dominant bacteria were *E. coli* and *K. pneumonia*. Antimicrobial susceptibility pattern of isolated bacteria were studied by using Kirby-Bauer disc diffusion method. The extended spectrum beta lactamases (ESBLs) genes were detected by performing PCR. The ESBL production was screened by a combined disk diffusion method (CDDM). The antibiotic susceptibility testing of bacteria showed high resistance to Ampicillin (80%), Co-trimoxazole (82.9%), Cefipime (75.6%) and Cefixime (73.2) while the most effective antibiotics were Amikacin, Imipenem and Meropenem for the treatment of urinary tract infection.

Development of Topically Applied Films for Curing Burn Patients

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Burn injuries accounts for the most devastating form of skin trauma. Breaching of intact skin barrier may lead to susceptibility to various infections. Nonetheless, Methicillin Resistant Staphylococcus aureus is the most prevalent pathogens in burn injuries. Emerging antibiotic resistance has made it resistant to most of the commonly used treatments. Therefore, the most effective treatment for curing infections caused by MRSA is vancomycin. However, vancomycin can only be administered parenterally. Current study has been designed to fabricate novel chitosan-based composite films containing vancomycin for wound healing applications. The developed vancomycin-chitosan films were evaluated for various quality attributes and were subjected to anti-bacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and wound healing efficacy study in rat model. The prepared vancomycin-chitosan film 2 (VCF2) physically displayed a substantial tensile strength and swelling ratio. Pharmacologically, VCF2 exhibited sustained vancomycin release, excellent antibacterial activity and improved wound healing efficacy in rats. The superior wound healing potential was ascribed to the enhanced levels of reduced glutathione, glutathione-S-transferase, catalase and decreased lipid peroxidation. Furthermore, improved angiogenesis, granulation, epidermal regeneration and down regulation in the expressions of tumor necrosis factor, cyclooxygenase-2 and nuclear factor kappa B were the reasons of improved wound healing as confirmed by histopathological and molecular techniques. Thus, it is plausible to say that VCF2 could provide a potential therapeutic approach in burn wounds.

Detection of *Helicobacter pylori* through Microscopy and PCR in Gastric Biopsies

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Helicobacter pylori infection is most prevalent in developing countries. It is an etiological agent of peptic ulcer, gastric adenocarcinoma, and mucosal-associated lymphoid tissue (MALT) lymphoma. Despite the development of different assays to confirm *H. pylori* infection, the diagnosis of infection is challenged by precision of the applied assay. Hence, the aim of this study was to detect *H. pylori* in the gastric biopsy specimen from patients having gastric complications through microscopy and PCR Methods. A total of 32 patients with gastric disorders underwent gastrointestinal endoscopy with biopsy. *H. pylori* infection in gastric biopsies was identified after

examination by microscopy and 16S rRNA species specific PCR. The agreement between two test results were analysed by McNemar's test and Kappa coefficient. *H. pylori* infection was confirmed in 7 (21.8%) patients by both assays, 15.6% in antral gastritis, 26% in gastric ulcer, 100% in gastric ulcer with duodenitis, 56% in gastric ulcer with duodenal ulcer, and 35 % in severe erosive duodenitis with antral gastritis. Out of 7 *H. pylori* infection confirmed patients, 2 patients were confirmed by microscopy and 5 patients by PCR. In case of two patients, both microscopy and PCR assay confirmed the *H. pylori* infection. The agreement between two test results was 88.45% and disagreed by 11.55% (p value > 0.05). We found that PCR assay to detect *H. pylori* is more sensitive than microscopy. However, we advocate for the combination of both assays to increase the strength of diagnostic accuracy due to the absence of the gold standard assay for *H. pylori* infection.

The Molecular Basis of Extensively Drug Resistant *Salmonella* typhi Isolates from Pediatric Septicemia Patients

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Sepsis is a syndromic response to infections and is becoming an emerging threat to the public health sector, particularly in developing countries. *Salmonella* Typhi (*S*. Typhi), the cause of typhoid fever, is one primary cause of pediatric sepsis in typhoid endemic areas. Extensively drug-resistant (XDR) *S*. Typhi is more common among pediatric patients, which is responsible for over 90% of the reported XDR typhoid cases, but the majority of antibiotic resistance studies available have been carried out using *S*. Typhi isolates from adult patients. Here, we characterized antibiotic-resistance profiles of XDR *S*. Typhi isolates from a medium size cohort of paediatric typhoid patients (n = 45, 68.89% male and 31.11% female) and determined antibiotic-resistance-related gene signatures associated with common treatment options to typhoid fever patients of 18 XDR *S*. Typhi representing all 45 isolates. Their ages were 1–13 years old: toddlers aging 1–2 years old (n = 9, 20%), pre-schoolers aging 3–5 years old (n = 17, 37.78%), school-age children aging 6–12 years old (n = 17, 37.78%), and adolescents aging 13–18 years old (n = 2, 4.44%). Through analyzing bla_{TEM1}, *dhfR7*, *sul1*, and *catA1*genes for multidrug-resistance, *qnrS*, *gyrA*, *gyrB*, *parC*, and *parE* for fluoroquinolone-resistance, $bla_{\text{CTX-M-15}}$ for XDR, and *macAB* and *acrAB* efflux pump system-associated genes, we showed the phenotype of the XDR *S*. Typhi isolates matches with their genotypes featured by the acquisitions of the genes bla_{TEM1}, *dhfR7*, *sul1*, *catA1*, *qnrS*, and $bla_{\text{CTX-M-15}}$ and a point mutation on *gyrA*. This study informs the molecular basis of antibiotic-resistance among recent *S*. Typhi isolates from pediatric septicemia patients, therefore providing insights into the development of molecular detection methods and treatment strategies for XDR *S*. Typhi.

Molecular Characterization of *E. coli* Associated Neonatal Sepsis in Pakistan

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Neonatal sepsis is an inflammatory response caused by different bacterial infections in neonates. Detection of neonatal sepsis involves identification of causative strain through biochemical testing, molecular analysis and phylogenetic analysis to give productive information. Pathogenic Escherichia coli (E. coli) is one of the most frequent cause of morbidity and mortality in newborns. Ineffective empirical therapy and the side effects of routine use of broad-spectrum empiric antimicrobial therapy cause excessive morbidity and mortality in neonates. We found a strong association of gram negative bacterium E. coli (involved in 14% cases) with neonatal sepsis in a tertiary care hospital in KPK. Neonatal sepsis is reported to be more common among male patients (65.1%). Most common E. coli phylogroups observed were F 23.07%, B2 23.07% and Clade Ⅰ Ⅱ, Ⅳ or Ⅴ 23.07%. Among strains of E. coli, 6 stains were classified as phylogroup F (3 strains) or B2 (3 strains), 4 among these strains were classified into phylogroup G, a phylogroup associated with greater virulence capacity. 7 strains of E. coli showed presence of GIM gene which encodes for metallo-beta-lactamase, confering resistance against β-lactum antibiotics. We checked presence of virulence genes and found 4 E. coli isolates were ecpA positive, 6 were papC positive, 8 were fimH positive and 7 were traT positive. To sum up, majority of the E. coli isolates show virulence potential with increased resistance against beta lactam drugs. This may have high impact in NICU setup in terms of treatment and management of neonates.

Genetic Diversity of *Staphylococcus aureus* Strains from A Tertiary Care Hospital in Rawalpindi, Pakistan

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Staphylococcus aureus is an important healthcare-associated bacterial species causing a multitude of infections in humans such as superficial skin and soft tissue infections, necrotizing pneumonia and food borne illnesses and postsurgical infections. Treatment of S. aureus infections has become more complicated due to the emergence of multidrug resistant strains of Methicillin Resistant Staphylococcus aureus (MRSA). The present study was aimed at characterization of S. aureus isolates from a tertiary care hospital in Rawalpindi district of Pakistan. S. aureus isolates were initially characterized by antibiotic sensitivity testing and antibiotic resistance genes were detected. Furthermore, Multilocus Sequence Typing (MLST), Pulsed-Field Gel Electrophoresis (PFGE) and spa typing was performed. The isolated strains were resistant to most of the tested antibiotics. The predominant sequence type was ST772 (24.2 %) followed by ST239 (22.7 %) and ST8 (10.6%), ST22 (9%), ST30 (7.5%) ST6 (7.5%), ST1413 (6%), ST272 (1.5%), ST238 (1.5%), ST207 (1.5%), ST1485 (1.5%), ST1(1.5%), ST45 (1.5%), ST526(1.5%). All MRSA isolates belonged to 29 different spa types, t632 and t657 were found to be most common *spa* types.

Transfusion Transmissible Infections Among Multi Transfused Beta-Thalassemia Patients

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Blood transfusion is the most common procedure in healthcare facilities which saves millions of lives every year. However, it is the main source of disease transmission among the people who undergo this procedure more frequently. The patients suffering from thalassemia are categorized among the most frequent blood recipients. Therefore, the chance of transmitting the transfusion-transmittable Infections (TTIs) such as hepatitis B, hepatitis C, acquired immunodeficiency syndrome, syphilis, and malaria are more common in these patients as compared to others. Due to increasing reports related to improper pre-transfusion screenings and the increasing number of hepatitis B and hepatitis C patients in Pakistan, it seems that the incidence of TTIs is also increasing especially among the patients experiencing multiple transfusions. Therefore, the present project was designed to study the prevalence of TTIs in patients suffering from thalassemia in Faisalabad Pakistan. A total of 200 blood samples from beta-thalassemia patients were collected from the Faisalabad region of Pakistan. Male patients were 130 (65%) and females were 70 (35%). The blood group of O+ve has a high frequency and rate of infection 47(33.09%) as compared to other ABO blood groups B+ve 40 (28.16%), A+ve 23 (16.19%), B-negative 13 (9.15%), AB+ve 10 (7.04%) and O-negative 07 (4.92%). Similarly, the prevalence rate of HCV was more detected as compare to other TTIs as 111(55.5%). HIV rate was 20 (10%). Malaria Parasites was 7(3.5%), Syphilis was 3 (1.5%) and HBsAg was 01 (0.5%) in beta-thalassemia patients. It also shows the high prevalence rate of infections in the age group of 6-10 years' patients that have transfused blood 50-100 pints 93 (65.49%). So, this rate of infections can be controlled by the proper screening of blood and educate people about thalassemia.

Occurrence of Ampc Beta-Lactamase Producing *Pseudomonas aeruginosa* Isolated from Skin Infection

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Pseudomonas aeruginosa is the opportunistic micro-organism and quite frequently associated with skin infections. AmpC-β-lactamase producing *Pseudomonas aeruginosa* is one of the main reason for devastating nosocomial infections among skin infected individuals and *P. aeruginosa* acquire multiple β-lactam mediated resiatance due to Class C of cephalosporinase. The goal of present study was to detect the wound infecting *P. aeruginosa* harboring AmpC beta-lactamases from the wound infections. Wound samples of 150 patients suffering from various wound infections were collected. Pseudomonas Cetrimide agar was used for selective isolation of *P. aeruginosa* which were further identified by biochemical testing according to standard protocols. Molecular characterization done using polymerase chain reaction. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method. Isolates were initially screened for AmpC production, phenotypically by cefoxitin disc resistance. Gene specific PCR was done for detection of AmpC beta-lactamase using specific primers under standard conditions. Total 48 (32%) *P. aeruginosa* were isolated and confirmed on the basis of molecular identification by PCR assay. Isolates showed maximum resistance towards Cefoxitin (83.33%), Imipenem and Gentamicin (81.25%), Amikacin (79.16%), Ceftriaxone (77.08%), Cefepime and Tobramycin (72.92%), Meropenem and Piperacillin (68.75%), Ticarcillin and Colistin (67%), Ciprofloxacin and Ceftazidime (62.5%) and Ampicillin (47.92%). Total 39 (81.25%) were Multi-drug resistant isolates and 25 (52%) were confirmed for AmpC beta lactamase production by using gene specific PCR. It is concluded that occurrence of AmpC β lactamase in *P. aeruginosa* responsible for skin infections was 52% respectively.

Sirt2 Gene Expression in Gastric Cancer Patients of Peshawar

Syed AliRaza Shah, Hazir Rahman

Helicobacter pylori gram negative microaerophilic rod and is the causative agent of gastritis and potent carcinogen for gastric cancer. Bacterial infections may alter *sirt2* gene expression in inflammatory tissues and cancer cells. In this study, *sirt2* gene expression in gastric cancer was surveyed with reference to *H. pylori* status. Stomach biopsies were collected from 20 gastric cancer patients, with *H. pylori* positive as determined by the rapid urease test. Gastric cancer was determined by Histopathology. After total RNA extraction from gastric cancer biopsy samples and cDNA synthesis, sirt2 gene expression level was determined by Real Time PCR and ΔΔCT methods. The expression of *sirt2* gene was increased by 2.6-fold. No statistical significance was found between *H. pylori* infection and *Sirt*2 gene expression in gastric cancer patients.

Occurrence and Antibiotics Sensitivity Profiling of Bacteria from Operation Theatres of DHQ Hospital, Narowal Punjab Pakistan

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The study was conducted to find out microbial load and characterize inherent strains of ESBL producing Gram-negative bacteria and also evaluate prevalence of $bla_{\text{CTX-MU}}$, bla_{SHV}, $bla_{\text{OXA-23}}$, and bla_{TEM} genes among bacterial pathogens in different sections of DHQ hospital of district Narowal including Operation Theaters and ICU's. In total, 200 environmental samples were taken and phenotypically identified performing different biochemical methods. Drug susceptibility testing was performed using Kirby Bauer Disk diffusion assay according to CLSI guidelines. PCR assay was used to confirm MDR isolates using specifically designed primers after DNA extraction. ESBL production was screened by a combined disk diffusion method (CDDM). Confirmed isolates were subjected to genotyping by conventional PCR for detection of $bla_{\text{CTX-MU}}$, bla_{SHV}, $bla_{\text{OXA-23}}$, and bla_{TEM} genes. The 77 (38.5%) samples exhibited growth upon cultures from which the majority of isolates were *Klebsiella pneumoniae* (24.67%), *Pseudomonas aeruginosa* (16.88%), *E. coli* (37.66%), and *Acinetobacter baumannii* (20.8%) respectively. The 74% of isolates were MDRs and 25.9% were XDR. 38.96% isolates were positive for ESBLs and showed maximum resistance against Ampicillin (57%), Cefepime (54%), Ceftriaxone (44%), Amikacin (57%), Meropenem (49%), and Trimethoprim/Sulfamethoxazole (62%). Distribution of ESBLs genes were $bla_{\text{CTX-MU}}$ (50%), bla_{TEM} (13%), and $bla_{\text{OXA-23}}$ (26.6%). The β-lactamase production in GNB was established as one of the main reasons for resistance to multiple drugs, and the most prevalent type of ESBL producing gene was $bla_{\text{CTX-MU}}$. Therefore, routine detection of ESBL-producing microorganisms should be done and implement strict rules and regulations for the prevention of such infections caused by ESBLs producing MDR species.

Green Synthesized Functionalized Microspheres for Targeted Delivery of Essential Oil Entrapped TiO_2 Nanoparticles Against MDR *S. aureus*

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It's critical to create new generation materials that can destroy multiple drug-resistant bacterial strains, which are a serious public health concern. This study concerns the biosynthesis of essential oil entrapped TiO_2 microspheres. We synthesized two types of calcium alginate based microspheres, TiO_2 nanoparticles functionalized with clove essential oil (CL-TiO_2-MS) and cinnamon essential oil (CI-TiO_2-MS). TiO_2 nanoparticles were synthesized by aqueous extract of *Nigella sativa seeds* whereas functionalized microspheres were formed by the ionotropic gelation method. Microspheres obtained were spherical, uniform sized, microporous, rough surfaced and were fully loaded with selected essential oils and TiO_2 nanoparticles. The synthesized microspheres were analyzed for antibacterial activity against clinical MDR strain of *Staphylococcus aureus*. Disc diffusion and flow cytometry analysis reveals strong antibacterial activity by CI-TiO_2-MS as compared to CL-TiO_2-MS. Both TiO_2 nanoparticles and modified microspheres were characterized through SEM/EDX, X-ray diffraction(XRD), and FTIR techniques. Results showed that TiO_2 nanoparticles were spherical and 99 nm-150 nm in size whereas functionalized microspheres were spherical and rough surfaced. Apoptosis analysis and SEM micrograph revealed that CI-TiO_2-MS have more bactericidal activity than CL-TiO_2-MS. The *In vitro* antibacterial experiments proved potential of CI-TiO_2-MS encapsulated microspheres as a prolonged controlled release system against MDR *Clinical S. aureus.*

Moxifloxacin Hcl Loaded Biodegradable Chitosan Nanoparticles for Potential Antibacterial and Wound Healing Efficacy

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The present work was aimed to formulate topical hybrid gel containing chitosan coated moxifloxacin HCl nanoparticles with enhanced antibacterial and healing activity. Materials and Methods Moxifloxacin HCl nanoparticles prepared by the ionic gelation method were loaded in a hybrid chitosan carbomer gel. Size analysis was done by SEM and Zeta sizer. The prepared gel was evaluated for biocompatibility and antibacterial property. In-vivo wound healing was calculated by percentage reduction in wounded area and histological parameters in Sprague- Dawley rats. Results Nanoparticles having average particle size 359 + 79 nm having 31.01 mV zeta potential with narrow size distribution (0.008) and high drug entrapment (63.5%) with maximum in-vitro drug release at pH 7.4 were prepared and loaded in a chitosan carbomer gel which showed good biocompatibility, antibacterial and In-vivo wound healing properties. Conclusion The prepared nanoparticles loaded chitosan carbomer gel can be an effective treatment for acute and challenged topical wounds.

Antibacterial Activity of TiO_2 Nanoparticles and Their Effect in Mice

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Metal oxide nanoparticles, a new class of materials, are increasingly being recognized for potential use in research and health related applications. In recent years, Titanium dioxide nanoparticles have been extensively used as an environmentally harmonious and clean photo catalyst, because of its optical properties, high chemical stability and nontoxicity. The main objective of this study was to observe the effect of TiO2 nanoparticles on bacteria as well as on eukaryotic system. TiO2 nanoparticles were synthesized by using extract of Trigonella foenum. The antibacterial as well as synergistic effect of TiO2 nanoparticles with β-lactam antibiotics were examined against gram positive and gram-negative bacteria. The antioxidant activity of nanoparticles was examined by α-α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity. Cytotoxicity was observed through brine shrimp assay. Kidney functioning of mice was examined by giving a single dose of TiO2 nanoparticles to different groups of animals. Results suggested that among bacterial strains E. coli gave the highest zone of inhibition 12mm and 20mm by disc and well diffusion assay respectively at 20mg/ml. The zone of inhibition increases on combination with antibiotics. TiO2 nanoparticles also possess antioxid ant potential, highest percentage scavenging activity was 51%. TiO2 nanoparticles were toxic towards Artemia salina with %age mortality of 3%. The serum BUN and CREA level did not change significantly as compared to control group after exposure to nanoparticles. The serum UA changed significantly as compared to control group at 650mg/kg/BW dose of nanoparticle. Results revealed that antibacterial, antioxidant and cytotoxic potential of TiO2 nanoparticles increases as the concentration increases.

Antibacterial Potential of Plants and Algal Extracts Against Clinically Important Bacteria and Fungi

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The development of antibiotic resistance is leading threat to human health. It stems from many factors including inappropriate use of antibiotics in human and animal health and their prolonged use as growth promoters at sub-clinical doses in poultry and livestock production. We were interested in investigating plants that could be useful in protecting humans or animals against most common infections in Karachi Pakistan. To conduct this study extracts of algae, leaves of annona reticulata (Custard apple), Flowers of Bauhinia variegata (Kachnar), Carica Papaya tree leaves, peels of grapes fruits and pomegranates. All these plant materials were collected and air dried and for further drying hot air oven was used. Dried materials were crushed and sieved for homogenous particle size.25 grams of each powder material were macerated for one week in solvents including ethanol, methanol, chloroform and di ethyl ether. After one week extracts were filtered prior setting up to rotary evaporator. Initially all extracts were tested against *Klebsiella pneumoniae, Staphylococus aureus, Pseudomonas aeruginosa, Vibrio spp, Escherichia coli, Salmonella typhi, Proteus vulgaris, Proteus mirabilis, Streptococcus epidermidis, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium solani, Rhizopus spp, Mucor spp,*

Trichophyton mentagrophytes, Microsporum gympseum, Candida albicans using disc diffusion method and their minimal inhibitory concentration (MIC) values determined using a microplate serial dilution technique. Results showed that algal extracts, papaya leaves and custard apple leaves have some noticeable antibacterial activity against *S.typhi*, however Kacnar does not showed positive results for this. Algal extracts also showed activity against *S. aureus and A. niger* while observed negative in rest of cases. Custard apple leaves and kachnar showed positive results for *E. coli.* It can be concluded from results that selected plant extracts possess antimicrobial potential against some of clinical isolates and can be exploited for medicinal purpose.

Resistance Modulation Through Nanoparticle Coupled Antibiotics Against Enteric *S. aureus* of Houbara Bustard Bird

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Staphylococcus aureus (*S. aureus*) is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters. *Staphylococcus aureus* were isolated from birds. It considered part of the normal intestinal bacterial flora of captive bustards and they were also isolated from the food items used to feed the captive bustards. Houbara bustard are free-living migratory birds that can be hinge almost solely in desert peninsulas in Asia. These birds are listed as vulnerable bird species by the IUCN as they are likely to become endangered due to many survival threats including microbes. Thus, present study is designed to investigate the prevalence of *S. aureus* in Houbara bustard kept at the Houbara Foundation International Pakistan (HFIP), district Bahawalpur. To this end, fecal samples from houbara bustard present at HFIP will be collected through convenient sampling technique. Collected fecal samples (n=72) will be preserved in container at 4°C that was collected from cloaca and transported to Central Diagnostic Laboratory, Cholistan University of Veterinary and Animal Sciences, Bahawalpur for further processing. Collected fecal samples was examined through macroscopic and microscopic examinations using standard protocols. Identification of bacteria was carried out through different confirmatory test. Probability as well as non-probability statistical tools using SPSS version 22 of statistical software at 5% probability were applied on obtained data. Current study found, 55.5% (40/72) of fecal samples positive for *S. aureus.* Retaliation of this bacteria towards septran (80%), ciprofloxacin and enrofloxacin was 70% which show sensitive and resistive towards cefoxitin and vancomycin which is 30% and intermediate response towards gentamicin and linezolid 40%. The highest to lowest minimum inhibitory concentration were as followed by gel nanoparticle alone (G), cefoxitin coated MgO nanoparticle (MC), ampicillin coated gel nanoparticle (GA), and gel tylosin coated gel and MgO nanoparticle (GMT), cefoxitin coated with gel and MgO nanoparticles (GMC), tylosin coated gel nanoparticle (GT), ampicillin coated with gel and MgO nanoparticles (GMA). The antibacterial action of these isolates was confirmed by microscopic examination. As a result, the study indicated that *S. aureus* is developing antibiotic resistance, and that nanoparticles combined with antibiotics are an effective method for combating resistance.

Probiotic Attributes and Bioactivity Potential of *Streptococcus thermophilus*; An Indigenous Isolate from *Capra Aegagrus* Hircus (Goat) Milk

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An increased demand for the control of pathogenic microorganisms and food quality & safety (from farm to end users) remains a continuous challenge. Bacteriocinogenic Lactic acid bacteria (LAB) and their bacteriocins are well known for their GRAS (generally regarded as safe) status. Bacteriocins are shown to inhibit the growth of pathogens and may be used as food supplement in natural way to influence the intestinal microbes which are useful for human and animal health. The aim of the study was to evaluate the probiotic characteristics and bioactivity of Streptococcus thermophilus isolated from goat milk against clinical multi drug resistant (MDR) strains. Morpho-growth manifestive considerations confirmed the isolate to be referred as Streptococcus thermophilus (ultimately confirmed as such by 16S rRNA Sequencing). Similarly, probiotic assessment of the Streptococcus thermophilus strain was under taken and it was also found to be bioactive against 14 clinical MDR strains. Bacteriocin characterization of this probiont was done including sensitivity to pH range, temperature, detergents (EDTA, Triton X-100, Tween 20, Tween 80, and urea) and enzymes (trypsin, chymotrypsin, lipase, pepsin, α- amylase). Maximum probiotic bioactivity was observed between 7-9 hours. Ammonium sulphate precipitated cell free neutralized supernatant (CFNS) of bacteriocin was found more active sagainst clinical MDR strains. Mode of action of this bacteriocin (thermophilin) was found to be bacteriostatic. Plasmid curing protocol suggested that the bacteriocin gene setup was on plasmid borne. Precipitated bacteriocin (thermophilin) was dialyzed through 10KDa cut off membrane, followed by SDS-PAGE for estimation of its molecular mass (found 11KDa accordingly).

Antibacterial Activity of Silver Nanoparticles Against Clinical Isolates of *Klebsiella pneumoniae*

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This study comprehends the evaluation of Silver nanoparticles as substitute of colistin against clinical isolates of MDR *K. pneumoniae*. Synthesized nanoparticles were characterized for their structural, morphological, compositional properties using various techniques. Collection of clinical isolates of MDR *Klebsiella pneumoniae* was done from Research laboratory of Department of Microbiology, Government College University Faisalabad. Their antibiotic resistance was determined through Kirby-Bauer disc diffusion assay which reveal that 6 isolates were MDR out of total of 25 isolates. Agar well diffusion assay used to determine antibacterial potential of different concentration of silver nanoparticles against MDR *K. pneumoniae* which revealed that these MDR clinical isolates of *K. pneumoniae* were found sensitive against silver nanoparticles at different concentrations i.e.

100ul, 75ul, 50µl and 25µl of 3mg/ml stock solution of silver nanoparticles. Maximum inhibitory zones were measured by 100ul of 3mg/ml solution of silver nanoparticles. MIC and MBC was executed to determine the antibacterial potential of silver nanoparticles. MIC results showed approximately similar values for MIC of 3mg/ml nanoparticles against MDR clinical isolates of *Klebsiella pneumoniae*. The synergistic effect of silver nanoparticle in combination with last antibiotic treatment choice (colistin) for *K. pneumoniae* was scrutinized to check whether the silver nanoparticles increase the effect of antibiotic or not. FIC index was calculated 0.39 and FIC value below or equal to 0.5 showed that they have a strong synergistic effect when employed after combination of both. The effect of silver nanoparticle against biofilm formation and preformed biofilm of *K. pneumoniae* was examined at different time intervals showed decreased effect of nanoparticles after 72 hours on biofilm formation while silver nanoparticles were less effective against preformed biofilm.

Antimicrobial Activity of Some Medicinal Plant Extracts Against Bacteria Causing Diarrhea

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Infectious looseness of the bowels is the second largest cause of mortality in children under the old historic period of five globally. Bacteria are responsible for most diarrheal episodes especially in developing countries, and progressive increase in antimicrobial resistance has given rise to the need to investigate other sources of therapy such as medicinal plants. According to fossil record, about 6000 years ago, Human use plants as medicines could be dated back to the Middle Age of Paleolithic. For many centuries herbal medicines have been known to man. Therapeutic efficacy of many endemic plants for several disorders has been delineated by practitioners of ancient drugs. Antimicrobial properties of medicative plants are being more and more from totally different parts of the planet. Plants have an abundant variety of secondary metabolites like glycosides, tannins, alkaloids, flavonoids and Terpenoids, etc., which have been found effective with their antimicrobial properties in vitro. To determine the susceptibility of *Escherichia coli, Staphylococcus aureus, Klebsiella oxytoca, Salmonella enterica,* and *Shigella sonnei* to the crude extracts of *Cedrus Deodar, Cupressus semepervirens, Papaver somniferum, Glycyrrhiza glabra, Zingiber officinale, Syzygium aromaticum,* and *Coriandrum sativum.* To determine the MIC and minimum bactericidal concentration (MBC) of *Cedrus Deodar, Cupressus semepervirens, Papaver somniferum, Glycyrrhiza glabra, Zingiber officinale, Syzygium aromaticum,* and *Coriandrum sativum* plant extracts on the test bacteria. To carry out cytotoxicity tests on plant extracts with most antimicrobial properties. The leaves, bark, rhizome, bulb, buds and seeds of *Cedrus Deodar, Cupressus semepervirens, Papaver somniferum, Glycyrrhiza glabra, Zingiber officinale, Syzygium aromaticum, and Coriandrum sativum* collected from Ly Market Karachi, Sindh, Pakistan and specimens were prepared and identified at University of Karachi from Agriculture Department. This study investigated the antimicrobial activity, MIC and MBC of all the plant extracts, as well as the cytotoxicity of extracts of *Cedrus deodar, Cupressus semepervirens, Glycyrrhiza glabra, Papaver somniferum, Zingiber officinale, Syzygium aromaticum,* and *Coriandrum sativum* against all test organisms. This study demonstrates that the methanol extract of *Glycyrrhiza glabra, Papaver somniferum, Zingiber officinale, Syzygium aromaticum,* and *Coriandrum sativum* were active against all the test organisms.

They exhibited inhibitory as well as bactericidal characteristics against all tested organisms with the exception of *Cedrus deodar*, and *Cupressus semeperviren*, which had low inhibitory property but high cytotoxty.

Role of Probiotics in The Reduction of Ochratoxin A from Poultry Feed

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Ochratoxin A (OTA) is a toxigenic fungal metabolite present in numerous sorts of foodstuffs and feed items. Presence of OTA in poultry feed may elicit health issues of poultry and humans and there is need to control the growth of toxigenic fungi and OTA production. In this study effectiveness of probiotics (*Lactobacillus spp.*) was evaluated against OTA producing fungi and their ability to bind with OTA. A total of (n=60) feed sample were collected and evaluated for the occurrence of OTA producing fungi. The fungi (*A. ochraceus*) were identified by observing their macroscopic and microscopic characters and further confirmed by polymerase chain reaction. The presence of OTA was detected by Thin Layer Chromatography (TLC) and toxin was quantified by High Performance Liquid Chromatography (HPLC). Effectiveness of probiotics (*Lactobacillus delbruecikii*, *Lactobacillus fermentum*) were observed against OTA producing fungi by well diffusion assay and toxin binding capacity of these probiotics was also evaluated. Highest level of mean fungal load was detected in feed samples was 4.42±.23 and the lowest was 3.14±.30. Fungal genera isolated from poultry feed were *Aspergillus, Penicillium, Fusarium Mucor* and *phaeoid* group fungi. The most common species were *A. flavus, A. fumigatus, A. niger, A. ochraceus, and A. terreus.* None of the probiotic showed inhibition zones against toxigenic fungi. Highest level of OTA reduction was detected by *Lactobacillus delbruecikii* (L5) which gave 100% reduction for OTA followed by *Lactobacillus fermentum* (L2) and lactobacillus (L4) gave 84% and 89% reduction for OTA respectively. It was concluded that probiotics did not have any effect on fungal growth but have ability to reduce the concentration of OTA in poultry feed.

Evaluation of Growth Promotion Potential of Chromium Tolerant PGPR Using *Zea Mays* L. In Chromium Contaminated Soil

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In this era of increasing industrialization, heavy metal pollution is increasing at alarming rate due to the release of untreated industrial wastewater. In agricultural soil, heavy metal contamination is a serious environmental issue which exerts many harmful effects on the agricultural lands and human health. Heavy metals (Cr, Pb, Cd etc.) are

toxic for both plants and animals. Some microorganisms have the ability to resist and degrade these heavy metals in less toxic form. In this study, five already isolated and identified bacterial strains i.e., *Pseudomonas protegens* (c1), *Pseudomonas fragi* (3b), *Pseudomonas* sp. (14), *Yersinia* sp. (21) & *Yersinia* sp. (3a) were used to check their growth promotion ability in the presence of chromium. The growth promotional traits of these bacterial strains was checked in the presence and absence of chromium using *Zea mays* thus, the strains were used to inoculate *Zea mays* seeds and enhancement in the growth parameters and biochemical contents of inoculated and non-inoculated plants were observed. The results have revealed that these bacterial strains have effective growth promoting potential under chromium stress and can be used for bioremediation in chromium contaminated areas.

Isolation of Bio Surfactant Producing Bacteria from Different Fuel Contaminated Sites of Sindh

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Biosurfactants are surfactants that are produced extracellularly or as part of the cell membrane by bacteria, yeasts and fungi. Biosurfactant applications in the environmental industries are promising due to their biodegradability, low toxicity and effectiveness in enhancing biodegradation and solubilisation of low solubility compounds. The detergents, personal care and consumer product sector utilize nearly 60% of all surfactants. This envisaged in the present study to collect 70 different soil samples from Hub road, Karachi port, Saeedabad, Site area, korangi industrial area, Gadani, Itehad town, Baldia. Soil samples were inoculated to mineral salt medium containing 0.1% crude oil and incubated on ambient temperature for 48 hours. After incubation samples were serially diluted by 10 folds' dilution up to four consecutive dilutions and last two dilutions was plated on nutrient agar. Screening of isolates were carried out on the basis of drop collapse assay, hemolysis, and oil spreading methods. Out of 233 only 11 found positive for drop collapse assay and oil spreading methods and among these only 5 were haemolytic. Identification of isolated strains were carried out on morphological and biochemical basis. Consortium of positive strains were develop using streak plate methods for increased biosurfactant production and its application in seed germination in plant growth promotion and oil emulsification were studied. Results have shown that isolated bacteria have the potential to produce biosurfactans of great importance hence it can be concluded that after further detailed investigation can be used for industrial scale.

Studying Diversification of Microbes in Soil Around Industrial Zones of Makori, Gurguri, Nashpa, District Karak, Khyber Pakhtunkhwa

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The most dangerous pollution in the environment are the unwanted hydrocarbon in form of oil and petroleum which is the result of leak from the coastal oil refiners. Which leads several researchers to investigate its distribution and effects in the environment and impact on human. This contamination effect almost every type of ecosystem (marine, fresh and terrestrial). Current study focus to evaluate the oil contaminant soil of the bhanda dhoud shah in terms of biological diversity (bacteria and fungi), and to evaluate them as potential degrader of the hydrocarbon present in that soil. Soil sample were collected from three different sites of district Karak: Makori CPF plant, Gurguri rig, Nashpa point and central city of district Karak. Samples were processed through microorganism isolation and identification. The gram staining and microscopy analyzation show that 50% bacterial isolates were gram positive and 50% gram negative bacteria. These bacteria were further confirmed by 16s rRNA molecular techniques. From the biodegradation test in bacteria, it was revealed that high degradation was recorded for the strain of the *Pseudomonas* spp (1.65×10^9). From the current study it was concluded that introduction of these portent bacteria can greatly reduce the petroleum pollution in the environment. Future study should be encouraged to dig out novel strains of bacteria, fungi and other microbes to control these pollutions.

Assessment of Microbe as Potential Bioplastic Producing Entrant

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Plastic effluence is a mounting distress to the terrestrial and aquatic community of environment. Though, majority of plastics that are used by world are non-biodegradable ones which accumulate and endangered the environment. These non-degradable plastics include polyethylene or polythene that is causing great environmental pollution. These environmental concerns thus leading researchers to develop eco-friendly bioplastics. The microorganisms are potential candidates for bioplastic production however; these bioplastics are categorized into: Biodegradable i.e. having biodegradable ability. The other type is Bio based plastics i.e. these plastics are being made from bio origin and also degradable. These include Polyhydroxybutyrate (PHB) and starch based plastics .Among them Poly hydroxy butyrate is a group of polymer which can be synthesize by certain micro-organisms in nutrient depleted condition. The degradation of these polymer also carried out by microorganism that produces depolymerase enzyme andthey are present in soil which make this polymer a good candidate for future bioplastic production. Our study focused on the selection of isolates which were capable of synthesizing polymer and their ability to produce PHB was determined by using Solvent extraction technique. For this technique isolates were

first grown in minimal media followed by using sodium hypochlorite and hot chloroform, they react and form the Crotonic acid which shows the production of PHB. Further, the analysis to determine production and degradation of PHB was performed by spectrophotometry. The results revealed the tremendous ability of Bacillus specie to produce PHB and thus it can be potential microorganism that can be used as an alternative for future bioplastic production.

Bioremediation Potential of Wastewater in Karachi, Pakistan

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Karachi is the largest city of Pakistan and has a population of 20 million, area of 3780km2. Karachi produces more than 9,000 tons of municipal waste daily. Nearly 400 million gallons untreated wastewater is disposed off in the Arabian Sea on a daily basis1. This wastewater is laden with plastics, which comprise 9% of the total waste1, heavy metals, while 30% of it is food waste2 which comprises mainly of fats, oil, grease (FOG), proteins and carbohydrates. Plastic strewn drains lead to clogged drainage system which causes them to overflow every rainy season flooding the city with sewage water. Single use plastic, particularly, prove to be a greater nuisance as it is their majority that ends up in landfills and drains. Malir River which passes through Korangi has about 200-million-gallon discharge of wastewater a day. Even waste holds a lot of potential which is unfortunately not fully realized. This waste possibly harbours bacteria; producing enzymes which can degrade plastic, carbohydrates, proteins, or lipids. Isolating such bacteria and adapting them to high concentrations of one or more of the above molecules can significantly improve the waste crisis at hand. Pakistan and many nations globally are suffering from pollution of various manners. Plastic, commercial, and industrial sewage and sludge are a major source of contamination of land and oceans. They are also the main source of spreading of infectious diseases in rural areas lacking adequate sewage systems. Pollution is also directly and indirectly contributing towards degradation of many plant and animal species. It has become a necessity of time to find environmentally friendly and economic methods to tackle this pollution. Our surroundings provide us with a plethora of microorganisms which can efficiently degrade multiple types of polymers, natural and synthetic both. This project aims to isolate such bacteria from the environment which can aid humans in combating the pollution crisis. They can be adapted to improve their efficiency under lab conditions and then used commercially to speed up the processes of degrading them. We performed this thesis in two phases. Physio-chemical analysis and microbiological analysis. For the latter, we measured temperature, electrical conductivity and other parameters to test the wastewater quality. For the next phase we prepared minimal salt media and supplied it with four different energy sources (starch, protein lipids and plastic) to check for presence of bacteria which were capable of utilizing them. After phase 1, it was established that the wastewater quality was not in compliance with national or international standards. For phase 2, we successfully isolated bacteria which were capable of degrading starch, protein lipids and plastic. A total of 11 colonies of amylase producing bacteria were isolated from sample 1 and 2. After 1 month of incubation, emulsified samples were obtained for lipase producing bacteria while we also observed that there was 0.64%-6.56% reduction in plastic weight inoculated with samples. This study is an attempt at finding ways to improve the adverse conditions of the environment suffering at the hands on mankind. Here I have tried to find natural ways and resources which can be

economical, commercial and are also environmentally compatible in degrading natural and synthetic polymers. Bacteria capable of degrading other polysaccharides can be used in multiple types of industries such as food and beverages, pharmaceuticals, cosmetics and agriculture. Besides their current use plant polymers such as starch, chitin, sucrose, maltose, etc can also be used for making animal and fish feed and also natural fertilizers. Bacterial enzymes responsible for degrading these polymers can be used for therapeutic intervention. Previous studies show presence of plastic degrading bacteria in the environment, the aim is to adapt such bacteria to degrade higher concentrations of plastic at a faster rate. Single use plastic are a huge nuisance to the environment as they are known to not degrade at their natural pace. Plastics, though break into smaller pieces through a process called thermooxidative degradation which uses UV Ultraviolet light from the sun to provide the activation energy required to initiate the incorporation of oxygen atoms into the polymer rays27. There are several organizations working towards cleaning plastic from oceans and land but they are only able to repurpose those plastic items and hundreds of tons of plastic find their way back to where they were originally disposed. We must find ways to completely degrade plastic and possibly acquire useful end products which can be used for other purposes. Sample 2 and sample 3 showed confident results where a good quantity of plastic was degraded, comparatively. The raw sample contained multiple species of bacteria and other microorganisms. We can safely assume this results were either due to a single, efficient specie or a multiple species which could both be bacterial and non-bacterial, working in coherence to utilize plastic for their growth. Such samples can further be analyzed to isolate single colonies for required bacteria and even other microorganisms to adapt them to degrade multiple types of plastics at higher pace and concentrations. We can also study the pathways of such bacteria, focusing on the enzymes involved in order to isolate genes responsible for plastic degradation. We can also genetically engineer other bacteria with short generation time to express such enzymes to boost up plastic degradation.

Bacterial Isolates and Their Antimicrobial Susceptibility Profile of Superficial and Deep-Seated Skin and Soft Tissue Infections

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Skin and soft tissue infections (SSTIs) are caused by microbial invasion of healthy or damaged skin. SSTIs are difficult to manage, contribute to chronicity and emergence of antimicrobial resistance. To ascertain the prevalence of bacteria causing SSTIs and their antimicrobial susceptibility patterns A prospective study from November 2020 to May 2021. A total of 592 samples from SSTIs were analyzed. A total of 467 samples revealed mono-bacterial growth, of which 65% were male. SSTIs are common among patients ranging in age 21-50 years with the dominance of Gram negative organisms (82%). *Escherichia coli*, *Klebsiella spp*., *Staphylococcus aureus* and *Pseudomonas aeruginosa* were predominant organisms. Gram negative bacteria were highly resistant (>65%) to ciprofloxacin and trimethoprim-sulfamethoxazole. For injectable, highest resistance was determined against ceftriaxone and least against amikacin. Resistance against carbapenem was highest among *P. aeruginosa* (57.8%), followed by *Klebsiella spp*. (32.6%), and *E. coli* (17.2%). *S. aureus* showed highest resistance against ciprofloxacin and least against clindamycin. Of 62 *S. aureus* isolates 79% were Methicillin-resistant *S. aureus* (MRSA). All isolates of *P. aeruginosa* and *S. aureus* were sensitive to polymyxin B and vancomycin respectively.

The prevalence of multidrug resistant gram negative bacteria was higher among deep-seated SSTI. High prevalence of bacteria among SSTIs was found. The predominant etiology of SSTIs are gram negative bacteria. Resistance against carbapenem is raising. High frequency of MRSA has emerged. MDR isolates are essentially involved in deep-seated SSTI.

Preparation & Characterization of *E. Coli* DH5α Bacterial Ghosts and Their Evaluation as a Drug Delivery Vehicle

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Bacterial ghosts are nonliving Gram-negative bacterial cell envelopes lacking cytoplasmic contents while conserving their cellular morphology. The study focuses on development of a new delivery platform. Bacterial ghosts can be prepared using genetic and chemical methods. In the following study, BGs of *E. coli* DH5α prepared by exposing the bacterial cells to tween-80 for an extended period of time followed by an immediate reduction of pH. Scanning Electron Microscope (SEM) results showed the excellent formation of bacterial ghosts with clear holes of 111nm size in their outer membranes. Furthermore, Release of DNA and protein content was confirmed by agarose gel electrophoresis and Bradford assay, respectively. Bacterial ghosts were loaded with anti-cancerous drug Doxorubicin Loading efficiency is determined using direct method which is 43µg/mg. Release profile is being studied using dialysis tubing for 11 days and loaded BGs showed slow release over a long period of time. Therefore, BGs could be represented as a powerful drug delivery approach and targeting vehicle for efficient delivery of anticancer drugs for the treatment of cancer. Subsequently, Doxorubicin dose could be considerably lowered on account of the delivery by BGs compared to free Doxorubicin. These findings will benefit the patient in many aspects like decreasing the dose, frequency of administration, and minimizing the cytotoxicity. Experimental validation will confirm the potential of bacterial ghost platform.

Ferulic Acid Production by *Lactobacillus acidophilus* Through Fermentation

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This study aims the production of Ferulic acid by using *Lactobacillus acidophilus* fermentation by using MRS Broth (Oxoid) at the temperature of 37 ºC and pH 5.0 under laboratory condition. The study was designed to observe the strain growth in MRS broth media, where samples were collected for qualitative and quantitative measurements of ferulic acid after fermentation of 72 hrs. The samples of strain were observed for optical density by using spectrophotometer at OD 600 nm the absorption was recorded at 1.539 Abs. Further, the samples were subjected for TLC for observing the presence of ferulic acid by color determination. The results were found with

the presence of ferulic acid in pink color in TLC method respectively. The result showed the highest activity of ferulic acid found at 0.65 gm/L in TLC method, whereas the pure standard of ferulic acid was used to compare the presence of ferulic acid at 0.7 gm/L in sample. It is concluded from the study that MRS broth is rich source for the growth and production of ferulic acid from *Lactobacillus acidophilus* and can be used in food and pharmaceutical industries as a rich source of aroma and antioxidant.

Control of Disease Caused by Phytopthora Capsici in Pepper Plant Using the Soil-Borne *Bacillus Spp* Isolated from Kohat Khyber Pakhtunkhwa

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Phytophthora blight disease is caused by Phytophthora capsici. It is responsible for severe damage to pepper plants, in humidified conditions. Many human-made toxic chemicals are used to control this disease, but they may often lead to fungicide resistance. pepper crops cultivated over an area of 1776 thousand hectares throughout the world, with a production rate of 7182 thousand tons per annum. Biological control being ecofriendly and sustainable is a good alternative for the effective control of P. blight disease. The study is designed to evaluate the efficacy of soil-borne bacterial isolates against the Phytophthora capsici. Phytophthora capsici was isolated from infected green pepper plant. Specific identification was done by special test through carrot agar media. Bacillus subtilis was isolated for the biocontrol of P. capsici. Invivo experiment was done, pepper plant was grown in a nursery, and the plant was infected purposely with P. capsici, bacteria was applied on the plant. After 15 days the fungus P. capsici growth was inhibited. All the isolates produced ovoid, papillate sporangia. Sporangial length of isolates ranged from 44.7 to µm while the width varied from 24 to 38.4 µm. Antagonism assay was performed to check the potential of rhizobacterial strains in mycelial growth inhibition against P. capsici in vitro. Significant activity against P. capsici was noted in dual culture assay on PDA. The activity of isolates was found against P. capsici and significantly inhibited the mycelial growth after 96 hrs. incubation. Fungal growth inhibition (cm) was ranged 63.7–90.3% over un-inoculated control. Maximum fungal mycelial growth inhibition was done by Bacillus cereus (90.3%), among all the tested bacterial agent's over untreated control. Bacteria were identified on the base of biochemical characteristics. Rhizobacteria with high antifungal potential were evaluated for disease suppression and plant growth promotion traits in pot trials under greenhouse conditions. All the tested bacterial strains enhanced the seed germination ranged (77.6–93.1%) as compared to control treatment (60.8%) and reduced the seed mortality caused by P. capsici. However, maximum seed germination was done (93.1%), Bacillus cereus (90.7%). Plant growth characters viz., shoot and root length (cm), fresh and dry shoot and root weight (g) were enhanced by the bacterial seed inoculation as compared to untreated control. All the rhizobacterial strains enhanced the shoot and root length in range (9.57–14.77 and 3.30–5.63 in cm) as compared to control where shoot and root length was 3.53 cm and1.09 cm respectively. This study concludes that B. subtilis showed activity against P. capsici. which also promotes plant growth.

Bioremediation of Hydrocarbons in Waste Water by Soil Isolates

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Karachi is overpopulated city of Pakistan produces tons of waste on daily basis, this untreated waste then disposes to Arabian sea, containing oil, grease, hydrocarbons, plastics, proteins, carbohydrate, lipids and some other hazardous products as well but this water and soil also contains the bacteria, these bacteria are nature friendly and they have the potential to degrade all such hazardous substances, the soil has rich flora of microorganism and participate in our ecosystem. Hazardous material from the surrounding continuously gets into the marine environment where they consume by the marine animal, from where they become the part of our food chain, on the other hand hazardous substances in soil decrease the soil fertility so we must identify such substances and the solution to resolve the problems. We are looking for such isolates that have the potential to degrade all these materials so that we can clean our soil. The aim of the study is to isolate hydrocarbon degrading bacteria from soil for treatment of oil in waste water, use of petroleum or hydrocarbon containing product increasing, causing damage to marine life. Oil polluted effluent contain polyaromatic hydrocarbons and other toxin that are lethal and inhibit plants and animal growth and lead to genetic mutation that causes harm to human and destroy marine ecosystem, but physical and chemical method both are expensive and has some unusual outcomes to certain extent like secondary contamination, that's why using micro-organism, and making them to adapt environment because they are nature friendly and didn't cause any secondary contamination. On the other hand, we are looking for the bacteria that can degrade starch, protein and lipid by producing amylase, protease, and lipase so that we can grow those bacteria is bulk and scale up our product for industrial purpose. Physiochemical analysis of Soil has been done to study the nature of soil and habitat of microorganism, then we make the dilution of the soil and spread it on NA medium to isolates bacteria. Then we incubate each isolates with enrichment medium infused with xylene and olive oil separately, we also screened for the bacteria that have the potential to produce lipase, amylase, and protease. It is multidimensional study on soil's isolates, they are potent in degrading hydrocarbons, in our study we planned to check their potential to degrade a wide variety of hydrocarbons including different oils like olive oil, crude oil, hexane and xylene, but only proceed experiment on olive oil and xylene, firstly it was decided to make soil bacteria to adapt environment that's why, incubate them for 2 days with olive oil and xylene separately, later on decided to spread it on NA agar containing hydrocarbons, but it was observed that both olive oil and xylene starts to degrade although it was physical observation but it was clear that olive oil on the surface starts to degrade, and it was then further incubated for 15 days on shaking incubator, results was very interesting , all the olive oil and xylene both disappeared from the surface.

Comparative Drug Susceptibility of Colistin Resistant *Escherichia coli* Isolated from Milk in District Mardan

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E. coli are prevalent in different environments and can cause various disorders i.e diarrhea, edema, and inflammation of serosa membrane, septicemia as well as mastitis. *E. coli* showed resistance to different antibiotics which represents a threat to humans and animals. Here we reported resistance of *E. coli* to colistin which is a last option of treatment. From local areas of Mardan milk samples were collected. According to standard guidelines of Clinical Laboratory Standard Institute bacteria were isolated. Out of positive isolates some showed multi drug resistivity. Vancomycin showed maximum resistance while minimum by Chloramphenicol. On other hand maximum susceptibility was found to Trimethoprim sulfamethoxazole and Cefipime. Colistin resistance genes were detected via Polymerase Chain Reaction. UDP was the most prevalent gene detected in isolates. It is concluded from this study that colistin resistance genes are dominant in milk and can be transferred to others via animal product i.e. milk and meat.

Characterization of Bacterial Pathogens from Commercially Available Ready to Eat (RTE) Salads and Vegetables Used in Salads Sold in Hyderabad, Sindh, Pakistan

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Vegetables used in salad are prepared at homes and in restaurants are consumed in raw form, the vegetables are handled and processed under unsafe conditions which favors the growth of pathogen. The main reason of an increasing outbreak of foodborne diseases through consuming ready to eat salads is the presence of pathogens. This study is therefore planned to isolate and characterize the pathogens present in ready to eat salads and vegetables used in salad. For this purpose, a total of 60 samples including 30 samples of different commercially available ready to eat (RTE) salads (vegetable salads, fruit salads, cream salads) and 30 different vegetables used in salad (cucumber, cabbage, lettuce) samples sold in Hyderabad, Sindh, Pakistan were purchased. Out of 60 samples analyzed, *Escherichia coli* was present in 27 samples (45%), *Klebsiella* were detected in 11 samples (18.3%), *Staphylococcus aureus* were detected in 9 samples (15%) *Streptococcus* were detected in 7 samples (11.6%) whereas *Salmonella* spp. was isolated from 5 samples (8.3%). The antibiotic sensitivity test of all the isolates was determined by Kirby Bauer-disk diffusion method with antibiotic containing discs on Mueller-Hinton Agar media. The results revealed that *Escherichia coli* were resistant to ampicillin, enrofloxacin, tetracycline, oxytetracycline and ciprofloxacin and sensitive to norfloxacin. *Klebsiella* were resistant to ampicillin, enrofloxacin, tetracycline, ciprofloxacin and oxytetracycline and norfloxacin. *Staphylococcus aureus* were resistant to ampicillin, norfloxacin tetracycline, oxytetracycline and enrofloxacin and sensitive to ciprofloxacin.

Streptococcus were resistant to ampicillin, enrofloxacin, ciprofloxacin, oxytetracycline and tetracycline and sensitive to norfloxacin. *Salmonella* were resistant to ampicillin, oxytetracycline, tetracycline, ciprofloxacin and enrofloxacin and sensitive to norfloxacin.

Evoluton of Indigenously Developed Rapid Identification of Medically Important Gram Positive Group of Cocci by Urea & Arginine

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The evolution of a Rapid identification system of gram positive group of cocci progress is a basic advantage of clinical purpose. The diagnosis of infections is conditional, to tremendous importance, on laboratory action and its estimation going on it can reduce the chances for bacteria to become resistant and enhance their mortality and morbidity data, and assay for diagnosing bacterial infections in the pathological laboratory. A commercially available systems operating these techniques for bacterial detection and identification have been proposed, the supervisor formulated the formulations for Gram-negative bacteria and she preferred us to work on formulating for Gram-positive bacteria as a portable role of her experiment lab. The rapid system was developed for the rapid identification of biochemically less active in comparisons with other groups of bacteria that covers 14 different bacterial species. Strips were not contaminated. In these strips, sugar fermentation and enzyme profile examination showed the ability of the microbes to metabolize in urea, and arginine of different Strain of Staphylococcus, Streptococcus, after evaluation some strain gives 100% concordant results and ensures the possibility to be used in clinical diagnosis. It gives effect in 3-4 hours. Total 14 bacterial strains which were used in research, Sample were collected from the pathological lab. All strains are known and isolated from various parts of the body. and cultures of 16 known strains add into 16 saline tube, now take strips and inoculate the prepared sugars into the strips and dry the sugars After drying, inoculate the culture. incubate at 37oC for 14-24 hours. After incubation check strips for colour change. Document the effects as colour revealed positive and negative results. I had performed this research in which I concluded 14 culture of gram-positive cocci involved 2 genera consist of staphylococcus and streptococcus and urea & arginine were used which gave the outcomes as all the species enormously fermented however some species do not ferment these sugars, whilst some of them give a negative result. If this rapid identification system of gram-positive cocci progress and its lower the risk of bacteria to become resistant and can easily be used in hospitals for clinical purposes

Effect of Exogenous Protease on Growth Performance, Microbial Count and Meat Quality of Broiler Reared on Fish Meal Based Diet

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This study determined the effect of protease supplementation in fish meal-based diets on growth, digestibility and meat quality of commercial broiler birds. Nine experimental diets were formulated using three levels i.e. 0, 25 and 50% of the protein source of fish meal, with or without alkaline protease (CIBENZA® DP100). Four hundred and thirty-two (432) day-old mixed sex broiler chicks were randomly divided into thirty-six experimental units of 12 chicks each. Feed consumption and body weight were measured weekly. On the last day of trial, two birds from each pen were picked up and processed for carcass parameters. Meat samples were analyzed for meat quality parameters includes pH, water holding capacity and cooking loss. Data collected were analyzed by analysis of variance technique under Completely Randomized Design in a factorial arrangement (3×2) (Diet × Protease) and mean values were compared using Tukey's test if value of $P<0.05$. Improved body weight gain and FCR were recorded in birds fed diet having protease enzyme in fish meal-based diet during day 1-42. Highest dressing percentage and thigh meat yield was observed in treatment groups replaced 25% of SBM with fish meal on protein equivalent basis with Enzyme as compared to 50% fish meal based diet with supplementation of enzyme. Relative organ weights heart weight showed significant *($P<0.05$)* difference. There was no significant difference among treatments regarding coliform count either supplemented with different levels of fish meal or with protease. No two-way interactions *($P>0.05$)* were found between protease and fish meal levels on water holding capacity, pH and cooking loss. An interaction *($P<0.05$)* between protease and fish meal levels on uric acid, serum albumin, cholesterol and triglycerides. Higher platelet count was measured for group formulated diet containing 50% fish meal. White blood count and hemoglobin levels were highest in treatment group having replaced 50% of SBM with fish meal on protein equivalent basis with enzyme. It can be concluded that the addition of protease in 25% fish meal based diet had improved growth performance, crude protein digestibility and carcass characteristics.

Analysis and Evaluation of Iron Chelating and Anti-Cancer Activities of Extract from Streptomyces Species

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Streptomyces are very promising source of natural compounds; more than 6000 different bioactive compounds have been isolated from this genus. Iron homeostasis is very important for normal physiology, but in some specific conditions such as in iron overload this balance is disturbed and free iron is available in plasma, this iron overload is called hemochromatosis and has been connected with several complications like diabetes, cardiac failure, liver cirrhosis and cancer. This study was focused on extraction of the bioactive metabolites from

Streptomyces sp. BD32, the ethyl acetate crude extract was assessed for their phytochemicals which revealed the presence of phenols, flavonoids, alkaloids, and tannins. After that, the chromatographic analysis of ethyl acetate extract using TLC and HPLC displayed different UV active metabolites which could be responsible for the given pharmacological activities. Moreover, GCMS analysis was carried out that unveiled the phenol and ester compounds as the main ingredients of the extract. The antioxidant potential of the extract was determined using DPPH assay, which exhibited potent IC50 of 0.034 mg/mL as compared to the positive control ascorbic acid 0.12 mg/mL. Furthermore, the iron chelation activity of the extract exhibited significant chelation potential by 250 and 125 µg/mL of the extracts, whereas 62.5 µg/mL displayed moderate chelation of the ferrous ion, similarly the cytotoxicity of the extract was evaluated using MTT assay with a range of extract concentrations, in which 51% cytotoxicity was exhibited by 350 µg/mL of the extract, whereas 65% of cells growth was inhibited by 700 µg/mL respectively. The current study demonstrates that the active compounds from microbes living in desert could be used for future potential therapy in the field of medicine.

Bioremediatory Potential of PGPR to Alleviate Acid Stress On the Growth of *Triticum aestivum*

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Plants are continually exposed to a variety of abiotic and biotic stresses in their natural surroundings, which can have negative impact on their productivity. Acid rain is one of such factors now-a-days due to increasing air pollution, which not only affects environment but also affects growth and development of plants. Plant Growth-Promoting Bacteria (PGPB) can enhance plant growth and protect them against several biotic and abiotic stress by utilizing a wide variety of direct and indirect mechanisms. In the current work, impact of various concentrations of sulfuric and nitric acid on the growth and development of *Triticum aestivum* L. was assessed. Moreover, the effect of single and co-inoculation of selected PGPB strains i.e., S5a and S12 on the growth was evaluated. Various growth and biochemical parameters were recorded such as germination percentage, shoot length, root length, number of leaves, fresh weight, chlorophyll content and protein contents of treated plants which were compared with control plants. The results showed that plants treated with low concentration of acids showed increment in growth parameters as micronutrients become more available to plants in optimum environmental conditions and in this way small concentrations of acids enhanced the growth. On the other hand, plants treated with higher concentrations i.e., 0.5, 1 and 2N concentrations of sulfuric acids not only showed decline in growth and biochemical parameters but also showed foliar injuries and bending and yellowing of leaves. However, it was observed that bacterial cultures treated plants showed resistant towards abiotic stress created by acids. It was observed that PGPB were more susceptible to nitric acid as compared to sulfuric acids.

Antifungal Susceptibility Profile of Invasive *Candida Glabrata* Isolates (2009-2020) from a Tertiary Care Hospital Laboratory in Pakistan

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Invasive infections with *Candida glabrata* are a global concern due to poor clinical outcomes and propensity to acquire resistance toantifungal agents. Monitoring emerging resistance and trends in *Candida glabrata*, an important agent of candidemia in Pakistan, is critical for patient management. Therefore, this study evaluated antifungal resistance and minimum inhibitory concentrations (MICs)distribution ininvasive *C. glabrata* isolates from Pakistan. This cross-sectional and descriptive study was conducted from Jan 2009-Mar 2020 at a clinical laboratory in Pakistan that has a nation-wide network Antifungal susceptibility of invasive *C. glabrata* isolatesagainst fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, micafungin, caspofungin and amphotericin B were performed using colorimetric broth microdilution and interpreted using CLSI criteria. Demographics, clinical history and outcome were studied. Chi-square test was used to demonstrate association between antifungal resistance andclinical characteristics of the patients. We identified 277 patients with invasive *C. glabrata* infection. Of which forty-eight (18.4%)isolates were resistant to fluconazole (MIC≥64 mg/L), one isolate each was resistant to amphotericin (MIC=2mg/L), anidulafungin (MIC=1mg/L) and micafungin (MIC=0.5mg/L). MIC_{90} for fluconazole was64mg/L and other triazoles 2mg/L, caspofungin 0.12mg/L, anidulafungin 0.06mg/L, micafungin 0.03mg/L and amphotericin 0.5mg/L.FluconazoleMIC≥64mg/L, caspofungin MIC>0.06mg/L and amphotericin MIC>0.25mg/L (above MIC_{50}) weresignificantly associated with patient being alive at the time of reporting, no use of healthcare devices, nor infection with other fungi. Fluconazole resistance was significantly associated with prior antifungal use by the patient. Surveillance data of antifungal resistance among common *Candida* species should be monitored closely for identification of resistant strains.

Prevalence of Needle Stick Injury and Nursing Practices Regarding Safe Injection and Sharp Disposal Working in Critical Care of Two Tertiary Care Hospitals

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This observational cross-sectional study design (Pilot) was conducted to assess the prevalence of needle stick injury and nursing practices regarding safe injection and sharp disposal in critical care units of two tertiary care hospitals from 1st July to 30th August, 2014. Data was collected using self-developed questionnaire about the prevalence of NSI and nurses' practices regarding safe injection and sharp disposal after thorough literature review, and then was given to the expert for review. Finally, the data was collected from the participants after verbal consent. The study result showing that about half of the nurses have no knowledge regarding disposal of

sharp and it has been found that 47.8% re-cap the needle prior to disposal. While 32.6% reported needle prick injury. Inadequate knowledge among nurses about safe nursing practices and lack of using preventive measures from Needle stick injury were identified. Lack of reporting is also a factor identified in this study.

Detection of Mycotoxigenic Fungi and Mycotoxins in Poultry Feed from Poultry Farms in Balochistan

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Mycotoxins are secondary toxic metabolites produced by fungi that occur naturally in agricultural commodities worldwide. Aflatoxins, Ochratoxin A, Fumonisins, Trichothecenes and Zearalenone are the most important mycotoxins types. Different species are responsible for the production of mycotoxins such as, Aspergillus, Fusarium, Penicillium and Claviceps genera. These mycotoxins can be carcinogenic, cytotoxic, mutagenic, teratogenic, neurotoxic, nephrotoxic, estrogenic, and immunosuppressant. A study was conducted for the presence of mycotoxigenic fungi and mycotoxins in poultry feed used by the poultry farmers of Balochistan. It was found that 40 out of the total 43 (93%) samples are contaminated with fumonisin tested by fumonisin detection Agra strip and ELISA tests. Whereas, the samples tested for aflatoxins showed that 29 out of 43 (66%) feed samples are contaminated with aflatoxin conformed by Agra strips and ELISA kits. The mycotoxins presence were also conformed with the help of thin layer chromatography compared with standard toxins. It was concluded that the majority of poultry feeds are contaminated with aflatoxin and fumonisin. The occurrence of these mycotoxins can be from the raw materials or form other sources during transportation and storage. This research was supported by the AIP-PARC-2017 project funded by USAID.

Synergistic Effect of *Lavendula angustifolia* L Oil on the Antimicrobial Activity of Gentamicin Against Methacillin Resistant *Stphylococcus aureus*

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The growth of resistance to diverse antimicrobial agents by bacteria, fungi, viruses, parasites, etc. is a great test to the medical field for the treatment of infections caused by them, and hence, there is a pressing require searching for new and new antimicrobials. The antimicrobial activity of essential oils and gentamicin drug is well known. The aim of this study will to verify the existence of the synergistic antimicrobial outcome of lavender essential oil and combined with the drug gentamicin. W investigated the efficacy of the combinations of gentamicin and lavender essential oil against the following strains: *Staphylococcus aureus* MRSA KBrn13, *Staphylococcus aureus* MRSA KBrn23 and *Staphylococcus aureus MSSA KBrn*31. In order to decide the sensitivity of these microorganisms, I determined the minimum inhibitory concentration (MIC – Minimal Inhibitory Concentration) and Fractional Inhibitory Concentration (FIC) and kinetic growth of bacteria like colony Forming Unit (CFU) and that gentamicin drug functionalized with essential oils have significant antimicrobial potential against MRSA and

MSSA. The study of the interaction of gentamicin with lavender essential oil will evaluated by the checkerboard method and Time kill assay. Synergistic interaction between lavender essential oil and gentamicin will be observed against *Staphylococcus aureus* KBrn 13 and *Staphylococcus aureus* MRSA KBrn 23 and *Staphylococcus aureus MSSA KBrn 31*. In particular, a very strong synergistic interaction will observed against *Staphylococcus aureus* MRSA (lavender essential oil and gentamicin drug MRSA 13 FIC index = 0.4; MRSA 23 FIC index = 0.2 and MSSA KBrn 31 FIC index = 1.2. Lavender essential oil combination with antibiotic appears as a new strategy to combat the various resistant strains the combination of essential oil with gentamicin verified to be effective against MRSA and MSSA. Therefore, lavender is appearing as one of the best option to be used in combination with antibiotic to increase effectiveness against resistant bacteria.

ABSTRACTS FOR POSTER PRESENTATIONS

Isolation, Genomic Characterization, *In Vivo* Efficacy of Lytic Bacteriophage against Extended Spectrum β Lactamase producing *Klebsiella pneumoniae*

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Klebsiella pneumoniae as commensal Gram-negative bacilli and a member of ESKAPE pathogens is common cause of bacteremia, endocarditis, wound, respiratory and urinary tract infections. Since the arrival of antibiotic therapy, there has been a parallel increase in the evolution of antimicrobial resistant pathogens. Due to emerging antibiotic resistance and halted antibiotic research demand to look for alternative option to cure MDR *K. pneumoniae* infection. The use of bacteriophage in the pre antibiotic era and recent development in phage therapy highlighted them as a potential alternate to cure K. pneumoniae specific infections. So, in this study, bacteriophage against ESBL producing *K. pneumoniae* were isolated, sequenced for identification or bioinformatics analysis, and evaluate their efficacy to eradicate biofilm and potency in in vivo infectious model. The lytic phages from environmental samples, were isolated and screened for clear plaques. One phage AYL was selected, based on its severe lytic action, had a significant impact on *K. pneumoniae* biofilm formation. Further research revealed that phage have specificity towards *K. pneumoniae* and incredible phage tolerability at various pH levels, good thermal stability, and a burst size of 161 PFU/cell. Based on whole genome sequencing of AYL phage it was found that it belongs to the *myoviridae* family. Protein profiling of phage showed 5 sharp bands and some light bands and phage structural proteins were predicted based on molecular weights. Furthermore, even at MOIs of 0.1, 1 and 10 phages co-culturing against this pathogen resulted in a considerable growth reduction. Moreover, AYL phage showed significant biofilm eradication as visualized via AFM, SEM, fluorescent and bright field microscopy (figure 2). These findings imply that the phage might be a good option for eliminating *K. pneumoniae* infections in clinical settings.
Keywords: ESKAPE pathogen; ESBL *Klebsiella pneumoniae*; antibiotic resistance; biofilm; phage therapy; Atomic force microscopy; SEM.

Evaluation of antibacterial activity of *Nigella sativa* against Multidrug Resistant Bacteria

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The antibacterial activity of Nigella sativa seeds extracts was evaluated against MDR bacteria using agar well diffusion method. Extracts of seeds in ethanol and methanol was prepared by simple extraction methods using grinded powder and made solutions of concentrations of 70%, 80% and 90%. Activity of N. sativa seed oil also checked in dilutions made in DMSO with concentration of 25%, 50% and 75%. For, S. aureus, 50% oil concentration in DMSO showed highest inhibitory zone of 15 mm followed by 90% ethanol extract showed clear zone of 13 mm and 12 mm zone of inhibition showed by the methanol based extract. For, E. coli 50% oil concentration in DMSO and 90% ethanol extract both showed clear zone of 12 mm and 11 mm zone of inhibition showed by the methanol based extract. However, MIC of 50% oil concentration is 0.4 µl followed by 90% ethanol extract showed 0.4 mg and 0.8 mg showed by the methanol based extract 90% on S. aureus. MIC of 50% oil

concentration is 0.4 µl followed by 90% ethanol extract showed 0.4 mg and also 0.4 mg showed by the methanol based extract 90% on E. coli. MBC all three extracts having bactericidal property was determined by taking samples from respected wells which show MIC and no growth was observed on MH agar plates. It was concluded that N. sativa has potential to be used as a medicinal plant as an alternate to antibiotics against MDR bacteria.

Bacteriological Quality Assessment of Some Selected Street Vended Food in Main Public Areas of District Hyderabad

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Street food vending is an exponentially increasing informal business sector provides ready to eat (RTE) meals at affordable prices and a valuable employment opportunity to individuals. Globally, poor implementation of good handling and manufacturing practices by street food vendors (SFVs) during food processing is alarmingly increasing the burden of foodborne illnesses (FBIs). On that account, the bacteriological quality of some selected street vended foods (SVFs) i.e. Biryani, Cholay chat, Dheleem, Dahibarey, Pakorey, Salad and Cane juice being sold near public areas in district Hyderabad, Sindh i.e. School, Hospitals and Bus stops were examined using standard procedures. The results revealed that a total of eight bacterial contaminants were detected from SVFs (i.e. *Acinetobacter calcoaceticus, Bacillus cereus, Bacillus safensis, Escherichia coli, Klebsiella pneumonia, Macrococcus caseolyticus, Pseudomonas montielii and Staphylococcus sciuri*) whereas among them *B. cereus* (27.77 %) remained the most frequently occurring bacterial contaminant followed by *E. coli* (14.28 %), *K. pneumonia* (12.69 %), *P. montielii* (9.52 %), *M. caseolyticus* (8.73 %), *A. calcoaceticus* (3.17 %), *B. safensis* (2.38 %) and *S. sciuri* (1.58 %). Among the SVFs, the Cholay chat (23.8 %) remained the most contaminated SVF followed by Biryani (11.9 %), Dheleem (11.9 %), Pakorey (10.31 %), Sugar cane juice (9.52 %), Salad (8.73 %) and Dahibarey (4.76 %). The results further declared that Bus stop areas (38.06 %) were involved in vending most contaminated SVFs followed by Hospital (22.20 %) and Schools (18.81 %). Present study concludes that SFVs are not focusing upon safe food handling and manufacturing practices. It is therefore suggested that street food buyers must consider overall hygienic status of SVFs prior to consumption in order to prevent themselves from foodborne illnesses.

Azadirachta Indica (Neem): Antibacterial Effects Against *Escherichia Coli* and *Salmonella*

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Neem is utilized as ancient medicine to treat various infections. Neem plant has a number of meditative properties such as antibacterial, antifungal, analgesic activities etc. that is why used for different therapies. Today, there are many applications of neem such as in therapeutics, pharmaceuticals and cosmetics. Aqueous extract of Neem leaves give therapeutic potentials such as anti-hyperglycaemic agent in insulin-dependent and non-insulin

dependent diabetes. Moreover, neem leaves are also used for treatment of various diseases along with skin infections like ringworm, acne, inflammation, chronic wound infections, diabetic foot and sphacelus. *Azadirachta indica* (neem leaves) is used in drugs for the treatment of polygenic disease and it shows the effective role in antidiabetic drug efficacy. The main objective of the study was to determine the antimicrobial activity of the extract obtained from the *Azadirachta indica* against *E. coli* and *Salmonella.* The extract was tested against gram negatives by agar well diffusion method. It has been seen that neem extract has showed activity against gram negative bacteria. The big zones of inhibition of 1.8 cm and 2.0 cm is obtained for *E. coli* and *Salmonella* that indicate that neem has activity against these two bacteria. It has also observed that gentamycin has the similar activity as neem. Therefore, neem extract can be used to treat infections by these two gram negative bacteria or as an alternative to gentamycin.

Identify The Fungal Species and to Optimize the Cultural Conditions for The Maximum Production of Xylanase by *Aspergillus Fumigatus*

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Hemicellulose is major constituent of plant biomass and xylanase hydrolyses ß-1-4, glycosidic bonds in the structure of xylan. The current study was aimed to identify the fungal species and to optimize the cultural conditions for the maximum production of xylanase by *Aspergillus fumigatus*. Enzyme was purified by ammonium sulphate precipitation and gel filtration chromatography. Purified enzyme was used for the characterization of temperature and pH. Maximum activity (2.121 IU/mL/min) of xylanase was observed after 96 hours of incubation, at 40℃ and pH 7. Wheat bran as a carbon source showed maximum xylanase production and activity (2.155 IU/mL/min), maize bran (2.001 IU/mL/min) than xylan (1.972 IU/mL/min) while saw dust showed least activity (0.124 IU/mL/min). Trypton as a nitrogen source supported higher production of xylanase (1.816 IU/mL/min) and beef extract (1.749 IU/mL/min). In case of carbon source (wheat bran), maximum production of xylanase was recorded in medium with pH 6 (1.977 IU/mL/min) and in case of nitrogen source (trypton) maximum xylanase production was recorded in medium with pH 5 (1.788 IU/mL/min). Maximum xylanase activity (1.171 IU/mL/min.) was observed at 20 % ammonium sulphate saturation and 2.224 IU/mL/min activity was observed in elution number 15 of gel filtration chromatography. Optimum pH and temperature of enzyme were 5 and 50℃ respectively. The activity of xylanase was inhibited by $ZnSO_4$ and $FeSO_4$ while it was stimulated in the presence $CuSO_4$ and $CaCl_2$. The activity of xylanase was enhanced in the presence of organic solvents like glycerol and methanol while decreased with ethanol and isopropanol. The activity was slightly decreased by 0.25% SDS. Xylanase activity was decreased with the increasing concentration of inhibitor (EDTA). The V_{max} and K_m of xylanase isolated from *Aspergillus fumigatus* were 4409.17 µM/mL /min and 1.982 mM respectively. These results showed that locally isolated *Aspergillus fumigatus* is suitable fungus for the production of industrial enzymes.

Medicinal Activity of Chromatographic Fractions of Selected Plants Against Multidrug Resistant Bacteria

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Therapeutic plants are rich source of traditional herbal medicine. Most of the plant's medicinal properties are due to the presence of secondary bioactive compounds. Numerous plants phytochemicals can also be used directly for the production of novel effective medicines. Some Indigenous plants were collected and used. Plants crude extracts were used against MDR GI bacterial pathogens. The most effective and maximum zone of inhibition was exhibited by aqueous extract against MDR GI S. Typhi. HPLC fractions were obtained from indigenous plants extracts. All the fractions showed activity against MDR GI bacteria. Aqueous fraction against MDR GI S. Typhi. Methanolic fraction against MDI GI E. coli and ethanolic against MDR GI S. flexneri showed best results.This study will be helpful for future to isolate and use the bioactive compounds of selected plants for therapeutic activity in pharmaceutical industry against MDR GI bacterial pathogens.

Bactericidal Activity and Biochemical Analysis of Skin Mucus of Cyprinids

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Fish skin mucus contains several bactericidal components that provide protection against invading pathogen. Skin mucus from several fish species were rich biochemically with strong bactericidal activity however information about skin mucus of members of Cyprinids are still very rare. We report the biochemical composition and bactericidal effect of skin mucus of five Cyprinids species against pathogenic bacteria isolated from infected fish. The bactericidal activities were evaluated in terms of zone of inhibition (ZOI) in mm and compared with Fosfomycin as a positive control. Variations in bactericidal activity have been observed among different Cyprinids species against same as well as different bacterial strains. The skin mucus from five selected Cyprinids showed strong bactericidal activity against all the pathogenic bacteria. Importantly, acidic skin mucus of all the species showed higher bactericidal activity than aqueous and organic extracts and antibiotic. The acidic extract of skin mucus from *L.rohita, G.catla* and *C.idella* exhibited higher ZOI against *A.hydrophila* (44±1; 44±1; 42.3±2.51 mm respectively), *S.aureus* (45.33±1.15; 40.33±1; 40.6±1.52 mm respectively) and *P. aeruginosa* (44±1; 40.6±0.57; 44±1mm respectively). Whereas the acidic extracts of *C. mirigala* and *H.molitrix* show least ZOI against *A.hydrophila* (29±2;35±1 mm respectively), *S.aureus* (31.6±1.52;32.66±0.577 mm respectively) and *P.aeruginosa* (39.6±1.52; 33.66±0.577 mm respectively). The biochemical characterization of acidic mucus extract showed the proteins as a major component in *L. rohita, C. idella* and *G. catla* (303.6±1.52, 250±1.53, 240±1.53 µg/ml respectively) followed by carbohydrate (100±1.52, 80±1.32, 67±1 µg/ml respectively) and then lipid content (4.07±0.05, 3.1±1.52, 2.52±1 g/ml respectively). Our results show that the skin mucus from Cyprinid species shows bactericidal effect, which may play a vital role in fish protection against bacterial pathogens. Generally, the study may hint for using skin mucus of fish as an alternate to antibiotics in animals and probably in human beings protection.

Bioterrorism of Today's World

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Today`s world is affecting by different tiny creatures that are viruses, bacteria protozoon and fungal spores and their chemical compartments. Which are used as bioweapons to destroy the weakest states. For different benefits like to destroy the economic level and health, mostly to destroy the humanity. So I have written an article on bioterrorism that has been used in a way that can make its possessors as the wondering state in the world. So for there is a huge void to protect our generations from the dangers of bioweapon, which steps we should take to protect our generation and upcoming ones. For this upcoming war which is of bioweapons we should take this steps to protect our beloved ones from this pandemic like (COVID19) (Asian flu) (anthrax) cholera, bubonic plague, are some of the most brutal killer in human history. And outbreaks of these pandemic diseases across international borders, are properly define as pandemic. Others bioweapon on which still researchers doing works to uses as powerful weapon in world, still they are in process to bring genetic mutation in it. Like an example of COVID-19 which is highly mutated structurally and genetically this include 6 mutations in the spike protein of which the virus uses to entry in to the host. Like these pandemic in which we loss million lives it's only due to bioterrorism act. How and when we will protect from this bio warfare.

Prevalence of Positive Blood Cultures and MDR in Patient Presented at Tertiary Care Centers.

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Multi Drug Resistance (MDR) is generated due to usage of massive amount of antibiotics for human therapy, resulted in the selection of pathogenic bacteria resistant to multiple drugs. Antimicrobial resistance is now a major challenge for clinicians to treat patients. Infections caused by MDR increase the mortality rate globally. In this study, positive blood cultures and their antibiotic resistance patterns has been observed. Data were collected from Children's Hospital Karachi and Shamsi's Ansari Diagnostic Laboratory. All samples were collected before starting empirical antimicrobial therapy. The isolates were identified by standard biochemical tests. Clinical & laboratory standard institute guidelines (CLSI) were followed for screening of antimicrobial resistance patterns. Total 1984 samples collected from January, 2020 to March, 2021. Statistics showed that in overall blood culture samples the positivity rate was 5.0% (50/984). The overall isolated cases of species showed 62% of Gram-negative while 38% of Gram positive bacteria were observed. Frequently identified Gram negative pathogenic species were Salmonella typhi and Escherichia coli and Gram positive pathogenic species were Enterococcus and Staphylococcus. They showed resistivity against most of the screened antibiotics. The most sensitive drugs for Gram-positive isolates were vancomycin, Gentamycin, linezolid, Quinolones and Tetracycline and for Gram-negative were aminoglycosides, and carbapenems. These identified MDR are reported to cause septicemia followed by death. Current study is a small piece of a big puzzle and need to be extended on broader level, but

findings of present study may enlighten the possible answers for future queries about treatment measures against MDR.

Medicinal Plant Extracts as A Potential Drug for The Treatment of COVID-19

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Global health is threatened by an ongoing outbreak of COVID-19, a life-threatening respiratory disease caused by the novel coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus). Treatment of COVID-19 is predominantly necessary, and the role of antiviral agents is yet to be established and people are dying at a very high rate each day. Natural products have been in constant use since ancient times and are proven by time to be effective. Medicinal plants extract have strong potential against many infections and are frequently used for the treatment of many severe diseases. Crude extracts of many medicinal plants have shown significant inhibitory effects against coronavirus. Medicinal plants have numerous compounds that could be used against COVID-19 as a potential drug. Moreover, in the future, these medicinal plant extracts could be used for potential drug discovery.

Monotheca Buxifulia Is Alternate Option for Sedative Drugs

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The medicinal plants possess wide range of pharmacological and biological activities, act as an alternate source of drugs to cure life threaten diseases. *Monotheca buxifolia* is an evergreen flowering tree of genus *monotheca* which belongs to family *Sapotaceae. M. buxifolia* specie is native in the hilly areas of Northern Pakistan, Afghanistan Oman, and in the south-east Saudi Arabia. In Pakistan it is mostly found in lower dir, Zhob, Gorakh Hills, Loralai, Kohat, Drosh Chitral etc. It is mainly used for fuel, railing around farms and fields, leave are also used as a fodder for animal mostly for cattle to increase production of milk, wood lumber, and roof and building shelters. The plant also yield small fruits locally called Gurgura (berry). This specie is widely studied for anti-inflammatory, antipyretic, anthelmintic antimicrobial, antioxidant, CNS depressant and ant nociceptive activities in various in vitro and in vivo experimental models. *M. buxifolia* contain high amount of phenolic and flavonoid content which is used as antipyretic agent, laxative, hematinic, purgative, vermicidal agent, vermifuge, and for the management gastro-urinary tract ailments. The leaves of *Monotheca buxifolia* contain more than 160 compounds. Flavonoids, anthraquinones (C_{14} H_8 $O_{2)}$, terpenoids (C_5 H_8), cardiac glycosides, saponins, reducing sugars, tannic acid and poly-phenolic compounds were found this plant. The leaves of *M. buxifolia* possess antibacterial and antifungal activities. *M. buxifolia* was used as a natural antioxidant agent due to the presence of high amount of phenolic and flavonoids. The current research was aimed to explore the *in vivo* acute toxicity and sedative activity of *Monotheca buxifolia* in experimental animals. *M. buxifolia* leaves methanolic extract was prepared using standard method with some modification. The leaves of *M. buxifulia* leaves were shade dried in dark at room temperature for at least 2-3 weeks. After drying the plant leaves were grinded and chopped for converting it into powder

form.Fifty grams (50gm) of powder material was soaked in flask containing 500 ml of solvent (methanol). Then, the flask was covered by a cotton and aluminum foil and shaked well until the plant powder mixed with the solvent. The extract was filtered using whatman filter paper no.1. Experimental animals (Rabbits) of uniform weight (1000-1500 g) were used. For acute toxicity two different protocols subcutaneous and orally administration both were used to determine *in vivo* acute toxicity of the plant extract. Rabbits were divided into 5 groups (each group consist of 3 rabbits). First group of rabbits were treated by distilled water as a control. Others Groups R2, R3, R4 and R5 were treated with 250, 300, 350 and 400 mg/kg doses respectively. After treatment, all groups were kept under continuous observation for 24 hours to find gross effect and mortality was observed in each group. Blood were taken from all animals to processed complete blood hematological examination). For sedative activity Rabbits were divided into 6 groups. Group 1 was treated by distilled water and considered as negative control while group 2 was treated by diazepam (0.3 mg/kg) as positive control (figure). R3 rabbits were treated by extract dose of 50 mg/g while doses of 100, 150 and 200 mg/kg were injected intra muscular to other groups respectively. Rabbits were observed for one hour continuously and after that pedal reflex, palpebral reflex and right reflexes were used as a standard. *M. buxifolia* was found safe on orally administration at all tested doses while subcutaneous administration caused acute toxicity at dose dependent manner, only 250 mg/kg dose group was survived while other high doses were found toxic. The *M. buxifolia* methanolic extract also showed sedative effect at dose depended manner. The subcutaneous administration can directly effect on hematology of experimental animals. *M. buxifula* exhibited significant sedative effect on dose dependent manner dose of 100, 150 and 200 mg/kg (intramuscularly injected) showed significant sedative effect as compared to positive and negative control. The findings of this study concluded that *M. Buxifolia* is good source for pharmacologically active compounds and might be useful in hypnotic medicine. It also concluded that *M. Buxifolia* methanolic leaves extract possessed significant *sedative* activity and it is safe in oral administration while it was toxic by using subcutaneous administration within the experimental tested doses and increased the ALT value and sub normally reduces temperature of the subject animals, a significant decrease in WBC and Platelets was also observed. it also concludes that this plant maybe use as alternate source of sedative drugs which can be helpful in the treatment of anosmia anxiety and depression.

Study of The Growing Incidence of Resistance to Antibiotics in Pathogenic Bacteria Associated with Patients of General Surgery

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Surgery or surgical procedures are medical practices that are used to treat or heal injuries, wounds, pathological conditions of patients that are normally not cured by simple procedures. It also includes other conditions of patients like cutting or suturing of tissues, insertion of implants or tissue grafting etc. Healthcare−associated infections(HAIs) are seen as a global public health threat. It has strong correlation with overall morbidity, mortality and has substantially increased burden on healthcare systems. Pakistan one of the developing country, is also facing the same challenges and there are numerous reports highlighting drug resistance. Scared data is available on SSIs from the Pakistan and few pilot studies have documented resistance issues being associated with SSIs. Expansion of surveillance, prevention of infection, and antimicrobial stewardship programs are some the steps that can be consider preventing or minimize the spread of resistant strains in surgical patients. In the present study, a total of one hundred clinical wound samples were obtained from DHQ hospital Kohat. *Pseudomonas*

aeurogenosa (35%) followed by positive S. aures (40%), Ecoli (15%), Bacillus alvei (5%), Proteus spp (5%), were found to be the predominant agent's isolates from the wound sample. All the isolates were identified based on their biochemical tests. Antibiotic sensitivity of all bacterial isolates were tested against (Vancomycin, Ampicillin, Oxacillin, Imipenem, Cefatazidime, Chloromphenicol, Kanamycin, Erythromycin, Clindamycin, Colistine sulphide, Levofloxacine, Cefixime) antibiotic discs. The most effective antibiotic against all the strains is Levofloxacine because it shows the highest zone for all of them. The least effective antibiotic for *E. coli* and *S. areus* is Colistine sulphide because it shows the lowest zone while for *Pseudomonas* the least effective antibiotic is chloramphenicol. VA, OX, E, and AMP are not effective against any of the strain because the show no zone.

Biological Activities of Crude Extract and Its Derived Fractions Obtained from *Cenchrus Biflorus* L.

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Cenchrus biflorus is a common medicinal plant widely used against numerous infectious diseases. The agrochemical potential of methanolic extract, n-hexane, chloroform, ethyl acetate, aqueous and n-butanol fractions were assessed to measure the antibacterial, antifungal, insecticidal and antiplasmodial activities of the plant. The crude, chloroform, and n-butanol soluble fractions showed excellent activities against Escherichia coli, Styphylococcus aureus, Salmonella typhi, and Klebsiella pneumonia and Pseudomonas aeruginosa but have no activity against Staphylococcus aureus. Similarly, the crude, n-hexane, and chloroform fractions were also found to have significant activity against fungal strains including Fusarium oxyporium, Aspergillus nigrus and Aspergillus flavus and Alterneria alternate have no activity against Aspergillus niger. Chemical pesticides have shown very good results at the beginning, but with the passage of time the need was realized to use the natural plant sources for the safe control of insects. Similarly, *Cenchrus biflorus* have also positive effect against plasmodium growth. The current study will provide minor contribution towards it. High mortality rate was recorded for the crude extract and chloroform fraction against ants. From our experiment it is informed that *Cenchrus biflorus* may be used to treat bacterial and fungal diseases and also as insect repellant and it is also possible to isolate antibacterial, antifungal, insecticidal and antiplasmodial drug from this plant.

Contamination in Drinking Water Sources of Lahore, Pakistan-A Review

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Water pollution is one of the serious threats to human health, particularly in developing countries. The main objective of this article is to highlight microbial and heavy metal contamination of drinking water in Lahore, its main sources and impacts on human health. Overall, Pakistan ranks at 80 among 122 nations regarding drinking water quality. The reason is that drinking water quality is poorly managed and monitored. The Lahore City for its drinking water supply depends upon groundwater sources and a number of challenges are faced by public due to

mismanagement of resources and an exploding population. Water sources including both surface and groundwater are contaminated with fecal coliforms and heavy metals. Human activities like improper disposal of municipal and industrial wastes are responsible for deteriorating water quality. Cross-contamination between sewer lines and municipal water supply system, due to old and leaking pipes and lack of water filtration and disinfection facilities also contribute to it. These toxic compounds get into the food chain through water, air and soil, and contribute towards many public health problems. The data reported in the review article is extracted from various studies, previously published in research journals and statistical reports released by the government and non-governmental organizations.

Optimization of Streptococcus uberis Vaccine Against Mastitis

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Mastitis decreases the productivity and quality of milk. Mastitis results in significant economic losses either due to low production or early culling of infected animals. There is no effective vaccine against S. uberis mastitis in dairy cows. The present study was conducted to optimize the vaccine against Streptococcus uberis. Formalin (0.4% v/v) inactivated S. uberis vaccines were prepared in three different concentrations i.e., 108, 1010 and 1012 CFU/mL and injected into three groups of adult rabbits (A, B, C) with a booster dose at day 14 and group D as control (Non-vaccinated). Serum samples were collected on day 21 and immune response against each vaccine was checked by Abbexa Rabbit Anti-Streptococcus uberis IgG ELISA kit. Maximum immune response was observed against 1012CFU/mL concentration in group C. After dose adjustment, two types of vaccines were prepared with 1012CFU/mL; oil-based and gel-based. To evaluate the antibody titer both of these vaccine preparations were injected into four groups of healthy adult rabbits. It was recorded that the oil-based vaccine (1012CFU/mL) produced higher titer than alum-based vaccine against S. uberis in rabbits. It was concluded that oil based S. uberis vaccine (1012CFU/mL) may be used to combat S. uberis mastitis in bovines.

Diabetic Epedimiology in Covid-19

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Diabetes has been recognized as a significant risk factor in the progression and prognosis of severe acute respiratory syndrome in patients with corona virus disease. The main aim of study was to determine the contribution of diabetes and related comorbidities to prognosis and progression of covid-19. This study also evaluates the biochemical examination of patients with diabetes which will be helpful in controlling mortality rate in covid-19. In this study, 100 diabetics and 100 non-diabetic patients were recruited after informed consent. These enrolled patients are all diagnosed with COVID-19. Common sign and symptoms, inflammation related biomarkers such as CRP, LDH, IL6, ESR and coagulation parameters such as D-dimer and fibrinogen were

estimated. Furthermore, the absolute count of neutrophils, lymphocytes and RBCs were observed. On the other hand, chest computed tomography was also observed. The correlation of all these parameters were also analyzed in between these two groups of COVID-19 patients. In this study, we found that the COVID-19 patients with diabetes were at higher risk of severe acute respiratory syndrome. The tissue injury related enzymes and the serum level of inflammatory biomarkers such as CRP, IL6 and D-dimer is significantly higher in diabetic patients as compared to non-diabetic group. On the other hand, the absolute count of neutrophils, lymphocytes and RBC's is lower in diabetic patients associated with dysregulaion of glucose metabolism. Furthermore, the chest CT imaging of diabetic group show more pathological changes as compared to non-diabetic group. This study proposed the mechanism associated with increased risk of negative COVID-19 outcomes in patients with diabetes. It is observed that there is a significant correlation of inflammatory biomarkers, tissue injury related enzymes, coagulation parameters and immune cells in corona virus infected patients of both diabetic and non-diabetic group. It is concluded from the study that diabetes has strong negative impact on COVID-19 patients as it weakens the immune system which is a major risk factor of prognosis and progression of corona virus disease and increases the mortality rate by rapid deterioration. It is also advised that more intensive attention should be taken in case of rapid deterioration. However, to definitely conclude this, further extensive studies would be required.

Phyto-Stimulatory Impact of *Bacillus* and *Serratia* Spp. On *Zea Mays* Using Hydroponic Technique

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In the current era of pollution and environmental hazards, growing crops need excessive use of land and greater amount of chemical fertilizers, hydroponic cultivation technique can be strategically used for production of crops in less area to give better yields in large quantity within a short period of time and also in all seasons with the use of plant growth promoting rhizobacteria (PGPRs) as biofertilizers instead of chemical fertilizers which are non-environmental friendly and pose health problems. Thus the current study was aimed to evaluate the effect of five PGPRs i.e., *Bacillus tropicus* (S12), *Brevundimonas diminuta* (S5a), *Bacillus cereus* (So3II), *Serratia marcescense* (S4c1), *Bacillus subtilis* (Mt3b) on physiological and biochemical parameters of treated *Zea mays* L. Two experiments were conducted under soil and hydroponic conditions. Results have revealed that bacterial inoculum significantly increased several plant characteristics (root length, shoot length, fresh weight) and biochemical parameters (chlorophyll content and protein content) in both soil and hydroponic conditions. Among all bacterial strains, *Bacillus cereus* (Sa3II) remarkably increased growth parameters of plants grown in soil experiment and biochemical parameters of plants grown in hydroponic experiment. *Bacillus subtilis* (Mt3b) responded well under hydroponic conditions and increased plant growth parameters and give best results for increase in total chlorophyll content of plants grown in soil condition. So, these strains could be used as biofertilizers to increase growth of *Zea mays* L. in both soil and hydroponic conditions.

Antimicrobial Resistance in Developing Countries Like Pakistan-A Critical Review

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The Antimicrobial resistance refers to resistance against drugs which are routinely used for treatment against diseases. The diseases may be bacterial, viral, parasitic or fungal in nature. Antibiotic resistance is a term used for diseases of bacterial origin. The bacteria isolated from glacial waters around two hundred years ago were having resistance against ampicillin. Therefore antimicrobial resistance was prevalent even before the discovery of antimicrobials. It is also true that AMR is increasing at an alarming rate which might be reason for this problem to be of more concern in recent times. It is now understandable that resistance against drugs is a consequence of misuse of antibiotics in humans, veterinary and agricultural industry. Misuse means using antibiotic without need, or without proper prescription, self-treatment, and dosage less than required, not at proper time or interrupted. Under one health perspective, AMR is considered as a biggest challenge which should be addressed at the earliest to avoid transfer of resistance from animals and environment to humans. The ban on the use of antimicrobial growth promoters in animals in developed countries is also a consequence of AMR spreading at an alarming rate. Trade and travel are also the reasons for organisms to spread at a rapid pace than ever before. In developing countries like Pakistan AMR is being monitored from the data generated at referral hospitals in big cities only. Resistance issue in these countries is more serious in case of Gram negative bacteria and also tuberculosis. Overall there is a higher burden of infectious diseases in poor economies than stronger rich world. There is little or no access to newer antibiotics, which might be crucial and life-saving in treating severe infections due to resistant microbes. Diagnosis of diseases needs to be strengthened to avoid overuse and misuse of antimicrobial agents. To address this threat to public health, the World Health Organization in 2015 had launched a Global Action Plan in the World Health Assembly to control AMR. In May 2017, Pakistan's National Action Plan to control AMR was released by Ministry of National Health Services. Antimicrobial Stewardship programs (ASPs) were launched in Health care systems to control Resistance problem. It was also decided that in the long term perspective poverty alleviation, improved and hygienic living conditions, clean water supply and easy access to Quality Health facilities including treatment and vaccination should be targeted which may play a vital role in preventing AMR. The comprehensive strengthening of Health care setup is essential to successfully implement the National Action Plan, controlling inappropriate antimicrobial usage and higher AMR rate in Pakistan. The clinical approach to address AMR includes infection control and their specific prevention protocol and antimicrobial management plans.

Prevalence of Multidrug Resistant *E. coli* isolated from Mastitic Milk samples of District Malakand and Dir Lower

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The antimicrobial resistance is rising in Pakistan at very high rate. The prevalence of extended spectrum beta lactamase (ESBL) E. coli is also increasing at high rate and is challenge to global health concerns. The current research is carried out to find out the multi drugs resistant (MDR) E. coli isolated from mastitic milk samples in district Malakand and Dir lower. We collected 100 milk samples from dairy cows and buffaloes infected with mastitis. The samples were streaked on Macconkey agar and then sub cultured on Eosin methylene blue (EMB) media. After confirmation of E. coli through biochemical tests and PCR the isolated colony were then cultured on muller Hinton agar to test against different antibiotics and to confirm MDR E. coli. These E. coli strains were tested against 10 to 15 different antibiotics including ciprofloxacin, ampicillin, cefotaxime, tetracycline, norfloxacin, chloramphenicol, meropenem etc. The isolates showed high resistance to cefotaxime (100%), cefaclor (92%) and to ampicillin (88%). High susceptibility rate shown to meropenem (100%). PCR was carried out to study the genome. Overall, 25 samples out of 100 were confirmed as ESBL producer E. coli. Three types of ESBL producing genes were found i.e CTX-M, TEM and SHV. Nucleotide analysis showed that CTX-M gene of ESBL was found maximum having frequency of 12/25 (48%), followed by TEM having frequency 8/25 (32%) and SHV having frequency 5/25 (20%). It is the very first study on mastitis in district Malakand and Dir lower and we concluded from our this study that CTX-M is the most frequent gene encoding ESBL enzyme followed by TEM and SHV. So the prevalence of these resistant genes in food producing animals is increasing at alarming rate which is a serious issue to our health. There is dire need of effective measures to control this serous issue.

Enhanced Solubilization and Purification of 3ABC Non-structural Protein of Foot-and mouth Disease Virus from Bacterial Inclusion Bodies

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Nonstructural 3abc protein of foot and mouth disease virus (fmdv) is used to differentiate vaccinated from naturally infected animals. It is a polyprotein which is cleaved into membrane associated 3a protein, three copies of 3b and 3cpro mediated by virally encoded 3c protease. The expression of this protein in e. Coli results into the formation of inclusion bodies which require solubilization in high concentration of chaotropes and extensive refolding process prior to purification of the native protein. Protein aggregation during refolding leads to the poor recovery of protein in functional form. Alternatively, mild solubilization methods have been proposed to recover the native and soluble protein from inclusion bodies present in e. Coli. In this study, 3abc protein was expressed predominantly as inclusion bodies using e. Coli host and solubilized in mild non-ionic detergent followed by purification through ni-nta chromatography. The protein recovery using this solubilization method, showed higher yield as compared previously described solubilization methods for 3abc protein. This method also favored higher stability of the 3abc recombinant protein stored at different temperatures. The reactivity of the proteins was

analyzed by western blotting and elisa which showed their ability to use them as antigen for the development of immunoassays. In conclusion, this study demonstrates an efficient and high yielding purification method of protein without refolding process than previously described methods involving renaturation steps. Keywords: fmdv, nsp, 3abc, inclusion bodies, non-ionic detergent, stability

Impact of Varying Temperature On the Plant Growth Promotional Potential of PGPR

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Plant growth promoting rhizospheric bacteria (PGPR) play important role in improving plant growth. These bacteria follow various strategies and mechanisms for enhancement of plant growth. The current study involves the isolation of psychrophiles from the rhizosphere of plant samples collected from Gilgit Baltistan region. These bacterial isolates were checked for their plant growth promotional activities through biological assay. *Triticum aestivum* seeds were inoculated with these isolates and the effect of these isolates were checked on the growth of *T. aestivum* using the temperature of 13^{o}C and 20^{o}C. Auxin production potential of the selected isolates was also checked. These isolates were further characterized morphologically and physiologically. The bacterial isolates were grown at different temperatures to check their growth response. Only plant growth promoting bacteria were screened for further study where the isolates were used for plant growth interaction studies to study their impact on the growth of plants at varying temperatures

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Ihtisham ul Haq, Muhsin Jamal

The antimicrobial resistance is rising in Pakistan at very high rate. The prevalence of extended spectrum beta lactamase (ESBL) *E. coli* is also increasing at high rate and is challenge to global health concerns. The current research is carried out to find out the multi drugs resistant (MDR) *E. coli* isolated from mastitic milk samples in district Malakand and Dir lower. We collected 100 milk samples from dairy cows and buffaloes infected with mastitis. The samples were streaked on Macconkey agar and then sub cultured on Eosin methylene blue (EMB) media. After confirmation of *E. coli* through biochemical tests and PCR the isolated colony was then cultured on muller Hinton agar to test against different antibiotics and to confirm MDR *E. coli*. These *E. coli* strains were tested against 10 to 15 different antibiotics including ciprofloxacin, ampicillin, cefotaxime, tetracycline, norfloxacin, chloramphenicol, meropenem etc. The isolates showed high resistance to cefotaxime (100%), cefaclor (92%) and to ampicillin (88%). High susceptibility rate shown to meropenem (100%). PCR was carried out to study the genome. Overall, 25 samples out of 100 were confirmed as ESBL producer *E. coli*. Three types of ESBL producing genes were found i.e CTX-M, TEM and SHV. Nucleotide analysis showed that CTX-M gene of ESBL was found maximum having frequency of 12/25 (48%), followed by TEM having frequency 8/25 (32%) and SHV having frequency 5/25 (20%). It is the very first study on mastitis in district Malakand and Dir lower and we concluded from this study that CTX-M is the most frequent gene encoding ESBL enzyme followed by

TEM and SHV. So the prevalence of these resistant genes in food producing animals is increasing at alarming rate which is a serious issue to our health. There is dire need of effective measures to control this serous issue.

Isolation and Enumeration of Members of Family Enterobacteriaceae from Pakistani Currency Notes.

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The currency notes are originated from banks run by states and circulated to public in routine purchasing**.** These notes may act as an environmental vehicle for transmission of pathogenic microbes and could cause major health hazards**.** Major cause of transmission of microbes through currencies is mainly due to lack of hygienic conditions and low environmental sanitation levels. So, the objective of this study was to enumerate the load of enterobacteriaceae organisms from Pakistani currency notes. About 25 currency notes of Pakistani 10 rupee were randomly collected from different places in Karachi, Pakistan. They were analyzed using pour plate method on VRBG agar. The study showed that the currency notes were heavily contaminated with enteric organisms. However, some of the samples showed no growth. The growth was ranging from 10^3-10^4 cfu/note. The colonies were further sub-cultured on MacConkey agar to determine the ratio of lactose fermenter with non-lactose fermenters. Most of the organisms were lactose fermenters and some of them were non-lactose fermenters. So, it was concluded that currency notes might act as a source in transmitting harmful microorganisms causing different diseases particularly food-borne illness or water-borne illness. To control the transmission of such disease-causing microbes we should take care of proper handling habits and should maintain hygienic conditions.

Mosquitocidal Activity of Indigenous Bacillus Strains Isolated from The Fields of District Kohat

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Dengue viruses and malarial protozoa are of increasing global concerns in public health. More than 80% of the global population is at risk of mosquito-borne diseases. Chemical insecticides are highly effective and convenient to use, but they are a potential threat for the environment and all kinds of life on earth. Therefore, the use of biological control agents for the control of insects such as mosquitoes is a safer and sustainable strategy. Bacillus-based biological control agents play a fundamental role in the field of bioinsecticides. Many Bacillus species like B. wiedmannii, B. thuringiensis, B. subtilisare effective against a broad range of insects. Total 30 samples were collected from the local fields of District Kohat. After morphological and biochemical characterization, three bacteria were identified i.e. B. wiedmannii strain FSL W8-0169, B. wiedmannii strain TD10 and B. subtilis. These isolates were further used for bioassay experiments. Mosquitoes and their larvae were collected from crops and water using different traps like Netting, Pipetting, aspirators, light traps, etc. Bioassay experiment was done to check mosquitocidal activities of the above-mentioned isolates. In supernatant, the mosquitocidal activity of B. wiedmannii strain FSL W8-0169 was observed to be the highest (72%) followed by B. subtilis (70%). B. wiedmanniistrain TD10showed the lowest mosquitocidal activity (67%), whereas in broth, B. subtilis showed

highest activity (69%) against mosquitos followed by B. wiedmanniistrain FSL W8-0169 (52%0. B. wiedmanniistrain TD10 showed the lowest activity (45%). From this study, it is concluded that the above-mentioned strains can be used as an alternate of chemical insecticide against mosquitos to control different mosquitos borne diseases.

Virulence Typing of Selected Bacterial Pathogens Associated with Neonatal Sepsis in Pakistan

Zainab Zahoor, Zia-Ur-Rehman Farooqi, Sundus Javed and Amna Mumtaz

Neonatal sepsis has high incidence with significant mortality as well as morbidity rate in Pakistan. In this study different bacterial pathogens causing neonatal sepsis in a tertiary care setup in KPK were included. It was found that *S. aureus* has a strong association with neonatal sepsis (frequency of isolation=43%). In addition to *S. aureus*, *C. freundii* (frequency of isolation=21%), *P. aeruginosa* (frequency of isolation=13%), *E. coli* (frequency of isolation=15%) and *S. enterica* (frequency of isolation=8%) were also isolated from blood and pus samples. Among the patients of neonatal sepsis, 61 (67.8%) were male and 29 (32.2%) were female patients. Molecular analysis revealed that majority of the *S. aureus* isolated from clinical samples of neonatal sepsis possess genes for the phantom valentine leucocidin toxin (as 76.9% isolates showed presence of Luk-PV gene). Similarly, most of the isolates were resistant to methicillin (as 74.4% isolates showed presence of MecA gene). Antibiotic resistance among bacteria causing sepsis is increasing at an alarming rate. Majority of *S. aureus* isolated were resistant to ciprofloxacin (71.7%) and penicillin (71.7%). *C. freundii* were mostly resistant to tigecycline (42%), cefixime (31.5%) and Fosfomycin (31.5%). *P. aeruginosa* was resistant to cefixime (61.5%) and ciprofloxacin (61.5%). While S. enterica isolates were sensitive to the all the antibiotics being tested. Hence it was concluded that S. aureus is the most common bacterium causing neonatal sepsis and antibiotic resistance has been found among the bacterial isolates causing neonatal sepsis.

Cross Sectional Study of Heptatis B and Heptatis C in District Baltistan

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A cross sectionl analytical study was conducted to detect the prevalence of Hepatitis B and Hepatitis C in district Baltistan, Gilgit Baltistan Pakistan. Association of different socio-demographic and its risk factors was also analyzed in this study. For this purpose, total (n=320) blood samples were collected equally from each tehsil of the district. All the information regarding the risk factors was gathered with the help of a questionnaire. The blood samples were tested initially with t Immunochromatographic test followed by confirmation with the help of ELISA kit. The result was showed that overall prevalence of HBV was 3.20% and HCV was 5.25% respectively. Higher prevalence of HBsAg was found in sub division Roundu (4.21%) and tehsil gamba (3.05%). While HCV

was more detected in tehsil gultari (5.24%). On the basis of age HBV was found in age group 20 -35 ears (3.20%) while for HCV 50-60 years (6.07%). HCV was found higher in females (7.32) and HBV was in male (2.25%). High level of these infections is an alarming situation and public awareness should be necessary among population regarding the transmission of these severe diseases.

Distribution of Biosurfactant Poducing Bacteria Near Sea Shore of Karachi, Sindh

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Biosurfactants are active compounds that are produced at the microbial cell surface or excreted, and reduce surface and interfacial tension. Microbial surfactants offer several benefits over synthetic ones, such as low toxicity and high biodegradability, and stay active at extreme pH and salinity. Biosurfactants are produced by bacteria, yeasts, and filamentous fungi. However, there are just a few publications that mention the presence of biosurfactant-producing microorganisms which have the potential to replace virtually any synthetic surfactant while also introducing some unique physio-chemical properties. Current study based on isolation of bacteria from sediments of sea shores of Karachi, Sindh. Isolation carried out on Zobell's media results in 84 bacterial isolates. All the 84 isolates tested for initial primary and secondary screening tests. Around seven different methods were used to check the biosurfactants including potentiality of the bacterial isolates are foaming activity, drop collapsing assay, Oil spreading test, Emulsification index test (E24), Hemolytic activity, Blue agar plate & CTAB agar plate method. Results showed variation in behavior and response to every test however 10 isolates showed positive results for all the methods of screening. Biosurfactants produced by bacteria, yeast, and fungi, are promising molecules for a wide variety of applications, including food additives, cosmetics, detergent formulations, agriculture, and wastewater treatment in combination with enzymes. Now it is right time to really consider ecofriendly biosurfactants.

Contribution of Microbiology in Nursing Theory and Practice

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The accomplishment of a basic microbiology course is a solid indicator of how students will perform in further nursing courses and clinical area. The students should give special attention to a microbiology course in nursing. A good nurse must have a sound knowledge of basic microbiology to integrate this knowledge in clinical practice. Nurses who are working in the hospital settings mostly use their knowledge for infection control, hospital-acquired infection, disease transmission and control of microorganism. Those who are mostly working in the community settings utilize microbiology knowledge in the collection and handling of specimens, understanding the medically important microorganisms, and combating infection in the immune- compromised host. This review synthesized all the studies of last 10 years focusing on Contribution of Microbiology in nursing Education. It is significant to discuss and highlight the main contribution of microbiology in the field of nursing practice. The findings in this literature review contributes to the understanding that microbiology in nursing has a beneficial effect on students' learning experience by enhancing their learning motivation and learning capacity. These

results also suggest that the academic nurse is the ideal educator to bridge the gap between the biosciences and nursing practice.Nurses must have sufficient education and training in microbiology to perform many roles within clinical nursing practice e.g. administering antibiotics, collecting specimens, preparing specimens for transport and delivery, educating patients and families, communicating results to the healthcare team, and developing care plans based on results of microbiology studies and patient immunological The high relevance assigned to infection control, hospital-acquired infections, and disease transmission reflects the importance of these topics in patient care.

Biocontrol of Disease Caused by *Meloidogyne incognita* In Okra Plant Using Plant Growth Promoting *Bacillus Spp*

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Okra (Abelmoschus esculentus) locally known as bhindi is a member of family Malvaceae. It is rich source of vitamins, minerals, proteins, and carbohydrates. The okra crop is grown on 15500 hectares area with total production of 117900 tons. Its yield decreases due to diseases caused by root-knot nematodes. Meloidogyne Incognita is the most destructive one which cause huge economic losses to okra production. Nematicides are generally used to control the nematodes however they have several adverse effects including toxicity, environmental contamination, and hazard. The PGPR have shown considerable potential as biological control agents. Bacillus species reduced population densities of the soybean cyst nematode in the greenhouse, micro plot, and field studies. Therefore, the present study was designed to determine the plant growth promoting traits and nematocidal potential of bacteria isolated from the fields of district Kohat for the management of root-knot nematodes (Meloidogyne species) in okra plant. Roots of infected okra plant and rhizospheric soil were collected from different locations in the District Kohat Khyber Pakhtunkhwa, Pakistan. The root-knot nematodes were isolated by using the Baermann funnel, and the root incubation methods and were identified based on their phenotypes. The bacteria were isolated from the rhizospheric soil by using serial dilution plating on LuriaBertani (LB) agar plates. Bacillus subtilis were identified based on morphological and biochemical characteristics. Plant growth promoting traits of B. subtitles were also investigated Finally, the strains, their cell pellet and their metabolites were applied to the juvenile and eggs of Meloidogyne incognita. It was observed that B. subtilis has biocontrol potential against Meloidogyne incognita. The juvenile survival was 98% without exposure to B. subtilis and decreased to 70% when exposed to B. subtilis broth culture after 24 hours. Moreover, cell pellet and metabolites of B. subtitles showed 55% and 65% mortality rate against the juvenile of M. incognita respectively after 24 hours. Furthermore, the B. subtilis broth culture, their cell pellet and their metabolites showed 50%, 30% and 45% activity against the eggs hatching of M. incognita. The B. subtilis also showed plant growth promoting traits by zinc and phosphate solubilization, ammonia and indole acetic acid production. From this study, it may be concluded that B. subtilis showed nematocidal activity against Meloidogyne incognita and produced nematocidal metabolites. It also promotes plants growth by zinc and phosphate solubilization, ammonia and indole acetic acid production.

Biosynthesis and Biocatalytic Activity of Zinc Oxide Nanoparticles Using *Zingiber Officinale* Legume Against Multi-Drug Resistant Bacterial Isolates

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Antibiotic resistance by microorganisms is a global issue that needs proper attention to be resolved as most of the deaths occurred due to infections caused by multi-drug resistant (MDR) bacteria. Different pathogenic organisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia, shigella spp., are responsible for bacterial infections in human being as well as animals. Nanoparticles exhibit novel properties due to variations in specific characteristics such as size, distribution and morphology of the particles. Zinc oxide nanoparticles are interesting because it exhibits high catalytic efficiency, strong adsorption and are used frequently as antimicrobial agent. Green synthesis using plant extracts are commonly preferred for the synthesis of ZnO-NPs due to the environmental friendly nature, cost effectiveness, and safe nature for human therapeutic use. The plant ginger is the most frequently consumed dietary item throughout the world. It has a wide variety of pharmacological properties such as anti-inflammatory, antioxidant, analgesic, hepato-protective etc. The present research study will be focused on to synthesize ZnO-NPs from the legume extract of Zingiber officinale and then determine its antibacterial potential to enhance the activity of commercially available antibiotics against MDR bacterial species. To fabricate and characterize ZnO-NPs using Zingiber officinal legumes. To determine the antibacterial activity of zinc oxide nanoparticles against MDR bacterial isolates. To evaluate of nanoparticles suffused antibiotic disc for enhancing antibacterial activity. Antibiotic sensitivity of all bacterial isolates were tested against (Ceftazidime, Clarithromycin, Cephradime, Metromidazol, Oxacilin) antibiotic discs. The result showed that the bacterial strains Shigella, Enterobacter, Pseudomonas aeruginosa, E. coli and Klebsiella were showed completely resistance against Oxacilin (100%), Metromidazol (100%), Cephradime (100%). The result showed that all tested bacterial strains were susceptible to ginger aqueous, n-hexane, and methanol extracts. Shigella, Enterobacter and *S. aureus* are more susceptible as compared to other bacterial strains against aqueous and methanol ginger extracts. Shigella spp., (27mm, 27.8mm), Enterobacter spp., (16.3mm, 21.3mm), *S. aureus* (23.6mm, 26.6mm) the zone of inhibition was observed with ginger aqueous and methanol extracts. SEM images of ZnO observed loose morphological aggregates, irregular and cubical with variable sizes. The UV-Vis spectra revealed that ZnO NPs can be prepared using natural reducing agent present in Zingiber officinal legumes extract. The UV Vis-Spectroscopy study showed sharp peaks that confirmed the synthesis of ZnO-NPs and also their involvement with natural reducing agents present in the leaf extract. The EDX profile gave information about the chemical composition of the nanoparticles such as; Oxygen was (39%), Zinc was (49.89%), and Carbon was (30.79%). The data of the antibacterial assay exhibit that most of the selected bacterial strains were highly susceptible to the ZnO NPs. The bacterial strains show different inhibition zones against various concentrations of ZnO nanoparticles. Klebsiella pneumonia was found to be the most susceptible strains among all strains by showing the highest inhibition zone (12 mm) against 10 mg of ZnO nanoparticles, for 5 mg (10 mm), for 4 mg (9.8 mm), at 2 mg (8.9 mm) and for 1 mg is (7.9 mm) respectively.

Antimcrobial Activity of Different Plant Essential Oils Against MRSA

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Staphylococcus aureus is a common pathogen causing infections that range from minor skin lesions to life threatening conditions. Methicillin Resistant *S. aureus* (MRSA) have limited treatment options since these strains are resistant to antibiotics. Alternative treatments could be used for MRSA and one of them are use of essential oils. The aim of this study is to evaluate the antibacterial activity of different plant essential oils against MRSA isolates. In current study, extraction of plant essential oils of eucalyptus, clove, cinnamon, oregano, sandal, thyme, cumin, turmeric, tea tree and ginger were done by hydrodistillation method. Antibacterial activity of essential oils was evaluated against MRSA isolates by agar well diffusion assay and Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined by broth micro-dilution method. The zone of inhibitions was showed only by eucalyptus, clove, cumin and thyme oil. The MIC of eucalyptus, clove, cumin and thyme oil were 0.38, 0.38, 1.54 and 3.123µg/ml respectively for MRS-01, 0.28, 0.22, 1.60, 3.14µg/ml for MRS-02, 0.457, 0.43, 1.74, 3.15 µg/ml for MRS-03, 0.47, 0.54, 1.627, 3.17µg/ml for MRS-04, 0.53, 0.58, 1.384, 3.11µg/ml for MRS-05. MBC of eucalyptus, clove, cumin and thyme came out to be 0.188, 0.2001, 0.778 and 1.563µg/ml respectively for MRS-01, 0.181, 0.176, 0.828 and 1.586µg/ml for MRS-02, 0.198, 0.198, 0.883 and 1.606µg/ml for MRS-03, 0.206, 0.203, 0.918 and 1.723µg/ml respectively for MRS-04, 0.218, 0.205, 0.707 and 1.513µg/ml respectively for MRS-05. Plant essential oils could be an alternate to synthetic antibiotics and demand further research and scientific trials. Hence PEOs can be used as promising antibacterial agents in future.

Prevalance of Antibiotic Resistance in Uropathogenes Associated with Diabetic Patients in District Kohat

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Antibiotic resistance is the ability of bacteria to neutralize the effects of antibiotics. Resistance to antibiotics imparts strength to microbes to cope with harassing situations. Such pathogens are not easily controllable. These microbes can cause infection in Urinary tract infections (UTIs). UTIs may lead to other disorders such as kidneys, bladder, urethra, and prostate. These infections cause more than 7 million medical visits and about 100,000 hospitalizations each year globally. Diabetic patients have a higher incidence of UTI. The main pathogen responsible for cystitis and pyelonephritis is *Escherichia coli* followed by *Proteus mirabilis* and *Klebsiella pneumonia.* Few studies are being conducted on prevalence of uropathogens associated with diabetic patients in some regions of Pakistan. But there is scarce data available on the prevalence of UTI among diabetic patients in District Kohat. Our recent study will focus on the characterization of resistant uropathogenic among diabetic patients in the Kohat region. In this research 130 Urine samples was collected from type-2 diabetic patients having urinary tract infection at DHQ Hospital Kohat. All clinical samples were inoculated by standard pour plate technique on CLED Agar and MacConkey agar and incubated at 37°C for overnight. The colonies were classified by performing Gram staining and biochemical tests. The antimicrobial susceptibility tests were performed by

using Kirby-Bauer disc diffusion method. Genomic DNA was extracted using the Phenol-Chloroform method and was stored at 4°C. After antibioghram profiling resistant strains was subjected for Molecular detection of antibiotic- resistant genes like TEM, NDM, OXA and SHV. Out of 130 tested urine samples, 108 (85%) were with positive urine culture report while in 22 (16.67%) cases no significant growth of any microorganism was obtained, so overall frequency of microbiologically confirmed UTI was 83.33 %. Out of these 108 positive cases *E. coli* was seen in 91 (85%) samples, isolated *E. coli* showed sensitive to piperacillin plus tazobacatom and imipenem but 100 % resistance was observed to erythromycin and ampicillin. *Klrbsiella pneumonia* was seen in 12 (9.4 %) samples, isolated *Klrbsiella pneumonia* showed resistance to deoxycycline and erythromycin while sensitive to imipenem and ceftazidime. *Proteus mirabillis* was seen in 5 (3.9%) samples, isolated *Proteus mirabillis* showed resistance to ceftazidime and choloramphenicol and sensitive to fosfomycin and imipenem. *E. coli* was found to be the most predominant isolate, showing high drug resistance followed by *Klrbsiella pneumonia* and *Proteus mirabillis.*

Antibiofilm Activity and Inhibition of EPS Production of Multidrug Resistant *Acinetobacter baumannii* by Aluminium oxide nanoparticles

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Acinetobacter baumannii is a biofilm forming multidrug resistant (MDR) pathogen responsible for respiratory tract infections. In this study, aluminium oxide nanoparticles (Al_2O_3 NPs) were synthesized and characterized by TEM and EDX that revealed spherical shaped nanoparticles with diameter < 10 nm. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for Al_2O_3 NPs was ranged between 125-1000 µg/mL. Exposure to NPs caused cellular membrane disruption indicated by an increase of cellular leakage contents. Biofilm inhibition was 11.64-70.2 %, whereas attachment of bacteria to polystyrene surfaces was reduced to 48.8-51.9 % in the presence of NPs. Nanoparticles also reduced the extracellular polymeric substance production and biomass of established biofilms. Our data revealed the non-toxic nature of Al_2O_3 NPs upto the concentrations of 120 µg/mL in HeLa cell lines. These results demonstrate an effective and safer use of Al_2O_3 NPs against MDR *A. baumannii* by targeting biofilm formation, adhesion and EPS production.

Assesment of Moringa oleifera Extract as Handwash

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In recent times, the use of plants as a source of vital compounds to fighting microbial infection has a gained prominence. Moringa oleifera is a common plant known as drumstick for various medicinal properties and very useful tree in tropic countries. All these parts of moringa tree were used in different healing procedures for different disease. Plants leaves was very good nutrient supplement and also used as an antibiotic. Moringa oleifera was an exceptionally good source of vitamin A , vitamin B , vitamin C, mineral (in particularly iron) Hands are needed to protect from bacterial pathogens as they are most prominent part of the body. Proper hand hygiene is the single most important, simplest, and least expensive means preventing from infections. It's required to protect

human from disease, to save life of person. The present research work was conducted to investigate phytochemical analysis and antimicrobial activity of aqueous leaves extract of moringa oleifera. The research was carried out to formulate and evaluate the herbal hand wash. The purpose of this study is to evaluate the antimicrobial activity of formulated herbal hand wash containing aqueous leaves extract of moringa oleifera against microorganism. The phytochemical analysis of sample showed the presence of tannins, saponins, reducing sugar, flavonoids, and phenolic flavonoids. The clinical isolates were used in this study for antimicrobial activity of moringa oleifera was tested against bacterial pathogens i.e. *Escherichia Coli*, *Pseudomonas aeroginosa*, *Kliebsella pneumoniae* by agar well diffusion & also compared with commercially prepared antibiotic disc by Kirby disc diffusion, also the efficiency was checking by using the hand wash on volunteers, also performed the antimicrobial activity of hand wash against above gram –ve clinical pathogens. The entire test organism had shown sensitivity against herbal hand extract. Result revealed that Moringa oleifera leaves as a miracle drug trees against aliments and useful for the treatment of many diseases and also the source of natural antibacterial and antifungal infection, also showed that the aqueous leaves extract of moringa oleifera contain hand wash formulation was more efficient in reducing the number of organisms. Thus it can be used as antimicrobial hand wash with less or no side effects.

Ants as Vectors of Bacteria in Hospital Environments

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The main objective of this study was to isolate the bacteria from the ants present in the hospital environment and how it can take part in the spread of HAIs. For this study, ants were collected from around 10 different areas of the hospital. I also recognized the genera of the collected ants. The isolated bacteria were gram-negative and gram-positive group, from the ants. Among the gram-negative *Escherichia coli*, *Citrobacter spp*, *Hafnia spp*, *Yersinia spp* and two *Shigella spp* and *Proteus spp* were isolated and among gram-positive only *Staphylococcus aureus* and *Staphylococcus saprophyticus* were observed. Bacteria from ants were isolated using Brain Heart Infusion broth (BHI), MacConkey Agar (MAC) and Mannitol Sugar Agar (MSA) and specie identification was done by IMVIC test, Coagulase test and Gram staining. Susceptibility was tested towards chloramphenicol (for gram negative) and vancomycin, cefoxitin, oxacillin (for gram positive). The gram-negative isolates i.e. *E .coli*, *Citrobacter freundii*, *Shigella flexineri* were resistant to chloramphenicol, while *Hafnia alvei*, *Shigella sonnei*, *Proteus merabilis*, *Proteus vulgaris* and *Yersinia spp* were susceptible to chloramphenicol. Resistant gram-positive microorganism against vancomycin, oxacillin, and cefoxitin, included both, coagulase-negative *Staphylococcus saprophyticus* and coagulase-positive *S. aureus*. The ant genera spotted were *Lasius spp*, *Solenopsis spp* and *Camponotus spp*. The study revealed that the ants carry pathogenic resistant and sensitive bacterial strains with them and can spread them into hospital environment. Not only contaminated surgical instruments are responsible for the spread of HAI's but these vectors can also play a part in the spread of nosocomial infections in hospital environments.

Thumb Impression Analysis: Isolation and Prevalence of the Microbial Strains by Analyzing Biometric Unit

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Millions of people around the globe suffer from various infectious diseases per annum. Most of the infection is hand acquired infections. World health organization suggests hand hygiene as a primitive precaution focus to decrease the infectious rates. Biometric devices are nowadays very commonly used both in the public and private sectors to record attendance. This study aims assess the risk of transmission of pathogenic bacteria through biometric devices by isolating the bacterial flora which may be present in biometric device. The isolation and identification of the bacterial isolates was done by gram staining, biochemical reactions and antibiotics testing. Gram positive cocci were found in abundance followed by Gram positive rods. The antibiotics resistance profile showed that the isolated bacterial strains were mainly sensitive to ciprofloxacin but resistant to cefotaxime. The study indicates that in our community the mostly found organisms are *Staph aureus*, *Bacillus* spp among gram positive strains whereas in gram negative *E. coli* and *Salmonella* were detected.

Antimicrobial Evaluation of Bacterial Isolates from Pus Samples in Khyber Teaching Hospital, Peshawar

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Pus is characterized as a greenish-yellow or brown fluid that is thick and rich in proteins. It may have foul smell. In our study gram negative bacteria (62.5%) were dominant than Gram positive bacteria (37.5%). Out of 147 samples only 40 samples showed positive growth and the rest were characterized with No growth. The most dominant microbe found was *Escherichia Coli* 14(35%) and *Staphylococcus Aureus* 14(35%). Other prevalent species included *Pseudomonas aeruginosa* 6(15%), *Enterobacter species* 5(12.5%) and the least prevalent isolate was *Staphylococcus Epidermitis* 1(2.5%). Infection was more prevalent in males 23(57.5%) than in females 17(42.5%). Pus infection was more prevalent in age group 0-10 (30%) and 21-30 (25%). Antibiotic susceptibility results show that Gram Negative bacteria showed absolute sensitivity towards Meropenem (100%). Piperacillin-Tazobactum (96%), TGC (88%), Amikacin (88%) were also found highly effective against infection. High resistance was observed Cefotamixe (56%). Gram positive bacteria show resistance towards Trimethoprim+Sulfomethoxazole (80%). Effective drugs included Amikacin (100%), Doxycycline (100%), Fusidic acid (80%) and Gentamycin (93.4%). The results of different studies compared with our study shows that predominant isolates in pus samples can vary among different regions of the globe. This study was conducted for the isolation and identification of different bacterial isolates predominant in pus samples. Gram negative

dominated gram positive bacteria. In current study infectious pathogens were *Staphylococcus aureus, Escherichia Coli, Pseudomonas aeruginosa, Enterobacter species and Staphylococcus Epidermitis. Staphylococcus Aureus and Escherichia Coli* were dominant isolates. All the bacterial isolates showed sensitivity towards Amikacin and resistance was developed against CTX and SXT. Strategies can be made to reduce the rate of resistance Much better therapeutic treatments can be designed against antibiotic resistant microbial species.

Identification and Antimicrobial Susceptibility Profile of Bacterial Pathogen Isolated from Wound Infections at RMI, Peshawar, Pakistan

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Wound infection mainly occurs by pathogenic bacteria that enters the skin through any cut causes pain and redness at that area and become responsible for pus or drainage. Contaminated equipment, air, careless handling or poor hygienic are the main causes of infection, which in turn long the process of wound to heal. The aim of this current study was to identify the prevalent pathogenic bacteria and to determine an antibiotic susceptibility of the isolates from infected wounds, to find out the wound microbiology. Wound swabs and pus drainage, from 362 patients was aseptically taken. All the specimens collected was processed by standard microbiological techniques for identification of pathogenic bacteria and the Kirby-Bauer method was used for an antibiotic susceptibility pattern. The most common isolates were *Staph. aureus (n=97, 37.74%)* and *E. Coli (n=61, 23.73%)* followed by *P. aeruginosa (n=25, 9.72%), Enterococcus spp. (n=19, 7.39%), Acineto. baumannii (n=16, 6.22%), K. pneumoniae (n=16, 6.22%), P. mirabilis (n=15, 5.83%), P. vulgaris (n=3, 1.16%), Entero. cloacae (n=3, 1.16%), Entero. aerogenes (n=2, 0.78%). Staph. Aureus* and *Enterococcus spp.* was highly sensitive towards linezolid (65.5%) and vancomycin (66.3%) and resistant towards Penicillin (62.0%). *Pseudomonas aeruginosa* was sensitive towards Piperacillin/Tazobactam (36%) and resistant towards Aztreonam (20%). Gram-negative were highly sensitive towards Amikacin (52.5%) while highly resistant towards Ceftazidime (53.4%). From this study we concluded that regular inspection of antibiotic susceptibility and awareness of drug resistivity is important along with proper hygienic care.

Bioactive Components Evaluation of *Berberis baluchistanica* from Ziarat Balochistan

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The present study was aimed to evaluate the bioactive components, antioxidant potential, and antimicrobial activity of crude ethanol extracts of bark, leaves and roots of *Berberis baluchistanica* Ahrendt. All extracts were analyzed for total phenolic, total flavonoid, and (DPPH; 1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, antibacterial and antifungal activity. Bioactive components analysis revealed the presence of alkaloids, tannins, glycosides, anthraquinones, saponins, flavonoids, coumarin, quinon, steroids, terpenoids and phlobatannins. The hydrogen donating ability was determined on the basis of their concentration providing 50%

inhibition (IC_{50}). Smallest IC_{50} value 0.6788 mg/mL was found for bark extract with highest antioxidant potential and highest was seen for roots 1.125 mg/mL having lowest antioxidant potential. Total phenolic content value of the bark extract was 38.174 ± 16.028 mg GAE/g and that leaves was 28.733 ± 13.724mg GAE/g. The lowest value was 19.897± 11.8141mg GAE/g for root extract. Flavonoid contents in bark, leaves and root were 6.1098± 1.071, 10.137 ± 1.843 and 2.9876± 1.8388 mg QE/g respectively. All the extracts have been tested against a wide group of bacteria and showed the highest inhibitory activity against the bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined by the two-fold serial dilution method. All the plant extracts were found to be effective against all the tested bacteria. As the extracts of bark, leaves and roots of *Berberis baluchistanica* showed a prospective source of antioxidant and antimicrobial activities, this medicinal plant can be useful in considerable biomedical, biological and agricultural fields.

Development and Evaluation of Rapid System for The Identification of Medically Important Gram-Positive Cocci

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Gram positive bacteria are becoming increasingly implicated in human diseases. The members of this group of bacteria are comprising many important genera which are actively involved in a number of outbreaks of human, animal and food born infections in many parts of the world including Pakistan. The emerging antibiotic resistant of these bacteria due to misuse of antibiotics is also causing big problems in patient healthcare and treatment. Identification of this groups of bacteria poses serious problems as most of the members of these groups of bacteria are biochemically less active in comparison with of other group of bacteria. The conventional methods developed for the rapid identification of these groups of bacteria are quite labor intensive, slow and time consuming. Number of commercial identification kits/assays have been developed for the rapid identification of these group of bacteria and are available in international and local markets. These kits are very expensive and unaffordable for the suffering poor patients' population of Pakistan. Unfortunately, these types of indigenous rapid identification systems are not available in Pakistan. In search of more reliable diagnostic methods and self-sufficiency, we initiated a research project to develop indigenous rapid diagnostic systems. During this study various parameters were optimized. The technology developed during previously developed system (QTS-24, QTS-12, QTS-NE) will be used in the development of the present rapid identification system. The R and D work will be used in the developments of present rapid identification systems.So,we can developed and evaluated rapid id strip for rapid identification of medically important gram positive cocci but this work will be under evaluated because we work only on 20 strains of gram positive cocci due to lack of time and for the development and evaluation, we needs to work on 1000 of strains as much as possible to become 100% surety for developing such type of strips as my supervisor Dr mahmooda developed a rapid identification strip for gram negative Enterobacteriaceae organisms. The modified conventional formulations of sugar fermentation and the enzymatic detection system were optimized by using live clinical bacteria isolates of these groups of bacteria (gram positive cocci). Unfortunately, these type of indigenous system are not available in Pakistan therefore in search of more reliable diagnostic method and to give self-sufficiency to the country, our supervisor has initiated various gram negative identification system project in past. These processes are patented by Government of Pakistan in her name and her

previous organization DESTO Karachi labs. Likewise, when she joined JUW as professor then she started a new project with her ten-research student of BS. She allocated the development of modified formulations of single identification test out of total of thirteen test to each of BS students.

Prevalence of Diabetes Mellitus in Hepatitis C Patients in Wazirabad Tehsil of Gujranwala District of Pakistan

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Hepatitis, a condition of liver's inflammation that can be self-limiting or, in certain chances, it may lead to liver cancer, fibrosis or cirrhosis. Hepatitis viruses mainly cause hepatitis in the world. People with hepatitis C have predominant chances to develop diabetes as HCV virus participates in causing type 2 diabetes. HCV virus causes pathogenesis in two ways: it either directly destroys the β cells of pancreas or contributes to the specific autoimmunity of β cells. The present cross sectional study was done in Wazirabad Tahsil of Gujranwala District to analyze the percentage of patients suffering from hepatitis C who had the risk of diabetes mellitus. For this research work, demographic information and data about any other medical history were collected by using a questionnaire. Blood samples were collected from hospital and real time PCR was performed to measure the viral load and blood sugar was measured by using glucometer. Data were then analyzed by using statistically designed software. A total of 29.33% patients, having hepatitis C, were found to be diabetic in Tehsil Wazirabad. 14.70% male and 38.59% female patients having hepatitis C were diabetic. From results shown that the patients of hepatitis C are at higher risk to develop diabetes, therefore; it is supposed that persons having hepatitis C should regularly visit doctors for routine check-up of diabetes and change their life style to reduce the risk of developing diabetes.

Thymus vulgaris Associated Bioactivity Against MDR Microbial Strains, Biofilm Formation and Bacterial Viruses

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Thymus vulgaris water extract and thyme oil extracted from the dried plant (by steam distillation) were found antibacterial, antibiofilm and antiphage. Multiple drug resistant (MDR) strains including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* were found sensitive (using agar well diffusion approach) to the water extract. Further, biofilm formation by bacterial strains was arrested by the *Thymus vulgaris* extract. Mechanism of bioactivity was studied by scanning electron microscopy. Accordingly, bacterial cell distortion and biofilm dislodge affects were witnessed in the treated bacterial strains. Thyme oil was fractionated by column and thin layer chromatography. One of these fraction (a phenolic compound) with maximum manifested stronger bioactivity compared to water extract, with maximum bioactivity against *S. aureus* and *C. albicans* (MIC 200µg/ml) followed by *E. coli* and *P. aeruginosa*. Thyme water extract treatment with

indigenously isolated coliphage (suspension) was followed by a dose dependent reduction in phage titre (in term of pfu) thereby indicating the antiviral potential of this plant.

Characterization of *Staphylococcus aureus* Isolated from Market Milk

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Staphylococcus aureus is a common microflora present on skin of the animals, if present in milk may produce toxins. Consequently, consumption of *S. aureus* contaminant milk may result in Staphylococcal food poisoning. Therefore, this study was planned to check the prevalence and to characterize the *Staphylococcus aureus* from market milk. A total of 50 market milk samples were collected randomly from different locations of Hyderabad city. The *S. aureus* were isolated on Mannitol Salt agar, among the presumptive *S. aureus* isolates an overall, 35 milk samples were found to be contaminated with S. aureus showing a prevalence rate of 70%. For genotypiccharacterization DNA was extracted from the isolates and all the isolates were identified by species specific primers targeting 16S rRNA that showed 750bp PCR amplified product. Antibiogram profile showed susceptibilities of the isolates against 10 antibiotics using the Kirby-Bauer disc diffusion method. The antibiotic ciprofloxacin showed higher susceptibility rate followed by norfloxacin, tetracycline, gentamicin, tobramycin, levofloxacin, erythromycin, vancomycin, oxytetracycline and ampicillin.

Probiotic Properties of Lactic Acid Bacteria Isolated from Traditional Yoghurt

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The aim of this study was to investigate the probiotic properties of Lactic acid bacteria isolated from traditional yoghurt (Dahi). Out of 84 isolates, 23 *L. acidophilus*, 21 *L. casei*, 09 *L. delbrueckii* subsp. *bulgaricus*, 11 *L. helveticus*, 09 *L. delbrueckii subsp. lactis*, 03 *L. viridescense* and 02 *L. plantarum* were identified. All of these identified species were examined for antimicrobial activity against selected pathogens, acid and bile tolerance and antibiotic susceptibility. It was observed that *L. acidophilus* S26, *L. casei* S66, *L. delbrueckii* subsp. *bulgaricus* S65, and L. *plantarum* S19 produced antimicrobial activities against the test strains and tolerated pH 2.5 and survived the 0.1, 0.2, and 0.3 % bile salt concentration. It was resistant to antibiotics ciprofloxacin, kanamycin, penicillin and vancomycin.

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Ayesha Umer, Tayyaba Asif, Dr. Safia Mushtaq and Aisha Qureshi

Among shellfish products the crab's meat becomes the most demanding seafood around the globe due to its nutritional value. In general, crab meat is a rich source of proteins contains appreciable quantities of digestible proteins, essential and non-essential amino acids and bioactive peptides. In Pakistan, the fish contribution to animal protein intake in 2013 (2.2 percent) was lower than the Southern Asian (13.7 percent) and world (16.3 percent) averages. Therefore, present study was designed to promote seafood consumption among masses through highlighting nutritional significance of seafood protein especially in crabs in the local community. The commercially important edible crab species from Pakistani waters are *Portunus pelagicus, Portunus sanguinolentus, Scylla serrata and Charybdis feriatus.* The principle aim of this study was to set baseline data of protein contents of four different edible commercial crab species by evaluating their amino acids contents. Among all the species the protein contents was higher in hard shell crab (*S. serrate* than those in soft shell crabs (*P. pelagicus, P. sanguinolentus and C. feriatus*) respectively which suggests that the Mud crab (*S. serrata*) are significantly different from other species. In terms of amino acid profiling total eighteen amino acids were detected in which arginine and lysine have shown the highest value with variations in all crab samples. The results revealed that all crab species contain good amount of amino acid composition, essential for many biochemical reactions in body processes.

Biological Extraction of Chitin from Crab waste using *Lactobacillus* and *Bacillus spp.*

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Crustacean shells are mainly rich in protein, calcium carbonate, asthaxanthin and chitin. These shells after the consumption of meat are generally degraded as waste. This rich and valuable resource is dumped for natural decay while the essential and important end products were also considered as waste. The chemical extraction approach was adopted previously to overcome this issue but the end product extraction is slightly tricky and not environmental friendly. Biological extraction was considered as an alternative approach for the extraction of chitin. It is an environmental friendly approach which generate lesser waste. In the current study, bacterial isolates with efficient production of lactic acid and protease were isolated from yoghurt and tin crabs. The isolated strains were further identified on morphological and biochemical characteristics. The co-fermentation approach technique was used for the extraction of chitin. In this approach two microbial cultures were simultaneously utilized for the extraction of chitin. The basic media components include Tryptone, Dextrose, Sodium Chloride and Whole Shell (WS). It was observed that the co-fermentation helps in efficient degradation of crustacean shell into chitin and other related components. The protein rich liquid was further used as the agricultural supplement in plants and increases the growth of plants. The extracted chitin was further used as chitosan in manufacturing of value added components.

The post COVID-19 Strategy to treat Crustacean Waste: Biological Extraction of Chitin from Crab Waste using *Lactobacillus* and *Bacillus spp.*

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The initial phase of COVID-19 focused on the spread of virus through Crustacean fishes. Crustacean shells are mainly rich in protein, calcium carbonate, asthaxanthin and chitin. These shells after the consumption of meat are generally degraded as waste. This rich and valuable resource is dumped for natural decay while the essential and important end products were also considered as waste. The nutrient rich nature of these shells make them highly contagious for the spread of diseases. The chemical extraction approach was adopted previously to overcome this issue but the end product extraction is slightly tricky and not environmental friendly. Biological extraction was considered as an alternative approach for the extraction of chitin. It is an environmental friendly approach which generate lesser waste. In the current study, bacterial isolates with efficient production of lactic acid and protease were isolated from yoghurt and tin crabs. The isolated strains were further identified on morphological and biochemical characteristics. The co-fermentation approach technique was used for the extraction of chitin. In this approach two microbial cultures were simultaneously utilized for the extraction of chitin. The basic media components include Tryptone, Dextrose, Sodium Chloride and Whole Shell (WS). It was observed that the co-fermentation helps in efficient degradation of crustacean shell into chitin and other related components. The protein rich liquid was further used as the agricultural supplement in plants and increases the growth of plants. The extracted chitin was further used as chitosan in manufacturing of value added components.

Derivative of Benzophenone Semicarbazone as a Potential Biofilm Inhibitory Compound Modulating Gene Expression in *Candida parapsilosis*

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As a commensal, *Candida parapsilosis* colonizes the skin, mucous membranes, and the gastrointestinal tract shortly after birth as a part of the normal flora (Andrade *et al.,* 2019). *C. parapsilosis* is found in the interdigital spaces of the hands, and can colonize temporarily the human integument. The most frequently found fungal species from the nails of psoriatic patients was *C. parapsilosis*. The susceptibility to biofilm - related fungal infections is increased due to the use of long term use of indwelling catheters, heart valves, ocular lenses, transplantation procedures, long stay in ICUs, and immunosuppressants (Katragkou*et al.,* 2008). Biofilm-associated infections caused by *Candida* spp. are the fourth leading cause of nosocomial diseases, mainly caused by contaminated implanted medical devices (Crawford et *al.,* 2009). Microbial resistance against available therapeutic drugs is on a rise due to biofilm forming microorganisms. Despite the availability of antifungal drugs, mortality due to invasive fungal infections is as high as 40% (Alcazar-Fuoli*et al.,* 2014). In this study we identified very first time a benzophenone semicarbazone derivative, 2((4-hydroxyphenyl)(phenyl)

methylene)*N*(4(trifluoromethyl)phenyl)hydrazinecarboxamide), as a lead candidate for *C. parapsilosis* - associated biofilm inhibition. It induces its inhibitory potential by modulating some of the genes expressions involved in cellular adhesion. Our transcriptomics analysis explained that the genes responsible for cell adhesion were significantly down-regulated which most likely associated with inhibition of initial attachment to the surface. Similarly, genes involved in SAPs, and iron metabolisms were found to be significantly up-regulated. Our study will be able to contribute substantially to antibiofilm research.

Medicinal Activity of Chromatographic Fractions of Selected Plants Against Multidrug Resistant Bacteria

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Therapeutic plants are rich source of traditional herbal medicine. Most of the plant's medicinal properties are due to the presence of secondary bioactive compounds. Numerous plants phytochemicals can also be used directly for the production of novel effective medicines. Some Indigenous plants were collected and used. Plants crude extracts were used against MDR GI bacterial pathogens. The most effective and maximum zone of inhibition was exhibited by aqueous extract against MDR GI *S.* Typhi. HPLC fractions were obtained from indigenous plants extracts. All the fractions showed activity against MDR GI bacteria. Aqueous fraction against MDR GI *S.* Typhi. Methanolic fraction against MDI GI *E. coli* and ethanolic against MDR GI *S. flexneri* showed best results. This study will be helpful for future to isolate and use the bioactive compounds of selected plants for therapeutic activity in pharmaceutical industry against MDR GI bacterial pathogens.

Prevelence and Antibiotic-Sensitivity of Gram-Negative Bacteria in Hospital Drinking Water

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Antibiotic resistance is a major public health problem. Various factors contribute to the proliferation of microbes in the environment. This study aims to determine the prominence and screening of the Antibiotic resistant Gram negative bacteria in hospital drinking water. Bacteria were isolated from drinking water of various hospitals taps of Karachi. Isolates were grown on MacConkey Agar, and EMB Agar Identification of microorganism was done by biochemical test. The predominant strains were identified as *Klebsiella*. Antibiogram testing was performed by using Ampicillin, Cefepime, and Levofloxacin. *Klebsiella* strains showed maximum sensitivity against levofloxacin but moderately resistant to Cefepime and ampicillin. The results indicate high level of antibiotic resistance in hospital drinking water in Karachi which may cause multi resistance infections and difficulty in treatment. Controlling the quality of drinking water in hospitals remains a major challenge. Resource protection, management and distribution of all strategies are important in maintaining and improving water supply. This raises the need to educate people about the practical use of antibiotics and the safe disposal of waste-containing antibiotics.

Pseudomonas aeruginosa: A Potential MDR & A Global Threat in The Near Future

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The main objective of the study was to look for the MDR; as drug resistance has been increasing tremendously and also analyzing the association between the virulent determinants and the antibiotic resistance in *Pseudomonas aeruginosa.* Microbial resistance is always being a threat in the treatment of such MDRs having versatile drug resistance mechanism making them resistant to most of the higher drugs which make the options limited for the treatment and in selecting antimicrobial therapy. This prospective study was conducted related to drug resistance in *P. aeruginosa*. For this purpose, random samples of the clinical isolates were collected having the sample size 50 isolates. All the clinical isolates were first subjected to a series of conventional tests to aid the identification of the required bacterium by performing Gram staining, growth on MacConkey and Cetrimide agar as well as by performing biochemical tests that included TSI and citrate. After that the isolates were examined for the presence of the virulence factors by different phenotypic methods including growth on Egg Yolk Agar, Blood Agar, DNase Agar, and Trypticase Soy Broth. The antimicrobial susceptibility was identified by Kirby Bauer method. The isolates were 100%, 90%, 40% positive for the phospholipase, DNase and biofilm formation respectively. 90% of the isolates showed α- hemolysis whereas the rest of them were γ-hemolytic. The findings of antimicrobial susceptibility showed that 70% of the isolates were positive for ESBL production while only 30% were MBL producers. The study speculated that some of the virulent factors may contribute to the antibiotic resistance so these can be given the more consideration and the researchers have to look forward in developing the methodologies to detect these determinants efficiently.

Resistance in *Klebsiella pneumoniae*- An approach for the Treatment of Pneumoniae

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Pneumonia is a major lung disease, especially in children and infants. One of the major causative agents of pneumoniae is *Klebsiella pneumoniae*. It causes mortality due to the impaired immune systems. About 151 million annual fatalities caused by pneumonia globally. Pakistan is among the worst affected countries with child mortality due to pneumonia, and acute lower respiratory infections, 9.2 million cases are reported per year. The emergence of resistance in *Klebsiella pneumoniae* has caused a global concern, especially developing countries. Current study deals with the systematic screening of large libraries of compounds against Multi drug resistant strains of *Klebsiella pneumoniae.* During the first phase the antibacterial susceptibility was evaluated with

standard drug colistin sulphate and nitroquinoline derivatives by micro-plate alamar blue assay (MABA) in order to validate drug resistance nature of available strains. Nitroquinoline derivatives i.e. compound 5-nitroquinolin-8-yl 3-phenylpropanoate, 5-Nitroquinolin-8-yl 3-isocyanobenzoate, and 5-Nitroquinolin-8-yl 3-nitrobenzoate were found to be potent inhibitors with MIC values of 10-30 μg/mL. The effect of compounds on bacterial cells were investigated by using AFM, and SEM techniques that showed the destructive nature of compounds. The selected non-toxic compounds were subjected to mechanism-based studies in order to find out their mode of action, cytotoxicity, and hemolysis. Molecular docking studies indicated that compounds 5-nitroquinolin-8-yl 3-phenylpropanoate, 5-Nitroquinolin-8-yl 3-isocyanobenzoate, and 5-Nitroquinolin-8-yl 3-nitrobenzoate were capable of interacting with the amino acid residues of topoisomerase IV of *K. pneumonia* such as Glu1084, Ser422, and Asp421.

Synthesis of Effective Hand Sanitizer Against Skin Pathogen

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Hand Sanitizer and their utilization is always mandatory in Health Care industries and Health care facilities. However, during the COVID-19 pandemic a sharp increase was observed in the utilization and demand of hand sanitizer increase and due to which health care sector effected badly. Therefore, by keeping in view the increase demand three different Lab scale Hand Sanitizer formulation were prepared and their antimicrobial potential was also explored for finding the efficacy against variety of pathogenic microbial cultures. It was observed that the hand sanitizer "A" which contains Ethanol, Aqua, Isopropyl myristate, Aloe barbadensis leaf extract, sunflower seed oil, Lavendor oil, Tea tree oil, Fragrance showed satisfactory performance against all tested pathogens while the efficacy of other B and C two formulas were compromised against *Staphylococcus aureus*. The Hand Sanitizer "A" was found to be skin friendly, non-allergenic, smooth fragrance and effective against variety of pathogenic microbial cultures. The formula "C" which contain organic components needs to be optimized furthure for increasing its antimicrobial ability. Formula "C" also need to increase the amount of moisturizing agent for smoothing effect. It was also observed that Hand Sanitizers A, B and C were also effective against *Listeria monocytogenes* and *Serretia marcescens.* Therefore, it is suggested that all the formulation is quite effective but the formulation "A" is effective against a skin pathogenic organism which increase its consumption for multipurpose use.

Attenuation of UCMS Induced Behavioural Deficits by Repeated Administration of Thymol in Rats

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Nowadays neurobehavioral disorders are a global concern, as the prevalence of illness has increased by Crisis like Coronavirus Disease 2019 (COVID-19) pandemic, has had a major effect on our lives. Young people and parents are facing challenges amid COVID-19 pandemic that has increased prevalence estimate of depression (28%),

anxiety (26.9%), post traumatic symptoms (24.1%), stress (36.5%), psychological distress (50%) and sleep problems (26%). In the light of recent studies, the present study was design to evaluate the antioxidant and neuroprotective role of thymol on UCMS induced increased oxidation and neurodegeneration in rats. Thymol is a monoterpene phenol with many Pharmacological and neuroprotective propoerties. It is one of the most studied compound nowadays. Twenty-four male Albino Wistar rats were initially divided into two groups Control and Thymol (10mg/kg) and further subdivided into unstressed and stressed groups. Memory function was assessed by Morris water maze (MWM) and Elevated plus maze (EPM) tests. To determine the effect of supplementation on neurotransmission brain biogenic amines serotonin (5-HT) and 5-HIAA levels were analyzed. The antioxidant properties were analyzed by the levels of lipid peroxidation (LPO) and cytoprotective enzymes activities namely catalase (CAT) and superoxide dismutase (SOD). Collectively our data suggests that supplementation of Thymol significantly attenuated UCMS induced increased oxidation and neurodegeneration by improving the activities of antioxidant enzymes and biogenic amines in brain.

Virulence Typing of Selected Bacterial Pathogens Associated with Neonatal Sepsis in Pakistan

Zainab Zahoor, Zia-Ur-Rehman Farooqi, Sundus Javed and Amna Mumtaz

Neonatal sepsis has high incidence with significant mortality as well as morbidity rate in Pakistan. In this study different bacterial pathogens causing neonatal sepsis in a tertiary care setup in KPK were included. It was found that *S. aureus* has a strong association with neonatal sepsis (frequency of isolation=43%). In addition to *S. aureus*, *C. freundii* (frequency of isolation=21%), *P. aeruginosa* (frequency of isolation=13%), *E. coli* (frequency of isolation=15%) and *S. enterica* (frequency of isolation=8%) were also isolated from blood and pus samples. Among the patients of neonatal sepsis, 61 (67.8%) were male and 29 (32.2%) were female patients. Molecular analysis revealed that majority of the *S. aureus* isolated from clinical samples of neonatal sepsis possess genes for the phantom valentine leucocidin toxin (as 76.9% isolates showed presence of Luk-PV gene). Similarly, most of the isolates were resistant to methicillin (as 74.4% isolates showed presence of MecA gene). Antibiotic resistance among bacteria causing sepsis is increasing at an alarming rate. Majority of *S. aureus* isolated were resistant to ciprofloxacin (71.7%) and penicillin (71.7%). *C. freundii* were mostly resistant to tigecycline (42%), cefixime (31.5%) and Fosfomycin (31.5%). *P. aeruginosa* was resistant to cefixime (61.5%) and ciprofloxacin (61.5%). While S. enterica isolates were sensitive to the all the antibiotics being tested. Hence it was concluded that S. aureus is the most common bacterium causing neonatal sepsis and antibiotic resistance has been found among the bacterial isolates causing neonatal sepsis.

An *In-Vitro* Investigation of the Antimicrobial Activity of Green Synthesized Silver Nanoparticle Using *Moringa Oleifera* Leaf Extract Against MDR Strains

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In our study we follow Green synthesis approach of silver nanoparticles from fresh leaves of *Moringa oleifera* that have high content of biomolecules i.e xanthonoids, terpenoids, flavonoids and phenolic compounds which

carry out the reduction of Ag^+ ions into AgNps. Leaf extract was get ready by boiling method and mixed with 3mM concentration of silver nitrate ($AgNO_3$). The reaction mixture was turned into dark brown from light yellowish which compelled the transformation of Ag^+ to Ag^o. Formation of AgNps was encouraged by performing Uv-visible spectrophotometer which shows surface plasmon resonance (SPR) band at ~477 nm. Synthesized nanoparticles are spherical in shape by TEM & SEM analysis and the average size is about 25.235±0.694 nm which is close approximate to XRD. XRD results demonstrated purity as well as crystalline nature of AgNps which is endorsed by EDX result that shows no impurity peak. Thermal stability of our samples was corroborated by TGA/DSC. Antibacterial activity was performed in-vitro against *Acinetobacter baumannii*, *Pseudomonas Aeruginosa*, *E. coli*, *Klebsiella pneoumoniae*, and it is concluded that AgNps could be the acceptable choice to treat infections caused by MDR bacterial strains. Antibacterial tests include Agar-well diffusion assay, MIC and MBC

Bio-catalytic activity of Musa paradisiaca **peel mediated Silver Nanoparticles Against Antibiotic Resistant Pathogenic Bacteria**

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Nanotechnology is rapid growing field with its wide range of application in science and technology. Biosynthesized nanoparticles are widely used in combination with existing antibiotics to reduce the chances of antimicrobial resistance. In the present study, banana peel extract is used as a capping and reducing agent in the synthesis of silver nanoparticles. The synthesized nanoparticles are characterized by UV–visible spectroscopy, Fourier transform infrared (FT-IR), and scanning electronic microscope (SEM). The biosynthesized nanoparticles are coated with commercially available antibiotics (Meropenem, Amikacin, Ampicillin, and Amoxicillin-clavulanic acid). AgNPs alone showed potent antimicrobial activity against *Klebsiella pneumonia & E. coli* the inhibition zones were 26±1.73 & 17±1.54 mm respectively. Meropenem alone showed highest antibacterial activity (ZI=23mm) against both the strains followed by amikacin (ZI=15±1.5) against *E. coli* and 20±0.5mm against *Klebsiella pneumoniae,* while both the strains were found resistant to ampicillin and amoxicillin-clavulanic acid. Furthermore, it is observed that AgNPs coated antibiotics showed significant antimicrobial potential than antibiotics alone. AgNPs coated Ampicillin and Amoxicillin-clavulanic acid showed inhibition zones of 15±1.2 mm & 15±0.9 respectively against *E. coli* while 12±1.4 mm & 14±0.6 mm against *K. pneumonia.* It is concluded that AgNPs have a synergistic effect in combination with antibiotics and can be used alone or with antibiotics to overcome the antimicrobial resistance.

Surveillance and Molecular Characterization of Carbapenem Resistance isolates of Gram Negative Bacteria

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Increasing bacterial resistance to antibiotics is a critical problem worldwide. Carbapenem resistance, mainly among Gram-negative pathogens, is an ongoing public-health problem of global dimensions and in the recent

years colistin resistance has also been reported. The objectives of the study were to determine the current situation of carbapenem and colistin resistance in Gram negative bacteria, antibiotic resistance profiles of these strains and identification of genes involve in resistance to carbapenems and colistin. Complete patients' detail regarding age, gender, type and site of infection etc. was recorded from September 2019 to January 2020. Bacteria were identified by using API 10S and 20E kits. Antibiotic sensitivity testing was performed by Disc diffusion method and micro dilution assay. Carbapenem and Colistin resistant genes were identified by using PCR. Frequency of carbapenem resistance isolates were 100% out of 110 gram negative bacterial isolates and that of colistin was 0.00% out of 110 bacterial species. Carbapenem and colistin resistance in each type of Gram-negative bacterial species was calculated to be higher by this study which creates a global health problem because colistin is drug of last resort to treat infections caused by antibiotic resistant bacteria. There is limited published data available of the current situation of carbapenem and colistin resistance from Pakistan. This research study determined the antibiotic resistant profiles in Gram-negative isolates that would be helpful to control the spread of antibiotic resistant bacterial strains and enhance treatment options for different bacterial infections.

Platelet Rich Plasma (PRP) and Stromal Vascular Fraction (SVF) in Patients to Treat with Osteoarthritis

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Osteoarthritis (OA) is a degenerative disease involving joint damage, an inadequate healing response and progressive deterioration of the joint architecture that commonly affects the knee and/or hip joints. Platelet-rich plasma and stromal vascular fraction is an autologous concentration of growth factors. The purpose of this study is to evaluate the effect of the PRP and SVF in the treatment of knee joint cartilage injuries and degenerative meniscus lesions as well as pain relief. The n=35 patients were selected in clinical trial. During study the patient revealed the history of pain and stiffness. All the baseline investigation was carried out to rule out rheumatoid arthritis, any autoimmune disease and gout. The Knee x-ray was also done and evaluated with Kellgren-Lawrence scale. The PRP and SVF was prepared and these were used to treat the patients. After every treatment the inflammatory parameters and change in the knee x-ray were noted. The PRP and SVF procedure was performed on 35 patients at Mussavir Stem Cell Clinic in Karachi, Pakistan. We included 60% females and 40% male patients in our study. The patients were evaluated according to the Kellgren-Lawrence scale before and after applying PRP and SVF for 3 to 6 months and vast difference was noted in x-ray. This treatment is a cost effective, safe with no side effect. The technique is good functional with pain relief, improvement in movements and mobility, and quality of life with early osteoarthritis of the knee

Activity of Syzygium cumini against Multi-Drug Resistant *Clostridium Perfringens* Type A

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The study was conducted for the antibiogram of *Clostridium perfringens* type A and evaluation of ethanolic extract of *Syzygium cumini* against multi-drug resistant isolates of *C. perfringens* type A. Swab samples of small intestines of chicken suspected for necrotic enteritis were processed for isolation of *C. perfringens* under anaerobic conditions. The isolates were identified on the basis of morphology, microscopy and biochemical profile. For confirmation, alpha toxin gene (324bps) was amplified by polymerase chain reaction (PCR). These isolates were evaluated for antibiotic resistance against six antibiotics following recommendations of CLSI. The multi-drug resistant isolates were subjected to susceptibility against ethanolic extract of *S. cumini* by well diffusion method followed by minimum inhibitory concentration (MIC). Out of ten samples, three isolates of *C. perfringens* type A were confirmed by biochemical and molecular characterization. Highest resistance (100%) was observed against erythromycin, spectinomycin, and colistin followed by neomycin (66.6%) and tetracycline and ampicillin (0%), respectively. All the MDR isolates were found sensitive to ethanolic extract of *S. cumini*. The MIC of ethanolic extract of *S. cumini* was recorded as 5.2 mg. It was concluded that ethanolic extract of *S. cumini* may be used as a substitute for antibiotics to cure necrotic enteritis in poultry.

Antimicrobial Resistance: A Plight beyond COVID-19 Pandemic

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Resistance traits are not a recent microbial invention to confront the antibiotics; rather they have been around for the many millennia of micro-organism existence. Worldwide, 700,000 die every year due to drug resistance and predicted to reach 10 million deaths per year by 2050 by healthcare organizations. The bat virus also resembles with SARS-CoV more than it does with MERS-CoV or SAR-CoV, indicating that it is a unique virus for humans. The bacterial and fungal infections also occur along with coronavirus with some resistant to drugs and maligancies are closely associated with worse outcomes. Clinical trials suggested that antibiotics do not have any impact on viral diseases. On the other side, a number of antimicrobial drugs are given to either exploit their antiviral activities potentials or resolve bacterial infections co-existing with virus infections. The current therapies regime against COVID-19 pandemic forefolded the threat of antimicrobial resistance, as many of patients positive with infection are treated with antibiotics to lower the chances of counteracting secondary bacterial infections, making resistant bacteria common. In addition to indiscriminate use of antibacterial drugs, the disaster were magnified by proliferation of adulterated antimicrobial medicines in developing countries, healthcare financing and agricultural issues, international travels, rapid spreading of disease and climate change are determinants

intersect with drug resistance at various societal level. Hence, global concerted targeted interventions to disrupt these multi-faced, interrelated and interdependent factors reduce the misuse of antimicrobials and discovery of an effective alternative treatment are urgently required at all levels of society.

Formulation and Evaluation of Antibacterial Lip Gloss

Areeba Kaleem and Dr. Saira Kamal Khan

Cosmetic products are one of the major causes of skin infections such as acne, pimples, discoloration etc. The microbial load present in cosmetics causes the skin diseases. This microbial load can be reduced or eliminated using antimicrobial agents such as essential oils in cosmetics. Essential oils are now widely incorporated in cosmetic products, perfumes, and other household products because of their analgesic, antiseptic, antimicrobial, carminative and diuretic properties as well as for their pleasant odour. The aim of this study was to investigate the antimicrobial activity of four essential oils (*Lavender oil, Eucalyptus oil, Lemon oil & Sandalwood oil*) added to lip gloss and tested against gram positive bacteria (*Staphylococcus aureus and Enterococcus spp*) and gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus spp, and Pseudomonas aeruginosa*). The antagonistic activity of these oils were assayed by the disc diffusion method and agar well diffusion method. The results showed that the essential oils have antimicrobial activity against gram negative bacteria and gram positive bacteria. These oils decreased the growth of micro-organisms. The conducted research results indicates that the lip gloss containing essential oils of *Lavender, Eucalyptus, Lemon and Sandalwood* can be used to prevent infections and can be used as a treatment for several skin diseases. Infections that spread due to sharing cosmetics can also be reduced using antimicrobial lip gloss. The shelf life of this germ-free product is more than normal lip gloss. As this product does not contain chemicals so it can be used by kids as well.

Antibacterial Potential of Silver-Oxide Nanoparticles Against Multidrug-Resistant *Pseudomonas aeruginosa*

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Multidrug resistant *Pseudomonas aeruginosa* is a leading nosocomial pathogen, conferring resistance to a variety of antimicrobial agents. The threat of drug-resistant *Pseudomonas aeruginosa* requires great efforts to produce highly effective and safe bactericide. In this regard the researchers move towards the new strategies including nanotechnology to fight against MDR pathogens. Nanotechnology offers the application of nanomaterials such as silver oxide nanoparticles, iron oxide nanoparticles, zinc oxide nanoparticles etc. Keeping in view, the present study has been designed to determine the activity of silver oxide nanoparticles against MDR *P. aeruginosa.* A total of (n=120) wound samples will be collected from patients having wound infections from different hospitals of Faisalabad. The samples will be processed on selective media named as cetrimide agar. Identification of *P. aeruginosa* will be done based on different bacterial characteristics like colony morphology, biochemical tests. Antimicrobial susceptibility testing will be performed using different antibiotics to confirm the MDR *P. aeruginosa* as per CLSI guidelines 2019. Antibacterial activity of Silver oxide nanoparticles will be determined by using minimum inhibitory concentration and minimum bactericidal concentration by broth micro-dilution. Data will be analyzed by applying suitable statistical tools.

Physicochemical Factors for Optimization of Biomass and Toxins of *Clostridium perfringens* Toxinotype B

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Alpha, beta and epsilon are the potent major toxins involve in pulpy kidney disease. To combat with this disease, proper vaccination of animals is necessary. For an effective vaccine production, bacterial biomass and toxins production optimization is necessary. For this purpose indigenous isolates were procured from Institute of Microbiology, UVAS, Lahore and characterized as *Clostridium perfringens* type B viz accession number MW332247.1, MW551887.1 and MW332060.1. Optimization of biomass and toxins production of the type B was performed under the influence of various physical and chemical conditions. Higher biomass (48.58±0.52 mg/mL) was produced at 37ºC after 48 hours of incubation. A higher biomass (40.22±0.00 mg/mL) was produced at 0.3% sodium acetate, respectively. *C. perfringens* type B produced higher hemolytic units of alpha (2.73±0.06 HU/mL) and epsilon (6.31±0.02 HU/mL) toxins at 37ºC after 24 hours of incubation. Higher cytotoxic units (6.26±0.23 CU/mL) of beta toxin were produced at 37ºC after 36 hours of incubation. A higher hemolytic unit (36.11±0.02 HU/mL) of alpha toxin and higher cytotoxic units (20.41±0.18 CU/mL) of beta toxin were produced at 0.5% ammonium chloride. Higher hemolytic units (34.23±0.15 HU/mL) of epsilon toxin were produced at 0.2% glucose. Optimized conditions for higher biomass and toxins production could be used for vaccine production.

Molecular Characterization of Antibiotics Resistance in Campylobacter *jejuni* Isolated from Retail Chickens in Karachi – Pakistan

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Gastrointestinal tract infections are among the leading causes of morbidity and mortality in Pakistan. Campylobacter is considered as most common cause of bacterial gastroenteritis, specially in children, defeating other enteric pathogens such as *Salmonella* and *Shigella*. *Campylobacter* is the part of normal intestinal flora in chicken. However, contaminated poultry is a transmission factor in humans for which *Campylobacter* is most common cause of gastroenteritis. Therefore, the presence of drug resistant strains in poultry might provide a niche for the spread of antibiotic resistant human infections. This study describes the antimicrobial resistance mechanisms of chicken isolates of *Campylobacter jejuni*. A total of 600 chicken cloacal swabs, collected from different poultry farms were screened. Isolates were characterized for antibiotic susceptibility and analyzed for integron analysis and resistant determinants of commonly available drugs. 81% samples were found positive with *Campylobacter jejuni*. Majority of the isolates exhibited high level of resistance against tetracycline, nalidixic acid, ciprofloxacin, sulfonamides and erythromycin. In agreement with R-phenotype, they possessed class I integron with 1650 bp variable region assorting R-cassettes for tetracycline and sulfonamide. Further

characterization revealed amino acid substitution at 6 different places in QRDR and downstream region of gyrA gene pertaining to fluroquinolone resistance. Out of them 3; L133F, V149I and A157 V are first time observed. Erythromycin resistance (MIC > 32 l g/ml) was observed in 18% cases with 3 different mechanisms including A2075G mutation in DMV region of 23SrRNA, V80I and V121Ala amino acid substitutions in rplD gene leading to structural modification in L4 ribosomal protein which provides the first evidence for the role of L4 ribosomal protein modification equally important with change in DMV region for R-type. The study provides useful information about prevalence and mechanism of drug resistance in *Campylobacter* isolates of poultry origin and their possible role in emerging drug resistance in food borne human pathogens.

Discovery of Novel Substrate Analogs Against R5pi-B Protein of *A. Phagocytophilum* Through Subtractive Proteomics Approach

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Human Granulocytic Anaplasmosis (HGA), a serious infectious disease, is caused by an obligate intracellular, gram-negative, pathogenic bacterium named *Anaplasma phagocytophilum*, transmitted through vector belongs to the Ixodes spp, hard-black-legged ticks. In vertebral host, *A. phagocytophilum* targets granulocytes whereas, in invertebrate host midgut, hemocytes and salivary gland are the targets. Currently, 42 countries including the United States, Europe, Africa, and Asia ticks having *A. phagocytophilum* have been documented. In an endemic area, about 30% of the population in the endemic area, has been exposed to this pathogenic organism. Symptoms can be associated with fever, headache, myalgias, arthralgias, and malaise. To identify potential drugs against *A. phagocytophilum* because vaccines are not available for prevention and control of pathogenic infection, the subtractive proteomic approach including DEG analysis, KAAS, and KEGG investigations was carried out to discover potentially druggable proteins. Further, from BLAST protein analysis, a protein Ribose-5-phosphate isomerase B (R5pI B) was selected as the drug target protein. Against the predicted active site residues of a particular protein, ligand-based virtual screening was performed using its substrate. We identified four substrate analogs (ZINC78106912 and ZINC86443272) with high docking energies, possible absorption via the gastrointestinal tract and blood-brain barriers, and also dependable drug-like properties. Hence these substrate analogs might be used as effective & potential drugs which leads to the better understanding and treatment of HGA.

Detection and Characterization of Local FMDV Isolates using One Step RT PCR

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Foot and Mouth Disease is known to infect animals which are cloven hoofed and considered as a contagious disease. The Foot and mouth disease in Pakistan is endemic, is caused by the Virus Foot and Mouth Disease serotype A, O and Asia-1. It has been reported that this disease has an outbreak rate in a continuous manner annually despite of regular vaccination. For an effective strategic vaccination program, detection and characterization of the prevalent serotypes caused by Foot and Mouth Disease Virus is now a crucial need. The

present study was designed to optimize a One-Step RT-PCR technique for the Genotyping of FMDV serotypes prevalent among cattle in Landhi Dairy Colony, Karachi for the selection of most appropriate vaccines to have an accurate knowledge of circulating virus serotypes in the country. One step RT-PCR was effectively established and optimized using the extracted RNA of reference Foot and Mouth Disease Virus for the genotyping of Foot and Mouth Disease Virus Serotype O with different PCR conditions, Volume of the Master Mix and thermal cycling conditions. One step RT-PCR was performed on 100 ELISA positive samples collected between the years of 2014 and 2015.Universal Primer Pairs IF/IR was used for the primary diagnosis of Foot and Mouth Disease Virus and serotype specific Primer Pairs were used for the Foot and Mouth Disease Virus serotype detection. Of the 100 field samples, 85% were Foot and Mouth Disease Virus positive. Out of 100, 85 samples were confirmed for Serotype O in the Foot and Mouth Disease Virus infected samples of the year 2014 and 2015. It may be concluded that the one step RT-PCR optimized in this study could be used for field's samples for the isolation and characterization of Foot and Mouth Disease Virus isolates. For a reliable and an effective Foot and Mouth Disease control program in Landhi Dairy Colony, we suggest introducing Foot and Mouth Disease control vaccination program on large scale for all cattle and buffaloes in Landhi dairy Colony annually.

Impact of Acute Febrile Infection (Malaria, Dengue and Typhoid) On Lab Parameters in CBC Report

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The clinical diagnosis of acute febrile illness such as malaria, dengue and typhoid, is challenging because of the non-specific and some overlapping signs and symptoms. Acute febrile illness is major health problem in tropical and temperate regions of the developing world which poses a significant burden on health expenditure. Accurate diagnosis is critical towards the effective management. The hematological abnormalities that have been reported to invariably accompany with these infections include anemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis. There have also been reports of leucopenia and leukocytosis in tropical regions. This is cross sectional hospital based study based on routinely used laboratory findings such as hemoglobin, RBC indices, white cell count, platelet count in screened patients with dengue by IgG, IgM or NS1, malaria by ICT and typhoid by typhidot. provide a diagnostic clue in a patient with acute febrile illness in single center trial. Total 1290 samples have been evaluated, 60 diagnosed with dengue, 100 with Malaria, 39 with typhoid fever. Hb, platelet and leucocyte were dramatically decreased in malaria as compare to other febrile infections while increase values of red cell distribution width were observed. In case of dengue fever raised values of lymphocytes, hematocrit with neutropenia and thrombocytopenia were reported. While patients diagnosed with typhoid showed that Hb, Platelet and leucocyte count may not be a good hematological marker for the diagnosis of typhoid.

Seroprevalence and Risk Factors Associated with Crimean Congo Hemorrhagic Fever Virus (CCHFV) in Human and Livestock Population in District Malakand

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Crimean Congo Hemorrhagic Fever (CCHF) is a viral zoonotic tick-borne disease mainly caused by CCHF virus and distributed in domestic and wild animals. The transmission of CCHFV is mainly occurs via bite of tick (Hyloma Sp) to human and animals. The present study was performed to determine the seroprevalence of IgG antibodies to CCHF in human and livestock's population and associated risk factors among both populations. A total 10 union councils were selected in District Malakand in which 54 households/ Farms were visited. Total 541 serum samples were collected from both populations. All farmers were extensively interviewed to identify major risk factors. A total 401 serum samples were screened for the presence of IgG antibodies in both Populations. A total 29% prevalence was recorded in livestock population. Highest ratio was detected in tehsil Batkhela (36.6%). Lowest ratio was recorded in tehsil Dargae. No positive sample was detected in farmers. Goat was highly exposed to CCHFV in present study (43%). Among the major risk factors of CCHF, it was observed that Hyloma ticks being circulating in all households are on the top. All individual was in closed contact with livestock. Most of the participants were unaware of CCHF and its transmission which increased risk of CCHF. On the basis of above facts, it is concluded that there is a high ratio of CCHF in livestock population. If proper preventive measures were not adopted on time, it may cause a huge outbreak.

Determination of Sub Inhibitory Concentration of Methicillin on Transforming *Methicillin Sensitive Staphylococcus aureus* To *Methicillin Resistant Staphylococcus aureus* By Giving Drug Doses

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The present study was conducted to evaluate the performance of cefoxitin disc diffusion method and oxacillin broth microdilution method for detection of methicillin resistant *S. aureus* (MRSA), taking presence of *mecA* gene as reference. In addition, inducible clindamycin resistance and beta-lactamase production were studied and minimum inhibitory concentration (MIC) of vancomycin for *S. aureus* isolates was determined. A total of 711 nonrepeated pus/wound swab samples from different anatomic locations were included in the study. The *Staphylococcus aureus* was identified on the basis of colony morphology, Gram's stain, and biochemical tests. A total of 110 (15.47%) *S. aureus* isolates were recovered, of which 39 (35.50%) isolates were identified as MRSA by cefoxitin disc diffusion method. By oxacillin broth microdilution method, 31.82% of the Staphylococcus aureus isolates were found to be MRSA. However, *mecA* gene was present in only 29.1% of the isolates. Further, beta-lactamase production was observed in 71.82% of the isolates, while inducible clindamycin resistance was found in 10% of *S. aureus* isolates. The MIC value of vancomycin for S. aureus ranged from 0.016 µg/mL to 1 µg/mL. On the basis of the absolute sensitivity (100%), both phenotypic methods could be employed for routine

diagnosis of MRSA in clinical microbiology laboratory; however, cefoxitin disc diffusion could be preferred over MIC method considering time and labour factor.

Development and Formulation of Rapid System for Rapid Identification of Medically Important Positive Cocci

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The rapid testing system, the purpose of my research study is to check the position or utility of a strain or a particular reaction or to isolate and identify the living beings that cause contamination. Conspicuous verification identity identification the realistic usage in fact of motion standards to understand sure animals from others. To see the location or software of a stress or a specific reaction, or to isolate and understand the dwelling being that reasons contamination. The hugeness of small scale dwelling being in microbiological labs. The leader made subtleties for gram-terrible small scale dwelling being as a touch part regular with her responsibility due to the fact the growing anti-contamination obstruction of those microbes due to abuse of anti-contamination marketers moreover motive risky problems in sufferers diagnosed with human offerings and treatment. Distinguishing evidence of this collecting of microscopic organisms has had problems as maxims of the people from those gatherings of microorganisms are biochemically much less dynamic in correlation with the opposite collecting of microbes. This request will make use of the found out traces assembled from an in depth through obsessive studies center. The collected traces proper off the bat applied in unconventional method and furthermore are going to be implemented to Quick Testing Strips (QTS). These strips are going to be hired through maker and manual due to the fact it offers rapid outcomes. This evaluation surveys the infinitesimal lifestyles bureaucracy for the advancement of latest strips for gram tremendous microbes. Generally, the traces of Staphylococcus and Streptococcus have been incorporated. After the evaluation, a pair of traces out of the 17 tested fashionable consequences. These consequences will moreover be applied bacterial investigations inside the lab. The scraps might require extra valuation that's impossible due to the absence of your time.

Antibacterial Potential of Garlic and Moringa Extract on *Klebsiella* Isolates from Hospital Drinking Water.

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The usage of the Moringa Oleifera seeds for hospital water treatment has been documented in several half of the globe. This study was conducted to assess the quality of antibacterial activity of Moringa seeds extract against Microorganism isolated from hospital drinking water. The two hundred and fifty (ml) of the sample was collected in a sterile bottle from the hospital. Isolation of microorganism from the water sample was done by using SPC Method and MPN technique. The identification of the microorganism isolates was created by performing gram staining, biochemical test and other microbiological testing. The Moringa Oleifera seeds extract were used for screening of antibacterial activity of the extract against Klebsiella isolated from hospital drinking water. We have made the comparison between garlic and Moringa extracts. The finding of the study on the antibacterial potential property of the extract demonstrated that the extract possessed antibacterial effect against the isolate and the activity of the extract was examined. The sensitivity of the isolate to the extract of garlic and Moringa was

different. Higher activity was shown by garlic extract as compare to Moringa extract. Statistical analysis of the result showed that Klebsiella is more sensitive to the garlic extract with a zone of inhibition of 40mm while Moringa extract shows less sensitivity towards Klebsiella 36 mm among the strains. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties.
Keywords: Moringa oleifera; Garlic extract, Antibacterial activity, Klebsiella, Hospital water.

Emergence of Extensively Drug Resistant *Salmonella* Typhi, it's Clinical Manifestation and Treatment Outcomes in Children in Karachi

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Salmonella Typhi is one of the leading health problems in Pakistan. With the emergence of extensively drug resistant (XDR) *Salmonella* Typhi, treatment options are limited. In the present study, we have reported the clinical manifestations and the response to treatment of patients with XDR Typhoid fever. A retrospective study was performed at Children Hospital Karachi from January 2018-January 2021. The data was retrieved from the hospital record system. Clinical presentation and treatment outcome of patients were evaluated and analysis was performed using SPSS. A total of 1600 cases with febrile episodes were screened, out of which 100 were clinically suspected for typhoid fever. Sixty were culture confirmed typhoid cases whereas, ten were extensively resistant. Blood culture and sensitivity showed highest resistance toward penicillin's and cephalosporins whereas carbapenems showed sensitivity. Fever, vomiting and abdominal pain were the most common symptoms among typhoid patients. For treatment of XDR typhoid oral azithromycin and intravenous meropenem were used. Almost all patients recovered and there was no recurrence of symptoms in any of the patients following treatment with an average time to defervescence was 5-7 days.: The emergence of extensively resistant salmonella typhi is an alarming sign and a matter of serious concern for developing nations such as Pakistan, where antimicrobial surveillance is poor. So, children presenting with fever and gut involvement should be screened for typhoid and culture must be sent before starting antibiotics.

Prevalence of HIV, HBV, and HCV in Chronically Transfused Patients

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Transfusion transmitted viral infections (TTI's) such as HIV, HBV and HCV could be prevented by following standard operating protocols of safe blood banking. The aim of this study is to observe the occurrence of TTI's in chronically transfused patients. 1500 male and female were integrated from 1 year to 80 years along with multiple transfused patients. Data were collected from the Children Hospital Karachi Research Institute for Genetics, & Bone marrow Transplant and its branch Shamsi and Ansari Diagnostics. It was observed that the rate of HBV was 0.42%, HCV 1.1%, and HIV 0.14% in chronically transfused patients. However, the prevalence of HCV from age 20-40 found 2.4%, HBV 1.6%. In 40-60 age group rate of HCV and HBV were detected 1.7% and 1.06

respectively. The prevalence rate of HCV and HBV is 0.26% and 0.4%. respectively which were comparatively low in age group between 60-80 years. Followed significance difference ($p<0.03$) among all groups. However, no evidence of presence of HIV antibodies were observed in investigated group lies between 20-80 years. The prevalence of blood transfusion viral infections found not much high due to safe blood transfusion. Safe blood banking play crucial role in prevention of TTI's. Strict screening and precautionary measures could prevent the magnitude of the disease significantly throughout the country. Although nucleic acid testing is increasing in trend and more recommended for blood scanning but chemiluminescence technology is still sharing better scanning for safe blood banking practices in developing world scenario.

Identification, Characterization and Pathogenesis of the Type Six Secretion System in *Helicobacter Pullorum* Poultry Isolates

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Helicobacter pullorum (H. pullorum) is a relatively less characterized Helicobacter species that causes vibrionic hepatitis in chickens. The infection may be transmitted to humans via contaminated meat where it is associated with colitis, IBD (inflammatory bowel disease) and Crohn's disease. Although a few virulence factors in *H. pullorum* have been reported, recently research has shown presence of the Type VI secretion system (T6SS) in *H. pullorum*. Studies on T6SS from different bacteria show that it plays a major role in bacterial pathogenesis and adaptation. Hcp and VgrG are the important proteins which play a central role in the structural formation of the T6SS pilus, as well as act as effector proteins in certain bacteria. In this study, *H. pullorum* isolated from liver and caecum samples of broiler chickens showed the presence of T6SS pilus protein Hcp. We predicted the in-silico *Helicobacter pullorum* Hcp (*HpuHcp*) structure and identified *C. jejuni* Hcp (*CjHcp*) as it's nearest homologue. Analysis of the predicted structure shows several common bacterial hcp motifs especially the presence of unique microbodies C-terminal targeting signal domain in *HpuHcp* which was seen for the first time in *CjHcp.* This could indicate that Hcp is a structural protein as well secretory protein and also helps in bacterial internalization as it depolymerises the membranous actin by deamidation of the host cell. This finding is supported by the evidence ofincreased invasion of hepatocytes by Hcp Positive *H. pullorum* isolates.

Phytostimulatory Impact of *Bacillus* and *Serratia* spp. on *Zea mays* L. using hydroponic technique

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In the current era of pollution and environmental hazards, growing crops need excessive use of land and greater amount of chemical fertilizers, hydroponic cultivation technique can be strategically used for production of crops in less area to give better yields in large quantity within a short period of time and also in all seasons with the use of plant growth promoting rhizobacteria (PGPRs) as biofertilizers instead of chemical fertilizers which are non-environmental friendly and pose health problems. Thus the current study was aimed to evaluate the effect of five PGPRs i.e., *Bacillus tropicus* (S12), *Brevundimonas diminuta* (S5a), *Bacillus cereus* (So3II), *Serratia*

marcescense (S4c1), *Bacillus subtilis* (Mt3b) on physiological and biochemical parameters of treated *Zea mays* L. Two experiments were conducted under soil and hydroponic conditions. Results have revealed that bacterial inoculum significantly increased several plant characteristics (root length, shoot length, fresh weight) and biochemical parameters (chlorophyll content and protein content) in both soil and hydroponic conditions. Among all bacterial strains, *Bacillus cereus* (Sa3II) remarkably increased growth parameters of plants grown in soil experiment and biochemical parameters of plants grown in hydroponic experiment. *Bacillus subtilis* (Mt3b) responded well under hydroponic conditions and increased plant growth parameters and give best results for increase in total chlorophyll content of plants grown in soil condition. So, these strains could be used as biofertilizers to increase growth of *Zea mays* L. in both soil and hydroponic conditions.

Evaluation of Indoor and Outdoor Fungal Pollution in University of Swabi

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The presence of airborne fungal spores plays a very important role to determine the future threat of mycotic infections as well as allergenic diseases. This study was designed to trap fungal spores from selected locations of the university main campus. A total of twenty-five sampling sites including 13 outdoor and 12 indoor were selected. The aerial fungal spores were trapped on Potato Dextrose Agar, used as culture medium. In this study, 15 fungal species belonging to 11 different genera were identified. Maximum colony count was in main canteen (30 colonies, 18.18%), and minimum colony count was one colony (0.60%). The study revealed that quantitatively *Alternaria alternata* was the dominant species as 61 colonies (36.96%) counted from all sites followed by *Aspergillus flavous (31 colonies, 18.78%), Alternaria solani (23 with 13.93%), Clydosporium herbarum* (19 colonies with 11.51%), *Geotrichum candidum* (7 colonies with 4.24%), *Alternaria brassicae, Aspergillus fumigatus and Helminthosporium solani* (4 colonies each with 2.42%), *Cochliobolus specifer (3 colonies with 1.81%), Curvularia lunata, Dreschleria specifer and Monilia spp.* (2 colonies each with 1.21%), *Fusarium oxysporum, Penicillium chrysogenum and Penicillium frequentans* (each have one colony with 0.60%). Variation was found in occurrence from site to site. No fungal species was isolated from all sampling sites. Outdoor air is comparatively more contaminated than indoor, and air of campus area is contaminated with some human pathogenic fungal spores that are linked with allergenic diseases and therefore it should be noticed. A detail study is recommended to evaluate aeromycoflora, and its seasonal occurrence to avoid any mycotic infection in campus area.

Isolation and Identification of Microbial Contaminants Associated with Commercial Poultry Feed

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Poultry feeds are food materials used in raising poultry birds. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat. The diseases of poultry is like the disease of other animals. They may be caused by pathogenic organisms, nutritional deficiency and from wound. This study was designed and carried out to determine the load of bacteria contaminating poultry feeds and source of feeds inside poultry farm and market feeds. The isolates were identified according to their cultural, microscopic and biochemical properties to the following gram negative bacteria as such as *Proteus spp*. It is concluded that poultry feeds, especially those inside farms are harboring potential pathogenic bacteria and fungi loads that are far above the acceptable levels, thus constituting a public health hazard and necessitate the application of the standard measures for production of feeds by manufacturers and health authorities. Therefore, the study recommends that hygienic production of poultry feed is a public health issue, proper treatment of feed ingredients and application of hygienic measures such as HACCP, starting from harvesting of feed ingredients to the storage, processing of feeds, packaging, transporting and eventually marketing of the bagged feeds is need of the hour.

A Survey and Evaluation of Breast Cancer Among Women in Local Area of Karachi

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Breast cancer is the propulsive cause of cancer mortality and morbidity around the world. The awareness of breast cancer is to energize monitoring of breast cancer for women. Females with family histories of breast cancer and older women have a much higher risk as compare to other women. Due to mutation in the genes there is the huge risk of breast cancer. The purpose of this study is to analysis the current conditions of awareness, prevention, precautions, advance detection techniques, management and symptoms of breast cancer. This is the priority for women with initial stage of breast cancer who may have never happening symptoms associate with the cancer undergo unneeded treatments. Sample size of our study is around 200. This was a cross-sectional study to evaluate the breast cancer and it is quantitative type of study. Half of the participants 62.5% did not know about the early diagnosis of breast cancer. Around 22.5% respondents knew the screening method of breast cancer. 7% women had done a mammogram, 9.5% of participants had a family history with breast cancer and 17% with other sorts of cancer like lungs, ovarian, intestinal cancer. Small number of participants of women have knowledge about the mammography which need to be performed once about two years after the age of 40. The study is to spread the knowledge about the breast cancer. The main motive is that early detection of breast cancer is important, breast self-examination require to address regarding breast cancer with more information spread through social media, seminars, participation in paramedics, welfare worker or public servant.

Prevelance of Antibiotic Resistant *Salmonella* Isolated from *Solanum Lycopersicum* Farms Nearby Poultry Farms

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Salmonella is the major food-borne pathogen associated with food products and causative agent of salmonellosis. Discharge of untreated wastes and leakage of poultry drainage in irrigation water might be the significant source of contamination in fields. The aim of this study was to investigate the presence of Salmonella in the rhizosphere and phyllo sphere of tomato following irrigation with ditch water contaminated with poultry drainage. Total 11 tomato fields in and around Faisalabad (Pakistan) were selected nearby the poultry farm area. Irrigated water, rhizosphere and leaves were analyzed for presence of Salmonella. A total of 130 samples were collected from different fields. Samples were cultivated on SS agar media and incubated at 37^{o}C. Out of 130 samples, 38 showed positive growth for bacterial contamination. 24 isolates were confirmed as *Salmonella* by morphological and biochemical characteristics. Our results indicated the presence of Salmonella isolates from irrigated water (n=13), from rhizosphere (n=7), from phyllo sphere (n=2) and from roots (n=2). Antibiotics susceptibility pattern of Salmonella isolates against routinely used antibiotics had indicated that 71% isolates were resistant to tetracycline and Amikacin, 61% resistance to Cefuroxime. All the isolates were sensitive to levofloxacin, and tobramycin. From obtained result it is confirmed that *Salmonella* spp. have been found in irrigation water mixed with poultry drainage and could be a source of salmonella contamination to the crops located near the poultry farms.

Keywords: Drainage, irrigated, Phyllo sphere, rhizosphere, Tomato

Bio-Plastic from Microalgae: The Potentials to Develop Biodegradable Face Masks to Combat COVID-19 Exacerbated Plastic Pollution

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Amid COVID-19, usage of face mask intensified as it is considered as the primary protection against SARS-CoV-2 virus to prevent the spread and aerosols. However, discarded face masks can potentially cause irretrievable damages to marine and terrestrial ecosystem. Millions of contaminated face masks are unsafely dumped in the waste each day posing a major environmental menace. Environmentalists have estimated that these facemasks will approximately take 450 years to break into micro-plastic. The use of non-toxic and organic facemask is inevitably required to overcome COVID-19 emerged plastic pollution. The study is proposed to develop biodegradable facemasks by using microalgae produced bio-plastic. Microalgae is rich in polysaccharides, lipids and proteins while cellulose is the most abundant polysaccharide that can be obtained from microalgae to produce organic fabric. Ligno-cellulosic fibers, cellulose nano-fibers, and regenerated cellulose films need to be contemplated to initiate facemask production due to their sustainability biodegradability and renewability. Present study focused on the microalgal source of cellulose that can be utilized to produce cost-effective, eco-friendly and biodegradable face masks. Pakistan shares southern territory with Arabian Sea which makes it enriched with

marine microalgae. These include *Spirulina platensis, Synechocystis sp., Nannochlorum sp., Nostoc sp., Synechococcus sp.* and various other microalgal species are aquatic inhabitants of Pakistan. Beside marine algae, Pakistan has diverse fresh water algal flora that washed off without being explored. Indigenously produced face masks by consuming natural or engineered fermented microalgae could be beneficial for small industries as well as to sustain country's GDP.

Sero-Prevalence and Molecular Characterization of Brucellosis Caused by *Brucella* Species in Goats and Humans

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Brucellosis is a major zoonotic threat to public health due to high contagious nature caused by various species of the genus *Brucella*. Livestock play a significant role in economy and employment of a country. *Brucellosis* have negative impact on the production of goats and also cause zoonotic *Brucellosis* in human having close contact with goats. In spite of its vital importance, there are limited comparative studies available on brucellosis in goats and humans in Pakistan, especially in Swat. The current study showed sero-prevalence of *Brucellosis* in humans and goats based on Rose Bengal Plate Test (RBPT) and Serum Plate Agglutination Test (SPAT) were determined, the results were also confirmed by species-specific PCR. A total of 24.00% samples were positive for *B. abortus* and 11.33% were positive for *B. melitensis* in human samples. In samples of goats, 26.66% were positive for *B. abortus* and 16.66% were for *B. melitensis* respectively. In age-group wise sero-prevalence, the maximum prevalence of brucellosis was recorded for 21–30 age-group (17.33%) followed by 11–20 age-group (10.67%). The least prevalence was recorded in age-group 31–40 (7.33%). There was no significant difference (as $P>0.05$ for all parameters) noted for both human and goat samples sero-prevalavce of brucellosis. The species-specific PCR confirmed *B. abortus* in 24% human samples and 26.66% goat samples by targeting the IS711 locus in the genome by obtaining an amplicon size of 498bp. The remaining sero-positive samples (11.3% human and 16.66% goats) were confirmed as *B. melitensis* by obtaining 731bp amplicon size. The 16S rRNA gene sequencing showed closed relationship with *Brucella abortus* NG-DG-01 (MZ020786.1), *Brucella abortus* strain IQ-milk No.2 (MN005940.1), *Ochrobactrum pectoris* strain T33M6 (MT367749.1) and *Ochrobactrum anthropic* strain DP5 (MT534533.1). The tree for human samples showed a closer relationship with *Brucella abortus* RB51-AHVLA strain RB51 (CP046720.1).

Prevalence and Economic Losses of Unnoticeable Bacterial Pathogens in The Raw Milk from Dairy Cattle with Subclinical Mastitis in Pakistan

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Mastitis is reported as the most common disease in dairy cattle. This disease manifests in two forms i.e. clinical and subclinical mastitis. Subclinical mastitis (SCM) affects the quantity and quality of milk though the animal is

apparently healthy. Thus, without any obvious symptoms, the diagnosis of subclinical mastitis is a challenge in dairy cow management and veterinary practice. Therefore, this study was designed to assess the prevalence and economic losses of unnoticeable bacterial pathogens in raw milk from cows with sub-clinical mastitis in different regions of Pakistan. We analyzed and collected data from various published research articles from 2005 to 2020, showing prevalence of subclinical mastitis and bacterial pathogens found in raw milk. A number of bacterial species such as *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Escherichia* coli, *Klebsiella spp*., and *Bacillus spp.* are reported as common causative agents of bovine mastitis. Our results show that the most important causative agent of subclinical mastitis amongst gram-positive bacteria is *Staphylococcus aureus* which is recorded approximately 40%, followed by *Streptococcus agalactiae 25%,* and a less severity is seen in *Escherichia* coli, Klebsiella, and *Streptococcus dysgalactiae* species. SCM causes loss to the dairy farmers in terms of reduction in milk quality and quantity, as well as losses associated with treatment and management etc. It also poses risk to humans from mild to life threatening ones; such as pneumonia, food poisoning, and several skin infections. Mastitis causes $35 billion loss to the world dairy industry. There are no current documented records of mastitis incurred to dairy industry in Pakistan. However, there is one study conducted in 1978 that has recorded a total economic loss of PKR 240 million per year in Pakistan. This study summarizes that SCM is an important disease of animal health, welfare and economic concerns and should be communicated to dairy farmers for improved hygiene management at farms.

Baby Skin Care Products a Potential Source of Infection

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Skin behaves as a natural immune barrier in neonates as the immune system of newborn is not fully furnished so, skin has to provide additional support to the baby, and it is more permeable and sensitive towards the environment. Weak immune system increases the risk of developing infections. In this connection the use of bacterial contaminated skin care product if it is manufactured under septic condition or by improper handling of batches during product processing makes the neonatal skin more prone to infections. Our study was focused on the presences of bacteria in skin care products and were examined in 25 baby products of different brands such as Baby mild, Johnsons baby, Mother care, Pigeon, Disney and Aveeno For this purpose we have cultivated these samples on Mannitol Salt Agar(MSA) agar. The results showed that the total % ratio of cultivated organisms is 20% whereas, 80% of product is free of contamination. Which includes 4% *Bacillus anthracis*, 8% *Staphylococcus aureus*, 8% *Streptococcus pneumoniae*, 28 % other *Streptococcus species* and 52% *Enterococcus specie.* As the result shows high amount of bacterial contamination in baby skin care products so, the authorities must pay attention to determine whether the aseptic conditions are applied during product processing and packaging or not and the product should be properly tested before being commercially available to minimize the risk of baby skin infections.

Preliminary Investigation for The Extraction of Lactase Enzyme from Lactobacillus for Lactose Intolerant Children

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The enzyme lactase is extensively present in nature such as in animals, plants and microorganisms. This research was conducted to report the isolation and extraction of lactase enzyme. Here in this study microbial source is used to produce lactase enzyme isolated from the simplest source i.e., fermented yogurt. Nine yogurt samples were taken from a local mart and various tests were performed to screen for economically important lactase enzyme producers. Upon biochemical test the organism showed negative catalase test. Further confirmation is done by streaking the organism on mMRS-BCP media and it turned from purple to yellow. Lactase enzyme is purified by three-phase partitioning method, the simple, quick and productive and usually one-step process. After the extraction and purification of enzyme, enzyme assay was performed to check the presence of enzyme on skim milk agar. The most important function of lactase is to breakdown the milk sugar lactose into glucose and galactose. The problem with the lactose sugar is that they cannot be hydrolyzed by many i.e., lactose-intolerant persons, as they lack the enzyme lactase. The main purpose of this study is to reduce the rate of lactose intolerant complications in children so they could enjoy same pleasures in life as lactose tolerant. Our study needs further animal testing and studying the animal response to our candies and as well as to check the candies modification in the animal physiology. The proper funding for our research can bring a new dimension to the lactose intolerant individual.

Escherichia ferugosni Isolated from Paharnag Drains Alleviate Cadmium (Cd) Stress Response by their Antioxidant Potentials

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Cadmium is highly toxic heavy metal that frequently contaminate the ecosystems thus posing hazards to the living species in such metal contaminated environment. In this study, the isolation and characterization of potent heavy metal resistant bacterial strain E. ferugosni was done from the polluted soil in Bawa Chak, Faisalabad. The selected bacterial E. ferugosni) strain has a minimum inhibitory concentration of 35mM for cadmium. E. ferugosni was cultured in MSM over a range of incubation parameter such as time (24 to 72hrs) pH (7.0), and temperatures (28-°C) in order to optimize the flocculant production. Surfactant production was during 24 hrs of incubation (97.62%), 48hrs (96.63%) and 72hrs (95.96%) respectively. Fourier transform infrared analysis (FTIR) of the biofloculant produced by the isolate showed the presence of carboxyl, hydroxyl and amino groups characteristic of polysaccharide and protein. In the present study antioxidant enzymes profile of isolated strain was measured. When E. ferugosni was subjected to stress of 15Mm Cd, the production of antioxidant enzymes varied such as SOD (118%) and APOX (28%) were increased while, CAT (86%), GST (13%), and POX (8%), decreased as compared to control.

Plant Growth Promoting Impact of Various Bioformulations On *Zea Mays* L.

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Plant growth promoting Rhizobacteria (PGPR) enhance plant growth and yield by using various phenomena like nitrogen fixation, phytohormone production and antibiotics as well as siderophores production. In this research work, bioformulations of four already isolated PGPR strains *Enterobacter* sp. (A7B), *Bacillus* sp. (AB8), *Enterobacter* sp. (A9G) and *Enterobacter* sp. (AM10) were prepared using organic carrier materials i.e., wheat bran and rice husk. The phytostimulatory activity of these cost effective bioformulations were monitored in various concentrations with soil on the growth of *Zea mays* L. A significant improvement was observed in growth (shoot length, root length, leaves number and fresh weight) as well as in biochemical parameters (protein content and chlorophyll content) of treated plants in all concentration treatment especially in 50% concentration of bioformulations with soil as compared to control treatment. Thus, the production and application of eco-friendly and cost effective bioformulations can be helpful to control environmental deterioration by reducing pollution and also will be helpful to support the agriculture industry and economy of developing countries like Pakistan.

Molecular Detection of ESBL-Producing *Klebsiella pneumoniae* Isolated from Mastitic Milk in Peshawar

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Klebsiella pneumoniae is a gram negative opportunistic bacterium which drives a number of diseases in both animals and humans including mastitis. Mastitis is the inflammation of mammary gland of animals. A major problem that is increasing day by day is the frequency of resistance produced by extended spectrum beta lactamases (ESBLs) Producing-K. Pneumoniae all over the world, due to ESBL-producing gram negative bacteria these B lactam antibiotics have no affect and become antimicrobial resistant especially in bovine mastitis. The main objective of this study is to isolate and identify the K. pneumoniae from mastitis milk and also to find out the molecular frequency of ESBL genes. We isolated a total of 100 milk samples from animals having mastitis from different areas of district Peshawar for screening of K. pneumoniae. Firstly 23(23%) K. pneumoniae were recovered after that ESBL resistance were checked among which 13 (56%) samples were confirmed as ESBL producing K. pneumoniae. The confirmed ESBL resistant K. pneumoniae were further checked for their antibiotic sensitivity against various classes of antibiotics. That showed highly resistance to Ampicillin, Vancomycin and Fusidic acid was (100%), while highly susceptibility was found to Amikacin, Azithromycin (100% each), Chloramphenicol (85%) and Tetracycline (80%). Also the ESBL positive K. pneumoniae were checked through PCR for the genes responsible for ESBL resistance that is blaCTX-M, blaSHV and blaTEM. All the three genes were detected in our isolates, according to our findings the percentages of these genes were blaCTX-M (24%), blaSHV (61%) and blaTEM (40 %) respectively. So in our study it is concluded that selective pressure for propagation as well as the occurrence of resistant isolates is applied due to the prolonged and common use of antibiotics.

Prevalence of Antibiotic Resistant Staphylococcus *Aureus* in Milk Samples from Different Areas of Karachi, Pakistan

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Antimicrobial resistance is an important health problem worldwide. *S. aureus* is reported to be one of the most common causative agents of food poisoning associated with the consumption of raw milk and milk products. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of food. As such, food products such as milk, cheese, yoghurt and other dairy products have been implicated as potential sources for the transmission of the pathogen to humans. Moreover, foods contaminated with antibiotic resistant bacteria represent ideal vehicles for the transmission of antibiotic resistant strains. The study was designed to determine the occurrence of *S. aureus* and MRSA strains in milk products obtained from some supermarkets, shops and farms of Karachi. Moreover, the possible health risks to consumers based on the presence of antibiotic resistance profiles of the isolates were also investigated. To carry out this study 60 samples from different areas of Karachi were collected. Sample were diluted 10 folds and plated on nutrient agar Isolated strains were identified on the basis of morphology and biochemical testing. Furhermore, they are tested for coagulase, catalase and hemolysis. About 60% isolates were coagulase positive. All the isolated *S. aureus* strains were tested against ampicillin, ceftriaxone, Trimethoprim, Cefixime, Ciprofloxacin, chloramphenicol and azithromycin antibiotics and resistance profile was developed.

Estimation of Microbial Load of Chemical Laboratory Quality Control of Saffron Pharmaceutical Private Limited

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S. aureus is an opportunistic bacteria; that is present as a normal flora on skin and is also present in upper reason of respiratory system. Staphylococcus aureus has entered the spotlight that causes a wide range of clinical infections and has become a leading cause of nosocomial and hospital-acquired infection. This study is designed to check the sterility of chemical laboratory sections of Saffron pharmaceutical whether the bacteria are associated with a defined type of environment and affect pharmaceutical production. For this purpose, Staphylococcus aureus cultures were isolated from environmental exposure to a chemical laboratory. They proceeded to identify Staphylococcus aureus by sub-culturing on different media and identified through standard methods using a gram

staining test. The result showed that the washing and record room had uncountable bacterial colonies, but other sections included balance room (056), instrument room (112), HPLC room (049), officer's room (035), and chemical hall no 1-2-3 (127, 098, 107) had observed different no of bacterial colonies and these bacterial colonies could make the pharmaceutical products more hazardous and objectionable. This study emphasizes the importance of decontamination and prevention from microbial contamination while performing lab experiments, clinical trials and diagnostic procedures.

From Waste to Value Added Product: Biosynthesis of Silver Nanoparticles Using Spoiled Fruit Extract

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Nanotechnology is an interdisciplinary field of study that incorporates key techniques from diverse fields such as chemistry, engineering, physics, and biology to develop unique methodologies for manipulating minute particles which results in the formation of nanoparticles (NPs). In recent times, the nanoparticles synthesized from plants are getting more attention because of its simplicity. In the synthesis of nanoparticles, biomolecules in extracts can act as both reducing and stabilizing agents. Although there are a variety of ways to synthesize nanoparticles but there is a growing demand for high yield, non-toxic, cost friendly, and ecologically acceptable methods. Therefore, we designed a green approach for the synthesis of AgNPs using spoiled fruit extract as a reducing agent and stabilizer in this work, as well as optimized the parameters that affect NP synthesis. For this study, fruit extracts were prepared by cutting the fruits into small pieces and mixed it with deionized water and heated for 1 h at 80°C. After heating, the extract was filtered, and filtrate was used for the synthesis of nanoparticles. The prepared fruit extract was added drop by drop into 18 mL aqueous AgNO3 solution. The mixture was stirred and heated at 80°C. To obtain maximum production of silver nanoparticles, 2 parameters were optimized: volume of extract (5, 10, 15, 20 and 25 mL) and concentration of AgNO3 (0.025, 0.05, 0.1, 0.2, 0.4 M). Results showed that in apple extract, nanoparticles produced within few minutes by giving the absorbance of 4.0. However, in banana extract the synthesis of nanoparticles was gentle. 0.1M of AgNO3 showed 0.498, 0.525, 0.523, 0.514, 0.470 absorbance at 380 λ, 400 λ, 420 λ, 440 λ, 460 λ wavelength respectively, whereas, 10 mL of volume of extract showed 0.705, 0.782, 0.835, 0.863, 0.850 absorbance at 380 λ, 400 λ, 420 λ, 440 λ, 460 λ wavelength, respectively. Therefore, the conclusion can be drawn that spoiled fruit extracts can be used for the synthesis of nanoparticles.

Effect of Hydrogen Sulfide On the Alleviation of Heavy Metals Toxicity in Spinach, Irrigated with Sewage Water from Malir River, Karachi

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Farmers are using the polluted Malir river water as a source of irrigation for their plant crops in Karachi, Pakistan. The amount of Cu and Fe are moderately higher in Malir River, Karachi. Heavy metals are highly toxic compounds that if consumed, can be harmful to human health. In this proposed study, experiments will be carried

out in order to examine effects of H2S gas on the alleviation of Copper (Cu), Arsenic (As) and Iron (Fe) present in Malir river water. In this study, NaHS will be used as H2S donor for the wáter treatment and then this treated wáter will be used for irrigation of spinach. The concentartion of heavy metals (Cu, As, Fe) before and after treating sewage water with H2S will be monitored by atomic absorption spectroscopy. Use of NaHS for the treatment of sewage wáter will result in improve germination rate and will enhance the plant growth.

Extraction of Lignin, Hemicellulose and Cellulose from Phragmite Karka & Conversion of Extracted Cellulose into PVA Based Porous Composite/Methylcellulose

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Halophytic biomasses are an abundant source of polysaccharides as they have a boundless supply of carbohydrates, which could be thought as the ultimate substrate and can be renewed into valuable chemicals and compounds. At present, saline soil and scarcity of agricultural fields are the most important non-biological worries. The cultivation of halophytes can serve as ideal feed stock and reasonably profitable biomass without interfering with the agriculture lands. *Phragmite karka* (PK) is reported as the herbaceous woody culms that is composed of 26% cellulose, 29% hemicellulose, and 10.33% lignin. The proposed study is about the chemical extraction of lignin, and hemicellulose and, finally, the isolation of cellulose fibers from PK. Different cellulose derivatives have been produced from the chemically purified cellulose. Etherification of cellulose is one of the most important routes of cellulose derivatization and methylcellulose is one of the important cellulose ethers. Methylcellulose presents an increase in thermal stability and solubility in water associated with the increase of the degree of substitution (DS), which improve the commercial applicability of the polymer. Several products of considerable commercial importance can be developed from methylcellulose. For example, it may be used as thickener in the food industry, as matrix for controlled release of drugs in the pharmaceutical industry as admixture for concrete in civil construction. Therefore, the extracted cellulose will be converted into methylcellulose. Synthesized methylcellulose will enhance the value to this abundant halophytic plant, *Phragmite karka* and may extend its range of biomedical applications.

Honey: A Natural Compound to Treat Eye Related Diseases

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Due to the anti-inflammatory and antimicrobial properties of honey, combined with it's soothing abilities make it a surprisingly effective ointment for several eye conditions. Therefore, we can use this natural ingredient to overcome many eye problems rather than expensive eye treatments or major operations as honey has no side

effects. Topically applied honey can reduce inflammation and irritation in eye. It can also kill harmful bacteria that could be causing an eye infection. Honey is a mixture of carbohydrates, proteins, aminoacids, vitamins, minerals, antioxidants and other compounds. Honey also contains eighteen free amino acids, the most abundant of which is proline. In this proposed study, honey will be used to treat different eye conditions. To treat eye infection, honey will be boiled with water and then will be used as an eyewash. Honey will be mixed with carrot juice and consumed regularly to improve the eye sight. Furthermore, Organic Manuka honey will be diluted with distilled water in 1:5 ratios and will be used to change the color of eye. This study will provide a natural and safe way to treat eye related diseases.

Use of Cacti and Blueberry for The Formation of Berrycact Syrup: A Healthy Juice

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Cacti have been used by ancient civilizations for centuries to cure diseases and to heal wounds. *Cactus cladodes*, fruits, and flowers have been traditionally used in folk medicines in several countries. For Example, Nopal cactus is famous for offering health benefits due to its high antioxidant, vitamin, and mineral content. It is used as a medicinal plant in various countries and grows in the desert, semi-desert, tropical and sub-tropical areas. Blueberries are a very popular, tasty fruit. They're low in calories and incredibly healthy, potentially regulating blood sugar levels and aiding heart and brain health. They are mainly used for flavoring purpose. The berrycact syrup is a blend of extracts from the pads or cladodes of nopal cactus, fused with blueberry juice. The necessity of the berrycact juice is due to the nutritional value of cladodes of nopal cactus and the blueberry fruit which are rich in vitamins and minerals, act as natural fat blockers, can reduce serum low-density lipoprotein-cholesterol, and can reduce blood glucose levels in hyperglycemic individuals by increasing insulin sensitivity. Thus, there is a need for new dietary or nutritional food products containing natural ingredients which are effective to target different health issues and our berrycact juice is solution to those issues. Clinical trials will further ensure the efficacy of the juice.

Statistical Optimization of Bioremoval of Malachite Green by Immobilized Trametes Pubescens (MB89)

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Textile industry is a major consumer of synthetic dye which have relatively high stability and can lead to environmental hazards due to their toxic and carcinogenic nature. Malachite Green, a dye used in textile industry, is a triphenylmethane dye and is resistant to fading upon exposure to light and water. The most efficient microorganisms in breaking down synthetic dyes are the white-rot fungi (WRF) and are key regulators in the global C-cycle. Therefore, this study was designed to statistically optimize the process of bio-removal of malachite green dye by WRF. In this study, fungal culture *Trametes Pubescens* (MB89) was used for the degradation of malachite green. The fungus was immobilized on coconut coir and then immobilized fungi was

used for dye removal. *Trametes Pubescens* (MB89) produced 556.8 IU L laccase enzyme. Lacasse can degrade the aromatic structure of dye. Furthermore, dye removal process by immobilized fungi was statistically optimized by using central composite design. In central composite design three factors (temperature, reaction time and initial dye concentration) were optimized. Results showed that ~ 99% dye was removed under optimized conditions (temperature 30.9℃, reaction time 59.27 h, initial dye concentration 0.006%). Thus, bioremediation has proved to be an effective method to get rid of toxic dyes from the textile effluent.

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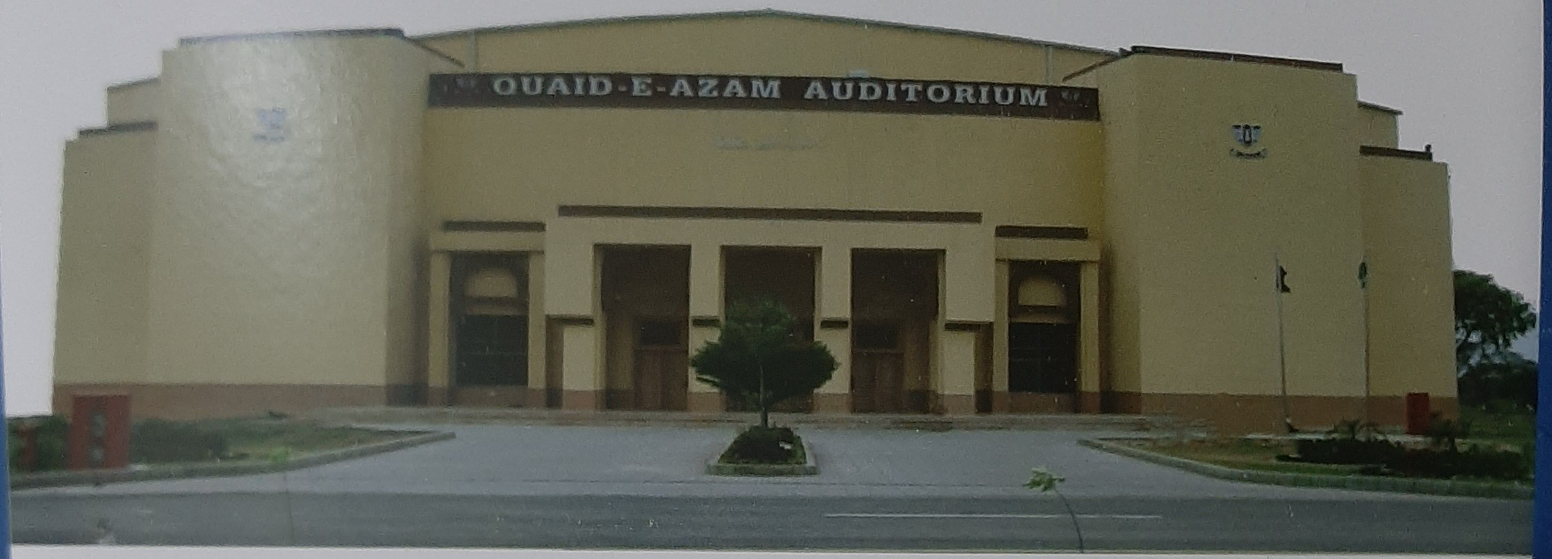

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