ORIGINAL ARTICLE

PAKISTAN JOURNAL OF MICROBIOLOGY
Official journal of Pakistan Society for Microbiology (PSM)

Prevalence of mupirocin resistant (*mupA*) gene in *S. aureus* isolates from two tertiary care hospitals of Punjab

Raza-e-Mustafa[1], Muhammad Shaheen Iqbal[*,1,2], Farheen Ansari[1], Muhammad Sohail[3], Karam Rasool[4], Muhammad Omer[1]

[1] Institute of Molecular Biology and Biotechnology University of Lahore, Pakistan
[2] Department of Bioscience, Comsats University Islamabad, Pakistan
[3] Department of Medical Lab Technology, Faculty of Rehabilitation and Allied Health Sciences, Riphah International University Islamabad, QIE Campus Lahore, Pakistan
[4] Chugtai Lab Jail Road Lahore, Pakistan

***Corresponding author:** Muhammad Shaheen Iqbal
Department of Bioscience, Comsats University Islamabad, Pakistan
Email: shaheen.microbiologist@gmail.com

ABSTRACT

Introduction: Mupirocin is a topical ointment and de-colonizing agent, used to reduce the burden of *Staphylococcus aureus* from nasal nares. Unjudiciary use of mupirocin has resulted in the emergence of resistance mediated by a gene *mupA* found to be associated with high-level mupirocin (HLMup R) resistance. The objectives of this study were to find out the prevalence of MRSA/MSSA, *mupA* gene, and antimicrobial resistance among burn patients and outdoor patients.
Material and Methods: A total of 238 non-repetitive specimens were collected from nasal nares and burn wounds of patients in two tertiary care hospitals. *S. aureus* was isolated by conventional biochemical and molecular methods. Prevalence of resistance gene and antimicrobial susceptibility were identified by PCR and Kirby Bauer disk diffusion method respectively.
Results: The prevalence of MRSA was 13% (32/238) and MSS 29% (70/238). We screened 102 isolates for the *mupA* gene and found 21 MRSA and 12 MSSA with a total of 33 isolates carrying this gene and the p-value was 0.0001. Most strains were found to be MDR.
Discussion and Conclusion: MRSA strains are prevailing in hospital environments whereas *mupA* gene carriage rate shows that antimicrobial use of mupirocin to decolonize bacteria needs to be minimized. Antimicrobial stewardship and closed surveillance may be regulated.

Keywords: *S. aureus*, mupirocin, *mupA*, HLMR

Pak J of Micro 2021; 1(02):19-24. DOI: https://www.doi.org/10.5281/zenodo.5745327
(Received 01 March 2021 – Accepted 23 May 2021)

INTRODUCTION

Antimicrobial resistance is the paramount complication of overuse of antibiotics, on the way to achieving successful therapy of infectious diseases. The emergence of antibiotic-resistant strains especially MDR and XDR failed the current antimicrobial strategies (1). This situation has posed a serious global threat and is increasing gradually. *Staphylococcus aureus* is a notorious pathogen in hospitals as well as in community settings (21). It is resistant against most of the currently available antibiotics and is a resident of approximately 30 % of people in their noses (2). However, its carriage is also not safe and it may lead to a high risk of getting

infectious or different diseases. (3). The methicillin-resistant form called MRSA is frequently found inside hospitals among patients as well as health care workers (22). Mostly it is asymptomatic when colonizes human nostrils and is called hospital-acquired infection (HAI) (4).

Mupirocin (Bactroban; SmithKline Beecham Pharmaceuticals), an antibiotic is effective for the eradication of nasal carriage of *S. aureus* however its prolonged usage has resulted in the emergence of a contaminated object or inhaling infected droplets spread by sneezing or coughing. However, the bacteria can move through the bloodstream (called bacteremia) and infect nearly any site in the body, predominantly heart valves (endocarditis) and bones (osteomyelitis) (7). The bacteria also tend to amass on medical devices in the body, such as artificial heart valves or joints (8). Staphylococcus aureus infections range from mild to life-threatening diseases. Despite its role as normal flora of the skin, this bacterium tends to infect include colonial morphology, DNase, coagulase, catalase, and PCR reactions. This study has been designed to find out the prevalence of MSSA and MRSA and mupA genes in hospital patients. As mupirocin is a front-line antibacterial to remove or eradicate nasal bacteria, resistance against this drug is of major concern among health care professionals. Its use in patients of thermal injury is necessary to prevent invasion from opportunistic bacteria. The study is very important to know the prevalence of genes in bacteria and the pattern of antimicrobial resistance in Pakistan.

MATERIAL AND METHODS

The study was conducted at the molecular biotechnology laboratory Pakistan Council of Scientific and Industrial Research Labs (PCSIR) and diagnostic microbiology laboratory Civil Hospital Gujranwala. The samples were collected from two tertiary care hospitals namely Mayo Hospital Lahore and Civil hospital Gujranwala. Mayo hospital Lahore has a pediatric burn unit where the patients were having burn wounds. They were given common antibiotics to kill bacteria in wounds and were also administered with mupirocin ointment to lower the burden of MRSA colonization hence minimizing the risk of infection and invasion of MRSA. Similarly, patients with minor burn wounds visiting the outdoor patient department (OPD) were also included in the study and nasal samples of anterior nares were collected. The research was carried out between June 2016 to May 2017. Samples were collected precisely and transferred to the laboratory for isolation and characterization of S. aureus strains by conventional methods and molecular identification by PCR using mecA primer.

staphylococcal aureus strains with high and low levels of resistance. Mostly, low-level (MICs between 8 and 256 mg/ml) mupirocin-resistant strains are isolated, while high-level (MICs greater than 500 mg/ml) mupirocin-resistant strains are occasionally isolated (5). Staphylococcus aureus is the leading cause of surgical site infections (SSI) osteomyelitis, skin and soft tissue infections (SSTI), in health care and hospital settings the skin regularly causes abscesses. It spreads by direct contact with an infected person or via

Initial Identification and Screening of Bacteria

Samples were collected with sterile cotton swabs and isolation and characterization of S. aureus were carried out by conventional biochemical methods. However, MRSA strains were isolated both phenotypically by Cefoxitin (30ug) and molecular identification by PCR using mecA primer.

Antimicrobial Susceptibility Tests

Antimicrobial resistance was detected by the Kirby-Bauer disk diffusion method according to clinical laboratory standards institute (CLSI 2016) recommendations. Mupirocin resistance strains were isolated according to recommendations of CLSI by disk diffusion method and also by broth dilution method. In the disk diffusion method disks of 200µg were used and the zone of inhibition was measured after 24-hour incubation. Vancomycin resistance was measured and for this single E-test strip was applied to each plate with sterile forceps. These plates were incubated for 24 hours, MICs were determined as the point of interception of the zone of inhibition with the E-test strip. (11) All the isolates were screened for the presence of mecA and *mupA* genes by the use of Polymerase Chain Reaction (PCR). Primers for genes were selected from already published sequences having Gene Bank Accession numbers X52593.1

RESULTS

Out of 238 clinical samples, we obtained a total of 102 isolates of *S. aureus* organisms. A total of 63 and 175 specimens were obtained from outdoor and indoor patients respectively. All these specimens were analyzed and we isolated 16 from OPD and 51 from indoor patients in Mayo Hospital Lahore whereas 11 from OPD and 24 from admitted patients in Civil Hospital Gujranwala. From the table below it was shown that the maximum resistance was formed against penicillin, amoxiclav, tetracycline, and erythromycin whereas vancomycin was highly effective but its exact performance cannot be detected without the MIC test. The organisms were not inhibited by antibiotics when applied on a lawn of 0.5 McFarland suspension of microorganisms.

PCR results of MRSA and MSSA isolates of *S. aureus* were visualized on gel and identified

according to the molecular weight of the gene. According to this, there are 07 *mupA* positive genes from MRSA and 03 *mupA* positive MSSA representing burn wound specimens. Similarly, 14 and 9 isolates of MRSA and MSSA respectively were positive from nasal swab specimens. Hence we obtained a total of 33 *mupA* positive isolates. Fig indicate the position of mupA and mecA positive isolates.

DISCUSSION

Pathogenic microorganisms are becoming a big threat now as resistance against antibiotics is on its rise and nearly all antibiotics are losing effectiveness against them (21). The presence of MRSA in burn patients had already posed serious issues in clinical therapy which is now an immense problem. In this regard, mupirocin is widely used in burn patients in hospitals and in health care homes for the eradication of nasal bacteria (23).

In hospitals of some countries, it is used for the staff in order to decolonize MRSA from nasal nares. We studied both, MRSA burden and resistance against different drugs of common use and the presence of *mupA* gene which encodes mupirocin resistance.

Table no. 1: Sequences of oligonucleotides used in the research

Gene	Oligonucleotide Sequence	Size	Ref
mecA	F: GTAGAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	310 bp	(9)
MupA	F: TAT ATT ATG CGA TGG AAG GTT GG R: AAT AAA ATC AGC TGG AAA GTG TTG	456 bp	(10)

Table no. 2: Number of resistant isolates with percentage against antimicrobial drugs

	NO OF RESISTANT ORGANISMS		
ANTIBIOTICS NAME	**BURN WOUND**	**NASAL**	**TOTAL (n=102)**
Amoxi-Clav (AMC) 30µg	39	46	85 (82%)
Cefoxitin (FOX) 30µg	18	14	32 (31%)
Imepinim (IMP)	36	25	61 (60%)
Chloramphenicol (C) 30µg	21	16	37 (36%)
Ciprofloxacin (CIP) 5µg	43	26	69 (68%)
Clindamycin (DA) 2µg	29	31	60 (61%)
Erythromycin (E) 15µg	34	44	78 (76.5%)
Fusidic acid FD) 10µg	24	21	45 (44%)
Mupirocin (MUP) 200µg	23	10	32 (35%)
Penicillin (P) 10µg	43	54	102 (100)
Sulf/Trimeth (SXT) 25µg	36	22	58 (57%)
Tetracycline (TE) 30µg	41	50	91 (89%)
Vancomycin (VA) 30µg	03	00	03 (3%)

Table 3: *mupA* positive isolates in MRSA and MSSA strain

Category	*mupA* positive MRSA	*mupA* positive MSSA	TOTAL (%)
BURN WOUND	07	03	10 (21%)
NASAL SWABS	14	9	23 (42%)
Total	21	12	33 (32.3%)

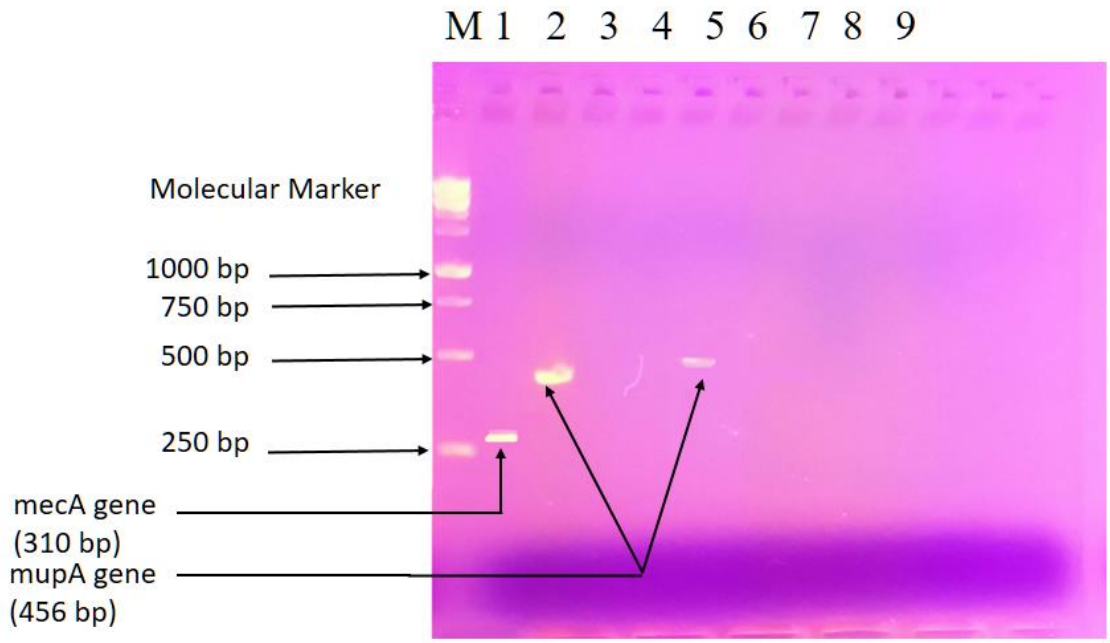

Figure:1 This picture depicts the PCR product on Agarose Gel electrophoresis analysis of *mecA* gene and *mupA* genes: Lane M shows molecular marker (range 250bp-1000bp); Lane 1: *mecA* gene (310 bp) Lane 2,5shows *mupA* gene product (456 bp) and lane 3, 4 are negative controls of wild type strains 25923; Lane 6, 7: negative control DNA free water.

Although many studies have been conducted to find out *mupA* genes in bacteria (12), these have reported different data based on different criteria set for research. However common thing among is the prevalence of *mupA* gene in pediatric patients in dermatology and burn units of hospitals. Most research has been conducted on mupirocin use in dermatology patients and skin and soft tissue infections (SSTI). However, we conducted our study on patients from dermatology burn wounds partially from the dermatology department and some were from burn patients.

According to Krishnan *et al* (2002), only one isolate was reported to be *mupA* positive from burn patients (12). In another study conducted by Canadian Nosocomial Infection Surveillance Program researchers, Andrew E *et al* isolated high resistant mupirocin (HLmup) strains from 198 *S. aureus* However there was an increase in this proportion from 1.6% in (1995-1999) to 7.0% for the duration of (2000-2004) (14). According to another study conducted on children at Texas children hospital USA, 20 isolates were resistant to mupirocin which were 14.7% however 11 % of strains were carrying *mupA* gene, the children were reporting for Skin and Soft Tissue Infections (SSTI) where the ratio of *mupA* was more common in MSSA as compared to MRSA strains (15). One study resembled to be matched with our results of *mupA* prevalence as recently conducted in Pediatric Dermatology Division New York City 31.3% patients were carrying *mupA* gene (16). One study from surgical intensive care unit mupirocin resistance was 13.2% out of 302 isolates of MRSA where 8.6% were high-level resistance using MIC method (17). Similarly, 14.1% *mupA* positive isolates were recovered from 193 MRSA strains in a dermatology center in Korea (18). A recent study conducted by Antonov NK *et al* (2015) from a pediatric care center can be similar to our study concerning the percentage of *mupA* gene presence where they identified 31.3% *mupA* positive isolates from 358 collected isolates. The children were being treated for skin and soft tissue infections (SSTI,s).

As we collected a total of 238 specimens from two hospitals where burn wound patients are treated. Mayo hospital Lahore is a pediatric burn unit where children are kept for treatment. The patients were on various antimicrobial and antiviral drugs as well as topical ointments and were also prescribed with mupirocin ointment to reduce the burden of colonized bacteria. This is a strategy to minimize the infection which might be prevailing in the hospital environments with healthcare-associated MRSA. Similarly, patients in Civil Hospital Gujranwala had also been prescribed mupirocin ointment to combat hospital-acquired infection of MRSA. We know that there are also other opportunistic bacteria present in the environment that may be isolated but the scope of

this was only to isolate *S. aureus* harboring genes for mupirocin resistance. Hence we only isolated MRSA and MSSA from the specimens. But there are various reports about Coagulase Negative *S. aureus* (CoNS) which also carry this gene (19). The rate of indoor isolates of infectious *S. aureus* was 75 which is three times higher as compared to outdoor isolates where there were 27. This shows how hospital-acquired MRSA is common in hospitals and this burden is a great obstacle in the treatment of burn wounds. Since the use of ointment of mupirocin and resistance against this are both increasing, there is a need for a rapid, accurate test for high-level mupirocin resistance in the clinical laboratory. To our knowledge, this is the first report of the prevalence of *mupA* genes detected from Pakistan which encode high-level mupirocin resistance that was tested on a significant number of clinical isolates using the disk diffusion method. Our results showed a huge resistance profile against commonly used antibiotics by *S. aureus* and especially MRSA strains which we isolated in this study. B-lactam antibiotics which had already lost efficacy against MRSA are useless and is reflected by our antibiogram profile. The last hope in the antibiotic lineage is vancomycin however it also has lost some effectiveness and we found 03 strains resistant against this antibiotic. Penicillin showed ineffective against 100 percent isolates including MSSA, 82% of isolates were found to be resistant against amoxiclav (AMC) 30µg, 89% against tetracycline, 57% against sulf/trimeth (SXT), and 77% isolates showed resistance against erythromycin (E) antibiotics. The rate of resistance is increasing day by day and antibiotics are losing effectiveness.

Hospital-acquired infections (HAI) are now more prevalent as compared to the past. Health professionals are the key source of *S. aureus* reservoirs which is a danger to infection control. In the presence of these strains recovery of burn, patients may be prolonged and the cost of cure may increase which affects financial as well as health infrastructures.

If we compare our study with other studies in the region we can say that burden of MRSA and infectious bacteria prevailing in the health care environment are the big problem in the way of antimicrobial therapy courses.

In our study, there are many limitations that we are going to mention here. First, we only used the disk diffusion method to identify mupirocin resistance *S. aureus*. If we use minimum inhibitory concentration (MIC's) there are three states of mupirocin susceptibility have been described against colonizing *S. aureus*. These are mupirocin susceptibility with minimum inhibitory concentrations (MICs) 4 µg/mL, low-level mupirocin resistance with MICs from 8 to 64 µg/mL, and high-level mupirocin resistance with MICs 512 µg/mL (20). However, we only determined high-level mupirocin resistance instead of using minimum inhibitory concentration (MIC) and used antimicrobial disks of 200ug whereas on the other side to accurately identify resistance isolates we also used the PCR method for *mecA* gene and *mupA* genes which encode resistance.

CONCLUSION

Findings obtained from the study suggest minimizing the use of antibiotics such as mupirocin in order to stop dry resistant strains from emerging. Closed surveillance against antimicrobial resistance bacteria should be carried out in order to prevent future outbreaks and obstacles for successful therapy.

REFERENCES:

1. Iqbal, M. S., Saleem, Y., Ansari, F., Qamar, M. U., Mazhar, S., Hassan, A., ... & Syed, Q. (2018). Staphylococcus aureus carrying lukS/F Panton-Valentine Leukocidin (PVL) toxin genes in hospitals of Lahore city. *The Journal of Infection in Developing Countries*, *12*(09), 720-725.
2. Ringberg H, Petersson AC, Walder M, Johansson PJH. (2006) The throat: an important site for MRSA colonization. Scand J Infect Dis 38:888–893.
3. Foster, T. J. (2005). Immune evasion by staphylococci. *Nature reviews microbiology*, *3*(12), 948-958.
4. Lobdell, K. W., Stamou, S., & Sanchez, J. A. (2012). Hospital-acquired infections. *Surgical Clinics*, *92*(1), 65-77.
5. Shittu, A. O., Kaba, M., Abdulgader, S. M., Ajao, Y. O., Abiola, M. O., & Olatimehin, A. O. (2018). Mupirocin-resistant *Staphylococcus aureus* in Africa: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control*, *7*(1), 1-16.
6. Alicia, I. H., Edwards, J. R., Patel, J., Horan Teresa, C., & Sievert Dawn, M. (2008). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control and Hospital Epidemiology*, *29*, 996-1011.
7. Lowy, F. D. (1998). Staphylococcus aureus infections. N. Engl. J. Med.
8. Litzler, P. Y., Benard, L., Barbier-Frebourg, N., Vilain, S., Jouenne, T., Beucher, E., ... &

Bessou, J. P. (2007). Biofilm formation on pyrolytic carbon heart valves: influence of surface free energy, roughness, and bacterial species. *The Journal of thoracic and cardiovascular surgery*, *134*(4), 1025-1032.

9. Zhang K., McClure J.-A., Elsayed S., Louie T., Conly J. M. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. Journal of Clinical Microbiology. 2005;43(10):5026–5033. doi: 10.1128/jcm.43.10.5026-5033.2005
10. Anthony, R. M., A. M. Connor, E. G. Power, and G. L. French. Use of the polymerase chain reaction for rapid detection of high-level mupirocin resistance in staphylococci. Eur. J. Clin. Microbiol. Infect. Dis1999. 18:30-34.
11. Simpson I N., Gisby J, Hemingway C P., Durodie j, and Macpherson I. Evaluation of Mupirocin E-test for Determination of Isolate Susceptibility: Comparison with Standard Agar Dilution Techniques. Journal of Clinical Microbiology, 1995. p. 2254–2259.
12. Khoshnood, S., Heidary, M., Asadi, A., Soleimani, S., Motahar, M., Savari, M., ... & Abdi, M. (2019). A review on mechanism of action, resistance, synergism, and clinical implications of mupirocin against Staphylococcus aureus. *Biomedicine & Pharmacotherapy*, *109*, 1809-1818.
13. Krishnan P U, Miles K, Shetty N. Detection of methicillin and mupirocin resistance in *Staphylococcus aureus* isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA J Clin Pathol 2002;55:745–748.
14. Andrew E. Simor, Tammy L. Stuart, Lisa Louie, Christine Watt,1 Marianne Ofner-Agostini, Denise Gravel, Michael Mulvey, Mark Loeb, Allison McGeer, Elizabeth Bryce, Anne Matlow, and the Canadian Nosocomial Infection Surveillance Program. Mupirocin-Resistant, Methicillin-Resistant *Staphylococcus aureus* Strains in Canadian HospitalsAntimicrob Agents Chemother2007, Vol. 51, No. 11 p. 3880–3886.
15. McNeil JC, Hulten KG, Kaplan SL, Mason EO. 2011. Mupirocin resistance in *Staphylococcus aureus* causing recurrent skin and soft tissue infections in children. Antimicrob Agents Chemother 55:2431–2433.
16. Antonov NK, Garzon MC, Morel KD et al. High prevalence of mupirocin resistance in *Staphylococcus aureus* isolates from a pediatric population. Antimicrob Agents Chemother 2015; 59:3350–6.
17. Ng, S. M. S., Ching, H. S. V., Xu, G., Ng, F. M., Ong, E. H., Lau, Q. Y., ... & Chia, C. B. (2017). Screening for a potent antibacterial peptide to treat mupirocin-resistant MRSA skin infections. *International Journal of Peptide Research and Therapeutics*, *23*(4), 481-491.
18. Park SY, Kim SM, Park SD. The prevalence, genotype and antimicrobial susceptibility of high-and low-level mupirocin resistant methicillin-resistant Staphylococcus aureus. Annals of dermatology. 2012 Feb 1;24(1):32-8.
19. Desroches, M., Potier, J., Laurent, F., Bourrel, A.S., Doucet-Populaire, F., & Decousser, J.W. (2013). Prevalence of mupirocin resistance among invasive coagulase-negative staphylococci and methicillin-resistant *Staphylococcus aureus* (MRSA) in France: emergence of a mupirocin-resistant MRSA clone harbouring *mupA*. The Journal of antimicrobial chemotherapy, 68 8, 1714-7.
20. Eltringham I. Mupirocin resistance and methicillin-resistant *Staphylococcus aureus* (MRSA). J Hosp Infect 1997; 35: 1-8
21. Hiramatsu, K., Ito, T., Tsubakishita, S., Sasaki, T., Takeuchi, F., Morimoto, Y., ... & Baba, T. (2013). Genomic basis for methicillin resistance in Staphylococcus aureus. *Infection & chemotherapy*, *45*(2), 117.
22. Andersson, H., Lindholm, C., & Fossum, B. (2011). MRSA–global threat and personal disaster: patients' experiences. *International nursing review*, *58*(1), 47-53.
23. Khan, T. M., Kok, Y. L., Bukhsh, A., Lee, L. H., Chan, K. G., & Goh, B. H. (2018). Incidence of methicillin-resistant Staphylococcus aureus (MRSA) in burn intensive care unit: a systematic review. *Germs*, *8*(3), 113.