

DOI: <https://doi.org/10.47391/JPMA.177>

Nasal carriage of highly resistant methicillin resistant *Staphylococcus aureus* (MRSA) strains by hospital staff in Hazara region of Pakistan

Maria Rukan¹, Humaira Jamil², Habib Ali Bokhari³, Aamer Ali Khattak⁴, Allah Nawaz Khan⁵, Zahid Ullah⁶, Sarfaraz Hussain⁷, Muhammad Ali Syed⁸

1,2,4,8 Department of Microbiology, The University of Haripur, Pakistan; **3** Department of Biosciences, COMSATS Univrsity, Islamabad, Pakistan; **5** Department of Pathology, Gomal Medical College, Dera Ismail Khan, Islamabad, Pakistan; **6** Department of Medical Lab Technology, The University of Haripur, Pakistan; **7** District Head Quarter Hospital, Haripur, Pakistan

Correspondence: Muhammad Ali Syed. **Email:** mirwah2000@yahoo.de m

Abstract

Objective: To isolate and characterise multidrug resistant strains of *Staphylococcus aureus* from healthcare workers who are at potential risk of nosocomial infections.

Methods: The observational, cross-sectional study was conducted from November 2014 to April 2015 at different hospitals of Haripur and Abbottabad, Pakistan, and comprised ward and operation theatre staff. The isolates were identified on the basis of microbiological and biochemical tests and further confirmed by polymerase chain reaction. Disc diffusion method was used for antibiotic sensitivity testing, and panton valentine leukocidin and methicillin resistance *mecA* genes were detected using polymerase chain reaction.

Results: Of 208 isolates, 108(52%) were from the ward staff and 100(48%) were from the operation theatre staff. Overall, 167(80.3%) isolates were positive for *Staphylococcus aureus*, and 75(36%) were methicillin-resistant *Staphylococcus aureus*. The number of antibiotic-resistant isolates was 75(45%) cefoxitin, 60(36%) ofloxacin, 152(91%) erythromycin, 52(31%) doxycycline, 127(76%) lincomycin, 53(32%) amoxicillin-clavulanate, 67(40%) ciprofloxacin, and 89(53%) ceftriaxone.

Conclusion: A high number of hospital staff, including those working in operation theatres, were found to be carrying methicillin-resistant *Staphylococcus aureus* and multidrug resistant strains in their nasal passage that may be a source of infection to patients.

Key Words: Methicillin resistance, Nosocomial infections, Vancomycin, MecA gene, Pvl gene.

Introduction

Staphylococcus (S.) aureus is one of the most common causes of healthcare-associated bacterial infections among humans, causing a range of illnesses, including skin and soft tissue, vaginal, urethral and intestinal as well as gastrointestinal (GI) infections^{1,2}. *S. aureus* hemolyses blood, coagulates plasma and produces extracellular enzymes and toxins that contribute to their virulence³. *S. aureus* may also cause many epidemic and endemic infections and result in a high rate of mortality and morbidity^{4,5}. *S. aureus* is coagulase-positive which is considered the distinguishing characteristic for this bacterial species from other staphylococci².

They are normal human commensal, present on skin and nasal passage of a large population. It was found that the number of colonies isolated from nasal samples were higher in number than colonies isolated from skin swab samples, as nose is the main ecological niche of *S. aureus*⁶.

One of the leading causes of healthcare-associated infections is methicillin-resistant *Staphylococcus aureus* (MRSA)⁷. *S. aureus* nasal carriage has been identified as a risk factor for the nosocomial infections in general hospital population, surgical patients, general and haemodialysis patients among others⁸. MRSA emerged for the first time in the United Kingdom in the early 1960s and since then it is one of the major health problem globally⁹.

Penicillin was introduced for the first time in the 1940s and at the time of introduction, 95% isolates were susceptible to it¹⁰. Beta lactamase-resistant type of penicillin, called methicillin, was introduced in the 1960s with the hope that it would work better, but soon after its introduction, methicillin-resistant strains emerged and disseminated globally. Nosocomial acquisition of MRSA has been a worldwide problem and a leading cause of healthcare-associated as well as postsurgical infections¹⁰⁻¹¹.

Studies have reported that the MRSA carriage by hospital staff may be a risk of transmission to those receiving treatment⁸. During any surgery or injury, skin and mucous membrane barrier may be broken, and act as an entry point of skin bacteria into the body². MRSA infections have become more difficult to treat nowadays due to high levels of antibiotic resistance against almost all conventionally used antimicrobial agents^{9,10,11}.

The current study was planned to isolate, identify and test antibiotic sensitivity of *S. aureus* strains from those working in operation theatre (OT) or hospitals wards.

Materials and Methods

The observational, cross-sectional study was conducted from November 2014 to April 2015 at three hospitals of Haripur and two of Abbottabad, in the Hazara Division, Pakistan, and comprised full-time ward and OT staff working for at least a year, including physicians, surgeons, nurses, ward boys, cleaners, technicians etc. who volunteered to take part. Those working for less than a year

or those not willing to participate were excluded, and so were those who suffered from any sort of skin infection. The study was carried out after approval from the ethics review committee of The University of Haripur and the identities of both the participants and the hospitals were kept confidential.

Nasal samples were collected using sterile cotton swabs. Data such as age, gender, employment and medical history was also recorded. The samples were transported to the microbiology lab of the University of Haripur for further processing.

For the identification of *S. aureus*, different microbiological and biochemical tests were performed including microscopy, growth and cultural characteristics on selective medium i.e. mannitol salt agar (Oxoid, UK). Biochemical tests included catalase, coagulase, mannitol fermentation tests as well deoxyribonucleic acid-ase (DNase) test.

All the isolates identified as *S. aureus* were subjected to disc diffusion assay using different antibiotics, according to the Kirby Bauer method¹². The antibiotics included cefoxitin (FOX), erythromycin (E), ofloxacin (OFX), doxocyclin (DOX), lincomycin (LCM), amoxicillin-clavulanate (AMC), ciprofloxacin (CIP) and ceftriaxone (CRO). Strains resistant to FOX were considered MRSA. Clinical & Laboratory Standards Institute (CLSI) breakpoints were used to determine antibiotic resistance¹³.

In order to perform molecular characterisation of *S. aureus* isolates, polymerase chain reaction (PCR) was performed for panton valentine leukocidin (pvl) and methicillin resistance *mecA* genes, as they are antibiotic resistance and virulence genes respectively. Each PCR reaction consisted of 25µl reaction mixture. Each reaction contained 2µL DNA, 1µl of forward and reverse primers for pvl and *mecA* genes, 2.5µl magnesium chloride (MgCl₂), 2.5µl PCR buffer, 0.5µl deoxyribonucleotide triphosphate (dNTPs), 0.5µL taq polymerase and 13µL ultrapure deionised water. The annealing temperature for both primers was 55°. All samples were run on gel with 100bp ladder; *mecA* gene product

size was 310bp and pvl gene amplification product size was 433bp. Detail of all primers used in the study is given in Table ^{14,15,16}

Results

Of 208 isolates, 108(52%) were from the ward staff and 100(48%) from the OT staff. Overall, 167(80.3%) isolates were positive for *S. aureus*, and 75(36%) were MRSA (Table 2).

The number of antibiotic-resistant isolates were FOX 75(45%), OFX 60(36%), E 152(91%), DO 52(31%), LCM 127(76%), AMC 53(32%), CIP 67(40%) and CRO 89(53%) (Figure 1). The number of isolated strains positive for *mecA* gene was 75 (45%), while 48(29%) were positive for pvl gene (Figure 2).

Discussion

The findings of the present study are alarming with an overall detection of 80.3% *S. aureus* and 36.1% MRSA.

S. aureus is one of the most common causes of nosocomial infections worldwide. Penicillin was considered a drug of choice for the treatment of staphylococcal infections at the time of introduction in the 1940s, but, presently, over 90% strains show resistance to penicillin¹⁷.

Multidrug resistance (MDR) has been increasing in *S. aureus* since the introduction of antibiotics in the 1940s. According to the National Nosocomial Infection Surveillance (NNIS), a unit of the Centre for Disease Control and Prevention (CDC), methicillin resistance of *S. aureus* among hospitals in the United States increased from 2.4% in 1975 to 29% in 1991. MRSA strains were reported for the first time in England in the 1960s¹⁸. Since then its prevalence is on rise and in almost all countries of the world.

Nasal carriage of MDR *S. aureus* has previously been reported¹⁶. Of concern is their carriage by healthcare professionals who are in direct and close contact with patients receiving treatment at the hospitals. A study in India reported 8%

nasal carriage by healthcare professionals¹⁹. Another study performed on healthcare ward staff of a hospital in Rawalpindi, Pakistan, reported 18% detection of *S. aureus* whereas 1.5% of them were MRSA²⁰. A recent study conducted in Lahore, Pakistan, reported 23.4% *S. aureus* with 8% MRSA²¹. As such, the current study's findings represent the highest frequency of *S. aureus* and MRSA carriage by healthcare professionals in the region.

It is reported in studies that death toll and risk of nosocomial infection and sepsis is higher with MRSA compared to methicillin-sensitive *Staphylococcus aureus* (MSSA)²²,²³. Further, it is also reported that antibiotic resistance profiles of MRSA is higher compared to MSSA²⁴. In our case, 29% strains were carrying the *pvl* gene, which is also a risk for healthcare workers themselves.

Conclusion

S. aureus and MRSA prevalence was found to be alarming, as hospital staff may be a cause of both healthcare-associated infections as well as an outbreak by highly resistant strains of *S. aureus*. Appropriate infection control measures must be ensured at both public and private sector healthcare facilities.

Acknowledgements: We are grateful to Vice-Chancellor of the University of Haripur for approving financial assistance as well as to the staff and administration of the participating hospitals.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: The University of Haripur and COMSATS University, Islamabad.

References

1. Melzer M, Eykyn J S, Gransden R W, Chinn S. Is Methicillin resistant *Staphylococcus aureus* more virulent than methicillin susceptible

- 172 S.aureus? A comparative cohort study of British patients with nosocomial
173 infection and bacteremia. Clin Infect Dis. 2003; 37 : 1453-60
- 174 2. Harris G L, Foster J S, Richards G R. An introduction to *Staphylococcus*
175 *aureus* and techniques for identifying and quantifying S. aureus adhesions
176 in relation to adhesion to biomaterials. Eur Cell Mater2002; 4: 1473-2262
- 177 3. Cox R A. Strategies for the management of healthcare staff colonized with
178 epidemic methicillin-resistant *Staphylococcus aureus*. J Hosp
179 Infect1998;35:117-127.
- 180 4. Eiff V C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a
181 source of *Staphylococcus aureus* bacteremia. N Engl J Med 2001; 344(1):
182 11-6.
- 183 5. Coello R, Glynn JR, Gaspar C, Picazo JJ, Fereres J. Risk factors
184 for developing clinical infection with methicillin-resistant *Staphylococcus*
185 *aureus* (MRSA) amongst hospital patients initially only colonized with
186 MRSA. J Hosp Infect 1999;37:39-46.
- 187 6. Williams REO. Healthy carriage of *Staphylococcus aureus*: its prevalence
188 and importance. Bacteriol Rev 1963 ; 27(1) : 56-71.
- 189 7. Huang SS, Yokoe SD, Hinrichsen VL, Spurchise LS, Datta R, Miroshnik
190 I et al. Impact of routine intensive care unit surveillance cultures and
191 resultant barrier precautions on hospital- wide methicillin-Resistant
192 *Staphylococcus aureus* bacteremia. Clin Infect Dis 2006 ; 43 : 971-8
- 193 8. Qureshi AH, Rafi S, Qureshi S M Ali AM. The current susceptibility
194 patterns of MRSA to conventional antistaphylococcal antimicrobials at
195 Rawalpindi. Pak J Med Sci 2005; 20:361-4.
- 196 9. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, Tolson
197 JS et al. Methicillin-resistant *Staphylococcus aureus* in U.S. Hospitals,
198 1975-1991. Infect Cont Hospit Epidemiol. 1992 ; 13(10) : 582-586.

10. Nwankwo EOK, Sale A, Magagi A, Gabriel I. Methicillin resistant *S. aureus* (MRSA) and their antibiotic sensitivity pattern in Kano, Nigeria. Afr J Clin and Exp Microbiol. 2010; 11(1) : 129-136.
11. Kluytmans J, Belkum V A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997; 10(3) : 505-520.
12. Bauer AW, Perry DM, Kirby WMM. Single disc antibiotic sensitivity testing of Staphylococci. AMA Arch Intern Med 1959; 104:208–216.
13. Clinical Laboratory Standards Institute Document Performance Standards for Antimicrobial Susceptibility Testing (2017). 27th Edition.
14. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine Leukocidin—producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999; 29(5); 1128-1132.
15. Zhang K, Sparling J, Chow BL, El-Sayed S, Hussain Z, Church DL et al. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J Clin Microbiol 2004; 42(11): 4947-4955.
16. Poulsen A B, Skov R, Pallesen L V. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. J Antimicrob Chemother 2003; 51(2):419-21.
17. Ubukata K, Nonoguchi R, Matsushashi M, Konno M. Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. J Bacteriol. 1989 ; 171(5) : 2882-2885.

18. Adler A, Temper V, Block S C, Abramson N, Moses EA. Pantone-valentine leukocidin-producing *Staphylococcus aureus*. Emerg Infect Dis. 2006; 12(11) : 1789-1790.
19. Malini J, Harle A S, Padmavathy M, Umapathy B L. Methicillin-resistant *Staphylococcus aureus* carriage among the health care workers in a tertiary care hospital. J Clinl Diag Res. 2005; 6(5) : 791-793.
20. Akhtar NC. Staphylococcal nasal carriage of health care workers. J Coll Physicians Surg Pak. 2005 ; 20(7) : 439-443.
21. Asif A, Javed I, Mushtaq S, Anwar S. Nasal carriage of methicillin resistant *Staphylococcus aureus* among tertiary care hospital employees in a non-outbreak setting. Pak J Pathol 2017; 28(4); 171-175.
22. Melzer M, Eykyn S J, Grandsden W R, Chinn S. Is methicillin-resistant *Staphylococcus aureus* more virulent than methicillin-susceptible *S. aureus*? A comparative cohort study of British patients with nosocomial infection and bacteremia. Clin Infect Dis 2003 ;37(11):1453-60.
23. Rozgonyi F, Kocsis E, Kristof K, Nagy, K. Is MRSA more virulent than MSSA? Is MRSA more virulent than MSSA? Clin Microbiol Infect 2007; 13(9): 843-845.
24. Sharma N, K. Garg R, Baliga S, Bhat G K. Nosocomial infections and drug susceptibility patterns in methicillin sensitive and methicillin resistant *Staphylococcus aureus*. J Clin Diagn Res 2013; 7(10): 2178–2180.
-

Table 1: Primer sequences used for polymerase chain reaction (PCR).

S.No.	Target	Primer name	Primers Sequence	Source
1	pvl gene	PVL-F PVL-R	5`- ATCATTAGGTAAAATGTCTGGACATGATCCA- 3` 5'-GCATCAAGTGTATTGGATAGCAAAAGC-3'	14
2	mecA gene	mecA-F mecA-R	5`-GTAGAAATGACTGAACGTCCGATAA-3` 5`-CCAATTCCACATTGTTTCGGTCTAA-3`	15
3	Nuc 1	Nuc F NucR	5'-TCAGCAAATGCATCACAAACAG-3` 5'-CGTAAATGCACTTGCTTCAGG-3'	16

256

257 -----

258

Table 2: Frequencies of *Staphylococcus (S.) aureus*, methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) among staff of both medical and surgical wards

	<i>S. aureus</i>	MSSA	MRSA
Hospital ward (n=108)	86 (79.6%)	48 (44.4%)	38 (35.2%)
Surgical ward (n= 100)	81 (81%)	44% (n=44)	37 (37%)
Overall	167 (80.3%)	92 (44.2%)	75 (36.1%)

262

263 -----

264

265

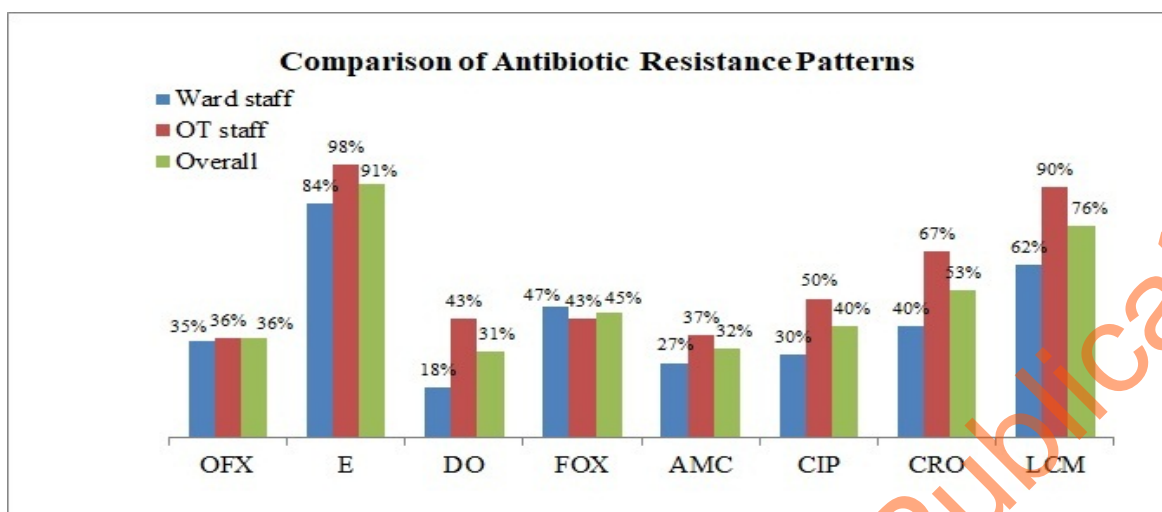
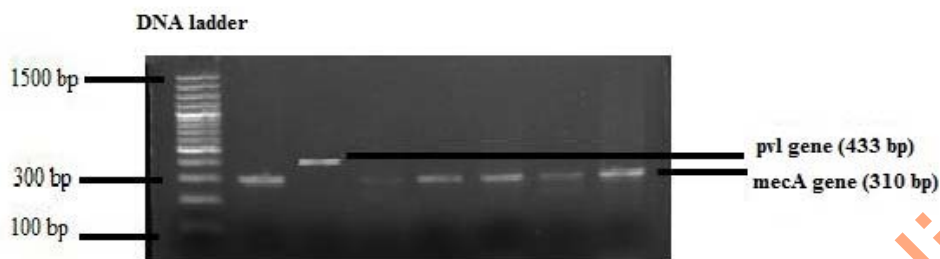


Figure 1: Antibiotic resistance profiles of *Staphylococcus* (*S.*) *aureus* isolates against different antibiotics. Each bar represents percentage of drug resistance in strains isolated from ward staff, operation theatre (OT) staff and overall respectively.

OFX: Ofloxacin, E: Erythromycin, DOX: Ddoxycycline, FOX: Cefoxitin, AMC: Amoxicillin-clavulanate, CIP: Ciprofloxacin, CRO: Ceftriaxone, LCM: Lincomycin.



286

287 **Figure 2: Agarose gel electrophoresis of polymerase chain reaction (PCR)**
 288 **products of mecA and pvl genes. MecA gene is responsible for resistance to**
 289 **methicillin, while the pvl gene encodes poly valentine leukocidin (PVL), a**
 290 **potent virulence factor of *Staphylococcus aureus* (*S. aureus*).**

291