Nasal Carriage And Antibiogram Of Staphylococcus Aureus Amidst Healthcare Personnel From A Teaching Hospital In Coastal Karnataka, India.

Fathimath Raseena, Sevitha Bhat, Archana Bhat, Radhakrishna M*

Department of Microbiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India

Abstract:Background: S.aureus is an increasing cause of infections in both immunities and hospitals. It is also a frequent colonizer of the skin and nose. The incidence of hospital-acquired and community-acquired S.aureus infection has risen associated with the simultaneous increase of drug-resistant strains called methicillin resistance S.aureus.Aims and objectives: To determine S. aureus and MRSA's nasal carriage rate among healthcare workers of a teaching hospital, detect the mecA gene, MIC of Vancomycin, and Mupirocin in MRSA isolates.Materials and methods: A total of 285 health care workers from a teaching hospital of coastal Karnataka has participated in this study. Both anterior nares were swabbed and processed in the microbiology laboratory. Standard bacteriological methods identified isolates. PCR confirmed MRSA strains. Results: A total of 285 healthcare workers in a teaching hospital were screened for nasal carriage of S.aureus. Out of 285, 45 (15.8 %) healthcare workers were screened positive for S.aureus. Fifteen (5.3%) were Methicillin-Resistant Staphylococcus Aureus (MRSA). All the MRSA strains were confirmed by mecA gene detection by PCR.Conclusion: The rate of prevalence of MRSA carriage (5.3°/c) in healthcare workers was relatively low. There was no vancomycin-resistant S.umum.t, and MIC creep to vancomycin to MRSA was not observed. Two mupirocin- resistant .S. aureus and significant numbers of Methicillin-Resistant Coagulase Negative Staphylococcus (MRCoNS) were identified. These findings highlight the urgency for application to infection control measures that aim to decrease M RSA transmission.

Keywords: Nasal carriage, MRSA, healthcare workers, drug resistance

Introduction:

Infections caused by S.aureus in community and health care settings continue to prevail. Adding to the existing problem is the emergence of superbug Methicillin Resistance Staphylococcus aureus (MRSA) [l]. Altered penicillin-binding proteins (PBPs) on the bacterial cell wall accounts for MRSA emergence [2]. Infections caused by MRSA are related to worst outcomes, prolonged hospital stay, higher costs of treatment, and increased mortality [3]. MRSA carriers constitute a significant source of infection in hospitals and the community. The transmission mode in patients and health care workers is through the contaminated hands, which leads to cross-contamination [4]. Antibiotic resistance occurs primarily when a microorganism acquires a gene that codes for antimicrobial inactivating enzymes. This drug resistance can be a spontaneous or genetic mutation or mediated by genetic elements such as transposon plasmid and integron or gene cassette. MRSA nasal carriers may be at higher risk of developing MRSA infection than methicillin-sensitive .S. aureus (MSSA) carriers and noncarriers, particularly in hospital settings. Therefore, strict vigilance is required to screen and prevent MRSA colonization in health care settings [5].

Treatment of MRSA infections continues to be a significant challenge, with vancomycin still being the drug of choice to date. With the increasing usage of vancomycin, Vancomycin-intermediate Staphylococcus aureus strains have cropped up. Mupirocin, a glycopeptide antibiotic, has been widely used for nasal decolonizer in health care workers [6, 7]. The mecA gene's presence, encoding for altered penicillin-binding protein 2a (PB P2a), accounts for MRSA. Coagulase-negative Staphylococci (CoNS) are present in the skin and anterior nares as normal flora. These organisms have relatively low virulence but are progressively recognized as agents of clinically significant infection of the bloodstream and other sites. The late worldwide distribution of Methicillin-Resistant CoNS (MRCoNS) is also seen [8].

The present study focused on estimating MRSA's nasal carriage rate among healthcare workers in a teaching hospital in Coastal Karnataka, India. The study's findings can be extrapolated to work out a policy for the control of MRSA in our hospital settings.

MATERIALS AND METHODS:

A Cross-sectional study was conducted in a Teaching Hospital and Microbiology Diagnostic Laboratory of Coastal Karnataka, India. All consenting healthcare workers were randomly recruited into the study. The Institutional Ethics Committee approved the present study. The data with details on age, gender, and years of work experience, lifestyle, H/O skin infections, or other illnesses about the study participants was obtained in a structured proforma. In this study, a total of 285 participants were enrolled. Both the anterior nares were swabbed with sterile cotton swabs moistened with sterile physiological saline. The nasal swabs were streaked onto Mannitol Salt Agar (MSA) plates & incubated at 37° C for 24 to 48 hours. The organism was identified as S.aureus by using standard biochemical tests [9]. The isolated strains of S.aureus were screened for methicillin susceptibility by the modified Kirby-Bauer disk diffusion method using cefoxitin (30µg) discs on Mueller-Hinton agar (MHA). Isolates that showed inhibition zone sizes of diameter ≥21 mm was considered as MRSA strains. MIC for vancomycin and mupirocin were determined by the E test [10]. Antibiotic sensitivity to all the S.aureus isolates against other antibiotics like penicillin(l0units), amoxicillin/clavulanic acid(20/l0µg),erythromycin(I5µg),clindamycin(2µg), chloramphenicol(30 µg),co-trimoxazole(25 µg), ceftriaxone(30 µg),gentamicin(l0 µg),linezolid(30 µg),teicoplanin(30 µg) and ciprof1oxacin(5 µg) were determined by modified Kirby-Bauer disk diffusion method. The standard strains as controls were S. aureus ATCC 25923, MRSA ATCC 29213, and MSSA ATCC 33591. Antibiotic susceptibility tests were interpreted as per CLSl guidelines [11].

Detection of mecA gene:

The bacterial DNA was extracted by the boiling method. A bacterial suspension (100ul) was boiled at 100^0C for 15 minutes. The suspension was later centrifuged, and the supernatant was used for PCR or frozen at -20^0C until further use. MRSA isolates were detected by conventional PCR using the mecA gene. PCR was performed in a 20µl reaction with 10X PCR buffer, 10mM dNTP, 10Mmof each primer, 5U/µl Taq polymerase, and 2µl of DNA template. Amplification was performed with the following cycling conditions. Initial denaturation at 94^{o}C for 2 minutes followed by 30 cycles of denaturation at 94^{o}C for 1 minute, annealing at 60^{o}C for 1 minute, extension at 72^{o}C for 1 minute, and a final extension at 72^{o}C for 5 minutes. The PCR products were analyzed on a 1.5% agarose gel in the 1xTE buffer. Ethidium bromide-stained DNA amplicons were visualized using a gel imaging system [12]. All the PCR components were purchased from Sigma Chemicals Pvt. Ltd.

Table 1: mecA gene sequence

Target Gene	Primer Sequence	Product size
mecA Forward	5' – TGC TAT CCA CCC TCA AAC AGG – 3'	286 bp
mecA Reverse	5' – AAC GTT GTA ACC ACC CCA AGA – 3'	

Statistical Analysis:
Results were compiled, tabulated, and all data were subjected to the statistical analysis SPSS version 17.0. The results were presented in the form of tables and graphs. Association of different factors done by using CHI-SQUARE TEST. p-value < 0.05 considered as significant.

Results:
A total of 285 healthcare workers in teaching hospitals were screened for nasal carriage of S.aureus and M RSA. Twenty-five (8.8%) were males, and 260(9 I .2%) were females. One hundred and fifty-five (54.38%) staff nurses and 65(22.8%) postgraduates constitute the majority of subjects screened (Table 2). Out of 285, 45 healthcare workers were S.aureus carriers, giving a carriage rate of 15.8%. Out of 45, 15 were MRSA giving a rate of 33.3% (Figure I), and it was confirmed by using PCR targeting the mecA gene (Figure 2). Overall, MRSA nasal carriage rate was 5.3% in this study. Coagulase-negative staphylococci (CoNS) were the predominant isolates 197(69.12%); (Table 2).
The antibiotic susceptibility pattern of S.aureus isolates was shown in figure 1. All the isolates of S.aureus were 100% resistant to penicillin. MRSA's 15 isolates showed 100% sensitivity to Linezolid, Chloramphenicol, Clindamycin, and Teicoplanin, respectively. MIC to vancomycin in all the 15 MRSA isolates was found to be <2µg/ml, and in Mupirocin 13 were found to be <4µg/ml, 2 were ≥512µg/ml by E test (Table 4). Detection of the mecA gene in MRSA isolates by PCR was depicted in figure 2.

Table 2: Profession related distribution carriage of *S.aureus* and MRSA status.

DESIGNATION	No. samples (%) n=285	No. positive for *S. aureus*	No. positive for MRSA
Staff Nurse	155(54.39)	35	11
Technical Staff	20(7.01)	0	0
PGs	65(22.81)	7	2
General Duty Workers	45(15.79)	3	2

Table 3: Nature of organisms isolated in anterior nares

ORGANISMS ISOLATED	Frequency(%) n=285
S.aureus	45(15.79)
CONS	197(69.12)
No Growth	28(9.82)
Candida	6(2.10)
Diphtheroids	9(3.17)

Table 4: MIC for Vancomycin and Mupirocin

Vancomycin MIC(µg/ml)	**No. of MRSA isolates(n=15)**
0.19	1
0.38	2
0.50	8

0.75	3
1	1
Mupirocin MIC(µg/ml)	**No. of MRSA isolates(n=15)**
0.25	5
0.38	4
0.50	4
≥1024	2

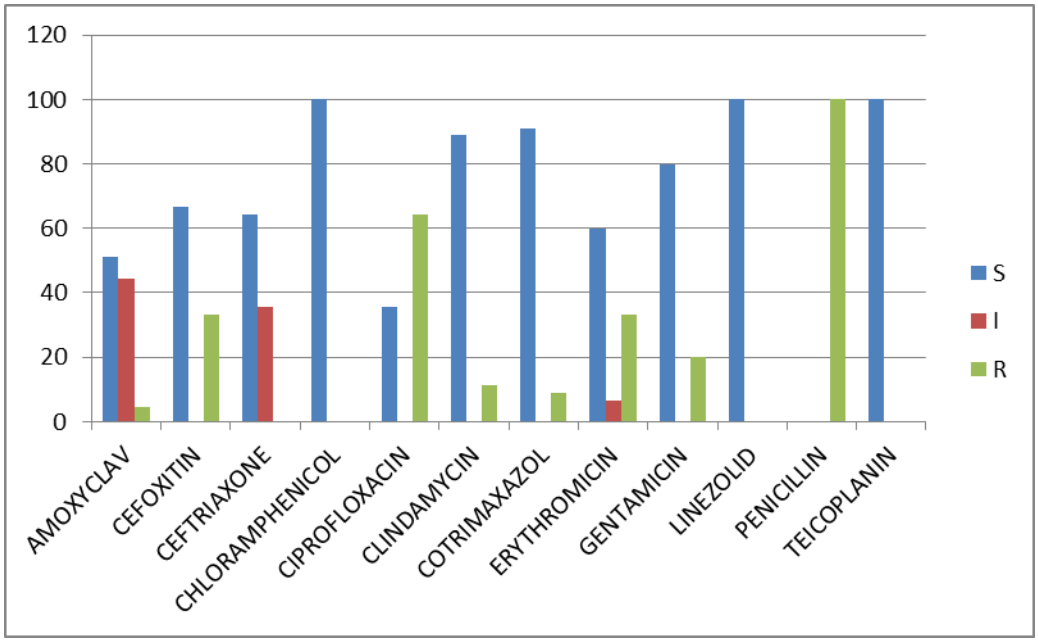

Fig.1: Antibiotic susceptibility pattern of *S.aureus* isolates

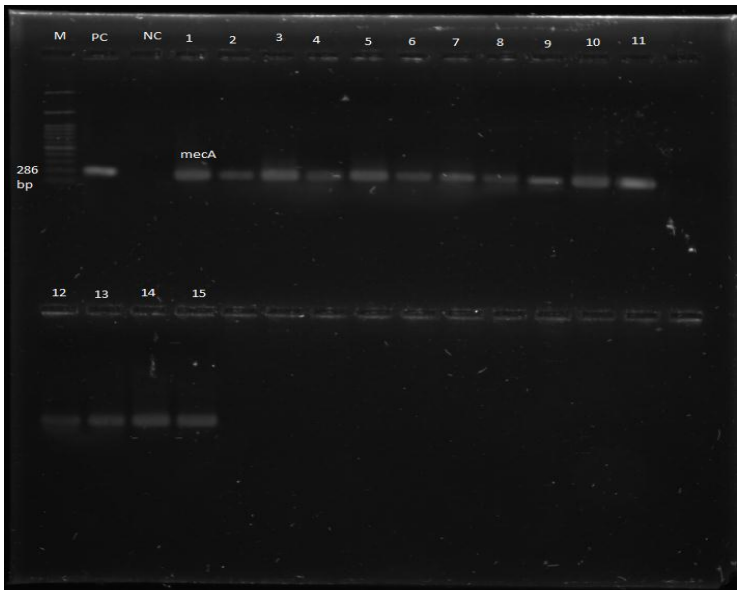

Fig. 2: Detection of the mecA gene. (Agarose gel Electrophoresis showing amplified PCR products, lane M: 100bp DNA ladder, PC: positive control MRSA ATCC 29213, NC: negative control, lane 1-15 positively amplified mecA gene).

Discussion:

The anterior nares of humans were found to be the primary ecological niches of S.aureus [13]. The carriage pattern is of three types; 20% of the population are persistent carriers. 60%

account for intermittent carriers and the remaining 20% were noncarriers. The difference in the nasal colonization of MRSA remains unexplained [14]. The prevalence of S. aureus and MRSA shows varied geographical distribution. The dissimilarity in MRSA distribution can be attributed to the design of the study undertaken [15]. MRSA carriers among healthcare workers (HCWs) in hospitals need to be screened. The MRSA carriers serve as a potential source of infection in health care settings.

This study highlighted a prevalence of 15.8% S.aureus carriage in healthcare workers in a teaching hospital of coastal Karnataka. Out of 45 S.aureus isolates, 15 were MRSA with a percentage of 33.3. The detection of the mecA gene confirmed all MRSA strains. The overall M RSA carriage rate was 5.26%. Further, it was observed that out of 35 S.aureus isolated from staff nurses, 11 (31 .4%) were found to be MRSA. Postgraduates followed this observation by having two MRSA out of seven isolates. The rate of S.aureus nasal carriage among technical staff was zero in our study. It was observed that risk factors like the use of antibiotics in the past six months and allergic disorders were statistically significant in the nasal carriage status of S. aureus among the healthcare workers ($p<0.05$). Sixty-one percent of the healthcare population who were the carriers of S. aureus had used antibiotics in the past six months, and 39% of the S. aureus carriers had allergic disorders. Whereas with the identified 15MRSA carriers, risk factors like allergic disorders were statistically significant ($p < 0.05$) to acquire carriage status.

Earlier studies from India revealed MRSA carriage rate of 10%, 1 .8%, 2.5% in Bengaluru, Pondicherry, and, Mangaluru, respectively [17, 18, 19]. An MRSA carriage of 3.4 % was reported in West Nepal [20]. Outside 1ndia, a very high carriage rate of 25.5% was reported from Palestine [21]. As mentioned above, the study detected an overall nasal carriage rate of MRSA 5.3% in healthcare personnel, which were lower than those reported by studies from Bengaluru (10%) but comparably higher than that of reviews Pondicherry 1 .8% and West Nepal 3.4 %. There was no vancomycin resistance MRSA in our study. All MRSA were sensitive to vancomycin, which showed MIC of <2µg/ml. MIC creep to Vancomycin to MRSA was not observed, whereas two mupirocin-resistant MRSA strains were reported (MIC of >1024µg/ml). The emergence of Mupirocin resistance strains, which is used as an active drug for decolonization, is also a cause for concern. All the isolates of S.aureus were 100% resistant to penicillin and 100% sensitive to linezolid. All MRSA isolates showed 100% susceptibility to linezolid, chloramphenicol, clindamycin, and teicoplanin. It was observed from our study that there was the emergence of mupirocin resistance strains of .S.aureus. Thereby decolonization by mupirocin may not be useful. The gradual increase in MRSA's rate of isolation recommends implementing strict control policies at the hospital level. However, this also suggests the importance of creating awareness among healthcare workers to eliminate the risk of nasal carriage and further decolonization. Sixty-five out of 197 CoNS were resistant to methicillin, indicating the emergence of MRCoNS. There is some concern that such strains pose any infection in a susceptible individual with predisposing factors [22].

The most critical factor for preventing nosocomial infections in healthcare professionals' is compliance with the standard guidelines on infection control. The risks implicated in health care infections due to MRSA has to be reinforced among them. Awareness of these implications will go a long way ahead if strict guidelines are adhered to. Simple preventive measures like practicing hand hygiene before and after examining the patient, using sterile PPE(personal protective equipment) in the postoperative wards, care of immunocompromised patients, and avoiding touching one's face during work can reduce the disease transmission rate considerably. All the health care workers should undergo periodic training in Infection control practice and antibiotic policy.

References:

1. Deresinski S. Methicillin-Resistant *Staphylococcus aureus*: An Evolutionary, Epidemiologic, and Therapeutic Odyssey. *Clin Infect Dis* 2005; 40(4):562-73.
2. Korn G, Martino M, Mimica I, Mimica L, Chiavone P, Musolino L. High frequency of colonization and absence of identifiable risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) in intensive care units in Brazil. *Braz. j. Infect. Dis.* 2001;5(1):1-7.
3. Ippolito G, Leone S, Lauria F, Nicastri E, Wenzel R. Methicillin-resistant *Staphylococcus aureus*: the superbug. *Int J Infect Dis*. 2010;14: S7-S11.
4. AlbrichWH, Arbarth S. Health-care workers: source, vector, or victim of MRSA? *The Lancet Infectious Diseases*. 2008;8(5):289-301.
5. Ledell, K., Muto, C., Jarvis, W. and Farr, B. (2003). SHEA Guideline for Preventing Nosocomial Transmission of Multidrug-Resistant Strains of *Staphylococcus aureus* and Enterococcus. *Infection Control & Hospital Epidemiology*, 24(09), pp.639-641.
6. Agarwal L, Singh A, Sengupta C, Agarwal A. Nasal carriage of Methicillin and Mupirocin-resistant *S. aureus* among healthcare workers in a tertiary care hospital. *Journal of Research in Pharmacy Practice*. 2015; 4(4):182.
7. David MDaum R. Community-Associated Methicillin-Resistant *Staphylococcus aureus:* Epidemiology and Clinical Consequences of an Emerging Epidemic. *Clinical Microbiology Reviews.* 2010; 23(3):616-687.
8. Stefani S, Varaldo PE. Epidemiology of methicillin-resistant staphylococci in Europe. *Clin Microbiol Infect.,* 2003; 9:1179-86.
9. Bauer, A.W., Q.M.M. Kirby, J.C. Sherns and M. Turik, 1966. Antibiotic susceptibility testing by standardized single disk method. *Am J. Clin. Path* 45: 493-496.
10. CLSI. Surveillance for MRSA: Principles, Practices, and Challenges; A Report. CLSI document X07-R. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
11. Clinical and Laboratory Standards Institute / NCCLS Performance standards for Antimicrobial disc diffusion tests; Approved standards. 9th ed. CLSI Document M2-M9. Wayne Pa: Clinical and Laboratory Standards Institute; 2006
12. Shahina S, Siddiqui S, Krishnan D. Nasal Carriage of Methicillin-Resistant Staphylococci with Inducible Clindamycin Resistance and Pvl Gene. *Int J Sci Res* 2012; 3(6):392-394.
13. Peacock SJ, Justice A, Griffiths D, De Silva GDI, Kantzanuo MN, Crook D. Determinants of Acquisition and Carriage of *Staphylococcus aureus* in Infancy. *J Clin Microbiol* 2003; 41(12):5718-25.
14. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997; 10(3):505-20.
15. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* 2001; 7(2):178-82.
16. Charles B, Alison S, Jean W, Lisa M. Nasal carriage of methicillin-resistant *Staphylococcus aureus* among students at a Louisiana medical university. *Braz J Infect Dis* 2013; 17(1):118-9
17. Malini J., Shruti A. Harle, Padmavathy M., Umapathy B.L., Navaneeth B.V., Keerthi Mannan J., Girish M.S. Methicillin-resistant *Staphylococcus aureus* Carriage among the Health Care Workers in a Tertiary Care Hospital. *J.Clin Diagn Res*. 2012; 6(5): 791-93.
18. Mathanraj S, Sujatha S, Sivasangeetha K, Parija SC. Screening for methicillin-resistant *Staphylococcus aureus* carriers among patients and healthcare workers of a tertiary care hospital in Southern India. *Indian J. Med. Microbiol.* 2009; 27:62-64

19. Radhakrishna M, D'Souza M, Kotigadde S, Saralaya K V Kotian M.S. Prevalence of Methicillin-Resistant *Staphylococcus aureus* Carriage amongst Health Care Workers of Critical Care Units in Kasturba Medical College Hospital, Mangalore, India. *J Clin Diagn Res*. 2013 Dec, Vol-7(12): 2697-2700.
20. Khanal R, Sah P, Lamichhane P, Lamsal A, Upadhaya S, Pahwa V. Nasal carriage of methicillin-resistant Staphylococcus aureus among health care workers at a tertiary care hospital in Western Nepal. *Antimicrob Resist Infect Control*. 2015;4(1).:39
21. Al Laham N. Detection and antibiotic resistance pattern of Staphylococcus aureus and (MRSA) isolated from healthcare workers Nares at three Gaza hospitals, Palestine. *Int Arab J Antimicrob Agents.* 2015:5(4)
22. Tashiro M, Izumikawa K, Ashizawa N, Narukawa M, Yamamoto Y. Clinical significance of methicillin-resistant coagulase-negative staphylococci obtained from sterile specimens. *Diagn Microbiol Infect Dis*. 2015 Jan; 81(1):71-75.