National Journal of Basic Medical Sciences ORIGINAL ARTICLE Volume - III, Issue-3

NASAL CARRIAGE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AMONG MEDICAL STUDENTS : COMPARISON OF CEFOXITIN AND OXACILLIN DISC DIFFUSION METHODS

Baragundi Mahesh C.[1], Sataraddi. Jagadeesh V.[2], Kulkarni. Ramakant B.[3]

ABSTRACT

Context: Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) is currently responsible for 50-63% of nosocomial infections. Nose is the most frequent carriage site for *S.aureus*. Medical students comprise a unique population at risk for acquisition of MRSA nasal carriage .

Aim: To know the prevalence of *S.aureus* and methicillin resistant *S.aureus* (MRSA) nasal carriage in medical students in different phases of medical education and to evaluate cefoxitin and oxacillin disc diffusion methods to detect methicillin resistance in nasal *S.aureus* isolates.

Materials and methods: The study was conducted on 104 medical students. Samples were collected from anterior nares using sterile cotton swab soaked in sterile saline and processed immediately. *S. aureus* isolates were identified by standard microbiological techniques. Cefoxitin and oxacillin disc diffusion tests were done by Kirby-Bauer method according to CLSI 2011 guidelines to detect MRSA.

Results: Over all *S.aureus* and MRSA nasal carriage was seen in 34.61% and 11.53% medical students. MRSA carriage was seen in 21.42% interns where as less than 4% MRSA carriage was seen in I and II phase students. Cefoxitin disc detected 12 and oxacillin disc detected 11 methicillin resistant *S.aureus* isolates.

Conclusions: Nasal carriage rate of MRSA was high among students exposed to patients and hospital environment. Medical students can be a potential source of nosocomial pathogens like MRSA. Cefoxitin disc diffusion was superior to oxacillin disc diffusion method in detecting MRSA. Continuous surveillance, decolonization of carriers and improvement in hygiene standards in hospitals should be adopted to break the transmission of MRSA.

Key words: *S.aureus*, MRSA, Nasal carriage, Medical students, Cefoxitin.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is an important cause of health care associated infections worldwide.[1] Hospital acquired methicillin resistant *S.aureus* (HA-MRSA) were first indentified in 1961. HA-MRSA are currently responsible for 50-63% of nosocomial infections.[2] Infections with MRSA are likely to be more severe and require longer hospitalization.[3] *S.aureus* can colonise the anterior nares as well as moist regions of skin (eg -groin, axillae etc) for relatively long periods of time, but nose is the most frequent carriage site for *S.aureus*.[4,5,6] The repeated exposure to *S.aureus* in the environment is considered to be an important determinant of *S.aureus* nasal carriage.[7] Exposure to microbes is an inherent risk of persons working in patient care settings. Colonised health care workers transfer such strains to patients or they transfer the organisms from one patient to another through their hands.[8] Medical students comprise a unique population at risk for acquisition of MRSA nasal carriage. During the first one year of medical course i.e during their I phase, most students have little or no patient contact. In contrast the exposure of medical students to patient and hospital environment gradually increases during their II phase, III phase and internship of medical course. There are few studies,[2,9,10,11.] which have reported on prevalence of MRSA in preclinical medical students. To our knowledge there are no studies which have analyzed whether the patient and hospital environment exposure is a risk factor for acquisition of MRSA nasal carriage in Indian medical

[1]Associate Professor, Dept. of Microbiology, S.N.Medical College, Navangar, Bagalkot- 587102 Karnataka. S.N.M.C. Bagalkot.
[2]Asst.Professor. S.N.M.C. Bagalkot. [3]Professor. S.N.M.C. Bagalkot.

students. Cefoxitin disc diffusion test is found to be superior test than oxacillin disc diffusion test for detection of methicillin resistance in staphylococcus aureus.[12]

Hence, the current study was undertaken to know the nasal carriage rate of S.aureus and MRSA in medical students, to know the effect of patient and hospital environment exposure on carriage rate, and to evaluate cefoxitin and oxacillin disc diffusion methods in detecting methicillin resistance in nasal *S.aureus* isolates.

MATERIALS AND METHODS

The present study was conducted on 104 medical students not suffering from any upper respiratory tract infection and who have not received any antibiotics in the past 30 days. The standards of ethical committee on Human experimentation were followed during the study. Ethical clearance was obtained by institutional ethical committee. Consent was taken from all subjects of the study. The study group comprised of 27 first phase, 26 second phase, 23 third phase students and 28 interns.

Nasal swabs were collected from all the participants with a sterile cotton swab soaked in sterile saline by rotating the swab in both the anterior nares consecutively. The swabs were processed immediately by inoculating on to sheep blood agar plates. The culture plates were incubated at 37°c for 24-48 hours. *S.aureus* isolates were identified by colony morphology, catalase, coagulase, mannitol fermentation and DNAse tests following standard microbiological techniques.

The *S.aureus* isolates were tested for methicillin resistance by disc diffusion method of Kirby-Bauer using cefoxitin (30µg) and oxacillin (1µg) discs (Hi Media LTD. Mumbai. India). Incubation was done at 33-35°c for 16-18 hours for cefoxitin and 24 hours for oxacillin. Interpretation was done according latest 2011 CLSI guidelines.[13] For cefoxitin $\leqslant$ 21mm and $\geqslant$ 22 mm diameter zones of inhibition were taken as resistant and susceptible zones respectively. For oxacillin $\leqslant$ 10mm and $\geqslant$ 13mm diameter zones of inhibition were taken as resistant and susceptible zones respectively.

Statistical analysis was done by Z test and P value <0.05 was taken as statistically significant.

RESULTS

The present study was conducted on 104 Medical students. Of the 104 students, 27(26%) were from I phase, 26(25%) were from II phase, 23(22.1%) were from III phase and 28(26.9%) were interns (Table-1).

Overall *S.aureus* nasal carriage was seen in 36(34.6%) students and MRSA carriage in 12(11.5%) students (Table-2).

S .*aureus* carriage rate was high among III phase students and interns when compared to I and II phase students. Overall carriage of *S.aureus* was more among male students when compared to female students. The difference in nasal carriage rates of *S.aureus* between I v/s II, II v/s III, III v/s interns and males versus females was statistically not significant(P>0.05). The difference in the nasal carriage of *S.aureus* between I and II phase together versus interns was also statistically not significant.

Methicillin resistance was less than 4% among I and II phase students and it rapidly increased to 17.39% and 21.42% in III phase students and interns respectively. MRSA carriage was more among male students when compared to female students. The difference in nasal carriage rates of MRSA between I v/s II, II v/s III, III v/s interns and males versus females was statistically not significant(P>0.05). The difference in the nasal carriage of MRSA between I and II phase together versus interns was statistically significant (Z=1.15, P<0.05).

Cefoxitin and Oxacillin disc diffusion methods in detection of MRSA are compared in table-3. Out of 36 *Staphylococcus aureus* isolated , Cefoxitin disc detected methicillin resistance in 12 *S.aureus* and oxacillin disc detected methicillin resistance in 11 *S.aureus* isolates. In the present study, sensitivity and specificity of oxacillin disc diffusion were 92.3% and 100% respectively when compared to cefoxitin disc diffusion method in detection of MRSA.

Table-1: Distribution of Medical students of the Study			
Medical Students	**Male**	**Female**	**Total (%)**
I Phase	17	10	27(26)
II Phase	13	13	26(25)
III Phase	17	06	23(22.1)
Interns	18	10	28(26.9)
Total	65	39	104(100)

Table-02: Nasal carriage of S.aureus and MRSA among Medical students

Medical Students	*S.aureus*carriage			MRSA carriage		
	Male (%)	Female (%)	Total (%)	Male (%)	Female (%)	Total (%)
I Phase	5(29.4)	3(30)	8(29.6)	1(5.8)	0(0)	1(3.7)
II Phase	4(30.8)	4(30.8)	8(30.5)	0(0)	1(7.6)	1(3.8)
III Phase	6(25.3)	3(50)	9(39.1)	3(17.6)	1(16.7)	4(17.4)
Interns	8(44.4)	3(30)	11(39.3)	4(22.2)	2(20)	6(21.4)
Total	23(35.4)	13(33.3)	36(34.6)	8(12.3)	4(10.3)	12(11.5)

*Z=2.15, p<0.05 for MRSA nasal carriage rate between I &II phase students v/s interns.

Table-03: Comparison of cefoxitin and oxacillin disc diffusion method for detection of MRSA.			
Disc	**Methicillin Sensitive (%)**	**Methicillin Resistant (%)**	**Total (%)**
Cefoxitin	24(66.7)	12(33.3)	36(100)
Oxacillin	25(69.4)	11(30.6)	36(100)

Sensitivity and specificity of oxacillin when compared to cefoxitin were 92.30 % and 100 % respectively.

DISCUSSION

Understanding the epidemiology of MRSA nasal colonization among health care providers is essential for development of effective MRSA infection control strategies. Present study, the first of its kind in India, was designed to determine the prevalence of *S.aureus* and MRSA nasal carriage among medical students in different phases of medical education course, to know the effect of patient and hospital environment exposure on carrier state and to compare cefoxitin and oxacillin disc diffusion methods in detection of methicillin resistance in *S.aureus* isolates.

The study group consisted of 104 medical students studing in different phases and interns. Over all *S.aureus* nasal carriage was seen in 34.61% students. Study done by Slifka KJ et al in Michigan,[14] Gualdoni et al in Vienna,[11] Knighton HT in Virginia[15] Chambarlain NR et al in USA,[2] and Adesida et al in USA,[16] have reported *S.aureus* nasal carriage rate of 34%, 25.3%, 46.1%, 47% and 14% respectively in medical students. To our knowledge, there are only two published reports of nasal carriage of *S.aureus* in medical students from India. One study from Manipal [17] and the other from Mangalore,[18] which have reported 23.7% and 68.0% of *S.aureus* nasal carriage rate respectively in medical students. The overall nasal carriage of *S.aureus* varies from 11-68% in different studies. The variations in carriage rate reported could be due to variations in geographical distribution of *S.aureus* or due to differences in methods used to detect nasal carriage in different hospital settings. *S.aureus* carriage rate gradually increased from I phase to internship. This could be due to increased exposure to patients and hospital environment as students pass from preclinical phase to para clinical and clinical phases. But this difference in carriage rates of *S.aureus* was statistically not significant.

Overall prevalence rate MRSA in present study was 11.5%. Studies from outside India have reported MRSA carriage rates from 2.5% to 11% among medical students.[2,10,11] Indian studies have reported 0-24% MRSA carriage rate among medical students.[17,18] The above cited studies have not compared MRSA carriage rates between different phases of medical education. Only one published study done outside India, has reported MRSA carriage rate of 2.1% in second year and 3.4% in third year medical students but without any statistical significance.[14] In contrast, in present study MRSA carriage rate among I and II phase students was less than 4% but increased rapidly to 17.4% in III phase

students and 21.4% in interns and this difference in carriage rate of MRSA was statistically significant (P<0.05). This implies that exposure to patient and hospital environment is associated with an increased carriage rate of MRSA. The difference in carriage rates of MRSA between males and females was statistically not significant, though there were more number of male carriers than females.

Accurate detection of methicillin resistance can be difficult due to presence of two subpopulations, one susceptible and other resistant, that may coexist within a culture of staphylococcus. All cells in a culture may have genetic information for resistance but only a few may express resistance in vitro. This phenomenon is called heteroresistance.[19] Cells expressing heteroresistance grow slowly than oxacillin susceptible population and may be missed at temperature above 35^{0}c. This is why CLSI recommends incubating isolates at 33-35^{o}c for full 24 hours when testing for methicillin resistance with oxacillin disc. MecA mediated methicillin resistance is the most common method of methicillin resistance and non mecA resistance is very rare. CLSI recommends cefoxitin 30µg disc to detect methicillin resistance medicated by mecA gene by disc diffusion method and it is considered as surrogate for mecA mediated methicillin resistance detection.[13] Various studies also have found cefoxitin disc diffusion method results in concurrence with PCR results for mec A detection.[20,21] In the present study we compared oxacillin disc diffusion method with cefoxitin disc diffusion taking cefoxitin as standard. Cefoxitin disc detected methicillin resistance in 12 *S.aureus* isolates where as oxacillin could detect in only 11 *S.aureus* isolates. False susceptibility with oxacillin in the present study was 8.3%. Such false susceptibility by oxacillin disc is reported by other workers[22] also and it was found well above CLSI recommended acceptability limit of $\leqslant$ 1.5%.[12] The false susceptibility with oxacillin disc diffusion is attributed to the fact that heteroresistant isolates grow slowly and appear susceptible with oxacillin disc where as cefoxitin is a better inducer of mecA gene and detects the resistance. Not only this, oxacillin zones are difficult to read because of frequent hazy zones. Sensitivity and specificity of oxacillin were found to be 92.3% and 100% respectively when compared to cefoxitin disc diffusion method. The findings in the present study are in agreement with findings of the Rao V. et al[20] who have have reported sensitivity and specificity of oxacillin to be 90% and 100% respectively when compared to cefoxitin in their study. With limitation that we did not do PCR for detection of mecA gene, we found cefoxitin disc diffusion to be superior test than oxacillin disc diffusion test due to its higher sensitivity and ease of reading zones.

CONCLUSIONS

The present study revealed that, the nasal carriage rate of MRSA was significantly high in students exposed patient and hospital environment. Medical students can be a potential source of nosocomial pathogens like MRSA. Cefoxitin disc diffusion method is superior to oxacillin disc diffusion for detection of MRSA. Continuous surveillance, decolonization of carriers and improvement of hygiene standards in hospitals should be adopted to break the transmission of MRSA.

REFERENCES

1. Handerson DK, Managing Methicillin-resistant staphylococci a paradigm for preventing nosocomial transmission of resistant organisms. J Am Med 2006;119:545-52.
2. Chamberlain NR, Singh VK. Prevalence of methicillin resistant Staphylococcus aureus in nasal samples from preclinical second year medical students. MO Med 2011; 18(5):373-6.
3. Medamin TA, Al-Abdulla AA, Al-Sanousi TM, Ghabrah SZ, Afandi, Bajjunid HA. Methicillin resistant Staphylococcus aureus in two tertiary care centres in Jeddah, Soudi Arabia. Infect Control Hosp epidemiol 2001;22:211-216.
4. Fakety FR. The epidemiology and prevention of staphylococcal infection. Medicine 1964;45:593-613.
5. Landerdale TLY, Wang JT, Lee WS, Huang JH, MCDonald LC, Huang LW etal. Carriage rates of methicillin resistant Staphylococcus aureus (MRSA) depend of anatomic location, the number of sites cultured, culture methods and the distribution of clonotypes. Eur J Clin Microbiol Infect Dis 2010;29:1553-59
6. Williams REO. Healthy carriage of Staphylococcus aureus; its prevalence and importance. Bacteriol Rev 1963; 27:56-7.
7. Wertheim HFL, Malles DC, Vos MC, Leeuwen WN, Belkum AV, Verbrugh HA etal. The role of nasal carriage in Staphylococcus aureus infections. The Lancet Infect Dis 2005;5:751-62.

8. Harbarth S, Liassine N, Dhavan S. Risk factors for persistent carriage of methicillin resistant Staphylococcus aureus. Clin Infect Dis 2001;31:1380-85.
9. Santosh DR, Shobha KL, Bairy I, Gowrish R, D'soyza J, Phenotypic detection and rate of nasal carriage of heterotypic Borderline oxacillin resistant Staphylococcus aureus in preclinical medical students from Malaysia. J Clin Diagnostic Res 2008; 2:985-90.
10. Ma XX, Sun DD, Wang S, Wang ML, Li M, Shang H etal. Nasal carriage of methicillin resistant Staphylococcus aureus among preclinical medical students. Epidemiologic and molecular characteristics of methicillin resistant S.aureus clones. Diagnostic microbiology and infectious disease 2011; 70(1):22-30.
11. Gualdon GA, Lingscheid T, Tobudic S, Burgmann H. Low nasal carriage of drug resistant bacteria among medical students in Vienna. GMS Krankenhaushyg Interdiszip 2012; 7(1):Doc4.
12. Brockena NM, Van TT, Manson JA, Marshall SA, Warshauer DM. Comparision of cefoxitin and oxacillin disc diffusion methods for detection of mec A mediated resistance in *Staphylococcus aureus* in a large scale study. J Clin Microbiol 2008; 465-70.
13. Clinical laboratory standards institute. Performance standard for antimicrobial susceptibility testing Vol 31No 1: M100-5-21. Clinical laboratory standards institute, Wayne, PA, USA.2011.
14. Slifka KJ, Nettleman MD, Dybas L, Stein GE. Is acquisition of methicillin resistant Staphylococcus aureus an occupational Hazard for Medical students. Clin Infect Dis 2009; 49(3):482-483.
15. Knighton HT. Relative constancy of specific bacteriophage patterns of staphylococci isolated from oral and nasal areas. J.D.Res 1961;41(3):701-706.
16. Adesida SA, Abioyi A, Banero BS, Brai BTC, Smith SI, Amisu KO etal. Associated risk factors and pulse field gel electrophoresis of nasal isolated S.aureus from medical students in a tertiary hospital in Logos, Nigeria. Brazillian J Infect Dis 2007;11(1):122-26.
17. Santosh DV, Shobha KL, Bairy I, Rao G, Anand KM, Dsouza J. Nasal screening and survey of preclinical medical students from Malaysia for nasal carriage of MRSA and rate of nasal colonization with staphylococcus species. Journal of clinical and diagnostic research 2007;1 (6):494-499.
18. Baliga S, Bansil R, Suchitra V, Bharati B, Vidyaniketan K, Shenoy S. Nasal carriage of MRSA in medical students. J Hosp Infect 2007; 91-92.
19. Bannaerman TL. Staphylococcus, Micrococcus and other catalase positive cocci that grow aerobically. In P.R. Murray, E.J Baron, J.H Jorgensen, M.A Pfaller, R H Yolken (eds). Manual of clinical microbiology 8th edn. ASM press, Washington DC.
20. Rao V, Bhat K G, Kugaji MS, Pai V, Shantaram M. Detection of Methicillin resistance in Staphylococcus aureus: comparision of disc diffusion and MIC with Mec A gene detection by PCR. Int J pharm Bio Sci 2011;1(4):518-21.
21. Anand KB, Agarwal P, Kumar S, Kopila K. Comparision of cefoxitin disc diffusion test, oxacillin screen agar and PCR for Mec A gene for detection of MRSA. Ind J Med Microbiol 2008; 27(1):27-29.
22. Pottumarthy S, Fritsche TR, Jones RN. Evaluation of alternative disc diffusion methods for detecting mec A mediated oxacillin resistance in an international collection of Staphylococci. Validation report from SENTRY antimicrobial surveillance program. Diagn Microbiol Infect Dis 2005; 51:57-62.