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Prevalence of Methicillin Resistant *Staphylococcus aureus* in Nasal Samples from Health Care Workers: Comparison of Cefoxitin and Oxacillin Disc Diffusion Methods

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The anterior nares is the most frequent carriage site for *Staphylococcus aureus*. Methicillin resistant *S.aureus* (MRSA) are now the major hospital acquired pathogens worldwide. To know the prevalence of nasal carriage of *S.aureus* and MRSA among health care workers (HCWs) and to compare cefoxitin and oxacillin disc diffusion methods in detecting MRSA. Study was conducted on 102 HCWs of a tertiary care hospital consisting of 12 laboratory technicians, 37 staff nurses and 53 nursing students. Samples were collected from anterior nares using sterile cotton swab soaked in sterile saline. Swabs were processed without delay. *S.aureus* were identified by standard microbiological techniques. Detection of MRSA was done by cefoxitin and oxacillin disc diffusion method according CLSI 2011 guidelines. Highest *S.aureus* and MRSA carriage of 54.05% and 45.94% respectively was seen in staff nurses. *S.aureus* and MRSA carriage was 41.66% and 33.33% in laboratory technicians and 41.50% and 22.64% respectively in nursing students. Cefoxitin detected 33 and oxacillin detected 30 MRSA. Prevalence of nasal carriage of *S.aureus* and MRSA is very high in HCWs. Cefoxitin disc is superior to oxacillin disc in detecting MRSA. Continuous surveillance, decolonization of carriers and improvement of hygiene standards in hospital should be adopted to break the transmission of MRSA.

Key words: *S.aureus*, MRSA, Nasal carriage, Cefoxitin, Health care workers.

Staphylococcus aureus is both human commensal and a frequent cause of clinically important infections[1]. The association between *S.aureus* nasal carriage and staphylococcal disease was first reported by Danbolt in 1931.[2] A causal relation between *S.aureus* nasal carriage and infection is supported by the fact that the nasal *S.aureus* strain and the infecting strain share same phage type or genotype[3,4]. Further more nasal application of an anti staphylococcal drug temporarily decolonizes the nose and body sites, which prevents the infection.[5] Although multiple body sites can be colonized in human beings, the anterior nares is the most frequent carriage site for *S.aureus*[6]. Methicillin resistant *Staphylococcus aureus* (MRSA) is an important cause of health care associated infections worldwide.[7] Hospitals represent a special environment providing health care to the patients and a work environment for the medical and other staff. Staphylococcus colonized health care workers (HCWs) transfer

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such strains to patients or they transfer the organisms from one patient to another through their hands leading to epidemics in hospitals.[8] People infected with antibiotic resistant organisms like MRSA are more likely to have longer and more expensive hospital stays, and may be more likely to die as a result of the infection. In most hospitals in developing counties like India, there is neither a surveillance system nor a control policy for MRSA. In India, the studies on prevalence of MRSA among HCWs are much lacking. CLSI[9] recommends the use of cefoxitin disc instead of oxacillin disc when using disc diffusion method to detect methicillin resistance in *S.aureus*. Hence the current study was under taken to know the prevalence of nasal carriage of *S.aureus* and MRSA among HCWs and to compare cefoxitin and oxacillin discs in detecting MRSA by Kirby-Bauer disc diffusion method.

MATERIALS AND METHODS

The present study was conducted on HCWs of a tertiary care hospital. 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 was conducted on 102 HCWs consisting of 12 laboratory technicians, 37 staff nurses and 53 nursing students posted in the hospital.

Collection of Nasal swab

Cotton swabs sterilized by hot air oven at 160°C for 1 hour and moistened with sterile saline were used for nasal swabbing. The swab was circled in both nostrils consecutively and placed back in the container.

Processing of Nasal swab

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 cefoxtin (30 µg) 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 following latest CLSI 2011 guidelines. For cefoxitin ≤21mm and ≥ 22 mm were taken as resistant and susceptible zones respectively. For oxacillin ≤10mm and ≥13mm diameter were taken as resistant and susceptible zones respectively.[9]

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

RESULTS

The present study was conducted on 102 Health care workers of a tertiary health care centre. Among the HCWs, 12(11.76%) were laboratory technicians,37(36.27%) were staff nurses and 53(51.96%) were nursing students (Table-1)

Highest nasal *S.aureus* carriage was seen in staff nurses (54.05%) followed by technicians (41.66%) and nursing students (41.50%). In all the three groups of HCWs, males showed higher carriage rates of *S.aureus* than females. The difference in carriage rate of *S.aureus* was statistically not significant between three groups of HCWs and between males and females (P>0.05).

MRSA nasal carriage was highest among staff nurses (45.94%) followed by technicians (33.33%) and nursing students (22.64%). Males showed higher carriage rates of MRSA among technicians and staff nurses where as females showed higher MRSA carriage rates among nursing students (Table-2). The difference in carriage rate of MRSA was statistically not significant between staff nurses and laboratory technicians and between males and females (P>0.05). The difference in nasal carriage rate of MRSA was statistically significant between staff nurses and nursing students. (Z=2.33, P<0.05).

Table-3 shows comparison of cefoxitin and oxacillin disc diffusion methods in detection of MRSA. Out of 47 *S.aureus* isolated, Cefoxitin detected 33 and oxacillin detected 30 MRSA.

There was one *S.aureus* which showed resistance by oxacillin disc but was sensitive by cefoxitin disc diffusion method.

The present study showed sensitivity and specificity of oxacillin disc diffusion to be 91.66% and 100 % respectively when compared to cefoxitin disc diffusion method in detection of MRSA.

Table 1. Distribution of Health care workers

HCWs	Male	Female	Total (%)
Lab-Technicians	10	2	12(11.76)
Staff nurses	20	17	37(36.27)
Nursing students	32	21	53(51.96)
Total	62	40	102(100)

Table 2. Nasal carriage of *S.aureus* and MRSA among HCWs

HCWs	*S.aureus* carriage			MRSA carriage		
	Male(%)	Female(%)	Total(%)	Male(%)	Female(%)	Total(%)
Lab Technicians	5(50)	0(0)	5(41.66)	4(40)	0(0)	4(33.33)
Staff Nurses	3(65)	7(41.17)	20(54.05)	10(50)	7(41.17)	17(45.94)
N.Students	14(43.75)	8(38.09)	22(41.50)	7(21.87)	5(23.80)	12(22.64)
Total	32(51.61)	15(37.5)	47(46.07)	21(33.87)	12(30)	33(32.35)

Z value was 2.33 and $p<0.05$ for MRSA carriage rate between Staff Nurses and N.Students

Table 3. Comparison of cefoxitin and oxacillin disc diffusion method in detection of MRSA

Disc	Methicillin sensitive(%)	Methicillin resistant(%)	Total (%)
Cefoxitin	14(29.78)	33(70.21)	47(100)
Oxacillin	17(36.17)	30(63.82)	47(100)

Sensitivity and specificity of oxacillin disc diffusion were 91.66% and 100 % respectively when compared to cefoxitin disc.

DISCUSSION

Health care workers are at the interface between hospitals on one hand and community on the other. They may serve as reservoirs, vectors or victims of MRSA cross contamination. The present study reports the nasal carriage of *S.aureus* in HCWs with special emphasis on methicillin resistant *S.aureus* and detection of methicillin resistance by cefoxitin and oxacillin disc diffusion methods.

The study group consisted of 102 HCWs. Over all nasal carriage of *S.aureus* was seen in 47(46.07%) of HCWs with highest carriage rate of 54.05% among staff nurses. Various studies from India and abroad have reported nasal carriage rate of *S.aureus* from 7.6% to 48.06% among HCWs.[10-19] The mechanisms leading to *S.aureus* carriage are multifactorial. *S.aureus* carriage rates vary between different geographical areas, ethnic groups, environmental factors and risk factors in the host.[20] The fact that staff nurses are more exposed to patients than nursing students and technicians explain the highest nasal carriage of *S.aureus* in them. The present study also revealed that *S.aureus* carriage rate was more in males than females in all the three groups of HCWs. Similar observation is reported by Bidya Shrestha *et al.,*[17] and Mahantaraj *et al.,*[13] Contrarily females had higher carriage rates in a study done by B shakya *et al.,*[15] The reason for sex differences needs further studies including possible role of hormones.

Overall nasal carriage of MRSA was seen in 33 (32.35%) HCWs, with highest carriage rate of 45.94% in staff nurses. The difference in nasal carriage of MRSA between staff nurses and nursing students was statistically significant ($Z=2.33$, $P<0.05$). This could be due to the fact that staff nurses are more exposed to patients than nursing students. Various studies have reported MRSA

nasal carriage rates from 1.5% to 38.9% among health care workers.[11-17] Variations in nasal carriage rate of MRSA among different studies could be due to variations in geographical distribution of MRSA or antibiotic policies or hygiene standards in different hospitals.

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.[21] Cells expressing heteroresistance grow slowly than oxacillin susceptible population and may be missed at temperature above 35°C. This is why CLSI recommends incubating isolates at 33-35°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.[9] Various studies [22-23] also have found cefoxitin disc diffusion method results in concurrence with PCR results for mec A detection. In the present study we compared oxacillin disc diffusion method with cefoxitin disc diffusion taking cefoxitin as standard. Cefoxitin disc detected methicillin resistance in 33 of *S.aureus* isolates when compared to oxacillin disc which detected methicillin resistance in only 30 isolates. False susceptibility with oxacillin in the present study was 9.09%. Such false susceptibility by oxacillin disc is reported by other workers also [24] and it was found well above CLSI recommended acceptability limit of ≤1.5%.[25] 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 better inducer of mecA gene and detects the resistance.[23] Not only this, oxacillin zones are difficult to read because of frequent hazy zones. In the present study, one isolate was resistant by oxacillin and sensitive by cefoxitin disc and this could be due to rare non-mecA mediated methicillin resistance or a false positive result by oxacillin. As MIC testing to confirm non mecA resistance(≥4µg/ml) or PCR to rule out mecA resistance was not done in the present study, this case was not included in the study.

Sensitivity and specificity of oxacillin disc diffusion were found to be 91.66% and 100% respectively when compared to cefoxitin disc diffusion. The findings in the present study are in agreement with findings of the Rao V. et al[22] who have have reported sensitivity and specificity of oxacillin to be 90% and 100% respectively when compared to cefoxitin in their study. With the limitation that we did not do PCR test for detection of mecA gene, we found that the cefoxitin disc diffusion test to be superior test than oxacillin disc diffusion test due to its higher sensitivity and ease of reading zone size.

In conclusion, the results of the present study suggest that the nasal carriage of *S.aureus* and MRSA is very high among HCWs, especially those exposed to patients and hospital environment. HCWs can be a potential source of nosocomial pathogens like MRSA to patients they take care of. Cefoxitin disc diffusion method is superior than oxacillin disc diffusion to detect MRSA. Hence continuous surveillance, decolonization of carriers and improvement of hygiene standards in hospitals should be adopted break to the transmission of MRSA.

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