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SCREENING FOR METHICILLIN- RESISTANT *STAPHYOCOCCUS AUREUS* NASAL CARRIAGE AMONG HEALTHCARE WORKERS IN A TERTIARY CARE HOSPITAL

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing significant morbidity and mortality in healthcare organization and in community. The aim of the study was to find out the current prevalence of MRSA and to demonstrate antibiotic resistance pattern of MRSA and Methicillin- sensitive *Staphylococcus aureus* (MSSA) among health care workers. Nasal swabs were collected from 300 healthcare workers (112 doctors and188 nurses). Identification of the isolate as *Staphylococcus aureus* was done using Gram stain, coagulase test and Catalase test. *Staphylococcus aureus* isolates were confirmed as MRSA using cefoxitin (30µg) disc by Kirby-Bauer disc diffusion method on Muller Hinton agar. From 300 nasal swabs, 250 coagulase negative *Staphylococci*, 40MSSAand 10 MRSA were isolated. Overall rate of isolation of MRSA and MSSA among healthcare workers was found to be 3% and 13% respectively. All the 10 MRSA strains screened from health care workers were resistant to Penicillin and Cefoxitin and were sensitive to four drugs viz. Vancomycin, Teicoplanin, Gentamicin and Linezolid. All 40 isolates of MSSA were resistant to Penicillin however, 100% sensitivity was observed to three drugs viz. Cefoxitin, Vancomycin and linezolid.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus* (MSSA), antibiotic resistance.

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INTRODUCTION

Staphylococcus aureus is the leading Gram positive bacteria causing hospital acquired infection. Methicillin-resistant *Staphylococcus aureus* (MRSA) by definition is a strain of *S. aureus* that is resistant to a large group of antibiotics called "the beta-lactams", which include the Penicillins and the Cephalosporins. MRSA is called a "super bug" because infections caused by these organisms are resistant to many commonly used antibiotics. The basic mechanism of resistance in most cases of MRSA is the production of Penicillin Binding Protein, PBP2 or PBP2a, mediated by the mec A gene in the cell wall of resistant strains. MRSA have emerged worldwide as a major cause of nosocomial infections. A considerable increase in the prevalence of MRSA has been observed globally during the last decade (Mehndiratta *et al*., 2012). MRSA causes superficial and mild infections on the skin, like sores or boils. The main mode of MRSA transmission is from a MRSA-colonized or infected patients to another patient by direct contact or through the touch of health care workers (de San *et al.,* 2007). Thus, patients may also acquire MRSA from colonized health care workers (Blok *et al.,* 2003). Nasal carriage is a major risk factor for *Staphylococcus aureus* infection, especially for MRSA as anterior nares are the major reservoir of *S. aureus*, 20% of humans are persistently and asymptomatically colonized, 60% are intermittently, while 20% are non-carriers (Gonzalez-Zorn *et al.,* 2005).

The role of MRSA carriers in the transmission of this pathogen is critical. Healthcare workers who are at the interface between the hospital and the community may serve as agents of cross-transmission of Hospital Associated-MRSA and Community Associated-MRSA (Fadeyi *et al.,* 2010). Thus the regular screening of healthcare workers in a hospital setup is necessary to prevent transmission and to avoid outbreaks of infection with MRSA in hospitals. Most patients with MRSA are asymptomatic and colonized, but the organism may sometimes cause life-threatening infections in a substantial number of patients (Casewell *et al.,* 1986, Ibelings *et al.,* 1998). As MRSA is typically multidrug-resistant, treatment options are limited to few antibiotics like teicoplanin and vancomycin. Since there are reports of reduced susceptibility and resistance of *Staphylococcus aureus* to vancomycin from different countries especially Japan and USA (Klevens *et al,* 2007), the study was conducted to determine current prevalence of MRSA as well as antimicrobial susceptibility patterns of isolates from

healthcare workers to adopt effective infection control measures.

MATERIAL AND METHODS

A cross-sectional study was conducted at a tertiary care hospital, in Western Maharashtra, India from June 2013 to May 2014. Three hundred healthcare workers were screened which included Doctors and Nurses working in intensive care units, pediatric wards, Medical wards, OPD of a tertiary care hospital. Informed consent was obtained from each of the healthcare worker (HCW). The study was approved by the institutional ethics committee and conducted in accordance with the ethical principles. Healthcare workers with diagnosed *Staphylococcal* infection and antibiotic usage within the last three months were excluded from the study. Premoistened sterile swabs were used to collect samples from the anterior nares of both sides. These swabs were then immediately taken to microbiology laboratory within 30 minutes to avoid use of any transport medium. Ensuring all sterile precautions, the samples were inoculated onto sheep blood agar plates and were incubated overnight at 37ºC. Suspected colonies of *Staphylococcus aureus* were confirmed by gram staining, mannitol fermentation and by both slide and tube coagulase test. Further Catalase test was also performed to differentiate between *Staphylococci* and *Streptococci*. Antimicrobial susceptibility testing was performed using Kirby-Bauer's disc diffusion method as per CLSI guidelines. The antibiotics that were used are listed in Table 1 below. The table also shows the concentration of antibiotics and the zone diameter used in interpretation of results. Methicillin resistance was checked for all isolates of *Staphylococcus aureus* using Cefoxitin 30 µg disc as well as Hi-Chrome MeReSA agar.

Table 1. Antibiotic disks used in the study

Antibiotic	Conc. (mcg/disc)	Zone diameter (mm)		Manufacturer
		Resistant	Susceptible	
PENICILLIN	10	≤ 28	≥29	Himedia
CEFOXITIN	30	≤14	≥18	Himedia
ERYTHROMYCIN	15	≤13	≥ 23	Himedia
VANCOMYCIN	30	–	≥15	Himedia
TEICOPLANIN	30	≤18	≥ 23	Himedia
TETRACYCLINE	30	≤18	≥ 23	Himedia
CLINDAMICIN	2	≤21	≥ 14	Himedia
GENTAMICIN	10	≤12	≥ 15	Himedia
RIFAMPICIN	5	≤16	≥ 20	Himedia
LEVOFLOXACIN	5	≤15	≥ 19	Himedia
COTRIMOXAZOLE	1.25/23.7	≤10	≥16	Himedia
LINEZOLID	30	–	≥21	Himedia

RESULTS

Characteristics of the healthcare workers

A total of 300 healthcare workers were screened from tertiary care health facility for present study. Table 2 presents the demographic profile of the healthcare workers in the present study. Of 300 participants, 141 (47 %) participants were from age group of 20 to 24 years. Ninety-two (30.7 %) participants were from 25 to 29 years age group. Whereas only 29 (9.7 %) and 38 (12.7 %) participants were from age group of 30 to 34 years and more than 35 years, respectively. Majority of the healthcare workers screened were females (Nurses) is 73.3 % and 26.7% were males. Of the total participants, 112 participants were doctors and remaining 188 participants were working as nurses in the hospital (Table 2).

Table 2. Demographic profile of the healthcare workers screened in the study

Parameters	Number	Percentage
Age groups (years)		
20 to 24	141	47.0
25 to 29	92	30.7
30 to 34	29	9.7
35 and more	38	12.7
Gender		
Female	220	73.3
Male	80	26.7
Profession		
Doctors	112	37.3
Nurses	188	62.7

Prevalence of MRSA and MSSA in healthcare workers

A total of 300 nasal swabs collected from healthcare workers were inoculated on sheep blood agar. Out of 300 isolates, 250 (83.4%) were coagulase negative *Staphylococci*, 40 (13.3%) were methicillin sensitive S*taphylococci* and 10 (3.3%) were methicillin resistant *Staphylococci*. Furthermore, table 3 presents detail picture of MRSA and MSSA according to the profession. Out of 112 doctors, one doctor tested positive for MRSA while 17 (15.2 %) doctors were MSSA positive. In case of nurses 9 (4.8 %) and 23 (12.2%) nurses tested positive for MRSA and MSSA, respectively (Table 3).

Percentage of MRSA and MSSA as per hospital units

Ten strains were identified as MRSA among the isolates. Of which six of them were from medical (Table 4), two from gynecology unit and one each from NICU and OPD. A total of 40 strains of MSSA were isolated from 300 samples. Healthcare workers from the OPD unit accounted for 42.5 % of the isolates followed by surgical unit (20.0 %), gynecology unit (12.5 %) and medicine unit (12.5 %).

Table 3. Percentage of MRSA and MSSA isolated from the anterior nares of doctors and nurses

Profession	MRSA	MSSA	CoNS	Total
Doctors	1 (10.0 %)	17 (42.5 %)	94 (37.6 %)	112 (37.3 %)
Nurses	9 (90.0 %)	23 (57.5 %)	156 (62.4 %)	188 (62.7 %)
Total	10 (100.0 %)	40 (100.0 %)	250 (100.0 %)	300 (100.0 %)

Resistance pattern

The antibiotic resistance patterns of MRSA and MSSA obtained from health workers are shown in Figure 1 and 2, respectively.

observed to three drugs viz. Cefoxitin, Vancomycin and linezolid followed by 97.5 % to Levofloxacin and Rifampicin, 95.

Table 4. Percentage isolation of *Staphylococci* from the anterior nares of healthcare workers from different units of hospital

Units	MRSA	MSSA	CoNS	Total
Central lab	0 (0.0%)	1 (2.5 %)	20 (8.0%)	21 (7.0%)
Gynecology	2 (20.0%)	5 (12.5%)	29 (11.6%)	36 (12.0%)
ICUs	0 (0.0%)	3 (7.5%)	34 (13.6 %)	37 (12.3%)
Medical/MF	2 (20.0%)	1 (2.5%)	26 (10.4%)	29 (9.6%)
Medical/MM	4 (40.0%)	4 (10.0%)	16 (6.4%)	24 (8.0%)
Medical/Paed	0 (0.0%)	0 (0.0%)	16 (6.4%)	16 (5.3%)
NICU	1 (10.0%)	1 (2.5%)	16 (6.4%)	18 (6.0%)
OPD	1 (10.0%)	17 (42.5%)	60 (24.0%)	78 (26.0%)
PICU	0 (0.0%)	0 (0.0%)	8 (3.2%)	8 (2.6%)
Surgical	0 (0.0%)	8 (20.0%)	25 (10.0%)	33 (11.0%)
Total	10 (100.0 %)	40 (100.0 %)	250 (100.0 %)	300 (100.0 %)

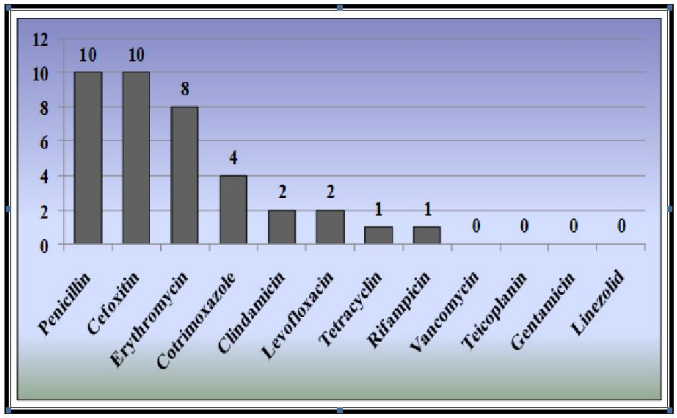

Figure 1. Antibiotic resistance pattern among MRSA isolated from the anterior nares of healthcare workers

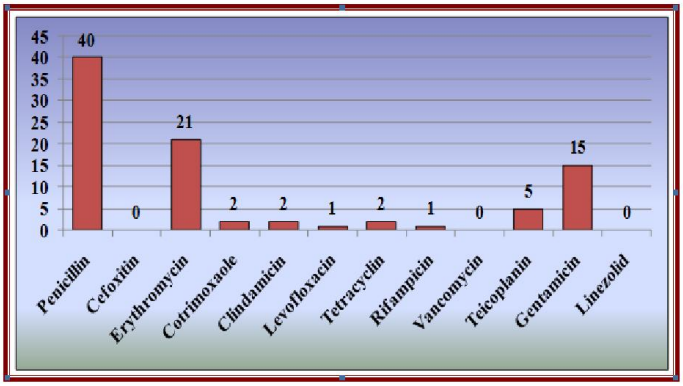

Figure 2: Antibiotic resistance pattern among MSSA isolated from the anterior nares of healthcare workers

The antibiotic resistance patterns of MRSA isolates were found to be highly variable. As indicated in Figure 1, all the 10 MRSA strains screened from health workers were resistant to Penicillin and Cefoxitin. Eight of the isolates were resistant to Erythromycin, four to Cotrimoxazole, two to Clindamicin and Levofloxacin and one to Tetracyclin and Rifampicin. All the ten isolates were sensitive to four drugs viz. Vancomycin, Teicoplanin, Gentamicin and Linezolid. Drug resistance pattern of MSSA isolates is given in Figure 2. All 40 isolates of MSSA were resistant to Penicillin. Of the 40 MSSA isolates, 21 (52.5 %) isolates were resistant to Eryromycin and 15 (37.5 %) to Gentamicin. However, 100 % sensitivity was 0 % to Cotrimoxazole, Clindamicin and Tertracyclin and 87.5 % to Teicoplanin.

DISCUSSION

The epidemiology of MRSA is gradually changing from being an occasional report to an established hospital acquired pathogen since its emergence was first reported. Recent studies report an increased prevalence of community acquired MRSA. Moreover, the association of multidrug resistance with MRSA has added to the problem. It is necessary to detect MRSA in health workers as they can act as carriers and thus, a potential source of infection. In healthy subjects, over time, three patterns of carriage can be distinguished: about 20% of people are persistent carriers, 60 % are intermittent carriers, and approximately 20% almost never carry *Staphylococcus aureus*. Also *Staphylococcal* carrier status can lead to nosocomial infections (Gonzalez-Zorn *et al.,* 2005). In this study the prevalence of MRSA carriers among healthcare workers and the resistance pattering of these strains were determined. In the present study, 10 (3.3%) health workers carried MRSA which included 1 doctor and 9 nurses. MRSA carrier rate among health workers found in our study is in concordance with findings of other studies (Mathanraj *et al.,* 2009, Vinodkumaraditya *et. al*., 2009, Johnston *et al*., 2007).

Blok *et al.,* (2003) reported less than 5% health workers to be colonized with MRSA. A higher MRSA carrier rate of 15% and 13.6% was reported by Bisaga *et al.,* (2008) and Suffoletto *et al.,* (2008), respectively in USA. de de Carvalho *et al.,* (2009) in their study isolated (4.1%) MRSA (14/340) from the saliva the of health professionals. Kumar *et al.,* (2011) reported 4.66 % MRSA carrier rate among health workers which included 83.4 % doctors and 16.6 % laboratory technicians. Methicillin resistance among *Staphylococci* is widespread in India. In a multi-centric study from India in 1996, prevalence of MRSA ranged from 27 % in Bombay to 47 % in Bangalore and 42.5 % in Delhi. The most recent data from India gives a figure of 66 % methicillin resistant CoNS among neonatal septicemia patients; isolated from blood and skin of these patients (Chaudhury *et al*., 2007). The ecological niche for the colonization of *Staphylococci* is the anterior nares, and hence greater amount of bacteremia cases recorded, have been due to *Staphylococcus aureus* of endogenous origin,

i.e from the nasal mucosa. Therefore, it is imperative that nasal colonization due to *Staphylococcus aureus* should be prevented in order to stem the rate of infection and in preventing the transmission of resistant strains of the organism from the health care workers to the patients. Although nasal carriage of *Staphylococcus aureus* is harmless in healthy individuals, they can become carriers, posing risk of spreading infection. Nasal carriage, particularly in case of healthcare workers can be a major risk factor in transmission of infection to patients because of their constant interaction and exposure to them. Health professional's compliance with sanitary and antibacterial guidelines is essential to prevent nosocomial infection. To achieve this, simple preventive measures like hand washing before and after patient examination, the use of sterile aprons and masks, and awareness while examining immune compromised patients should be implemented strictly. Simultaneous monitoring and surveillance of nosocomial infections by the hospital infection control committee can help to further reduce transmission. In this study, we have tested the susceptibility of the MRSA and MSSA strains with 12 different antibiotics. As expected, all the strains of MSRA were resistant to Penicillin and Cefoxitin whereas, all the strains showed susceptibility to four antibiotics viz. Vancomycin, Teicoplanin, Gentamicin and Linezolid. Out of ten, eight strains were resistant to Erythomycin. Gentamicin resistance is on the rise since 1996. An increase in gentamicin resistance from 0% before 1996 to 80% after 1996 has been reported.(154) Qureshi had reported a gentamicin resistance of 97.8% (Price *et al.* 1998), which is similar to the findings of our study. The clinically significant observation of this study is the moderate resistance shown by MRSA to other conventional antibiotics. In our study, moderate resistance is seen in case of Cotrimoxazole whereas low resistance is seen for Clindamicin, Levofloxacin, Tetracyclin. The other contemporary reports state higher resistance rates for aminoglycosides and fluoroquinolones. However, Pulimood had observed only 8% resistance of MRSA to Gentamicin (Qureshi *et al.,* 2004) as against 63.6 % in our study.

Conclusion

The study showed overall MRSA and MSSA carrier rate as 3% and 13% respectively. Both doctors as well as nurses were reported to be carriers for MSSA whereas, for MRSA largely nurses were the carrier. Accurate and early detection of these strains in hospitals and community will reduce the risk of transmission. Also, with a systematic review of isolation policy in hospitals with increased compliance with hand hygiene recommendation could lead to significant reduction in MRSA. Use of antibiotics like vancomycin, linezolid and teicoplanin may lead to further reduction of MRSA and MSSA as these were sensitive drugs identified in the present study.

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