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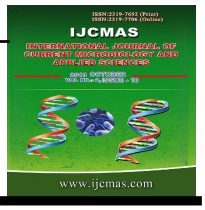

Original Research Article

Prevalence of Methicillin-Resistant *Staphylococcus aureus* colonisation and its antibiotic susceptibility profile among healthcare personnel in a tertiary care setup of northern India

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A B S T R A C T

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most common nosocomial pathogen. Colonisation of MRSA among the health care personnel (HCP) taking care of patients may lead to spread of disease among patients and co-workers. The present study is aimed to detect the methicillin resistance in *Staphylococcus aureus* isolates obtained from the health care staff from selected wards of a 2800-bedded tertiary care hospital setup of northern India. Nasal and hand swabs collected from health care personnel of Cardiothoracic and vascular surgery (CTVS) wards and Trauma ventilatory units (TVU). Detection of methicillin resistance was performed for *Staphylococcus aureus* isolated from the swabs as per CLSI guidelines using cefoxitin 30µg disc. Out of 166 health care personnel, *Staphylococcus aureus* isolates were detected in 76/166 (45.78%) and MRSA was detected in 22/166 (13.25%). Of the 22 HCP with MRSA, 2/22(9.09%) were carrying MRSA in both hand and nasal swabs, whereas 12/22(54.54%) were only nasal carriers and 8/22(36.36%) were only hand carriers. Rate of colonisation of *Staphylococcus aureus* among HCP was 45.78% and carriage of MRSA was 13.25% and thus the possibility of spread of MRSA among the patients in the hospital.

Introduction

Staphylococcus aureus has been a major cause of infections in humans for well over a century. *Staphylococcus aureus* is both a human commensal and a frequent cause of clinically important infections, ranging from skin and soft tissue infections such as metastatic abscesses, wound infections to septic arthritis, osteomyelitis and severe systemic infections including pneumonia, bacteraemia(Lowy, 1998). It is one of the most common causes of hospital-acquired infection throughout the world (McDonald, 2006). Advent of penicillin and sulphonamides had dramatically reduced the frequency of these infections.

However, penicillinase producing *Staphylococcus aureus* remain a major problem in hospitals worldwide till the introduction of semisynthetic penicillins such as methicillin or oxacillin. Unfortunately, methicillin-resistant *Staphylococcus aureus* has been reported soon after its discovery (Barrett *et al*., 1968). Since then, outbreaks of MRSA have been increasingly reported from various healthcare institutes worldwide (Torvaldsen S *et al*., 1999; Vidhani s *et al*., 2009; Vinodhkumaradithyaa A *et al*., 2009).

Carriage of MRSA in nose, axilla, perineum and hand of patients and health care personnel (HCPs), prolonged duration of hospital stay, intravenous drug abuse and irrational use or over-prescription of antibiotics are important risk factors for MRSA acquisition (Klutymans J *et al*., 1997). These factors along with the diversity in *mecA* gene (responsible for resistant mechanisms of MRSA isolates) for community and healthcare-associated infections pose a major challenge to prevent the spread of disease in both community and hospitals **(**Fey, et al., 2003**)**. Along with that, MRSA strains are becoming multidrug resistant and presently antibiotic used for treatment are systemic antibiotics such as vancomycin, linezolid, quinupristin or dalfopristin (Rivera, A.M. and Boucher, H.W., 2011). This has also raises the alarm to check the unprecedented use of antibiotics in hospitals as it may soon leave no treatment options. Nasal decolonisation of MRSA among HCPSs using 2% mupirocin ointment is the only modality to prevent the transmission of MRSA (Hudson I. R., 1994).

Therefore, knowledge of prevalence of MRSA among HCPs becomes necessary for planning a strategy to check nasal colonisation of MRSA and prevent the transmission of infections among patients and co-workers. This study has aimed to determine the current prevalence of MRSA colonisation among carriers and need of proper nasal decolonisation, along with *in vitro* susceptibility profile of isolates to various antimicrobial drugs to determine the susceptibility to common antibiotics used for treatment of *Staphylococcus aureus* infections in a major referral and tertiary care hospital setup.

Materials and Methods

Nasal and hand swabs collected from 166 HCPs from Cardiothoracic and vascular surgery ward, trauma ventilatory units and surgical wards of Gandhi Memorial and Associated Hospitals, King George Medical University, Lucknow, India during July' 2011 to June ' 2012 were included in this study.

Collection of swabs

All samples were collected aseptically. With gloved hands, sterile swab stick with transport tube was taken. For collecting nasal samples, swab was inserted approximately 2 cm (approximately ¾ inches) into the nares and rotated against the anterior nasal mucosa for 3 seconds. Using the same swab, repeated for other nares and placed back into the transport tube. End of the swab was pushed firmly to ensure that the swab is inserted up to the end of the transport tube ensuring that the swab tip is in contact with the moistened pledget. Transport tube cap was then secured. For hand samples, another sterile swab stick with transport tube was taken. Swab was removed from the tube and rubbed in palmer surface of hand three

times at 60°. Using the same swab, it was repeated for the other hand. The swab was then transferred back to the tube and secured properly. After collecting both nasal and hand swabs, it was labelled properly and processed.

Processing and identification

Specimens were inoculated on 5% blood agar, MacConkey agar and RCM (Robertson cooked meat medium) for further subculturing and incubated over night at 37°C. *Staphylococcus aureus* were identified on the basis of colony characteristics, Gram stain, catalase test and coagulase test. Coagulase test was performed by using both slide and tube coagulase method. *Staphylococcus aureus* ATTC-25923 of known coagulase production was included as control strain. Only those isolates which are positive for both the slide and tube coagulase test were included in the study.

Detection of methicillin resistance and antibiotic susceptibility profile

All confirmed non-duplicate *Staphylococcus aureus* are further tested for detection of methicillin resistance by Kirby-Bauer disc diffusion method using cefoxitin 30 µg discs (Himedia Labs, India) as per Clinical Laboratory Standards Institute (CLSI) 2012 guidelines. Inoculum was prepared in a 4 ml sterile saline from the colony to obtain a suspension of 0.5 McFarland standards turbidity. Mueller Hinton agar with 4% NaCl is inoculated by a sterile cotton swab by streaking the surface of medium by rotating at 60° three times. Sensitive control *Staphylococcus aureus* ATCC 25923 was tested with each batch of test. Cefoxitin 30 µg discs were applied on the surface of agar at appropriate distance from each other. Zone of inhibition were examined after incubating the plates at 37°C for 16-18 hours in good illumination to detect colonies within the zone. For cefoxitin, zone size of $\leq$ 21 mm was taken as resistant and $\geq$ 22 mm as sensitive.

Further, antibiotic susceptibility profile was determined by the modified Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitive, intermediate or resistance to different antimicrobials. The antibiotics used were ampicillin (10 µg); oxacillin (30µg); erythromycin (15µg); clindamycin (2µg) ciprofloxacin (5µg); levofloxacin (5µg); tetracycline (30µg); co-trimoxazole (25µg); vancomycin (30µg); linezolid (30µg). Finally, the data was recorded and analyzed at the completion of the study as per recommendations of the CLSI guidelines 2012 (Table No.1). *Staphylococcus aureus* ATCC 29213 was used as reference strain for the standardization of antibiotic susceptibility testing.

Result and Discussion

Out of 166 HCPs, 76 non-duplicate *Staphylococcus aureus* were isolated. Of these *Staphylococcus aureus*, only 12 isolates were isolated from both nasal and hand swab of same HCPs. While 48 and 16 *Staphylococcus aureus* were isolated from nasal swab and hand swab respectively (Table 2). Among these isolated *Staphylococcus aureus*, 22 isolates were showing resistance to cefoxitin 30 µg discs and were thus methicillin-resistant *Staphylococcus aureus* (Table 3). These 22 MRSA isolates were from 12, 8 and 2 nasal only, hand only and both nasal and hand swabs respectively (Table 4).

Table.1 Zone size Interpretative Chart (CLSI 2012)

S. No.	Antibiotic	Diameter of zone of inhibition in mm		
		Resistant	Intermediate	Sensitive
1.	Ampicillin	≤ 28	-	≥29
2.	Ciprofloxacin	15	16-20	21
3.	Clindamycin	≥ 21	15-20	≤14
4.	Erythromycin	≥23	14-22	≤13
5.	Levofloxacin	≥19	16-18	≤15
6.	Linezolid	≥21	-	≤20
7.	Oxacillin	≥13	11-12	≤10
8.	Tetracycline	≥19	15-18	≤14
9.	Septran	≥16	11-15	≤11
10.	Vancomycin	-	-	-

Table.2 Percentage of colonization of *Staphylococcus aureus* at two different site in 166 HCPs

Site	*Staphylococcus aureus*
Nasal Swab only	48 (28.91%)
Hand Swab only	16 (9.63%)
Both nasal and hand swab	12 (7.22%)
Total non-duplicate *Staphylococcus aureus* from both nasal and hand swabs	**76 (45.78%)**

Table.3 Distribution of MRSA and MSSA among the *Staphylococcus aureus* isolates

MSSA	54 (32.53%*)
MRSA	22 (13.25%*)
*** Percentage in parenthesis represent out of total HCPs**	

Table.4 Percentage of colonization of MRSA in nasal and hand swabs of the HCPs

Site	MRSA
Nasal Swab & Hand Swab	2 (9.09%*)
Nasal Swab only	12 (54.54%*)
Hand Swab only	8 (36.36%*)
***Percentage in parenthesis represent out of *Staphylococcus aureus* isolates**	

Table.5 Antimicrobial sensitivity pattern of MRSA and MSSA isolates

Antibiotics	Sensitive (%)		Intermediate (%)		Resistant (%)	
	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
Ampicillin	1 (4.5)	15 (27.8)		-	21 (95.5)	39 (72.2)
Ciprofloxacin	6 (27.2)	31 (57.4)	3 (13.6)	14 (25.9)	13 (59.2)	9 (16.7)
Clindamycin	10 (45.5)	37 (68.5)	3 (13.6)	6 (11.1)	9 (40.9)	11 (20.4)
Erythromycin	8 (36.3)	29 (53.7)	1 (4.5)	9 (16.7)	13 (59.2)	16 (29.6)
Linezolid	22 (100)	54 (100)	-	-	-	-
Septran	7 (31.8)	27 (50)	4 (18.2)	9 (16.7)	11 (50)	18 (33.3)
Tetracycline	12 (54.5)	37 (68.5)	3 (13.6)	4 (7.4)	7 (31.9)	13 (24.1)
Vancomycin	22 (100)	54(100)	-	-	-	-

The antibiotic susceptibility profile of both MRSA and MSSA isolates as determined by disc diffusion method is documented in Table 5. All MRSA and MSSA isolates were susceptible to vancomycin and linezolid. Most of the MRSA isolates were resistant to ampicillin (95.5%) followed by ciprofloxacin and erythromycin (59.2%). While in case of MSSA strains, resistant to ampicillin was seen in 72.2 %.

MRSA is one of the commonest nosocomial pathogen in hospitals worldwide and it is increasingly recovered causing significant morbidity and mortality (Helen and Ralph Corey, 2008). Carriers and infected patients are the important reservoirs of MRSA in hospitals/healthcare-institutions. Nasal and transient carriage on the hands of health care workers is the predominant mode for healthcare staff-to-patient transmission (McDonald , 1997). Outbreaks of MRSA were reported soon after its discovery worldwide but its significance in India was *Staphylococcus aureus* were isolated from not recognised till it emerges as a problem in the late 1980s. Along with that these strains are usually also resistant to other antibiotics of same group and other groups too. In last two decades, emergence of diversion in the phenotypic character of *Staphylococcus aureus* as health-care associated and community associated strains has also led to failure in preventing the spread of MRSA, which poses a major therapeutic problem in many hospitals (Kluytmans *et al*., 2006). Variation in clinical presentation, virulence factors and genotypic character has been a major concern to prevent the disease transmission.

In this study, the prevalence of MRSA colonisation and its antibiotic susceptibility profile in different HCPs subjects were determined. Of 166 HCPs, 76 (45.78%) non-duplicate *Staphylococcus aureus* were isolated. As high as 48 *Staphylococcus aureus* were obtained from nasal swab only while only 12 both nasal and hand swab of same HCPs.

Thus, anterior nares were found to be the most common site of colonisation as suggested by various studies (Wertheim *et al*., 2005; Klutymans *et al*., 1997).

Among the 76 *Staphylococcus aureus*, methicillin resistance were seen in 22 isolates. Thus, 13.25% of HCPs is harbouring the MRSA in their nares and hand, which could be a serious threat to spread the disease among other HCPs and the patients. No precise reason could be attributed to this apparent increase in carrier rate. Various studies shows highly variable carrier rate which ranges from 0% to 29% (Mehta *et al*., 1998; Sachdev *et al*., 2003; Pulimood *et al*., 1996). As nasal colonisation of *Staphylococcus aureus* was high nasal carriage of MRSA 12/22 (54.54%) was also the commonest presentation followed by hand carriage 8/22(36.36%).

In this study, all the isolates were susceptible to vancomycin and linezolid. As expected, most of the isolates were resistant to ampicillin. But the clinically significant observation of the study is the association of resistance shown by MRSA to other antibiotics used for treatment of staphylococcal infections. Other studies report a higher resistance rates for fluoroquinolones and aminoglycosides. Pulimood *et al*., 1996 has reported a high ciprofloxacin resistance of 90% and Qureshi *et al*., 2004 had reported a resistance of 98.9%. In contrast, this study has demonstrated 59.2% of the strains resistant to ciprofloxacin and a further lower resistant rate to clindamycin (40.9%) and tetracycline (31.9%).

As in this study most of the strains (95.5%) were resistant to ampicillin. Saxena *et al*., 2003 in contrast found higher susceptibility among the isolates obtained from carrier samples. Nevertheless, this study has demonstrated a high percentage (47.7%) of multidrug resistant MRSA from the all samples.

In conclusion, the degree of colonisation of MRSA in HCPs is a major problem to dealt with as MRSA are capable of causing serious disease in hospitalised patients. Also diversity in MRSA which depends upon the environmental condition is also a threat. Antibiotic susceptibility profile of MRSA has shown that vancomycin and linezolid are only drugs which were uniformly sensitive in all isolates. When treatment is done by vancomycin and linezolid are the only drugs *in vitro* susceptibility testing of MRSA isolates are absolutely needed in the laboratories. Multiple, prolonged use of antibiotics and prolonged hospitalization are other important factors which make hospital an ideal place of transmission and perpetuation of MRSA. Hence this study suggests a planning of strategies for determination of carriers and nasal decolonisation of HCPs in hospitals to prevent the spread of disease caused by *Staphylococcus aureus*.

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