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Staphylococcus aureus: Nasal-carriage in Health Care Workers and In-patients with Special Reference to MRSA

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Authors' contributions

This work was carried out in collaboration between all authors. Author PM designed the study and wrote the protocol. Author RN carried out the study and helped in literature searches. Author NB wrote the final draft of the manuscript, managed the analyses of the study and the literature searches. Author MA performed the statistical analysis. Authors helped in literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To find out the nasal carriage of *Staphylococcus aureus* in health care workers and in-patients in a tertiary care center.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Microbiology, Government Medical College and Hospital, Srinagar Kashmir. One year study (July 2013 to June 2014).
Methodology: Nasal swabs were taken from health care workers (HCWs) and in-patients and these were processed for the recovery of *Staphylococcus aureus* (*S. aureus*) and methicillin resistant

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Staphylococcus aureus (MRSA). Antimicrobial susceptibility of the isolates to various antibiotics was performed as per Clinical Laboratory Standards Institute (CLSI), guidelines and D-test done to ascertain constitutive macrolide-lincosamide-streptogramin B ($cMLS_B$) and inducible macrolide-lincosamide-streptogramin B ($_iMLS_B$) phenotype. Risk factors for their carriage were also analyzed. Statistical analysis was done using SPSS software version 16 and a P value of < 0.05 was taken as significant.

Results: Higher rate of isolation of *S. aureus* and MRSA was seen among 480 in-patients (47.1% and 32.7% respectively) compared with 256 HCWs (32.8% and 19.1% respectively). Significant resistance (P<0.05) to clindamycin, erythromycin, ciprofloxacin, tetracycline and co-trimoxazole was seen in MRSA isolates recovered from in-patients. MRSA isolates had higher $cMLS_B$ and $_iMLS_B$ resistance. Years of service and level of education in HCWs were significantly associated with MRSA carriage in them.

Conclusion: Nasal-carriage of *S. aureus* and MRSA is common in HCWs and in-patients in our hospital. Apart from periodic screening for MRSA carriage, strict adherence to existing infection control guidelines is mandatory.

Keywords: HCWs; in-patients; MRSA.

1. INTRODUCTION

Staphylococcus aureus is a major nosocomial pathogen, causing healthcare-associated infections worldwide [1]. Shortly after the introduction of penicillin, *S. aureus* strains resistant to the drug appeared on the horizon [2,3]. Methicillin was subsequently used to treat such infections but again strains with acquired resistance to this antibiotic; methicillin-resistant *S. aureus* (MRSA) emerged [4]. Nasal colonization with *S. aureus* is a dynamic process; various factors being responsible for the gain and loss of carriage. Risk of subsequent infection in a person colonized with *S. aureus* as well as MRSA, increases with time and remains persistently elevated [5].

A causal relationship between *S. aureus* nasal carriage and subsequent infection is supported by the fact that many a times nasal and the infecting strain have the same genotype. Colonizing strains may thus serve as endogenous reservoirs for overt clinical infections or may spread to other patients [6]. Active surveillance for patients colonized with MRSA is recommended to prevent infections due to this pathogen in health care settings. Healthcare workers (HCWs) are also known to carry pathogenic hospital strains in their nose and on their skin, thus facilitating the transmission of these to both patients and other HCW's [5].

Several studies worldwide have reported the rate of nasal carriage of *S. aureus* strains varying from 16.8% to 90% [7-9]. Emergence of MRSA strains, which are often multidrug-resistant, renders the treatment of such infections more challenging as options are limited.

The aim of this study was to describe the pattern of colonization with *S. aureus* especially MRSA among HCWs and in-patients, in a leading tertiary care hospital of this north Indian state as well as to delineate the antibiogram of these isolates.

2. MATERIALS AND METHODS

Nasal swabs were taken from 480 in-patients (of all age groups) admitted in various wards and 256 HCW's at Government Medical College and Hospital, Srinagar. In total 736 samples were collected from in-patients and health care personnel including doctors, nursing staff, sanitary attendants and laboratory technicians. Swabs were also taken from canteen staff, laundry attendants and administrative staff.

Sterile moistened cotton swabs were introduced 2 cm into the nasal vestibule and rubbed along the walls of the nasal cavity. These were put in a vial containing 6.5% NaCl and transported within 2 hours to the Microbiology laboratory of our hospital. These were inoculated onto blood agar and mannitol salt agar (MSA) plates. The swabs were also put in Robertson's cooked meat broth (RCM) with high salt concentration (7.5%) to aid in the recovery of small number of *S. aureus*. The media plates were incubated aerobically at 35℃±2℃ for 18-24 hrs and RCM tubes were incubated for 72hrs. In case the primary culture plates were sterile and RCM showed turbidity, it was sub-cultured on to the above mentioned media.

Gram positive, catalase positive cocci were identified as *S. aureus* on the basis of colony characteristics on blood agar, growth on MSA, slide and tube coagulase positivity, DNAase and phosphatase tests positivity as well as bacitracin (0.04 units) resistance and lysostaphin (20 µg) sensitivity. Antibiotic susceptibility testing was done for all the confirmed *S. aureus* isolates by Kirby Bauer disk diffusion method on Mueller Hinton agar (MHA) according to Clinical Laboratory Standard Institute (CLSI) 2013 guidelines [10]. Antibiotic discs used included penicillin (10 units), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), gentamicin (10 µg), amikacin (30 µg), clindamycin (2 µg), erythromycin (15 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg) and tetracycline (30 µg). Methicillin resistance was evaluated by using cefoxitin (30 µg) discs.

D-test to look for constitutive $cMLS_B$ or inducible $iMLS_B$ clindamycin resistance was done in all the isolates of *S. aureus*. It was done on the same plate on which antimicrobial susceptibility was performed. For the test, a 15 µg erythromycin disc was placed 20 mm apart from a 2 µg clindamycin disc on MHA plate and incubated at 35℃±2℃ for 16-18 hrs. Flattening of the zone of inhibition adjacent to the erythromycin disc (referred to as D zone) was taken as $iMLS_B$ whereas reduced zone of inhibition or hazy growth within the zone of inhibition with no D zone was taken as $cMLS_B$ phenotype [10]. *S. aureus* ATCC 25923 was used as a standard quality control strain for the disc diffusion test.

Ethical clearance for the study was sought from the institute's ethical clearance committee and written consent was taken from HCW's as well as in-patients. Details of the in-patients and HCWs such as age, gender, antibiotic intake in the previous 3 months, were noted on a pre-prepared proforma. Apart from these, years of service, level of education (for HCWs) and duration of hospital stay, history of prior hospitalization (last 1 year) and the place of admission, at the time of taking the nasal swab (for in-patients) was also recorded. Findings were analyzed using descriptive and analytical statistics using SPSS software, version 16. *P*-value of <0.05 was taken as significant.

All the discs, media and control strains were procured from Himedia Laboratories Pvt. Ltd., Mumbai.

3. RESULTS

A total of 310 (42.1%) isolates of *S. aureus* were recovered from the 736 nasal swabs collected from 480 (n=226; 47.1%) in-patients and 256 (n=84; 32.8%) healthcare workers (HCWs). Of the 310 *S. aureus* isolates, 226 (72.9%) were from in-patients whereas 84 (27.1%) were from HCWs. Methicillin resistance was seen in 74 (32.7%) *S. aureus* isolates recovered from in-patients and 16 (19.1%) *S. aureus* isolates recovered from HCWs (Table 1). The occurrence of MRSA among *S. aureus* was 29.0% (n=90).

A total of 32 (24.8%) *S. aureus* isolates recovered from in-patients showed $cMLS_B$ phenotype whereas 19 (14.7%) showed $iMLS_B$ phenotype, i.e. D-test positive. MS phenotype B (D-test negative) was seen in 78 isolates. Among *S. aureus* isolates recovered from HCWs, 17 (21.8%) depicted $cMLS_B$ phenotype, whereas 7 (9%) were D-test positive; $iMLS_B$ phenotype. MS phenotype B (D-test negative) was seen in 54 isolates. MRSA isolates recovered from both in-patients and HCWs depicted higher constitutive and inducible clindamycin resistance (Table 2).

MRSA were isolated more from male patients (n=41, 55.4%) than female patients (n=33, 44.6%). Higher isolation of MRSA was seen in patients belonging to the age group of 50-59 years (n=26, 35.1%). Specimens received from patients housed in the surgical intensive care unit (SICCU) yielded higher number of MRSA isolates (n=29, 39.2%), followed by plastic surgery ward (n=19, 25.7%). Prior history of hospitalization (last one year) was seen in 12 (16.2%) patients from whom MRSA were recovered whereas history of antibiotic intake in the last three months was noted in 23 (31.1%) patients, most common of which were fluoroquinolones (n=8) followed by co-trimoxazole (n=5), macrolides (n=4), cephalosporins (n=4), aminoglycosides plus an inhibitor combination (n=2). Details of the HCWs from whom *S. aureus* was isolated are given in Table 3. Higher carriage of MRSA (n=4, 25%) was seen in HCWs working in the SICCU. As with in-patients, more MRSA were recovered from male HCWs, 10 (62.5%). Nasal swabs taken from HCWs in the age group of 30-50 years yielded more MRSA isolates, 11 (68.8%). Those who had been working in the hospital from the last 1-4 years were found to have more MRSA carriage, 8 (50%). Nasal carriage of MRSA was more in HCWs who were college graduates, 11 (68.8%). Two

(12.5%) HCWs had taken fluoroquinolones in the last three months. MRSA carriage was seen more in nurses, 9 (56.3%) and doctors, 2 (12.5%).

Results of the antimicrobial susceptibility testing are given in Tables 4 and 5. Higher resistance to the antibiotics tested was seen in *S. aureus* isolates in general and MRSA isolates in particular recovered from both HCWs and in-patients. Hundred percent isolates of MRSA recovered from both the groups were resistant to penicillin, whereas all the isolates were uniformly sensitive to linezolid, teicoplanin and vancomycin. High resistance of MRSA isolates recovered from HCWs was seen against

Table 1. Characteristics of MSSA and MRSA isolates among in-patients and health care workers

	MSSA (n=220)	MRSA (n=90)	Total (n=310)	*P*-value
In-patients	152 (67.3%)	74 (32.7%)	226 (72.9%)	<0.05
Healthcare workers	68 (81%)	16 (19.1%)	84 (27.1%)	

Table 2. Pattern of clindamycin resistance in *S. aureus* isolates recovered from in-patients and HCWs

	In-patients			
	Clindamycin resistant MSSA (n=67)	**Clindamycin resistant MRSA (n=62)**	**Total (n=129)**	
$MLSB_c$	11 (16.4)	21 (33.9)	32 (24.8)	p>0.999
$MLSB_i$	6 (8.9)	13 (21)	19 (14.7)	
	HCWs			
	Clindamycin resistant MSSA (n=68)	**Clindamycin resistant MRSA (n=10)**	**Total (n=78)**	
$MLSB_c$	13 (19.1)	4 (40)	17 (21.8)	p>0.999
$MLSB_i$	5 (7.4)	2 (20)	7 (9)	

Table 3. Details of the health care workers from whom *S. aureus* were recovered

Characteristic	MSSA carriage (n=68)	MRSA carriage (n=16)	*P*-value
Gender			
Male	40 (58.8)	10 (62.5)	
Female	28 (41.2)	6 (37.5)	0.78
Age (years)			
<30	21 (30.9)	2 (12.5)	
30-50	34 (50)	11 (68.8)	0.29
>50	13 (19.1)	3 (18.7)	
Years of service			
1-4	13 (19.1)	8 (50)	
6-9	30 (44.1)	6 (37.5)	0.02
≥10	25 (36.8)	2 (12.5)	
Level of education			
Under graduate	8 (11.8)	1 (6.3)	
Graduate	24 (35.3)	11 (68.8)	0.05
Post graduate	36 (52.9)	4 (25)	
Antibiotic intake in the last 3 months			
Present	15 (22.1)	2 (12.5)	
Absent	53 (77.9)	14 (87.5)	0.39
Occupation			
Doctor	13 (19.1)	2 (12.5)	
Nurse	19 (27.9)	9 (56.3)	
Nursing orderlies	11 (16.2)	3 (18.8)	
Sanitary attendants	8 (11.8)	1 (6.3)	0.09
Laundry attendants	3 (4.4)	0	
Canteen staff	4 (5.9)	0	
Laboratory staff	6 (8.8)	1 (6.3)	
Administrative staff	4 (5.9)	0	

erythromycin (81.3%), ciprofloxacin (75%), co-trimoxazole (68.8%) tetracycline and clindamycin (62.5% in that order). On the other hand significant resistance among MRSA isolates recovered from in-patients was seen against erythromycin (91.9%), co-trimoxazole (85.1%), clindamycin (83.3%), tetracycline (82.4%) and ciprofloxacin (79.7%) respectively.

4. DISCUSSION

The current study highlights the carriage of *S. aureus* in HCWs and in-patients in our hospital. Higher isolation of *S. aureus* and of MRSA was seen from in-patients (72.9% and 32.7% respectively) in our study as compared to HCWs (27.1% and 19.1% respectively). Higher prevalence of carriage of *S. aureus* among in-patients in our study as compared to HCWs could be due to more number of in-patients screened for the same. A comparative study with equal number of subjects screened in both the groups can provide better insight into the prevalence of *S. aureus* carriage in them. Data available from India suggests that MRSA carriage rates are nearly 15.6% in in-patients and range from 1.8 to 25% amongst health-care workers in various hospital settings [11]. Al-Abdli et al. studied the frequency of *S. aureus* carriage among HCWs in 10 different hospitals in Libya and found the prevalence to be 47.5%; out of which 21.4% were MRSA [12]. On the other hand, higher carriage rates of *S. aureus* were reported in Yemen (85%) and Nigeria (52.5%) [13,14]. These difference can be attributed to factors like sample size, quality of sample collected, culture techniques used, population studied, geographical area and accompanying risk factors that contribute to the dynamics of *S. aureus* and MRSA carriage.

MRSA were isolated more from male in-patients (n= 41, 55.4%) as well as HCWs (n=10, 62.5%), in our study. This is in accordance to what was seen by Tenguria et al. [15] where the authors found higher prevalence of methicillin resistance in *S aureus* isolates recovered from male patients. Shibabaw et al. [16] in their study reported similar findings where higher numbers of MRSA were recovered from male HCWs (17.5%). Higher isolation of *S. aureus* from male HCWs was also seen by Shakya et al. Al-Humaidan et al. Rongpharpi et al. [17-19] in their respective studies.

In the present study higher number of MRSA isolates were recovered from in-patients in the age group of 50-59 yrs; n= 26, 35.1% and HCWs in the age group of 30-50 yrs; n=11, 68.8%.Our results are comparable to those reported by Tenguria et al. where authors found MRSA carriage of 30% among in-patients in the age group of 50-59 yrs and Al-Humaidan et al. where preponderance of MRSA carriage in the age group of 30-50 yrs (20%) was seen among HCWs [15,18].

Specimens obtained from in-patients (n=29, 39.2%) and HCWs (n=4, 25%) in the SICCU yielded higher number of MRSA isolates in our study. This is in contrast to what was reported by Mohajeri et al. [20] where high MRSA carriage rate was seen in the infant wards (80%) followed by intensive care unit (47.4%). On the other hand, Khanal et al. in their study reported MRSA carriage in 28.6% of the HCWs working in ICU [21]. Pan et al. [22] also reported high isolation of MRSA (38.1%) from HCWs working in the ICU. Askarian et al. [1] in a study carried out in Iran, reported higher prevalence of nasal carriage of MRSA in HCWs working in the surgical ward.

Table 4. Antimicrobial susceptibility profile of *S. aureus* isolates recovered form in-patients

Antibiotic	MSSA (n=152)		MRSA (n=74)		P-value
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)	
Penicillin	2 (1.3)	150 (98.7)	0	74 (100)	-
Cefoxitin	152 (100)	0	0	74 (100)	-
Clindamycin	71 (46.7)	81 (53.3)	12 (16.2)	62 (83.8)	<0.001
Erythromycin	39 (25.7)	113 (74.3)	6 (8.1)	68 (91.9)	0.001
Ciprofloxacin	62 (40.8)	90 (59.2)	15 (20.3)	59 (79.7)	0.002
Tetracycline	51 (33.6)	101 (66.4)	13 (17.6)	61 (82.4)	0.012
Amikacin	57 (37.5)	95 (62.5)	21 (28.4)	53 (71.6)	0.176
Gentamicin	50 (32.9)	102 (67.1)	18 (24.3)	56 (75.7)	0.187
Co-trimoxazole	105 (69.1)	47 (30.9)	11 (14.9)	63 (85.1)	<0.001
Linezolid	152 (100)	0	74 (100)	0	-
Vancomycin	152 (100)	0	74 (100)	0	-
Teicoplanin	152 (100)	0	74 (100)	0	-

Table 5. Antimicrobial susceptibility profile of *S. aureus* isolates recovered form health care workers

Antibiotic	MSSA (n=68)		MRSA (n=16)		p-value
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)	
Penicillin	0	68 (100)	0	16 (100)	-
Cefoxitin	68 (100)	0	0	16 (100)	-
Clindamycin	20 (29.4)	48 (70.6)	6 (37.5)	10 (62.5)	0.528
Erythromycin	12 (17.6)	56 (82.4)	3 (18.8)	13 (81.3)	0.917
Ciprofloxacin	33 (48.5)	35 (51.5)	4 (25)	12 (75)	0.088
Tetracycline	32 (47.1)	36 (52.9)	6 (37.5)	10 (62.5)	0.489
Amikacin	23 (33.8)	45 (66.2)	8 (50)	8 (50)	0.228
Gentamicin	20 (29.4)	48 (70.6)	8 (50)	8 (50)	0.116
Co-trimoxazole	35 (51.5)	33 (48.5)	5 (31.3)	11 (68.8)	0.145
Linezolid	68 (100)	0	16 (100)	0	-
Vancomycin	68 (100)	0	16 (100)	0	-
Teicoplanin	68 (100)	0	16 (100)	0	-

Whereas the carriage rate of MRSA was highest among the staff working in the orthopedics department in a study carried out in the northeastern part of our country [19].

Admission of critically ill patients in SICCU that have to undergo invasive monitoring and receive broad spectrum antibiotics as empirical therapy places them at a higher risk of being infected with multidrug resistant (MDR) pathogens. Nasal carriage of MRSA among HCWs involved in the care of such patients can serve as a source of infection in them if proper infection control practices are not followed. Routine surveillance of HCWs in high dependency areas of the hospital like ICUs and surgical wards for nasal carriage of MRSA and its eradication should be carried out, to reduce the risk of transmission and subsequent infections in patients.

Prior history of hospitalization (last 1 year) and antibiotic intake (last 3 months) was seen in 12 (16.2%) and 23 (31.1%) of in-patients respectively from whom MRSA were recovered. On the other hand 2 (12.5%) HCWs from whom MRSA were recovered had taken antibiotics in the last 3 months. However no significant association was found between these variables and MRSA carriage either in HCWs or in-patients. This is in contrast to what was reported by Rongpharpi et al. [19] where the investigators found a significant association between antibiotic intake and MRSA isolation among HCWs.

We found a significant isolation of MRSA; (P<0.05) from HCWs with < 5 year of service record (50%). This could be attributed to the lack of knowledge and experience regarding proper infection control practices in them, compared to more senior staff well versed with basic infection control measures. Our results are comparable to those seen by other investigators [1,16]. Interestingly with regards to level of education of the HCWs we found significant isolation of MRSA from those who were college graduates (P<0.05) in our study. The greatest challenge for the success of established infection control protocols is strict compliance rather than novelty hence continued training and capacity building of newer recruits in the field of health care should be carried out at regular intervals and their active participation sought in this regard. This can prove to be a simple and cost effective method of preventing transmission of drug resistant bacteria (e.g. MRSA) especially in patients with open wounds admitted to surgical units.

Highest carriage of MRSA was seen in nurses (56.3%) and doctors (12.5%) in our study, which could be due to their frequent contact with patients. Many studies conducted across the globe have reported more MRSA carriage rates among nurses and doctors [1,12,16,18,19,21].

Over all higher resistance to the various antibiotics tested was seen in MRSA isolates recovered from both HCWs and in-patients in our study. Significant resistance among MRSA isolates recovered from in-patients was seen to clindamycin, erythromycin, ciprofloxacin, tetracycline and co-trimoxazole (P<0.05), that represents an MDR phenomenon. Various studies in our country and across the world have reported high resistance rates to various anti-Staphylococcal antibiotics [1,12,15,19,20]. Both $cMLS_B$ and $iMLS_B$ phenotypes were seen more in MRSA than methicillin sensitive *S. aureus* (MSSA). These resistance patterns can be detected by simple and inexpensive tests like D-test which should be incorporated in the susceptibility testing of these organisms

routinely. Our results are similar to those seen by Fomda et al. where the authors reported significantly higher $cMLS_B$ and $iMLS_B$ phenotypes in MRSA isolates [23]. Results similar to ours have been reported previously as well [24,25]. Proper selection of antibiotics, infection control practices, antibiotic stewardship, proper de-escalation and regular surveillance to assess the local ecology so as to guide proper antibiotic therapy are simple measures that can go a long way in reducing the growing menace of antimicrobial resistance.

Our study has certain limitations to it. We did not investigate the persistence of MRSA carriage and its relation to subsequent infection in the admitted patients. Relatedness of the recovered MRSA isolates from in-patients as well as HCWs by any molecular method was not looked into, which could provide valuable insight into the infection control practices being followed in our hospital. In addition risk factors for colonization of *S. aureus* among in-patients were not looked into neither were important aspects like universal or targeted screening and decolonization of patients upon admission and the role of mupirocin and chlorhexidine body washes for such purposes evaluated.

5. CONCLUSION

The results of this study demonstrated that nasal carriage of MRSA was common among health care professionals and in-patients in our hospital. Since these isolates are highly resistant to common antibiotics, continuous monitoring of their susceptibility profiles cannot be over emphasized. The hospital staff and in-patients should be screened for MRSA and appropriate treatment measures instituted. All HCWs should as a matter of routine, be educated and trained about infection control practices (e.g. hand washing, wearing gloves, barrier nursing) and maintenance of adequate hygiene. Since the antibiotic armamentarium for the treatment of infections due to these pathogens is limited, we need to focus on simple and basic infection control practices that can go a long way in reducing nosocomial infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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