

Staphylococcus aureus including MRSA nasal carriage among hospital exposed and unexposed medical students

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ABSTRACT

Introduction: *Staphylococcus aureus* is one of the most common human pathogen causing a wide range of infections. It is estimated that *S.aureus* colonizes the anterior nares in approximately 31% of the general population at any given time. The incidence of community acquired & hospital acquired *S. aureus* has been increasing over the past few decades, predominantly due to continuous upsurge in the drug resistant isolates. Moreover, globally the incidence of methicillin resistant *S.aureus* (MRSA) is progressively increasing. Hence, it would be imperative to screen all healthcare workers, interns and admitted patients for MRSA carriage and to treat all those who are found positive for the same. With the above background, the current study was undertaken to investigate the carrier rate of *S. aureus* (including MRSA) among hospital unexposed & exposed medical students. **Methods:** A total of 181 medical students of Veer Chandra Singh Garhwali Government Institute of Medical Sciences & Research, Srinagar Garhwal, Uttarakhand. Study participants were broadly divided into two groups: hospital exposed group (n=107) and hospital unexposed group (n=74). Nasal swabs were obtained & cultured for the detection of *S. aureus*. Congo red agar and 0.1% Crystal Violet Assay were performed to observe the ability to form in vitro biofilm by *S. aureus*. **Results:** Out of total 181 medical students 29.28% were found to be healthy carrier of *S. aureus*. Among the hospital exposed group 37.38% and among hospital unexposed group 17.57% were found to be healthy carrier of *S. aureus*. Only one student (hospital exposed group) was found to be positive for MRSA. Beta-lactamase production was noted in 90.57% strains of *S. aureus* while the significant rate of slime layer production was observed in 73.58% of strains. **Conclusion:** Prevalence of *S. aureus* nasal carriage increases with the duration of exposure to the hospital environment. The nasal carriage of *S. aureus* in medical students indicate the potential danger of dissemination of *S. aureus* including MRSA from them to the hospitalized patients which in turn complicates the treatment of same.

Keywords: Biofilm, Congo red agar, mecA, Nosocomial infection, Penicillinase

Introduction

Staphylococcus aureus, one of the commonest pathogen, can cause various infections ranging from localized skin infections to fatal meningitis in humans.^[1] Staphylococcal infections occur regularly

in hospitalized patients and has serious consequences despite the antibiotic therapy. The virulence of this organism occurs through cross-infection by spread from patient to patient in hospitals and other institutional settings. In contrast, healthy individuals have a small risk of contracting as an invasive infection, but they can be carriers of *S. aureus*.^[2] The primary habitat of *S. aureus* in moist squamous epithelium of the anterior nares so the most invasive infections by this organism are assumed to arise from nasal carriage.^[3] Increased colonization of *S. aureus* in patients and

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healthcare workers frequently occurs in hospitals. Both hospital and community-acquired infections caused by drug resistant *S. aureus* has increased in the past three decades.

Antibiotic-resistant bacteria are an increasing problem in the world among infected patients; antibiotic resistance is associated with increase in length of stay, healthcare costs, and patient morbidity and mortality.^[4]

Swoopstake use and exposure of antimicrobials has led to antibiotic resistance of *S. aureus*.^[5] The incidence of community-acquired and hospital-acquired *S. aureus* infections has been rising with increasing emergence of drug-resistant strains like methicillin resistant *Staphylococcus aureus* (MRSA).^[5-7] The difference between MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) is resistance to beta-lactam antibiotics; this is often associated with resistance to multiple other antibiotics, which limits the therapeutic options.^[8] MRSA, once used to be confined to the hospital environment, has now circulated in the community among previously healthy patients.^[9] Numbers of infections due to MRSA are consistently high and remain a major risk to hospitalized patients.^[10] The major treatment option for MRSA remains Vancomycin, Teicoplanin, and Linezolid, however more recently decline in the MIC of Vancomycin and Linezolid in MRSA were reported.^[11] Even after treating MRSA infections with empirical anti-MRSA therapy the mortality rate remains high specially among the geriatric patients.^[12]

Medical students come in contact with patients in outpatients departments (OPDs), wards, intensive care units (ICUs), and in operation theaters. There is chance of disseminating *S. aureus* from medical students to the patients or vice-versa. Medical students during their first-year course do not visit the OPD, Wards, ICUs, and operation theaters while second year on wards they are regularly posted in clinics where they come in contact with patients. Clinical exposure of 1–2 years were reported not affecting the carriage rate of MRSA among the medical students of a Taiwanese University,^[13] though few studies has proven that exposure to the hospital environment of a prolonged duration may cause an increase of MRSA carriage among the health personnel.^[14-16] Therefore, this study was conducted to figure out the prevalence of *S. aureus* including MRSA among medical students exposed to hospital environment against those who are not exposed and the ability of the isolates to form biofilm.

Materials and Methods

Study design and population

An experimental based cross-sectional study, which included nasal swab of 181 medical students was considered. A written permission from Institutional Ethical Committee was obtained to conduct the study (IEC/VCSGGIMS&R/13/2013, Dated: 10.05.2013). The healthy medical students who had no history upper respiratory tract infection and/or had no antibiotic intake in past 1 month were included in the study.

Study setting

The study was conducted in the department of Microbiology & Immunology of Veer Chandra Singh Garhwali Government Institute of Medical Sciences & Research (V.C.S.G.G.I.M.S.& R) located at Srinagar Garhwal, Uttarakhand. Medical students studying in the same institute were included in this study.

Students were divided into two groups: hospital exposed and hospital unexposed. In hospital unexposed group, first year medical students were included, who were considered as representative of community; the hospital exposed group consisted of second year onward medical students having at least 6 months of hospital exposure in the form of clinical postings at Hemwati Nandan Bahuguna (HNBB) Base Hospital. All the subjects were sampled, after obtaining a written informed consent along with duly filled questionnaire.

Sample collection

Nasal swab was collected from the anterior nares with the help of pre-sterilized cotton swab and proceeded immediately in the bacteriology laboratory in the Dept. of Microbiology and Immunology.

Laboratory proceedings

Nasal swabs collected from both groups of students were inoculated on Mannitol Salt Agar and 5% sheep blood agar incubated at 37°C for 24 h. The isolates grown on culture medium were identified by Gram staining, Catalase test, Coagulase test (slide and tube method), DNase, and Phosphatase tests followed by antibiotic susceptibility test by Kirby & Bauer's disc diffusion test. Methicillin resistance was detected by taking cefoxitin (30 µg) as a surrogate marker and was confirmed by using PBP2a latex agglutination test (Oxoid Ltd., Hampshire, UK).

Beta-lactamase production

S. aureus strains were further tested for Beta-lactamase production. It was detected by following two different iodometric methods:^[17]

1. Test tube method - A loopful of heavy inoculums of 24 h old cultures from Muller Hinton agar were mixed well with 1.0 ml Penicillin solution containing 10,000 Unit per ml. The tubes were left for 60 min at room temperature, mixing in between every 15 min. Then two drops of 1% soluble starch solution was added followed by one drop of iodine solution. The tubes were mixed well and results recorded within 10 min
2. Agar plate method - Isolates were inoculated on Muller Hinton agar containing 1% soluble starch and incubated at 37°C. After 48 h of incubation, the plate was flooded with Penicillin solution containing 10,000 Units per ml and left at room temperature. After 30 min the penicillin solution was decanted completely and the plate was flooded with 1:5 dilution of iodine solution. After 3–5 min results were recorded.

Slime production

It was detected by using Congo red agar (CRA) method by Freeman *et al.*^[18] Brain heart infusion agar containing 5% sucrose, 0.08% Congo red was used for slime detection. The isolates were streaked to a length of 1.5 cm on CRA plate) and incubated at 37°C. Results were recorded after 24 h of incubation.

Biofilm production was determined by Crystal violet Assay as mentioned by Stepnovich *et al.*^[19] Isolated strains were cultured on Tryptic Soy Agar (TSA) medium for 24 h. Thereafter 3-4 bacterial colonies were inoculated in test tubes containing 10 ml. Trypticase Soy Broth (TSB) with 1% glucose and matched to 0.5 McFarland after overnight incubation at 37°C. Further triplicate of 100 µl bacterial suspension of each strains was dispensed into 96-wells sterile cell culture plate and incubated at 37°C. After 24 h of incubation the wells were drained and washed with 0.2 ml of phosphate buffer saline (pH 7.2) for four times. The wells were fixed by 2% sodium acetate and 0.1% crystal violet stain was added. The plate was incubated for further 5 min at room temperature before removing the Crystal violet. The wells were washed with deionized water twice and after drying 95% ethanol was added to each well. Thereafter the plate was kept at 4°C and using spectrophotometer the absorbance at 595 nm was measured [Figure 1]. Optical density (OD) value of ≥ 3.0 was taken as the upper limit. The OD of the test strains was compared with OD cut off value (ODc). The ODc was calculated as the average OD of negative control + 3x standard deviation (SD) of negative control. *S. aureus* ATCC 35556 strain was used as a positive control for biofilm production and sterile TSB medium as negative control. All dehydrated media, reagents and antibiotic disks were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India.

Statistical analysis

Data was entered and stored in M.S. Excel sheet and analyzed by using SPSS version 21. Statistical test (chi) 2 was used for analyzing qualitative variable and two tailed Student 't' test for quantitative variable. $P < 0.05$ was taken as statistically significant.

Results

All of the volunteers were found to be carriers of *Staphylococcus species* as revealed by culture characteristics, Gram

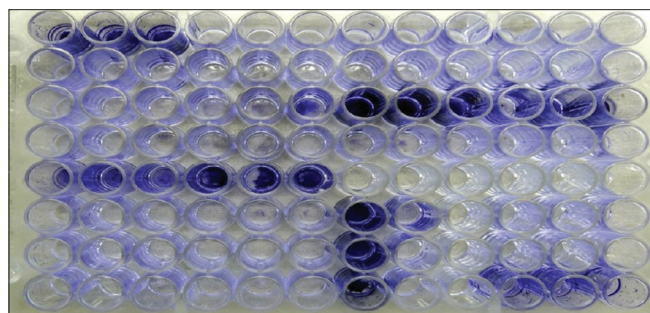


Figure 1: Microtitre plate after staining with 0.1% crystal violet assay

staining, and catalase test. Out of them 53 (29.28%) were carriers for *S. aureus* and 128 (70.72%) were carriers of CONS. The carrier rate of *S. aureus* was observed higher among the exposed group (37.38%) in comparison to unexposed group (17.57%).

High rates of resistance was observed against ampicillin, penicillin, cotrimoxazole, and erythromycin while amikacin, linezolid, teicoplanin, and vancomycin were susceptible to all the strains [Figure 2]. Cefoxitin disk (30 µg) was used to screen MRSA and was confirmed by latex agglutination test for detection of PBP2a.

The hospital unexposed group was represented by 74 (40.88%) students from first year batch of which 13 (17.57%) were found to be carrier of *S. aureus*. Out of 13 carriers, three (23.08%) were males and 10 (76.92%) were females. All the 13 strains did not show any resistance towards cefoxitin disc (i.e., no MRSA found).

Students studying in second year onward batches represented the hospital exposed group as they are posted in various wards and OPD where they come in contact with patients on day today basis. A total of 107 (59.12) students were included in hospital-exposed group. *S. aureus* nasal carriage were recorded in 40 (37.38%) students out of which 14 (35%) were females and 26 (65%) were males. Only one (2.5%) strain found to be resistant towards cefoxitin (i.e., MRSA). The student with MRSA nasal carriage was resampled. Second sample also resulted in the growth of MRSA with identical biochemical reactions, antibiogram and latex agglutination test for PBP2a.

Despite of their antibiogram, all the *S. aureus* isolates were tested for Beta-lactamase production by Test Tube and Agar Plate Iodometric techniques. In test tube iodometric technique an instant discoloration was observed and on Muller hinton agar containing 1% soluble starch >10 mm diameter discoloration around the culture was seen. Beta-lactamase production was found in 48 strains (90.57%) by both the methods.

Slime layer production on CRA was observed in 39 (73.58%) strains. Slime layer productions were recorded as negative ($n = 14$), weak positive ($n = 22$), positive ($n = 6$), and strongly positive ($n=11$) on the basis of color and consistency of colonies. Strongly positive results for slime layer production

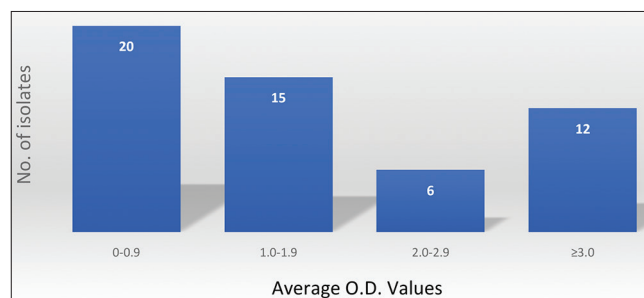


Figure 2: Number of *S. aureus* isolates in each average optical density value after testing in triplicates by 0.1% crystal violet assay.

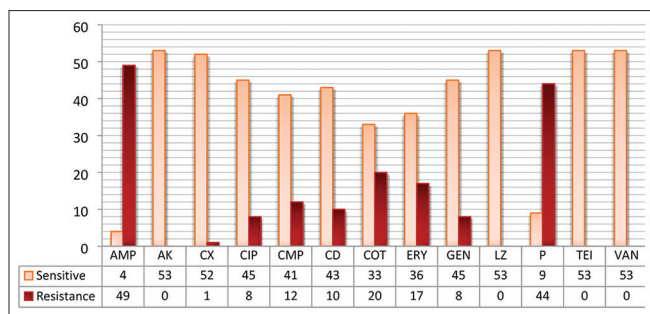


Figure 3: Antibiotic susceptibility and resistance pattern of isolated *S. aureus* strains

by CRA was seen in 11 strains and all of these strains also had high OD value of 3.0 and above by 0.1% Crystal Violet Assay [Figure 3]. There were 12 strains having average OD value between 2.0 and 2.99 out of which three were positive by CRA, five were weakly positive while four strains tested negative.

Discussion

For first year of their course, medical students are not posted for clinical duties. Second year onwards they are posted in different clinical departments during which they get exposed to hospital environment. Medical students included in present study have daily clinical exposure at HNB base hospital until the completion of their course.

The present study reveals that 29.28% of medical students in Garhwal region, unexposed (17.57%) and unexposed (37.38%) to the hospital environment, harbored *S. aureus* in their nares. Many published data on nasal carriage of *S. aureus* among normal healthy population presents the carrier rate of 10–30% and is similar to present study. The overall nasal carriage rate was observed higher in males (32.58%) in comparison to females (26.09%). The gender ratio of *S. aureus* nasal carriage was seen opposite among hospital exposed and unexposed group. Among the hospital unexposed group females (24.39%) harbored *S. aureus* comparatively higher than males (9.10%) while among the hospital exposed group the rate of *S. aureus* nasal carriage was very high in males (46.43%) than females (27.45%). Since the unexposed group represents the community, the results of present study suggests that the nasal carriage of *S. aureus* in general population/community (17.57%) is quite less and it increases with more and more exposure to hospital environment, as suggested by Chen CS *et al.*^[13] Similarly, in the present study the nasal carriage rate increases with longer duration of hospital exposure. There were 38 medical students from second year batch who participated in this study, out of which 11 (28.95%) were found to harbor *S. aureus* in their anterior nares. From third year batch 57 medical students volunteered among whom 27 (47.37%) were observed as nasal carriers of *S. aureus*. The Sample size was too less in fourth- and fifth-year batches (5 & 7 students respectively) to comment on.

Slime layer production was observed to be more prevalent in *S. aureus* isolated from hospital unexposed group suggesting them

to be hospital strains which indicates the potential possibility of implant associated infections, if such strains get transmitted from carrier medical students to the patients.

The *in vitro* biofilm production quantified by 0.1% Crystal Violet Assay was correlated with slime layer production by CRA. All the 11 strains of *S. aureus* producing very strong slime layer on CRA were seen having OD value of more than equal to 3.0 by Crystal Violet Assay. Among the 12 strains having OD value between 2.0 and 2.99, three were positive in CRA while five were weak positive and four were negative. This result suggests that strong biofilm producing strains can have good result in CRA method but strains with weaker ability to form biofilm may be missed by CRA method. The strains having the ability to form weak biofilm can more efficiently be detected by 0.1% Crystal Violet Assay. Sander Croes *et al.* suggested no relation between the results of CRA and Crystal Violet Assay as the sensitivity and specificity of CRA for *S. aureus* was approximately 9% and 90%, respectively.^[20]

The MRSA strain in the present study failed to form any slime layer on CRA while 0.1% Crystal Violet Assay has detected the biofilm production by it. It has been suggested by many authors that prevalence of slime layer production by MRSA is quite less than MSSA.^[20-22] So, Crystal Violet Assay remains a better method for the detection of biomass. CRA screening forms no alternative for Crystal Violet Assay to detect biofilm formation, as already strongly suggested by few authors.^[20-23] Though there was high consistency reported between CRA and Crystal Violet Assay by studies done by Grinholc *et al.* and Jain *et al.* 96% and 91%, respectively, but their definition of slime layer production was based on the color of the colonies and not on the morphology.^[24,25]

Among *S. aureus*, the methicillin-resistant strains make the management of patients difficult specially in critical care units. MRSA has also gained importance in geriatrics medicine as it has been associated with higher mortality rates in elderly patients even after using anti-MRSA therapy.^[12] In this study, the prevalence of MRSA among the anterior nares of the healthy medical students of was found too far lower than other studies on the medical students and healthcare workers from other parts of India.^[26-28] The student with MRSA nasal carriage was advised for mupirocin ointment treatment. After a period of 45 days, one more sample was collected and cultured, where no *S. aureus* was isolated.

The antibiotics susceptibility pattern of *S. aureus* isolated from anterior nares of healthy healthcare workers of Pokhara, Nepal and healthy Medical students Puducherry does not vary from the present study.^[26,29] The difference was observed only in the *in vitro* activity of Penicillin at Puducherry, where Penicillin was found more susceptible. As Srinagar is more proximal to Pokhara in spite of belonging to different countries, both towns share similar geographical conditions, the antibiogram pattern was compared with studies of Sah *et al.*^[27]

Beta-lactamase production was noted in 48 strains (90.57%). Similar finding was given by Shanmugam *et al.*^[26] Beta-lactamase production was seen higher among the hospital exposed students, as these students might harbor the hospital strains in their anterior nares. Beta-lactamase production was recorded higher than the rate of slime layer production by CRA.

Limitation

Molecular identification of *mecA* gene was not performed to confirm the methicillin resistance due to lack of molecular diagnostic facilities at our center. A long duration study with sampling at regular interval could be performed on a batch of medical students for better identification of the impact of hospital exposure time duration on acquiring *S. aureus* nasal carriage.

Conclusion

The nasal carriage rate of *S. aureus* in healthy community is low but the incidence of nasal carriage rate increases with the duration of hospital exposure. The prevalence of MRSA carriage is comparatively very low among medical students in the Garhwal region of Uttarakhand state.

High rate of beta-lactamase production by the *S. aureus* isolated in present study suggests these strains are acquired from hospital environment. The high prevalence of biomass formation in isolated strains indicates the potential possibility of acquiring implant associated infections, if such bacterial strains get transmitted from carrier students to their patients. Such strains are difficult to manage and can be a cause of higher mortality especially among elderly, immunocompromised and critically ill patients.

Hence, it would be imperative to screen all healthcare workers, interns and admitted patients for MRSA carriage and to treat all those who are found positive for the same.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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