

Surveillance Study on Nasal and Hand Carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Dental and Medical Staff and Students of a Medical University

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Abstract

Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterium which commonly colonizes the human body and is an important nosocomial and community acquired pathogen. Increasing reports of antibiotic resistance to various drugs and occurrence of methicillin resistant strains compound the problem.

This study was conducted on dental and medical staff and students (current and future health care providers) to detect the colonization of *S. aureus* and the methicillin resistant strains among them. A total of 147 participants were screened for colonization of *S. aureus* in the anterior nares and hands. The nasal and hand swabs were processed according to standard *Clinical and Laboratory Standards Institute* (CLSI) guidelines, namely formation of yellow colonies on Mannitol Salt Agar, catalase and coagulase test. Antibiotic susceptibility testing for common antibiotics was also performed according to CLSI guidelines. Data analysis was performed using SPSS version 21.

100 isolates of *S. aureus* (35%) were obtained which was confirmed by standard laboratory procedures of which 3 isolates were methicillin resistant. All the MRSA strains were confirmed by PBP2 latex agglutination test and E test. Varying levels of resistance to common antibiotics was noted in the study. The study showed 35% colonization by *S. aureus* on the hands and nares of health care providers. Three isolates were methicillin resistant (methicillin resistant *S. aureus*/MRSA) which was confirmed by E test and PBP2a latex agglutination test.

This study highlights the need to educate health care personnel on prevalence of *S. aureus* carriage and good practices for prevention of its spread. Colonisation of community acquired MRSA (CA-MRSA) in hospital personnel is best detected by screening and decolonization measures should be advised.

Introduction

Staphylococcus aureus is known as a Gram-positive bacterium that is very frequently implicated in several of infectious processes which ranges from a mild skin disease to serious skin manifestations. The organism possesses several properties that contribute to its ability to cause serious disease, including the production of toxins. Methicillin-resistant *Staphylococcus aureus* (MRSA) were first reported in 1961 [1] and have become one of the common pathogen worldwide. MRSA (Methicillin-Resistant *Staphylococcus aureus*) is a bacterium that causes infections in different parts of the body. Most often it causes mild infections on the skin, causing sores or boils, but it can also infect surgical wounds, the bloodstream, lungs, or even the urinary tract [2]. A methicillin-resistant *Staphylococcus aureus* (MRSA) carrier is a person who has the MRSA bacteria residing on the skin or in their nose but remain asymptomatic [2]. MRSA colonizers are potential source of infection, especially in hospital settings and can cause severe infections in persons with compromised immune system.

The goal of this research study was to analyse the prevalence of colonization of *S. aureus* as well as detect methicillin-resistant *Staphylococcus aureus* (MRSA) carrier state among the medical and the dental students and the staff.

Subjects and Methods

The study was conducted at the Faculty of Medicine and Faculty of Dentistry, SEGi University after obtaining approval

from the University Research and Ethics Board. Nasal and hand swabs were collected from medical and dental staff and first and second year students after obtaining informed consent by random convenient sampling. Subjects with fever, running nose, sore throat, infected open wounds or on antibiotics were excluded from the study. Swabs were directly inoculated onto mannitol salt agar plates which were incubated at 37°C for 24-48 hours. Yellow colonies of *S. aureus* were identified while white colonies which were identified as coagulase negative staphylococci were not processed further. The yellow colonies were confirmed by Gram stain, catalase test; slide and tube coagulase test. Strains confirmed as *S. aureus* were tested for resistance to methicillin using disc diffusion technique (CLSI guidelines) [3]. This was confirmed by testing for presence of penicillin binding protein (PBP2⁺) by latex agglutination test [4] as well as E test [5]. The data was further analysed using SPSS version 21. The characterized isolates were stored at -20°C. Subjects detected to be carriers of MRSA were advised appropriate decolonization measures.

Results

The study population of 147 subjects consisted of students and staff from the medical and dental faculties. 73% of samples were obtained from the medical faculty student and staff while dental faculty contributed 27 % of the total samples (*Figure 1*).

The students contributed to majority of the samples (72.5%) while the staff of both faculties provided 27.5% of samples. A

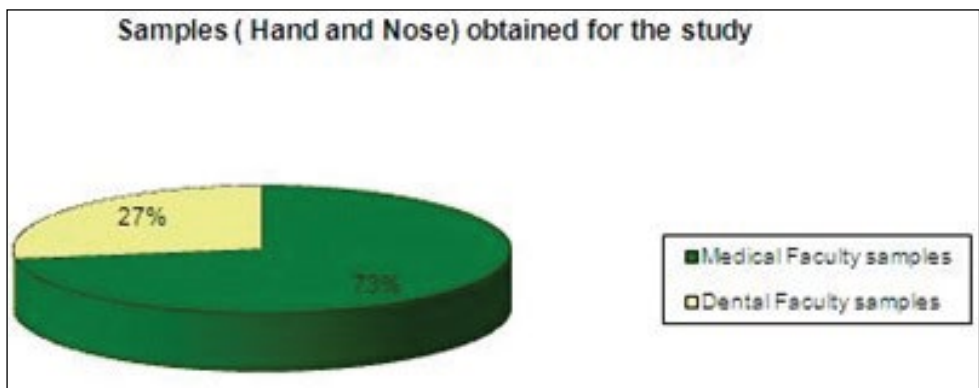


Figure 1. Distribution of samples obtained from medical and dental faculty.

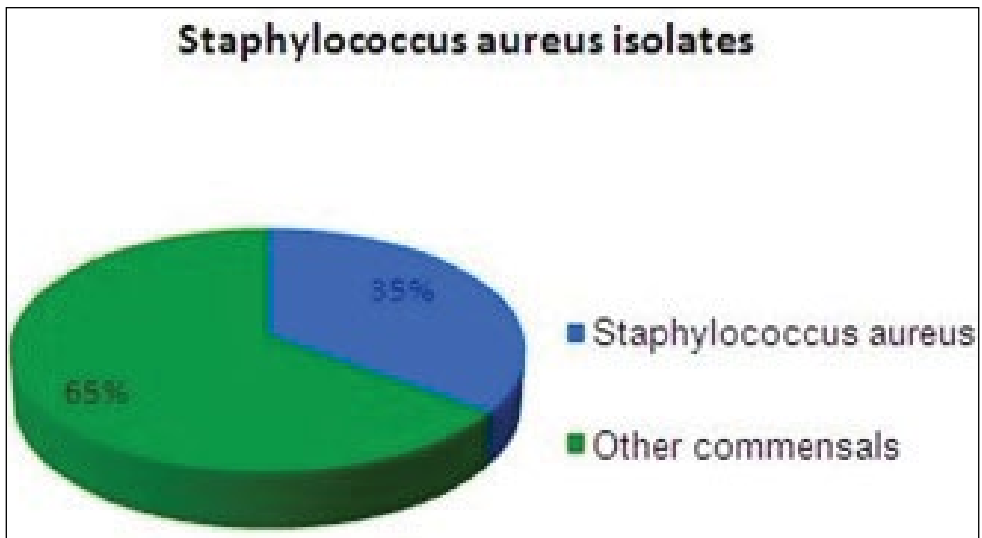


Figure 2. Staphylococcus aureus isolates.

total of 100 *S. aureus* isolates (35%) were obtained (Figure 2) from the samples of which 96 isolates were processed further as 4 isolates failed to grow on further subculture. Among the 96 *S. aureus* isolates, 79 (79%) were from medical faculty and 21(21%) were from the dental faculty (Figure 3) ($p < 0.05$). Sixty two (62) isolates were from medical students, 14 from medical staff, 12 from dental students and 8 from dental staff (Figure 4).

S. aureus was isolated from hand swabs of 77 participants (52.4%) and 23 isolates (16.8%) were from nasal swabs (Table 1) ($p = 0.0$). All the isolates were subjected to antibiotic susceptibility testing using Kirby Bauer’s disc diffusion method. Fifty five (55) isolates were sensitive to penicillin while 41 isolates were resistant by disc diffusion method (Table 2).

All the 96 isolates were screened using oxacillin disc diffusion technique and two of them were resistant; one isolate exhibited intermediate resistance with 95.2% of the strains being sensitive to oxacillin (Table 3). All the three resistant isolates were obtained from anterior nares of medical students. Further cefoxitin disc diffusion test was also done and all the 3 methicillin resistant strains were also resistant to cefoxitin (Table 4).

Other antibiotics tested included ampicillin, sulphamethoxazole, ciprofloxacin and novobocin. Overall, the isolates had a higher resistance to penicillin (41%) and ampicillin (56%) (Figure 5). Three isolates were presumptively classified as methicillin resistant *Staphylococcus aureus* (MRSA) due to resistance to oxacillin

and cefoxitin. Minimum inhibitory concentration by E test was done to confirm the MRSA isolates. Additionally one isolate though sensitive to methicillin and PBP2a negative, showed resistance to cefoxitin (Table 5).

PBP2a latex agglutination test was performed on all the isolates and the 3 isolates which showed resistance / intermediate resistance to oxacillin and cefoxitin were positive for PBP2a agglutination. One isolate which was oxacillin sensitive and PBP2a negative demonstrated resistance to cefoxitin on disc diffusion test. All the three subjects who were carriers of the MRSA strain of *S. aureus* were advised to perform decolonization using chlorhexidine body wash and intranasal application of mupirocin ointment.

Discussion

Staphylococcus aureus is part of the normal flora and colonization by it is common in the anterior nares. Its presence in medical health personnel is of concern especially those handling or caring for neonatal, elderly as well as immune-compromised people [6].

The study showed a higher prevalence of *S. aureus* colonization among the medical students than dental students ($p < 0.05$). Isolation of *S. aureus* from hand swabs was more than nasal swabs which indicated that hand colonization of *S. aureus* was more than nasal colonization ($p = 0.0$). The colonization rate of *Staphylococcus aureus* was 35% in this study of which 52.4% were isolated from hands and 16.8% were from nasal swabs. The rate of colonization in medical students in previous study by Aguilera et al also demonstrated



Figure 3. Total *S. aureus* isolates from dental & medical faculty.

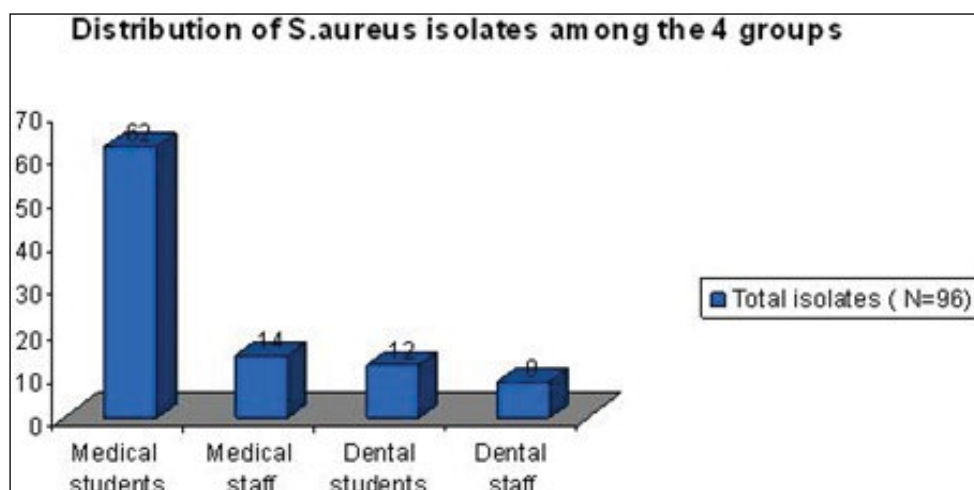


Figure 4. *S. aureus* isolate distribution among medical and dental staff and students.

<i>S. aureus</i> isolates	Total no. of samples collected	% of <i>S. aureus</i> isolation
Hand swab	77	147
Nasal swab	23	137

Table 1. *S. aureus* isolates obtained from total participants in the study.

<i>S. aureus</i> isolates	Medical students	Medical staff	Dental students	Dental Staff
Penicillin sensitive	36	9	7	3
Penicillin resistant	27	4	5	5
Total isolates (N=96)	63	13	12	8

Table 2. Susceptibility to penicillin among the *S. aureus* isolates.

a similar rate of 39.3% colonization by *S. aureus* with 3 isolates being methicillin resistant [7].

S. aureus colonization is primarily reported to be mostly in the anterior nares [8] but extra nasal sites are also implicated; e.g.: skin, oropharynx, axilla and rectum [9]. The presence of *S. aureus* on the hands is a risk factor for transmission to close contacts, especially if handled without proper hand washing techniques [10].

Antibiotic susceptibility study has shown resistance to penicillin (42.7%) and ampicillin (18%) with intermediate resistance to ampicillin in 22 isolates (25%) performed according to CLSI guidelines. Various studies have reported variable pattern of resistance to these antibiotics in community

	Medical students	Medical staff	Dental students	Dental staff
Oxacillin sensitive	59	14	12	8
Oxacillin intermediate	1	0	0	0
Oxacillin resistant	2	0	0	0
Total isolates (N=96)	62	14	12	8

Table 3. Susceptibility to oxacillin among the *S. aureus* isolates.

	Medical students	Medical staff	Dental students	Dental staff
Cefoxitin sensitive	59	14	12	8
Cefoxitin resistant	3	0	0	0
Total isolates (N=96)	62	14	12	8

Table 4. Susceptibility to cefoxitin among the *S. aureus* isolates.

acquired *S. aureus* strains; while resistance is very high in hospital acquired strains/ hospital personnel who are actively administering health care and in direct contact with patients over a longer period of time [11-14].

The isolation of 3 strains (3.13%) which were methicillin resistant was further confirmed by PBP2 latex agglutination test and E test. Previous studies have shown the variable results ranging from 0-9% [15] to 16% [16] with highest recorded colonization being 42% [17]. One isolate was resistant to cefoxitin but not to oxacillin; with negative PBP2a latex agglutination result. Cefoxitin disc diffusion is considered a more reliable predictor of methicillin resistance. PBP2a

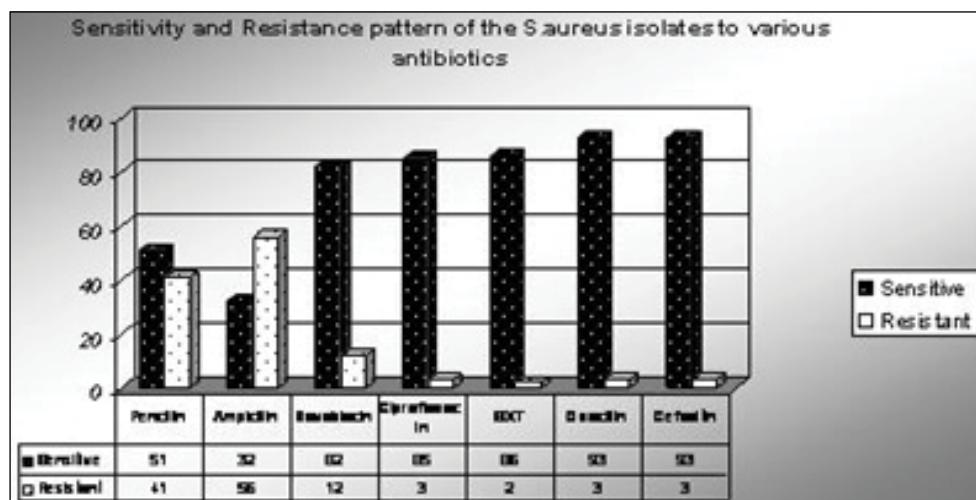


Figure 5. Antimicrobial sensitivity pattern of the *S. aureus* isolates.

	Case 1	Case 2	Case 3	Case 4
Oxacillin	R	R	R	S
Cefoxitin	R	R	R	R
PBP2a	R	R	R	S

R=resistant; S=susceptible

Table 5. *S. aureus* isolates with resistance to oxacillin and cefoxitin.

latex test was negative which could indicate that the levels of PBP2a are too low to be detected by latex agglutination test. This isolate needs to be further tested by PCR for presence of *mecA* gene to confirm genotypic resistance which is more reliable [18].

Studies have shown that colonization isolates are less likely to possess staphylococcal cassette chromosome *mec* type IV, Panton-Valentine Leukocidin, or *agr* type 1 and hence colonization with specific strain types, rather than methicillin-resistant *Staphylococcus aureus* colonization in general, increases the risk for CA-MRSA disease [19]. This further requires polymerase chain reaction study to characterize the staphylococcal cassette chromosome *mec* type and pulse field gel electrophoresis to determine genetic relatedness between strains.

Since the 3 isolates were from medical students in their preclinical years that had no previous exposure to hospital setting, the presence of MRSA colonization was considered significant. To confirm CA-MRSA, they have to be have been pedigreed by their antimicrobial susceptibility profiles, their DNA fragment patterns by pulsed-field gel electrophoresis

(PFGE), protein A (*spa*) gene typing, carriage of PVL genes, multi-locus sequence typing (MLST), and the type of *SCCmec* element carried by them. The carriers were advised to perform decolonization measures with intranasal application of mupirocin and chlorhexidine antiseptic soap [20,21] and reexamination for the presence of *S. aureus* in nasal swab was negative after a period of 6 weeks.

Conclusion

In this study, *S. aureus* colonization rate among medical and dental staff was detected to be 35% of which three isolates were methicillin resistant (MRSA). Screening for MRSA and subsequent decolonization in patients and health care workers is advised especially in high risk cases. The epidemiology of *S. aureus* and MRSA colonisation is highly variable and the efficacy of screening-decolonization is yet to be convincingly demonstrated. Molecular studies to further characterize the strains and identify its source would be beneficial. The presence of MRSA colonization in future health care providers is best detected early and appropriate infection control measures should be administered timely. This can help to prevent outbreaks in high risk units, and rapid tests to detect MRSA can be quite handy. The effectiveness of decolonization with mupirocin is facing current problem of emerging resistance, especially by MRSA strains [22-24]; hence precaution should still be taken to prevent cross-transmission and re-colonization.

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