Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among medical students in Jeddah, Saudi Arabia

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ABSTRACT

الأهداف: دراسة مدى انتشار الحمل الجرثومي الأنفي للمكورات العنقودية الذهبية المقاومة للميثيسيلين وذلك لدى طلاب الطب خلال مناوباتهم في العمل.

الطريقة: اعتمدت هذه الدراسة المقطعية على التحليل الجزيئي من أجل التحري عن مدى انتشار المكورات العنقودية الذهبية المقاومة للميثيسيلين بين أطباء الطب في جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية. ولقد قمنا بجمع المسحات الأنفية من 150 طبيب مقيم وطلاب السنة السادسة وذلك خلال الفترة من سبتمبر 2014م إلى يناير 2015م، بالإضافة إلى مجموعة الشاهد المكونة من 32 طالباً الذين لم يبدؤوا العمل السريري. وقمنا بإجراء تفاعل البوليميريز المتسلسل من أجل التحري عن جين المكورات العنقودية الذهبية (nuc gene)، فيما تم إعادة إجراء هذا الاختبار على عينات المكورات العنقودية الذهبية الموجبة من أجل التقصي عن جين المكورات العنقودية الذهبية المقاومة للميثيسيلين (mecA gene).

النتائج: أشارت نتائج الدراسة إلى ظهور الحمل الجرثومي الأنفي للمكورات العنقودية الذهبية بين 38 طالباً من أصل 150 طالب. ولقد كانت نسبة انتشار حساسية المكورات العنقودية الذهبية 18.7% (العدد=28). غير أن نتائج التحليل قد أشارت إلى ظهور جين mecA لدى 10 طلاب (6.7%) مما يعني حملهم للمكورات العنقودية الذهبية المقاومة للميثيسيلين. كما وكانت نسبة حمل الأطباء المقيمين للمكورات العنقودية الذهبية المقاومة للميثيسيلين أكثر من الطلاب الغير معرضين للعمل السريري ($p<0.05$)، في حين كانت حساسية المكورات العنقودية الذهبية لدى الطلاب الغير معرضين للعمل السريري أكثر من غيرهم ($p<0.01$).

الخاتمة: أشارت الدراسة إلى ظهور المكورات العنقودية الذهبية المقاومة للميثيسيلين بين طلاب جامعة الملك عبدالعزيز، مما يوضح مدى تأثير هذه الفئة في نقل العدوى للمرضى المنومين في المستشفيات. ولهذا فإنه يجب على طلاب الطب تلقي التعليم الكافي حول طرق التحكم بالعدوى من أجل تجنب انتشارها في المستشفيات.

Objectives: To identify *Methicillin-resistant Staphylococcus aureus* (MRSA) nasal carriage status among medical students during their clinical rotations.

Methods: This cross-sectional study detected the prevalence of MRSA among medical students at King Abdulaziz University (KAU), Jeddah, Saudi Arabia, using molecular approaches. Nasal swabs were collected from 150 internship and sixth-year medical students between September 2014 and January 2015, and compared with the control group of 32 third-year medical students who were not exposed to clinical work. Polymerase chain reaction (PCR) screening was performed to identify *Staphylococcus aureus (S. aureus) nuc* gene, and an additional PCR was performed on *S. aureus* positive samples to detect the presence of *mecA* gene.

Results: Out of 150 students screened, 38 were nasal carriers of *S. aureus*. The prevalence of methicillin-sensitive *S. aureus* (MSSA) carriers was 18.7% (n=28), whereas 10 students (6.7%) were *mecA*-positive, representing MRSA carriers. Interns carry MRSA more than 6th year students and students who were not exposed to clinical work ($p<0.05$), while MSSA is found more in students who were not exposed to clinical work ($p<0.01$).

Conclusion: We found MRSA carriers among medical students at KAU, which showed a possible contribution of this group to transmit infection to hospitalized patients. Medical students must receive sufficient knowledge regarding control measures to avoid spread of this infection in hospitals.

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Staphylococcus aureus (S. aureus) is a key pathogen, which is implicated in nosocomial and community acquired infections.[1,2] Infection caused by *S. aureus* can be endogenous, where the infectious organism is found in the patient's body, or exogenous, where the organism is transmitted from an external source. The organism is normally found as a commensal in the anterior nares of healthy individuals. Immuno-compromised patients are at high risk of acquiring infection. Therefore, nasal colonization of hospital staff, students, and visitors who are in direct contact with this type of patients can be a potential source of transmitting infection.[3,4] More clinical attention has been given to staphylococcal infection due to the ability of this organism to rapidly develop resistance to a wide range of antibiotics.[5] After the identification of ß-lactamase, the core cause of penicillin resistance in *S. aureus*, newer semi-synthetic penicillinase-resistant ß-lactams, exemplified by methicillin, were introduced to counteract the penicillinase-producing *S. aureus* strains.[6] However, very shortly after its introduction, strains of *S. aureus* resistant to methicillin were reported.[7] These are known as methicillin-resistant *Staphylococcus aureus* (MRSA). Since the early 1960s, multi-resistant strains of *S. aureus* have emerged in hospitals and the community, which are now resistant to methicillin and a wide range of currently available antibiotics. According to national nosocomial surveillance system in 2003,[8] 60% of nosocomial infections caused by *S. aureus* from the intensive care units were resistant to methicillin. This limits the therapeutic options to very few agents such as vancomycin and teicoplanin. However, the overuse of vancomycin resulted in emergence of MRSA that shows decreased susceptibility to these agents.[9,10] The prevalence rate of MRSA had reached 50% in the United States hospitals.[11] In the United Kingdom, MRSA accounted for 44% of *S. aureus* isolated from health care workers. In Japan, MRSA accounted 60-70% of *S. aureus* isolated from inpatients.[12,13] In Saudi Arabia, the MRSA nasal carriage rate among healthcare workers was reported as 76%.[14] However, a 20-year literature search did not reveal any data for the prevalence of MRSA carriers among medical students in Saudi Arabia. Thus, healthcare workers are at higher risk of colonization by MRSA than the general public, apparently due to increased exposure to this organism. Moreover, they can be a major source of transmission during contact with their patients if infection control measures are not complied. The presence of MRSA in health institutes is directly proportional to high rate of infections caused by this strain. This may lead to a relative increase in treatment cost and length of hospital stay. Therefore, screening for MRSA in hospitals is an important factor for building up successful infection control strategies.[15,16] Medical students would be a key target group to introduce awareness of hospital-acquired infections. Therefore, prevalence studies need to be carried out to screen this group to assess their carriage status during their clinical rotations. In Saudi Arabia, such studies have not been frequently carried out. These students can be exposed to patients and other healthcare workers during their clinical rotation and can be potential nasal carriers for spreading MRSA within hospitals. Therefore, this study aims to identify the MRSA nasal carriage status among medical students during their clinical rotations at King Abdulaziz University (KAU) Hospitals, Jeddah, Saudi Arabia.

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Methods.

Study design. This cross-sectional study was executed between August 2014 and January 2015, at the Department of Medical Microbiology and Parasitology, Faculty of Medicine, KAU, Jeddah, Saudi Arabia. The number of students participating in the study was 182, of which 150 were from the sixth year and internship year, and 32 control samples were obtained from third-year medical students who were not exposed in the hospitals. All of the third-year students who were tested negative for *S. aureus* nasal colonization were excluded from the study. Students who were hospitalized in the past 6 months, and students who had used antibiotics in the past 3 months were excluded from the study. An informed consent form was signed by students who agreed to participate in the study. Also, a questionnaire for demographic data and health history was completed by the students. The questionnaire targeted variables such as year of study, gender, recent antibiotics consumption, and smoking status. The study was approved by the Unit of Biomedical Ethics at the Faculty of Medicine, KAU.

Sample collection. Nasal swabs were collected from all participants, one swab used for both nostrils, after been moisturized into sterile normal saline, and inoculated into Amies transport media (Copan, Italy). Samples were then processed within 1-3 hours according to a

previously described method.[17] To summarize, samples were inoculated into mannitol-salt agar, which is a selective medium for *S. aureus* and incubated aerobically at 37°C for 24 hours. Single yellow colonies were then sub-cultured into blood agar and incubated at 37°C for 24 hours. *Staphylococcus aureus* was identified by Gram stain, catalase test, and slide coagulase test from single colonies grown on blood agar.

Antibiotic susceptibility tests. *Staphylococcus aureus* strains were subjected to antibiotic susceptibility tests by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI).[18] Antibiotics included in this study were; 1 µg oxacillin (Oxoid Limited, Hampshire, UK), 30 µg vancomycin (Bioanalyse®, Ankara, Turkey), 5 µg rifampicin (Oxoid), and 5 µg ciprofloxacin (Bioanalyse®). Phenotypically, *S. aureus* strains were considered as MRSA if they were resistant to oxacillin, and methicillin-sensitive *Staphylococcus aureus* (MSSA) if they were oxacillin-sensitive. The *S. aureus* strains ATCC 33591 (MRSA) were used as positive and ATCC 25923 (MSSA) were used as negative controls.

Molecular examination. Genomic DNA extraction. All samples included in the study were subjected to genomic DNA extraction following the previously described protocol.[17,19] Briefly, 10 fresh colonies of *S. aureus* grown on blood agar were suspended into 1 mL of Tris (0.5 M, pH 8.0), centrifuged at 13,000 rpm for 5 minutes. Supernatants were discarded, and pellets were resuspended into Tris ethylenediaminetetraacetic acid (TE) buffer (10 mM Tris, 1 mM Ethylenediaminetetraacetic acid, pH 8.0), boiled at 100°C for 30 minutes. Samples were then incubated at 37°C for 20 minutes and 65°C for 10 minutes, and finally centrifuged at 13,000 rpm for 15 minutes. Supernatant containing genomic DNA was collected and stored at -20°C for further assays.

Detection of mecA and nuc genes. Genomic DNA of all positive *S. aureus* strains identified by microbiological methods was used as DNA templates for 2 separate screening PCRs. The first was carried out to detect the *nuc* gene, which is a unique gene that identifies *S. aureus*, which codes for nuclease production. The second PCR was performed to determine the presence of *mecA* gene, which codes for altered penicillin-binding protein responsible for methicillin resistance. For the *nuc* gene, primers used were *nuc_F* (TAAGTGCTGGCATATGTATG), and *nuc*_R (CAATTTTMTTTGCATTTTCT) to amplify a 425 bp DNA fragment of the *nuc* gene. For *mecA* gene, primers used were *mecA*_F (GTGGAATTGGCCAATACAGGAAC) and *mecA*_R (GTTAGTTGAATATCTTTGCCATC) that amplifies a 502 bp DNA fragment of the *mecA* gene. Genomic DNA was subjected to initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for one minute, annealing at 54°C for one minute, and extension at 72°C for one minutes, and a final extension at 72°C for 5 minutes. The DNA was visualized by 2% agarose gel electrophoresis. *Staphylococcus aureus* strains ATCC 33591 were used as MRSA and ATCC 25923 were used as MSSA controls.

Statistical data analysis was applied to compare the prevalence of positive MRSA carriers among medical students to those of MSSA carriers. Grouping of results was based on gender, year of study, health, and smoking status. Statistical analysis was performed by the IBM SPSS Statistics for Windows version 16.0 (SPSS Inc., Chicago, IL, USA) to determine the significant relation among these variables.

Results. A total number of 150 samples were collected from medical students and interns carrying out clinical rotation at different departments of the hospital. A control group of 32 samples were obtained from medical students at their pre-clinical years, who were not exposed to hospitals. Seventy-seven samples were collected from male students (51.3%, of which 33 [22%] were from internship year and 44 [29.3%] from sixth year), and 73 samples were obtained from female students (48.7% of which 23 [15.3%] from internship year and 50 [33.3%] from sixth year) (**Table 1**). *Staphylococcus aureus* was isolated from 38 samples (25.3%) of the 150 participants; 10 (6.7%) samples were positive for MRSA (26.3% of all *S. aureus* were MRSA), and the other 28 (18.7%) samples were MSSA (**Table 2**). There is a statistically significant correlation between the prevalence of MRSA in interns compared with sixth-year students and the control group ($p<0.05$). Moreover, there is statistically significant correlation between the prevalence of MSSA in the control group compared with interns and sixth-year medical students ($p<0.01$). Furthermore, there was a statistically significant correlation between the prevalence of MSSA and both recent antibiotic consumption and smoking ($p<0.01$). However, there was no statistically significant correlation between the prevalence of MRSA and gender ($p=0.46$) in males and ($p=0.57$) in females.

All the 38 *S. aureus* strains were subjected to phenotypic analysis including Gram staining, catalase, coagulase tests, and antibiotic susceptibility tests. Also,

Table 1 - Frequency of methicillin-sensitive *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) nasal colonization according to the year of the study and gender among internship and sixth year medical students in comparison with the control group (third year medical students) who were not exposed to clinical work, at King Abdulaziz University, Jeddah, Saudi Arabia.

Gender		Year of study		
Male	Female	Internship	Sixth year	Third year (control)
77 (51.3)	73 (48.7)	56	94	32
			MRSA nasal carriers	
6 (7.8)	4 (5.5)	6 (10.7)	4 (4.3)	0 (0)
			MSSA nasal carriers	
14 (18.2)	14 (19.2)	12 (21.4)	16 (17.0)	32 (100)

Table 2 - Prevalence of methicillin-sensitive *Staphylococcus aureus* (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) nasal carriage among medical students at King Abdulaziz University, Jeddah, Saudi Arabia identified by molecular methods.

Variables	Sample group (interns and 6th year)	Control group (3rd year)
Number of samples	150	32
MRSA	10 (6.7)	0 (0)
MSSA	28 (18.7)	32 (100)

genotypic analysis was performed by PCR screening for the *mecA* gene. Oxacillin resistance was found in only 2 isolates by disc diffusion method (5.3% of all *S. aureus* strains). None of the strains demonstrated resistance to vancomycin, rifampicin, cefoxitin, or ciprofloxacin. After screening all *S. aureus* strains by PCR-based analysis for the presence of *nuc* gene, which was found in all *S. aureus*, a specific PCR screening for the *mecA* gene was carried out. This showed that 10 strains (26.3% of all *S. aureus* strains) were carrying the *mecA* gene, representing methicillin-resistant strains. The number of *mecA*-positive isolates detected by screening PCR was higher than the number of oxacillin-resistant isolates. However, it was not statistically significant (p=0.71). None of the *S. aureus* isolates obtained from the control group of the third-year students who were not exposed to clinical work showed positive MRSA nasal carriage, neither by phenotypic resistance to oxacillin nor by PCR screening. Compared with the sample population, it was a significant finding that all samples from the control group who are nasal carriers of *S. aureus* tested negative for MRSA (p<0.01).

Discussion. Methicillin-resistant *S. aureus* is an important hospital and community acquired pathogen. Aside from the resistance of this organism to a wide range of antibiotics, its ability to infect hospitalized patients, immune-compromised in particular, is very high.[20] A published study[21] provided evidence that healthcare workers and medical students can be important vectors for spreading the organism, especially when infection control measures are compromised. Therefore, limiting the spread of MRSA can be highlighted by stressing proper hand hygiene and educating patients and healthy carriers on MRSA colonization. From the past 20-years, literature did not show any reports on the nasal colonization status of MRSA in medical students in Saudi Arabia, and few reports on healthcare workers.[14,22] This study shows that 6.7% of students carry MRSA strains in their nares, providing evidence that these strains are present in KAU Hospital. Although the rate of MRSA carriers among medical students in their clinical years was relatively low, it remains higher than internationally reported surveillances,[17,23-26] and can be threatening due to the frequent and direct contact with patients. In Colombia, a study carried out by Bettin et al[17] shows that the MRSA carriage status by medical students in their clinical rotations was 1.6%. In Turkey, Baliga et al[23] reported that MRSA carriers accounted for 4.4% of medical students in clinical practice.

Interestingly, in this study, no MRSA colonization was found from the 32 control students who were not exposed to clinical work, whereas MRSA was found in those who were at clinical years. This can provide evidence that frequent exposure to MRSA in hospitals may play a critical role in gaining nasal colonization by MRSA. This finding is very similar to several studies performed on medical students who are at preclinical years. For example, Kitti et al[24] found that only 1% of university students were colonized by either MRSA or MSSA in Thailand, proposing that individuals who are not exposed to the pathogen are at low risk of nasal colonization. Also, Peichowicz et al[25] compared students who are at clinical years to those at preclinical years. They found that 21% of clinical students were colonized by MRSA, while all preclinical students were negative. In a Hungarian study by Laub et al[26] on university students, MRSA nasal colonization was found in only 0.7% of students. All these data provide clear evidence that medical students who are frequently in contact with hospitals can acquire the MRSA pathogen during their hospital rotations. Thus, it is essential to identify MRSA carriers and apply educational sessions to limit the spread of this pathogen via this group of MRSA carriers.

Polymerase chain reaction screening demonstrated that *mecA* gene was found in 10 isolates, whereas

only 2 isolates showed phenotypic resistance to oxacillin. Although molecular analysis showed more strains carrying the *mecA* gene in their genome than the phenotypic expression as shown by antibiotic susceptibility tests, it was statistically not significant. A recent study by Pu et al[27] tested 103 *S. aureus* isolates from different farms and found that 49 (47.6%) were positive for *mecA* gene. However, only 12 isolates were resistant to oxacillin when they performed antimicrobial susceptibility tests, considering these isolates as oxacillin-sensitive MRSA (OS-MRSA).[27] This may explain the low number of phenotypic oxacillin-resistant *mecA*-positive strains, whereas all these strains were susceptible to cefoxitin. Moreover, the *mecA* gene can possibly be present in the genome of *S. aureus* but in an inactive or inhibited form. Further research is needed to investigate the possibility of inhibition or inactivation of the *mecA* gene in *S. aureus*.

Routine decolonization of MRSA in healthcare workers and medical students is not common. For instance, international guidelines suggest the use of mupirocin for nasal decolonization of healthcare workers and patients who are colonized with MRSA.[28] Nevertheless, mupirocin can only eliminate the organism from the nasal cavity for a few weeks, and relapse can commonly occur a few months later.[29] Moreover, there is an increasing concern regarding resistance to mupirocin. Therefore, it has been suggested to avoid decolonization of this group of MRSA carriers, and increase awareness on MRSA colonization for the students who were screened positive of MRSA carriers by attending educational sessions on hand hygiene, patients safety, and infection control managements, to limit the risk of spreading the MRSA to susceptible patients.[21,30] Although the sample number in this study was relatively low, it can provide an initial indication on MRSA colonization in this sample group. Further research may be executed to assess MRSA colonization among medical students during longer periods. Moreover, molecular typing of these strains by using pulsed-field gel electrophoresis (PFGE), or by PCR restriction fragment length polymorphism (RFLP) and DNA sequencing in order to identify different types of this organism.

In conclusion, our findings indicate that some medical students who are practicing in their clinical training at KAU hospital carry MRSA strains in their nasal cavities. The presence of these strains in this sample population, and its absence in the control group of pre-clinical students clearly indicates that these strains may be acquired from the hospital during clinical training. Such findings suggest that more prevention and control precautions, aside from educational sessions on patients safety, and hand hygiene may be required for medical students prior to starting hospital training to increase awareness of hospital-acquired MRSA and other infections.

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