Brief Original Article

High incidence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers in Saudi Arabia

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Abstract

Introduction: Nosocomial infections are normally hospital acquired. Nasal carriage of *Staphylococcus aureus (S. aureus)* is very common and may be transmitted via a hand-to-nose route. The objective of the present study was to screen healthcare workers for the colonization of their nasal cavities with MRSA.

Methodology: The study group included hospital staff such as nurses, doctors, and technicians. The control group included university students. For isolation, nasal swabs were taken from the volunteers and cultured on mannitol salt agar media selective for *S. aureus*. Suspected colonies were confirmed by PCR using specific primers for the coagulase and *mec*A gene. Typing of the coagulase-positive strains was done using restriction fragment length polymorphism (RFLP).

Results: The results indicated an incidence rate of 76% among healthcare workers. This is in comparison with students who served as control and were negative for MRSA. Using RFLP, four different types of MRSA were confirmed.

Conclusions: The results of this study are alarming. Effective control measures must be formulated and implemented to avoid indiscriminate use of antimicrobials and the spread of these infectious agents in the region.

Key words: MRSA; nosocomial infections; *mecA* gene; coagulase; RFLP; burn unit

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Introduction

Nosocomial infections are hospital acquired and caused by bacteria and/or other microorganisms. They may be endogenous, arising from an infectious agent present within a patient's body, or exogenous, transmitted from other sources within the hospital. Most at risk are patients who are immuno compromised. In addition to patient-to-patient spread, staff, students, visitors, and voluntary workers may be affected [1]. Fomites also play an important role in outbreaks. Nonporous fomites were involved in a recent outbreak of CA-MRSA in California, where the duration of transmissibility was not accounted for. In these CA-MRSA strains, different fomites caused contamination of the skin with MRSA, and transmission continued for many weeks after initial contamination [2]. Staphylococci and enterococci are major causes of nosocomial infections. They cause superficial skin lesions such as boils and more serious infections such as pneumonia, phlebitis, meningitis, mastitis, and urinary tract infections, as well as deep-seated infections such as osteomyelitis and endocarditis [3]. MRSA is a strain of *S. aureus* which, by definition, is resistant to the semi-synthetic penicillins (*i.e.* methicillin, nafcillin, and oxacillin) [4]. As such, it is resistant to all other beta-lactam antibiotics (including penicillins, cephalosporins, and cephamycins). Additionally, MRSA is often resistant to other classes of antimicrobials, including aminoglycosides, macrolides, and quinolones. Thus, MRSA is not only methicillin-resistant, but is also multidrug-resistant [5]. MRSA colonization and infection in acute and non-acute care facilities have increased dramatically over the past two decades, evidenced by the increasing number of reported outbreaks in the medical literature. Because of its

resistance to antibiotics, management of MRSA infections requires more complicated, toxic, and expensive treatment [6]. It is important for healthcare professionals to understand the difference between colonization and infection. Colonization indicates the presence of the organism without symptoms of illness. *S. aureus* permanently colonizes the anterior nares of about 20% to 30% of the general population. Hospital workers are more likely to be colonized than persons in the general population, presumably because of increased exposure [7]. The resistance to antibiotics is due to a gene called *mecA*, which is part of the staphylococcal cassette chromosome. It codes for a penciclin-binding protein (PBP2a) that prevents the action of beta lactam antibiotics [8]. This study focused on rapid detection of MRSA using gene-specific primers designed to detect the *mecA* gene. Recently, coagulase gene typing has been used as an important tool to characterize pathogenic staphylococci. Its discriminatory power relies on the heterogeneity of the region containing the 81 bp tandem repeats at the 3' ends of the coagulase gene. PCR amplification of this particular region produces DNA fragments of different sizes, which can then be further differentiated by *Alu*I digestion [9]. In this study, coagulase gene (*coa*) PCR and RFLP were used to type the MRSA strains from various populations.

Methodology

Sample collection

Samples were collected from hospital workers including doctors, nurses, and technicians. The sample group consisted of volunteers from various hospitals. A total of one hundred and fifty samples were included in this study, of which 100 were from healthcare workers. The healthcare workers were from different hospitals that had various sectors such as outpatient departments, intensive care units, burn units, pediatric units, and maternity units. Samples from healthcare workers from only three units – the outpatient departments, intensive care units, and burn units – were collected. Of the 100 healthcare workers, 65 were men and 35 were women, all of different nationalities. A control group of 50 students not exposed to hospitals was also used for the study. The students were undergraduate students at King Abdulaziz University. Before participating in the study, it was confirmed that the students had not been recently hospitalized or exposed to any clinical conditions. All volunteers signed a consent form and ethical approval was obtained for the study Swabs were taken from both the anterior nares using sterile swabs moistened with saline.

Isolation, growth and identification of bacteria

Each swab was immediately placed in an enrichment broth, processed in the microbiology laboratory on the same day of sampling, and incubated at 35.8ºC overnight. The enrichment broth for *S. aureus* consisted of 37.5 g NaCl, 1.25 g yeast extract, 5.0 g tryptone in 500 mL distilled water. Each 10 µL of incubated enrichment broth was inoculated in mannitol salt agar (HiMedia Labs, Mumbai, India), which is selective for *S. aureus*, and incubated at 35.8ºC between 24 and 48 hours; yellow colonies were selected and confirmed to be *S. aureus* following catalase, coagulase, and DNAse tests [10], and were finally confirmed by PCR using specific primers as shown below.

Antimicrobial resistance testing

Tests for methicillin resistance were performed using the Kirby-Bauer disc diffusion method, using oxacillin (1 µg) disc on Mueller-Hinton agar (HiMedia Labs, Mumbai, India) with 24-hour incubation at 35.8ºC. Results were interpreted according to the criteria of CLSI (2007) [11]. Methicillin resistance was confirmed by agar screen test using a Mueller-Hinton agar plate supplemented with 4% NaCl and oxacillin (6 µgm/mL). *S. aureus* ATCC 700699 was used as a control methicillin-resistant strain.

PCR amplification

Colonies were resuspended in 100 µL of lysis buffer containing 1% Triton X 100 and boiled at 95°C for 15 minutes. The suspension was centrifuged at 10,000 rpm for 5 minutes, and 10 µLl of the supernatant was used as template for PCR. Primers specific for the *mec*A gene were used to amplify the methicillin gene, which codes for a modified penicillin-binding protein that confers resistance to the beta lactam class of antibiotics. *MecA*-positive strains were then screened for the presence of the coagulase gene using primers specific for the coagulase gene. Primers used and PCR conditions are shown in Table 1 [12,9]. PCR products were visualized by electrophoresis using 1% agarose gel. *Mec*A-positive strains were subjected to PCR for the coagulase gene. The different size coagulase gene products were subjected to restriction digestion using *Alu*I restriction enzyme for one hour at 37°C. ATCC 700699 strain was used as an MRSA-positive control.

Table 1: Primers and PCR conditions used for detection of *mec*A and coagulase genes.

Gene	Primers used	PCR conditions	Number of cycles	Product size
*mec*A	FP: 5′AAAATCGATGGTAAAGGTTGGC 3' RP: 5′ AGTTCTGCAGTACCGGATTTGC 3’	94°C – 30 secs 55°C – 30 secs 72°C – 1min 72°C – 5 min (final extension)	40	533 bp
Coagulase	FP : 5′ATAGAGATGCTGGTACAGG 3’ RP : 5′GCTTCCGATTGTTCGATGC3′	94°C – 30 secs 60°C – 1 min 72°C – 1min 72°C – 5 min (final extension)	40	350 bp 430 bp 570 bp 630 bp

Table 2: Percentage distribution of nasal carriage of MRSA amongst the health care workers

Age		Sex		Place of work (hospital unit)		
30-40 years	40-50 years	Male	Female	Burn Unit	Intensive care unit (ICU)	Out patient (OP)
22%	54%	40%	36%	47%	21%	5%

Table 3: Distribution of coagulase PCR products among MRSA positive subjects

PCR product size	Distribution of coagulase PCR product size amongst the 73 MRSA positive subjects
350 bp	40
430 bp	15
570 bp	10
630 bp	8

Table 4: Coagulase gene products and their RFLP pattern

Coagulase gene product size	RFLP bands obtained by digestion with *Alu*I
370 bp	260 bp and 110 bp
430 bp	190 bp and 240 bp
570 bp	100 bp and 470 bp
630 bp	160 bp, 240 bp, 330 bp

Statistical analysis

Basic statistical analyses were conducted to determine the percentage of subjects positive for nasal carriage of MRSA. The positive subjects were grouped according to place of work, age, and sex. SPSS version 17 software was used for statistical analyses to check if there was significant relation between place of work and the nasal carriage of MRSA. Significance was calculated in terms of p value such that p values of less than 0.05 were considered to be significant. The subjects positive to MRSA were also classified according to the percentage distribution of the varying size of coagulase gene product as a measure of typing the MRSA isolates.

Results

Out of 100 healthcare workers screened, 73% were found to be positive for MRSA in the anterior nares based on culture results and antimicrobial susceptibility to oxacillin. Colonies isolated from 73 subjects were resistant to oxacillin. The classification of the positive subjects based on location of work (hospital unit), age, and sex is shown in Table 2. None of the students in the study tested positive for MRSA nasal carriage. The absence of MRSA carriage in students was a very significant finding when compared to the healthcare workers.

PCR-based screening for suspected carriers of MRSA showed that most of the healthcare workers (staff, nurses, and doctors) were MRSA-positive but asymptomatic. Out of 100 samples of healthcare workers screened, 73 tested positive for nasal carriage of MRSA. Statistical analyses showed that there was no significant relation between MRSA carriage and age and sex of the study population. Among the healthcare workers, a high number of positives was found in the burn unit, where, out of 58 samples, 47 were positive. The p value was 0.04 and considered to be significant at the 95% confidence level. In the intensive care unit (ICU), 18 out of 24 samples were positive; here, too, a significant p value of less than 0.05 at the 95% confidence level was obtained. On the contrary, in the outpatient department (OPD), only 8 out of 18 samples were positive; the p value of 0.09 was considered insignificant.

*Mec*A-positive strains were subjected to PCR for the coagulase gene. Four different strain types were found based on polymorphisms in the size of the coagulase gene – 350 bp, 430 bp, 570 bp, and 630 bp. The 76 subjects that were MRSA-positive were classified according to the coagulase gene product size, as indicated in Table 3. It was found that the 570 bp PCR product was most abundant, accounting for 53% (40 out of 76 subjects), while the 630 bp product accounted for 32% (24 out of 76 subjects). A total of 11% had the 430 bp product (8 out of 76 subjects), and only 4% had the 350 bp gene product. The PCR products also yielded different restriction patterns on digestion with the enzyme *Alu*I as depicted in Table 4, indicating that amongst the strains isolated from this study there were four different types.

Discussion

MRSA colonization and infection in acute and non-acute care facilities have increased dramatically over the past two decades, evidenced by the increasing number of reported outbreaks in the medical literature [13]. Because of its resistance to antibiotics, management of MRSA infections requires more complicated, toxic, and expensive treatment. It is important for healthcare professionals to understand the difference between colonization and infection. Colonization indicates the presence of the organism without symptoms of illness. *S. aureus* permanently colonizes the anterior nares of about 20% to 30% of the general population. Hospital workers are more likely to be colonized than persons in the general population, presumably because of increased exposure. Estimates of healthcare worker (HCW) carriage from the worldwide literature vary widely depending on the country, hospital specialty, and setting (endemic, non-endemic, or outbreak). Recent studies conducted in endemic hospital settings reported non-outbreak carriage rates of zero to 15%. The role of HCW carriage in the transmission of MRSA is not well understood. Persistent carriage could act as a reservoir for infection, and HCWs have been implicated as the source in a number of published outbreak reports [14]. A 2009 study by Mathanraj *et al.* [15] in an Indian hospital reported that 1.8% of healthcare workers had colonization of MRSA in the anterior nares. A study by Ferreira de Silva *et al.* in 2008 [16] evaluated the epidemiological and sensitivity profile of *S. aureus* lineage isolated in healthcare workers (HCW) of a university hospital in Pernambuco state, Brazil. From the 202 HCW evaluated, 52 were colonized by *S. aureus* (25.7%). The factors associated with colonization by *S. aureus* were age group, professional category, and use of individual protection equipment. In our study, it was found that 76% of the healthcare workers screened tested positive for nasal carriage of MRSA, though they were asymptomatic. This indicates a very high incidence of MRSA. Of the 73%, it is important to

note that the highest incidence, 47%, was noted in the burn unit, followed by 21% in the intensive care unit and just 5% in the outpatient department. On conducting statistical analyses using the Chi square test, significant p values were noted for the burn unit and ICU compared to OPD. This distribution is very much expected because MRSA is known to cause high rates of infections in burns and wounds units [17], and the finding explains the fact that healthcare workers in this unit acquired MRSA due to constant exposure to infected patients. It is well known that the burn unit is a particularly fertile environment for MRSA because of open wounds, frequent dressing changes requiring handling by multiple healthcare workers (HCW), the use of intraluminal devices, and the inherent immuno-compromised status of burn patients [18]. In 2000, Preetha *et al.* [19] screened healthcare workers of the burn unit of a tertiary care hospital and found that 71% of the healthcare workers were positive for nasal carriage of MRSA; this is very much in line with our findings. It is, however, important to draw attention to the fact that in our study, the number of samples from the burn unit was much higher compared to samples from other units such as ICU and OPD. Even then, 47 of 58 samples from the burn unit were positive for MRSA and showed a significant p value, clearly indicating that the burn unit is a high-risk area for exposure to MRSA. In this study, there was also sufficient significance in the ICU; this can be explained by the fact that healthcare workers in the ICU could have acquired the pathogen from patients in the ICU who had wounds, drains, and invasive monitoring devices that breached the skin and increased the risk of developing infections. On the other hand, there was no statistical significance in the outpatient samples, probably because there was no risk of long-term exposure. We did not find any significant difference in MRSA colonization between male and female workers. As far as age is concerned, we found that the subjects in the age group of 40 to 50 years had almost double the rates of MRSA colonization with figures of 54% vs. 22% in age group of 30 to 40 years. This finding is very much in agreement to a 2007 report by Elixhauser and Steiner, [20] which documented that MRSA infections occur at a higher incidence in older people owing to a weaker immune system. In our study, none of the 50 students screened were positive for MRSA colonization, indicating clearly that non-exposure to the infectious agent plays a major role in avoiding nasal carriage. In a 2011 study by Kitti *et al.* [21] on nasal colonization of MRSA and MSSA among healthy young Thai adults, the authors found only 1% colonization in university students, which is very similar to our results, suggesting that people who are not exposed to the pathogen have a very low risk of nasal carriage. Another study by Laub *et al.* in 2011 [22] on Hungarian students showed that only 2/300 students were positive for MRSA nasal colonization. On the contrary, a study carried out on medical students by Avial *et al.* in 2012 [23] showed a significant increase of 27% to 48% over the six-year period of the students' medical course. In another study by Peichowicz *et al.* in 2011 [24] comparing student populations with clinical exposure and without clinical exposure, the authors found that MRSA colonization was positive only in the clinical students, with an occurrence of 21%, while there was no occurrence in non-clinical students. These findings clearly indicate that medical students, being constantly in touch with hospitals and inpatients, do stand a higher risk of colonization, further proving that it is absolutely essential to develop rapid and regular screening programs for transmission of MRSA in healthcare settings. In most cases, culture methods are used to identify MRSA, but the results take at least two days [25]. It is necessary to develop faster and more precise methods for identifying MRSA. This study shows that rapid identification of MRSA can be done using the PCR specific for the *mecA* gene. The technique can be used as a regular screening program in hospitals to avoid the spread of MRSA and also to educate healthcare professionals about the importance of frequent and proper hand sanitization methods to prevent MRSA infections from spreading.

PCR-restriction fragment length polymorphism (RFLP) typing of the coagulase gene (*coa*) can be used to differentiate *S. aureus* strains on the basis of sequence variation within the 3′ end coding region of the gene [9]. In their 2007 study, Ishino *et al.* [26], used PCR-RFLP typing of the coagulase gene for typing 678 isolates of *S. aureus*. The sizes of the PCR products ranged from 350 to 917 bp in increments of 81 bp, reflecting the number of 81 bp repeat units contained in the *coa* gene. After digestion with AluI, 31 *coa*-RFLP types were detected and numbered to allow them to be distinguished from each other. In our study, we found four different sized PCR products for the coagulase gene, and RFLP characterization showed that at least four types of MRSA strains were detected in our sample size of 150. Of the 73 MRSA-positive subjects, we found that the 570 bp product was most frequently occurring; this could possibly be an indication of the extent of the spread of this

particular MRSA clonal type. These findings also lead us to presume that if this study could be extended to a wider number of samples, we would get a better spectrum of types of MRSA isolates.

Conclusions

In conclusion, our study on a limited population comparing healthcare workers who were constantly exposed to the pathogenic MRSA and students who were not exposed to the pathogen clearly shows that people in a healthcare setting are constantly at risk of acquiring, colonizing, and spreading MRSA. Although the volunteers who tested positive for colonization were asymptomatic, they are high-risk groups for transmission and dissemination to non-infected people. The percentage of nasal carriage of the healthcare workers was very much associated with the high-risk patient groups with whom they are in constant contact. Given the irrational use of antibiotics and the failure to comply with dosage regulation of antibiotics in the community, there is a high risk of transmission of nosocomial infections to the community, leading to outbreaks of community-acquired MRSA (CAMRSA). This clearly shows the need for formulating and implementing regular screening programs in hospitals and clinics to prevent the spread of nosocomial infections. PCR, a rapid tool for faster and definitive detection of the pathogen, can be used as a routine screening test in healthcare settings. It is important to formulate and implement awareness campaigns, hand sanitization practices, and regular screening programs to prevent outbreaks of MRSA infection.

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