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DETECTION OF METHICILLIN RESISTANT *Staphylococcus aureus* ISOLATED FROM NASAL CARRIAGE OF HEALTH CARE WORKERS BY POLYMERASE CHAIN REACTION

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KEYWORDS

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative Staphylococci (MRCoNS) are major health problems worldwide. There is a growing concern about the rapid rise in resistance of nosocomial infections to antimicrobial agents. The aim of the present study was to determine nasal colonization of MRSA and MRCoNS among healthcare workers (HCWs) in King Abdulaziz University hospital. A total of 100 respondents were selected for this study and were classified into two groups: HCWs group which included 50 technicians and nurses, and the control group which included 50 university students. Nasal swabs from anterior nares were cultured on selective media for the presence of *mecA* gene that is common to these bacteria. Polymerase chain reaction (PCR) and specific primers were used to determine *mecA* and coagulase gene (*coa*). The identified isolates were tested by restriction fragment length polymorphism (RFLP). Results of current study revealed that among 50 HCWs, 21 (42%) were nasal carriers for MRCoNS and 12 (24%) for MRSA. The control group was negative for MRSA and MRCoNS. The results of the current study will help in making decision to prevent the spread of infections among workers in the field of health care.

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1 Introduction

Nosocomial or hospital acquired infections (HAIs) are one of the most common problems in hospitals throughout the world, these are the infections that develop during hospitalization and are neither present nor incubating at the time of the patients' admission (Revelas, 2012). The source of nosocomial infections can be either endogenous (e.g. from the patient's own flora) or exogenous (e.g. from a source other than the patients). HAI is an infection whose development is favored by environment, such as one acquired by a patient during hospital visit or one developing among hospital staff (Shrestha et al., 2009). About 20% of patients undergoing surgery acquire at least one nosocomial infection, leading to increased morbidity, mortality, hospital stay and costs. *Staphylococci* and *Enterococci* are major causes of nosocomial infections (Boyce et al., 1992).

Methicillin resistant *S. aureus* (MRSA) and coagulase negative *Staphylococci* (MRCoNS) which by definition are resistant to the semi-synthetic penicillin (i.e. methicillin) (Van Hal et al., 2007). The prevalence of MRSA infections have increased dramatically over the past two decades (Williamson et al., 2014). According to Baiu & AL-Abdli (2015), due to antibiotics resistance, MRSA infections required more complicated treatment. Resistance due to *mecA* gene, which encodes an alternative *Penicillin Binding Protein* (PBP2a), prevents the action of beta lactam antibiotics (Jousselin et al., 2016). In addition, coagulase gene (*Coa*) is usually used to identify and compare *S. aureus* because it consists of 81-bp tandem repeats region at the 3' ends (Hookey et al., 1998). Although, most of the MRSA infections do not take a serious clinical course in healthcare workers, some infections tend to become chronic and can cause severe health problems, depending upon the strength of the individual's immune system. For healthcare facilities, surveillance is an important and accepted method to assess the incidence of infection due to multidrug-resistant bacteria and if necessary, to improve infection control measures (Deurenberg et al., 2007). The aim of the current study was to determine the frequency of MRSA and MRCoNS among HCWs using PCR-RFLP analysis.

2 Materials and Methods

2.1 Sample collection

Samples were collected from King Abdulaziz University Hospital

(KAUH) and King Abdulaziz University (KAU) students. HCWs samples included nurses and technicians from different wards: Medical Intensive Care Unit (MICU), Surgical Intensive Care Unit (SICU), Cardiac Care Unit (CCU), and Outpatient Departments (OPD) of the same hospital. The control samples included the students. A total of 100 respondents were selected for this study, among this 50 were from HCWs while the other 50 were control students. The positive subjects were grouped according to place of work, age and gender.

2.2 Isolation, growth and identification of bacteria

Each swab was immediately placed in an enrichment broth and incubated at 37°C for 24 h; these collected samples were processed in the microbiology laboratory on the same day of sampling. The enrichment broth that shows the presence of the *mecA* gene of *Staphylococci* was MeReSa Agar Base. The selective broth was prepared by suspending 40.06 g of the medium into 500 ml of the distilled water and boiling. The medium was cooled (45-50°C) and MeRsEa selective supplement (FD299) was added with 5 ml distilled water and mixed well. The final volume was 2 mg of methicillin in each vial.

Soon after, the medium was poured into sterile Petri plates and cooled to check for sterility by keeping at 37°C overnight. In this study, the detection of MRSA and MRCoNS were determined by direct culture of each swab on chromogenic medium. All cultures which were showing light pink colored growth selected and confirmed to be *mecA* positive isolates following coagulase, and were finally confirmed by PCR using specific primers as shown below, and all others are recorded as MSSA isolates (HiMedia Labs. Products, India)

2.3 PCR amplification

Bacterial DNA was extracted and stored at -20°C using GeneJET Genomic DNA Purification Kit #K0721 (Thermo Scientific, USA) according to the manufacturer's instructions. For PCR, *MecA* gene primers were used to amplify *mecA* gene and *MecA*-positive strains were screened for *coa* gene using specific primers. The primers sequences are shown in Table 1. PCR products were detected using 2% agarose gel electrophoresis and visualized with ethidium bromide dye. *Coa* gene products were subjected to restriction digestion by *AluI* restriction enzyme (Hookey et al., 1998).

Table 1 Primer sequence and PCR conditions used for detection of *mecA* and *coa* genes

Gene	Primer sequence	PCR conditions	Number of cycles	Product size
<i>MecA</i>	FP: 5'AAAATCGATGGTAAAGGTTGGC 3' RP: 5' AGTTCTGCAGTACCGGATTTCG 3'	94°C – 30 s	40	533 bp
		55°C – 30 s		
		72°C – 1min		
		72°C – 5 min (final extension)		
<i>Coa</i>	FP: 5'ATAGAG ATGCTGGTACAGG3' RP: 5'GCTTCCGATTGTTTCGATGC3'	94°C – 1min	30	350 bp
		60°C – 1min		430 bp
		72°C – 1min		570 bp
		72°C – 5 min (final extension)		630 bp

2.4 Statistical analysis

Statistical analysis was performed using SPSS for windows v. 20.0. Chi square test was used to compare frequency distribution of MRSA and MRCoNS among HCWs. P values of < 0.05 were considered to be statistically significant.

3 Results

Out of 50 HCWs participating in the study, 21 (42%) were nasal carriers of MRCoNS and 12 (24%) for MRSA in the anterior nares based on culture results and coagulase test. Table 2 summarizes the organization of the positive subjects according to the unit, age, and gender. The results showed that none of the students, who are participating as controls, were positive for MRSA and MRCoNS nasal carriage. The absence of MRCoNS and MRSA nasal carriage in students was an actual important result when compared to the HCWs.

As previously described, all MRSA isolates ($n=12$; 24%) and MRCoNS ($n=21$; 42%) were obtained from 50 HCWs from four different units at KAUH (MICU, SICU, CICU and OPD). The highest incidence of MRSA (12%) and MRCoNS (26%) was recorded in MICU from 28 HCWs. Furthermore, 8% of MRSA and 14% of MRCoNS isolates in SICU were from 16 HCWs. While in CCU, 2% of MRSA and MRCoNS were from two HCWs, respectively. The lowest nasal carriage was isolated from 4 HCWs OPD by 2% in MRSA and (0%) of MRCoNS. Results of this assay demonstrated that all MRSA and MRCoNS isolates detected by chromogenic method showed the presence of amplified bands of *mecA* gene (533 bp) (Figure 1).

Statistical analysis of results revealed that there was no significant difference between the units and MRSA and MRCoNS using chi-square test ($P>0.05$). The age of HCWs infection ranged from 23 to 51 years (mean age 33 ± 5.82 years). Among these, 31 females were (62%) and two were males (4%). With respect to nasal carriage of MRSA, 11 females and one male were colonized. While in case of MRCoNS, 20 females and one male were

Parameters		Total	Colonization	MRSA		MRCoNS	
				NO.	%	NO.	%
Unit	MICU	28	19	6	12	13	26
	SICU	16	11	4	8	7	14
	CCU	2	2	1	2	1	2
	OPD	4	1	1	2	0	0
Age (years)	20-29	12	8	2	4	6	12
	30-39	33	21	8	16	13	26
	40-49	3	2	1	2	2	4
	50-59	2	1	1	2	0	0
Gender	Female	48	31	11	22	20	40
	Male	2	2	1	2	1	2
Total		50	33	12	24	21	42

colonized. No significant difference was reported between age and MRSA or MRCoNS, individually. Moreover, there were no significant differences between gender and MRSA or MRCoNS separately, as shown in table 3.

Coa gene is important for the identification of *S. aureus*, with sizes ranging from 400 bp to 900 bp. *Alu I* restriction

Figure 1 Agarose gel electrophoresis (2%) of *mecA* gene PCR products where lane 1: 100 bp DNA ladder and lane 2-9: PCR product

Table 3 Chi-square test for MRSA and MRCoNS colonization disaggregated by age group, gender, and units

Parameters		χ^2	df	P value
MRSA	Age	0.756	3	0.685
	Unit	0.854	3	0.837
	Gender	0.772	1	0.38
MRCoNS	Age	3.256	3	0.815
	Unit	3.195	3	0.363
	Gender	0.055	1	0.169

P value is not significant ($P > 0.05$)

Figure 2 Agarose gel electrophoresis (2%) of *coa* gene PCR products where lane 1: 100 bp DNA ladder and lanes 2-9: PCR product.

enzyme digestion of the PCR-amplified of *coa* gene yielded one distinct PCR-RFLP pattern. The PCR products of *coa* gene (Figure 2) of all MRSA strains and controls were cut by *Alu* I enzyme and gave one fragment as shown in Figure 3.

Figure 3 Agarose gel electrophoresis (2%) of *coa* gene *Alu* I restriction enzyme digestion PCR products, where lane 1: 100 bp DNA ladder, lane 2: control sample and lanes 3-10: *Alu* I restriction digestion pattern.

4 Discussion

Staphylococci are the major causes of nosocomial infections. MRSA and MRCoNS are resistant to all β -lactam antibiotics and are considered as the most important causes of nosocomial infections around the world. One of the most effective methods for preventing the spread of MRSA and MRCoNS, requires detection of colonized HCWs and measuring the associated risk factors of colonization.

Results of this study revealed low prevalence rate of MRSA nasal carriage (24%) among HCWs but high prevalence rate of MRCoNS nasal carriage (42%). Although, these rates are lower than those reported in other areas of the world (Lu et al., 2005; Shibabaw et al., 2013; Hussein et al., 2017). The prevalence has decreased gradually from 38% in 2002 to the current rate of 24% in same hospital (Madani, 2002). There were considerable differences among main regions for the rate of MRSA nasal carriage in Makkah, Dahrn and Riyadh cities (Yousef et al., 2013). Therefore, it seems that the difference in the incidence of MRSA reflects host or environmental factors. Additionally, high variation may be due to the epidemiology of MRSA in transition period and infection control rules may be most effective. Comparing the results of this study with other regional countries, it appears that Saudi Arabia has a higher MRSA prevalence rate compared to other neighbor counties (Aly & Balkhy, 2012). These different rates among MRSA from different countries may be attributed to variations in patient populations, the biological characteristics of the *S. aureus* strains, and/or infection control practices (Orrett & Land, 2006)

In this study, the frequency of MRSA carriage varied between different departments. The prevalence was highest in MICU and it was followed by SICU, which was close to the results that were obtained by Altunbas et al. (2013). In a study accomplished in Libya, Zorgani et al. (2009) reported that the incidence of MRSA were 42% and 27% within MICU and SICU, respectively. This is markedly higher than those reported from other countries (Goyal et al., 2002). On the other hand, the least carriage rate in HCWs was reported in CCU and OPD (Akhtar, 2010).

MRSA nasal carrier rate was high (20%) in age group of 20 to 40 years and less (4%) in age groups above 40 years. These finding was contrary to that observed in the study done in Saudi Arabia which showed infection in the 'extremes of age' group (younger than one or older than 60 years) (Madani, 2002). Another study by Iyer et al. (2014) from Jeddah found the double rates of MRSA colonization in age group of 40-50 years, which documented that MRSA infection, occur at a higher incidence in older people owing to a weaker immune system.

MRCoNS nasal carriage was seen in 42% of the HCWs in the present study. Results of this study are in agreement with the findings of Mahesh et al. (2015) those who reported that overall nosocomial infection rates were 42 (41.17%) of the HCWs. In this study, prevalence of MRCoNS rate was lower than other previous reports done in other countries such as France, Germany, Greece, Italy and Turkey (Sader et al., 2007; Mehdinejad et al., 2008). The variations in nasal carriage prevalence of MRCoNS might be due to the variations in geographical distributions of bacterial strains or due to the infection control practices.

There was a remarkable difference in MRCoNS carriage rate in HCWs of MICU (26%), SICU (14%), CCU (2%) and OPD (0%). In accordance with the results of these studies, length of ICU stay was a significant risk factor for ICU-acquired infection both in univariate and multivariate analyses in this study. Each invasive procedure performed poses a risk of infection; it is in agreement with the results of other studies (Ak et al., 2011).

In case of gender study, no significant differences were reported in the rates of nasal carriage in males and females. This finding was contrary to that observed in the study done in Nigerian where females harbored bacteria significantly more often than males (Lamikanra et al., 1985). Further, findings of present study are in agreement in the findings of Majumda et al. (2009).

In this study, none of the screened students (50) were found positive for MRSA or MRCoNS colonization. Similar to results of this study, a study carried out by Iyer et al. (2014) on nasal colonization, suggesting that people who are not exposed to the pathogen have a very low risk of nasal carriage. On the other hand, Mollaghan et al. (2010) reported regarding the globally increased prevalence of MRSA nasal colonization in the community. Risk factors that increase the prevalence of MRSA nasal colonization in Asia may be misuse of antibiotic and lower socioeconomic status.

These findings indicate the need to develop rapid and regular screening programs for transmission of MRSA in HCWs settings. This study clearly showed that rapid identification of MRSA isolates can be done using the PCR specific for the *mecA* gene.

A study conducted by Tiwari et al. (2008) suggested that *coa* PCR was sensitive and specific in confirming the fact that this gene is present in all *S. aureus* isolates. *Alu I* restriction enzymes was used for *coa* gene PCR-RFLP, aiming at a more detailed characterization of MRSA isolates, since it generated one RFLP band pattern. Our results are in accordance with Lawrence et al. (1996) who isolated MRSA strains from various hospitals and showed that the strains were closely related and had a unique RFLP pattern when analyzed by *coa* gene typing. In contrast to the findings of these results, Kobayashi et al. (1995), who found that MRSA was classified into 6 RFLP patterns, with 5 patterns detected frequently in MRSA. Eed et al. (2015) showed that *Alu I* digestion of the *coa* gene PCR products of 58 MRSA strains yielded nine different RFLP patterns. In conclusion, the results of the current study showed that 42% of the HCWs were nasal carriers for MRCoNS and 24% for MRSA. The results of the current study might help in decision making to prevent the spread of infections among workers in the field of health care.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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