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Research Article
Methicillin-resistant *Staphylococcus aureus* Nasal Carriage Among Patients Admitted at Shaqra General Hospital in Saudi Arabia

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Abstract

Background and Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) have been causing increasing problems in hospitals and nursing homes worldwide. Limited number of studies in Saudi Arabia has attempted to investigate infection and risk factors associated with nosocomial acquired MRSA. The present study was undertaken to determine the occurrence, prevalence, antibiotic susceptibility pattern and genetic characteristics of MRSA among admitted cases at Shaqra General Hospital (Saudi Arabia). **Methodology:** This study was conducted from October, 2014 to March, 2015. Nasal swabs were taken from 220 patients (105 males and 115 females) admitted at Shaqra General Hospital. The isolates were identified as *S. aureus* based on morphology, Gram stain, catalase test, coagulase test and mannitol salt agar fermentation. Antibiotic susceptibility testing of MRSA was performed with standard disk diffusion method. All methicillin-resistant isolates were examined for the existence of the *mecA* gene by PCR technique. **Results:** Of the 220 patients, 90 (40.91%) were found to be nasal carriers of *S. aureus*. Among these 90 *S. aureus* isolates, 48 (21.82%) were MRSA. A statistically significant difference was only found for antibiotics usage between those with and without MRSA colonization. Antibiotic susceptibility pattern of isolated MRSA showed high susceptibility to vancomycin, linezolid, rifampicin, teicoplanin, complete resistance to penicillin, ampicillin, oxacillin and cefoxitin and intermediate resistance to amikacin, ciprofloxacin, teicoplanin, tetracycline and vancomycin. **Conclusion:** A high prevalence of multidrug-resistant MRSA nasal carriage was found. The identification of MRSA carriers is a step towards establishing a control policy for MRSA and helps to identify measures needed to reduce colonization pressure.

Key words: Resistance, methicillin, nasal, nosocomial, MRSA

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is cross-transmitted in hospital settings and has a high impact not only on patient morbidity and mortality but also on hospitalization costs. Worldwide, it has been endemic in many healthcare facilities since 1990s[1]. The MRSA remains a major pathogen in nosocomial infections in developing countries[2] and in Latin America, according to SENTRY[3]. The fact that a patient can harbour MRSA at hospital admission has consequences not just for the choice of patient treatment: It also impacts on the effectiveness of infection control in the hospital. The MRSA reservoir at hospital can make other measures of infection control not as effective, thereby causing pathogen transmission to continue[4]. Guidelines aimed at controlling the spread of MRSA therefore propose to systematically search for colonized patients and then to isolate and decolonize them[5]. This policy has not been tested rigorously in methodologically-sound randomized trials and most of the evidence comes from observational and quasi-experimental studies[6]. There is also concern about the cost of such measures and the lack of available rooms for isolation[7].

Many published studies have arrived at different results regarding identification and isolation of MRSA colonized patients. These differences may be attributable to many factors, ranging from differences between the settings and patients to methodological issues and the multifaceted nature of infection control practices. In one study, universal MRSA surveillance reduced the infection risk during hospitalization and 30 days after discharge[8], while another study evaluated MRSA screening at admission in surgical patients in an endemic setting of MRSA and found no decrease in surgical site infections and nosocomial acquisition of MRSA[9]. Yet another study in an Intensive Care Unit (ICU) compared two interventions to kreduce transmission of MRSA, after identification of colonized patients by the pathogen: Cohort-isolation o single room isolation and found no difference in cross-transmission between the two periods[10].

The present study aims to estimate the prevalence of MRSA colonization and infection in patients at the time of admission to hospital, the incidence of colonization and infection during hospitalization and the potential risk factors for both, in Shaqra General Hospital in KSA, in order to obtain information to support infection control planning for MRSA policy in the hospital. As well as to detect nasal carriage of MRSA, its antibiotic susceptibility pattern and genetic characteristics.

MATERIALS AND METHODS

Cases: This study was conducted from October, 2014 to March, 2015. Nasal swabs were taken from 220 patients (105 males and 115 females) admitted at Shaqra General Hospital. Nasal swabs were collected from all patients, within 48 h of admission. Oral consent was obtained from all participants prior to specimen collection. Proposed risk factors for carriage of MRSA were evaluated after a thorough history obtained by the admitting staff. The proposed risk factors included age, gender, hospitalization in the past 12 months, residential care in the preceding 6 months, antibiotic usage in the preceding 6 months and history of underlying diseases, such as hypertension, Ischemic Heart Disease (IHD), Chronic Obstructive Pulmonary Disease (COPD), recent dialysis and Diabetes Mellitus (DM). Statistical analysis was done using the chi-square test for non continuous variables and the student t-test for continuous variables ($p<0.05$ was considered significant).

Samples collection: Initial screening and identification of *S. aureus* were according to standard laboratory protocols. Briefly, sterile cotton swabs were rubbed over the anterior nares of both nostrils of all studied patients, both nostrils were sampled using the same swab. The swabs were sent to the laboratory streaked on mannitol salt agar plates (MSA) (bioM'erieux, France) and incubated at 37°C for 48 h. Mannitol-fermenting colonies (i.e., those that were yellow or gold) were identified as *S. aureus.* The isolates were confirmed as *S. aureus* by Gram stain and positive results for catalase and coagulase tests and isolates of *S. aureus* were tested for methicillin resistance using a standard oxacillin salt agar screening plate procedure and cefoxitin susceptibility as indicated by the International Nosocomial Infection Control Consortium Report: 2002-2007[2].

Antibiotic susceptibility testing: The susceptibility of MRSA isolates to different antibiotics was carried out by the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines[11].

Detection of *mecA* gene by PCR: *Staphylococcus aureus* isolates resistant to oxacillin by the disk diffusion technique were confirmed as MRSA by PCR detection of the *mecA* gene according to Geha *et al.*[12]. These isolates were stored on agar slopes at -4°C for PCR assay.

DNA extraction: The DNA was extracted from overnight broth of the isolates using Omega Bio-Tech, DNA kits (D3395-01/02), following the manufacturer's instructions.

Primers: Oligonucleotide primers used were purchased from The Midland certified reagent company Inc. (Midland, Texas), oligonucleotides sequences for MRSA were *mecA* 1-F (5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and *mecA* 2-R (5'-CCA ATT CCA CAT TGT TTC GGT CTA A- 3'). The expected product of amplification of the target sequence with these primers was 310 bp in length.

PCR amplification: Amplification was carried out in thermocycler (Biometra, Germany) using 50 µL reaction volume containing the following: 0.5 µM of each primers, 200 µM concentrations of each deoxynucleoside triphosphate, 1x reaction buffer (50 mM KCl, 10 mM tris-HCl (pH 8.3), l.5 mM $MgCl_2$ and 0.001 % w/v gelatin), 1.25 units of Taq polymerase (Promega, Madison, USA) and 2 µL of extracted DNA. Cycling conditions were as follow: Initial denaturation at 94°C for 4 min, annealing. At 50°C for 45 sec and extension at 72°C for 1 min for 25 cycles. A final extension step of 2 min at 72°C was added.

Detection of amplified PCR products: Following PCR amplification, 5 µL were taken from PCR products and subjected to conventional agarose gel electrophoresis (l.5% agarose, lx tris-borate-EDTA buffer) at 100 V for 70 min[13]. Gel was stained with ethidium bromide (Sigma) then, visualized under UV illumination and photographed.

RESULTS

During the study period, 220 patients (105 males and 115 females) admitted at different wards of Shaqra General Hospital were screened for *S. aureus* nasal carriage. Of 220 patients, 90 (40.91%) were found to be nasal carriers of *S. aureus*. Of the 90 nares *S. aureus* isolates, 48 (21.82%) were identified as MRSA. Characteristics of the patients and risk factors for MRSA carriage are shown in Table 1. The mean age of participants was 32.4±8.3 years (range 19-74 years). Patients with MRSA colonization were significantly associated with antibiotics usage in the preceding 6 months compared to those who had taken antibiotics (p = 0.001). There were no significant differences regarding the age (p = 0.34), gender (p = 0.46 and 0.23), hospitalization in the preceding 12 months (p = 0.11) and history of underlying diseases between those with and without MRSA colonization. Overall, most colonized patients had at least one of the proposed risk factors, as did the non-colonized individuals.

The isolated strains were identified as MRSA by oxacillin resistance (Disc diffusion method) and confirmed by detection of *mecA* gene by PCR assay.

Antibiotics susceptibility patterns of MRSA isolates is shown in Table 2. All MRSA strains were resistant to penicillin, ampicillin, oxacillin and cefoxitin while susceptiblity to vancomycin, linezolid, rifampicin, teicoplanin were 97.9, 66.67, 58.33 and 54.17%, respectively. The rates of resistance to cotrimoxazole, amikacin, ciprofloxacin, tetracycline, erythromycin, clindamycin were 66.67, 77.08, 66.67, 60.42, 85.42 and 87.50%, respectively. The MRSA isolates has intermediate resistance as follows: To amikacin 2 (4.17%) and 1 (2.08%) to vancomycin, 4 (8.33%) to ciprofloxacin, 6 (12.50%) to teicoplanin and 5 (10.42%) to tetracycline.

DISCUSSION

The MRSA has emerged as the most important nosocomial pathogen worldwide. The MRSA is a notorious organism causing infections mainly in Health Care Institutions[14]. Outbreaks of such infection in hospitals are also accelerated with marked increase in morbidity and mortality specially outbreaks in ICU setting[15]. Nasal carriage of MRSA is an important risk factor for subsequent infection[16]. Most

Table 1: Risk factors for MRSA carriage in the studied population

	No. of patients (%)		
Variables	With MRSA colonization N = 48 (%)	Without MRSA colonization N = 172 (%)	p-value
Age (years) Mean±SD	33.2±9.3	33.2±9.3	0.34
Male	22 (43.0%)	80 (45.9%)	0.46
Female	26 (57.0%)	92 (54.1%)	0.23
Hospitalization in the preceding 12 months	7 (7.5%)	13 (4.0%)	0.11
Residential (non-hospital) care in the preceding 6 months	9 (9.7%)	25 (7.6%)	0.24
Antibiotics in the preceding 6 months	43 (49.2%)	87 (26.6%)	0.13
History of underlying diseases			
Hypertension	26 (28.0%)	78 (23.9%)	0.13
Ischemic heart disease	1 (1.0%)	4 (1.2%)	0.92
Diabetes mellitus	21 (22.6%)	66 (20.2%)	0.86
Recent dialysis	0 (0%)	2 (0.6%)	NA

NA: Non-applicable for statistical analysis due to the small sample size

Table 2: Antibiotics susceptibility patterns of MRSA isolates

	MRSA isolates (N = 46)		
Antibiotics	S	I	R
Penicillin	-	-	48 (100%)
Ampicillin	-	-	48 (100%)
Oxacillin	-	-	48 (100%)
Cefoxitin	-	-	48 (100%)
Cotrimoxazole	16 (33.33%)	-	32 (66.67%)
Amikacin	9 (18.75%)	2 (4.17%)	37 (77.08%)
Ciprofloxacin	12 (25.00%)	4 (8.33%)	32 (66.67%)
Teicoplanin	26 (54.17%)	6 (12.50%)	16 (33.33%)
Linezolid	32 (66.67%)	-	16 (33.33%)
Tetracycline	14 (29.17%)	5 (10.42%)	29 (60.42%)
Erythromycin	7 (14.58%)	-	41 (85.42%)
Clindamycin	6 (12.50%)	-	42 (87.50%)
Rifampicin	28 (58.33%)	-	20 (41.67%)
Vancomycin	47 (97.9%)	1 (2.08%)	-

S: Susceptible, I: Intermediate, R: Resistant, antibiotic disk concentrations per recommendations of Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards)[11]

MRSA-colonized patients are asymptomatic and remain unrecognized so, they serve as a source of dissemination of MRSA within the hospital. Therefore, Center for Disease Control has recommended active surveillance programs to detect MRSA carriers.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is not only resistant to β-lactam antibiotics but also frequently resistant to aminoglycosides, fluoroquinolones and macrolides[17]. Glycopeptides are the antimicrobials of choice for MRSA infection. However, the emergence of isolates with reduced susceptibility to vancomycin was also reported[18]. Rapid identification of MRSA carriers is important for controlling MRSA spread and therefore screening for MRSA is a key strategy for successful infection control[19]. Conventional culture and PCR are recommended method for MRSA .surveillance[20]. Our methodology and results were in line with earlier reports.

We agree that the identification of baseline rates and the associated risk factors of colonization are necessary to estimate the burden of colonization and the demands for isolation facilities[21] and to help adopt a cost-effective strategy of patient screening which would take into consideration the population prevalence of MRSA and the structure of the hospital[22]. Thus, use of own data, obtained from a study that uses local resources can help to propose a policy that fits the institutional needs and lead to satisfactory outcomes regarding MRSA control.

The present study showed that the overall MRSA nasal carriage among admitted patients at Shaqra General Hospital during the period of the study was 21.82%. This finding is comparable to (19%) that reported by Al-Rawahi *et al.*[23]. However, it is less than that reported (49%) by Atkinson *et al.*[24] but much higher than those previously reported (usually no more than 10%)[25-27]. These discrepancies in the rates of the nasal carriage of *S. aureus* trains-may be due in part to the differences in the quality and size of samples and the use of different techniques or may reflect a true higher rate of MRSA among hospital-acquired *S. aureus* infection in our hospital.

Risk factors significantly associated with MRSA colonization in previous studies were old age, male gender, contact with healthcare facility, hospital admission, surgical procedures within the last year, previous use of antibiotics, exposure to a patient colonized or infected with MRSA, nursing home residence and diabetes mellitus[28-31]. In the present study, antibiotics usage in the previous 6 months was identified as the only significant risk factor for MRSA colonization. This finding is consistent with Tacconelli *et al.*[32] who noted that the risk of acquiring MRSA is increased by severity of illness and the use of antimicrobials prior to hospitalization. Moreover, Vovko *et al.*[33] have also identified that current antibiotics therapy, male gender and the presence of pressure sores or wounds as risk factors associated with MRSA carriage. Abuse and misuse of antibiotics by the patients in our locality strongly contribute to this finding. The other studied variables were not identified as risk factors for nasal carriage of MRSA strains. Previous surgery, contact with healthcare facility and exposure to a patient colonized or infected with MRSA have not been included in the suggested variables.

Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries associated with increased medical costs as well as high morbidity and mortality of the patients[34]. Surveillance on the antimicrobial susceptibility patterns of MRSA is of utmost importance in understanding new and emerging resistance trends and in the management of both hospital and community-acquired infections. In the present study, all MRSA isolates recovered from nasal carriers were susceptible to vancomycin, linezolid, rifampicin, teicoplanin, possibly because of its limited use in our hospital. The high resistance rates of MRSA to cotrimoxazole, amikacin, ciprofloxacin, tetracycline, erythromycin, clindamycin were supported by other previous studies that reported a relationship between methicillin-resistance and resistance to other antibiotics[35-37] especially aminoglycoside resistance[38]. The reported intermediate and complete resistance of MRSA strains to to amikacin, ciprofloxacin, teicoplanin and tetracycline may be related to the disk diffusion technique utilized in this study which noted to be unreliable for the determination of susceptibility to these antibiotics, due to its low sensitivity[39].

Rapid detection of MRSA-colonized patients has the potential of improving patient care and positively affecting

hospital infection control practices. There are many screening methods available to detect MRSA based on culture, however, conventional culture methods for MRSA screening are time consuming, usually take about 2-3 days to produce a positive result. The PCR allows earlier identification (usually within one day) of MRSA carriers by detection of *mecA* gene thus, may subsequently reduce MRSA colonization and infection rates[40]. In this study, PCR testing confirmed that all nasal MRSA strains isolated from the participants were *mecA* gene-positive.

CONCLUSION

Although, MRSA infection is a very frequent pathogen in hospitals, there are few data regarding patient colonization. Even though the universal screening is not a resolved issue, knowledge of the magnitude of colonization is invaluable for planning to control the pathogen. In a heterogeneous sample of clinical patients admitted to a Shaqra hospital, a high prevalence of MRSA colonization was found and the results of the study provide the basis for better-targeted MRSA control policies aimed at specific groups of patients. The study also gives an estimate of the resources needed for a particular hospital to implement such interventions.

Staphylococcus aureus nasal colonization as well as methicillin-resistance among these isolates is common in our community. Routine screening of nasal carriage of MRSA of admitted patients at hospitals should be done as part of the hospital's infection control program. The general principles of infection control including patient isolation and appropriate cleaning and decontamination of clinical areas should be adopted for patients with MRSA. The inappropriate or unnecessary use of antibiotics should be avoided and this will also reduce the likelihood of the emergence and spread of strains with reduced susceptibility to glycopeptides.

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