

Full Length Research Paper

Prevalence and distribution of methicillin resistant *Staphylococcus aureus* (MRSA) among laboratory science students and laboratory staff from a single hospital in North Saudi Arabia

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***Staphylococcus aureus* causes morbidity and mortality in both community and hospital settings. Methicillin resistant *S. aureus* (MRSA) is being increasingly reported globally. This study aimed at finding out the prevalence and the distribution of the community-associated MRSA (CA-MRSA) or healthcare-associated MRSA (HA-MRSA) among 150 subjects. Of the 150 subjects, 125 were students of laboratory science and 25 were laboratory staff. Nasal swabs were collected aseptically and cultured using standard microbiological protocols. Antibiotic susceptibility was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Methicillin resistance was detected by resistance to oxacillin and ceftoxitin and confirmed by detecting *mecA* gene. Strain typing of MRSA strains was done by pulse field gel electrophoresis (PFGE). The distribution of CA-MRSA from all MRSA isolates were, 100% (2/2) from the first year laboratory science students, 100% (2/2) from the second year laboratory science students, 100% (2/2) from the third year laboratory science students, 67% (2/3) from the fourth year laboratory science students, 43% (3/7) from fifth year students and 40% (4/10) from the hospital staff, respectively. The PFGE results showed that out of total 26 MRSA isolates, there were two major groups; 15 were found to be of one group, consisting of all CA-MRSA with SCC_{mec} type IV; and 11 isolates were of second group, HA-MRSA with SSC_{mec} type III and IIIA on the other group. Additionally, 100% (15/15) and 20% (3/15) CA-MRSA isolates were found positive for Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin-1 (TSST-1), respectively. Furthermore, the CA-MRSA isolates showed a higher susceptibility pattern to non- β -lactam antibiotics as compared to HA-MRSA. Our study reports a high percentage of CA-MRSA isolates among the healthcare workers who have lesser or no exposure to the hospital environment as compared to those with high exposure. Also, the genetic relatedness, presence of Panton-Valentine leukocidin (PVL) and identical antibiogram of CA-MRSA makes this study interesting, as carriage of these isolates in the laboratory students of hospital setup may play a key role in the epidemiology and pathogenesis of infection in the hospitals in future.**

Key words: Methicillin resistant *Staphylococcus aureus* (MRSA), healthcare workers, antibiotic susceptibility, molecular typing.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a potential harmful pathogen associated with several

infections like, bacteremia, infective endocarditis, sepsis, toxic shock syndrome, and skin and soft tissue infections

(van Hal et al., 2012). Infections caused by MRSA lead to excess morbidity and mortality among hospitalized patients, exhibiting a serious healthcare issue worldwide (Ippolito et al., 2010). The first MRSA strain was isolated in hospitals in the United Kingdom in 1961 and thereafter, reported worldwide rapidly (Abujheisha, 2013). The resistance to methicillin among MRSA was reported to be due to *mecA* gene which encodes a penicillin-binding protein that has got a reduced affinity towards methicillin. As a result of production of this unique penicillin-binding protein, methicillin cannot bind to the bacterial cell efficiently, which in turn results in reduced capacity of methicillin to inhibit bacterial cell-wall synthesis. The *mecA* gene was shown to be present on a mobile genetic element called staphylococcal chromosome cassette *mec* (SCC*mec*) (Borbón-Esquer et al., 2014). Currently, MRSA is a cause of concern for the healthcare society globally because this strain has acquired resistance to several classes of antimicrobial agents, therefore, it commonly exhibits the multidrug resistance (MDR) phenotype, thus poses a continuous threat for failure of antimicrobial therapy (Cadilla et al., 2011). Initially, MRSA infections were reported in the community among individuals who had had recent exposure to health-care settings or had been in close contact with MRSA-infected individuals (Lowy et al., 1998), and therefore, MRSA was considered to be primarily a health-care-associated threat until the late 1990s. During mid-90's, a sudden change in the MRSA target population occurred, and healthy individuals in the community developed MRSA infections rapidly and these infections were called CA-MRSA (DeLeo et al., 2010; Otto, 2010). The first case of CA-MRSA was reported in 1993 from Australia (David and Daum, 2010) and shortly thereafter, CA-MRSA cases were reported worldwide (Chatterjee and Otto, 2013).

The CA-MRSA strains have been distinguished from their HA-MRSA counterparts by different molecular methods. HA-MRSA strains carry a relatively large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to types I, II, or III. All these cassettes contain the *mecA* gene signature, which is nearly universal among MRSA isolates. HA-MRSA strains are often resistant to many classes of non- β -lactam antimicrobials. In contrast, CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V (Miller et al., 2008), are often susceptible to many classes of non- β -lactam antimicrobials and carry the genes for the virulence like Panton-Valentine leukocidin (PVL) (David and Daum, 2010). Although, the distribution of CA-MRSA among hospital staff has been studied in detail globally, however, literature review in Saudi Arabia showed a lack of data. Therefore, the aim of this study was to study the distribution of CA-MRSA and HA-MRSA

among laboratory science students and laboratory staff of a single hospital in northern region of Saudi Arabia.

MATERIALS AND METHODS

Study design and swab collection

In this study, a total of 150 subjects (125 students of laboratory science and 25 laboratory staff) of a single hospital were screened for the presence of *S. aureus*. A single non repetitive nasal swab was collected from each individual for screening. The students of first and second year were in the preparatory year and did not visit the hospital; the students of third year had just started the hospital training. Further, the fourth year students had completed one year of hospital laboratory training; whereas the fifth year students had completed two years of hospital laboratory training.

Bacterial identification

The bacterial strains were phenotypically characterized by Gram stain, catalase test, determination of tube coagulase activity and an agglutination test with a Slidex Staph Plus kit (Biomérieux). Reference strains of MRSA (NCTC 10442); methicillin sensitive *S. aureus* (MSSA) (ATCC 25923); and coagulase-negative staphylococci (CoNS) (ATCC 12228) were the control strains used. An isolated colony from each Columbia blood agar (Oxoid, UK) plate was picked, streaked onto two new Columbia blood agar plates, and incubated at 37°C for 24 h. All inocula were prepared from these subcultures. Further, the confirmation of identification of different types of *S. aureus* was carried out by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry MALDI-TOF MS (Bruker Daltonics, Germany) by direct method (Anderson et al., 2012).

Antimicrobial susceptibility testing

Five colonies were transferred into a tube containing sterile saline to prepare a suspension equivalent in density to that of a 0.5 McFarland standard. The detection of resistance was performed as per the Clinical and Laboratory Standards Institute (CLSI) 2011 guidelines, using oxacillin (1 μ g) and cefoxitin (30 μ g) (Oxoid, Basingstoke, UK). Further, the antibiotic susceptibility was performed by Microscan (Siemens Healthcare Diagnostics, Sacramento, CA, USA).

Molecular biology study

The confirmation of methicillin resistance among the selected bacterial isolates was confirmed by detection of *mecA* gene using polymerase chain reaction (PCR). In this method, a triplex PCR looking for *mecA* (a gene specific for methicillin resistance), *nuc* (a gene specific to detect *S. aureus*) and 16S rRNA (a genus-specific for *Staphylococcus* spp.) were used. The genes *mecA*; *nuc*; and 16S rRNA were detected by the triplex PCR using methods previously described (AlKhulaifi et al., 2014). The control strains used for the three genes are NCTC 10442; ATCC 25923; and ATCC 12228, respectively.

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Table 1. The prevalence of type of *Staphylococcus aureus* among the laboratory science students and laboratory staff.

Subjects	Number of individuals	<i>S. aureus</i> (percent)	MRSA from total <i>S. aureus</i> (percent)	CA-MRSA from total MRSA (percent)	HA-MRSA from total MRSA (percent)
1 st year	25	2 (8)	2 (100)	2 (100)	0 (0)
2 nd year	25	3 (12)	2 (67)	2 (100)	0 (0)
3 rd year	25	3 (12)	2 (67)	2 (100)	0 (0)
4 th year	25	6 (24)	3 (50)	2 (67)	1 (33)
5 th year	25	13 (52)	7 (54)	3 (43)	4 (57)
Staff	25	16 (64)	10 (63)	4 (40)	6 (60)
Total	150	43 (29)	26 (60)	15 (58)	11 (42)

MRSA, methicillin resistant *Staphylococcus aureus*; CA-MRSA, community-associated MRSA; HA-MRSA, healthcare-associated MRSA.

SCC*mec* typing

In this method, a multiplex PCR was used to detect the structural variations in the *mecA* element (Oliveira and de Lencastre, 2002).

Detection of Panton-Valentine leukocidin (PVL) and Toxic Shock Syndrome Toxin 1 (TSST1)

PVL and TSST1 toxins were detected by using PCR methods previously described in the literature (Becker et al., 1998; Sharma et al., 2000). The control strains used for the two genes were NCTC 13300 and NCTC 11693, respectively.

Pulsed field gel electrophoresis (PFGE)

PFGE was performed for 26 total MRSA isolates obtained in the study. It was performed according to Kaufmann method (Murchan et al., 2003), using *Sma*I-digested fragments of bacterial chromosomal DNA, with fragment separation achieved in 0.8% agarose. Electrophoresis conditions comprised using a constant voltage of 6 V/cm at 14°C and pulse times of 3.5-25 s increased linearly over 12 h (block 1), followed by 1-5 s increase over 8 h. Gel patterns were analyzed using BioNumerics software (Applied Maths) with the band tolerance set at 1.0%.

RESULTS

The results of the prevalence of *S. aureus* in this study are shown in the Table 1. Of the total nasal swabs collected, *S. aureus* were isolated from 52% laboratory science students and 64% from laboratory staff. The CA-MRSA was 100% among 1st, 2nd, and 3rd year laboratory science students and 40% among laboratory staff. The HA-MRSA were higher among laboratory staff (60%) and 4th and 5th year laboratory science students (57 and 60%, respectively).

Antimicrobial susceptibility

The results of antimicrobial susceptibility (Figure 1) showed that among CA-MRSA, 6.6% (1/15); 26 percent (4/15); 20% (3/15) and 60% (9/15) were found to be resistant to augmentin (amoxicillin and clavulanate); gentamicin; amikacin and tetracycline, respectively.

Additionally, 100% (15/15) CA-MRSA were found to be susceptible to ciprofloxacin. Among the HA-MRSA, 100% (11/11) isolates were found to be resistant to all the six β -lactam antibiotics and tetracycline. Furthermore, 9% (1/11); 9% (1/11) and 45% (5/11) of HA-MRSA were found to be resistant to gentamicin; amikacin and ciprofloxacin, respectively.

Molecular biology study

The triplex PCR looking for *mecA*, *nuc* and 16S rRNA correlated very well with the phenotypic tests carried out. Overall, 100 (15/15) and 20% (3/15) CA-MRSA isolates were found to be positive for PVL and TSST-1, respectively. The results of PVL and TSST-1 toxins detected using PCR methods are shown in Figure 1.

PFGE and SCC*mec* typing

The results of PFGE (Figure 1) showed that at 60% cut off there were two major groups. Out of 26 isolates, 15 were found to be one group consisting of all CA-MRSA with SCC*mec* type IV. The other group, 11 isolates were HA-MRSA with SCC*mec* type III and IIIA.

DISCUSSION

In hospitalized patients, MRSA has been a problem since the 1960s (Macal et al., 2014). *S. aureus* is a permanent colonizer in the anterior nares of about 20 to 30% of the general population. In comparison with general population, hospital workers are more likely to be colonized, most likely because of increased exposure (Iyer et al., 2014).

In a review, a total of 26 studies on MRSA prevalence in different regions of Kingdom of Saudi Arabia (KSA) were analyzed since 2002 to 2012. The MRSA prevalence in patients of King Fahad Medical City in 2011, in Riyadh was 50.4%, within a similar order of magnitude to other hospitals in Saudi Arabia. In a hospital in the Western region of Saudi Arabia, the MRSA prevalence was 38.9%. The prevalence of CA-MRSA in a

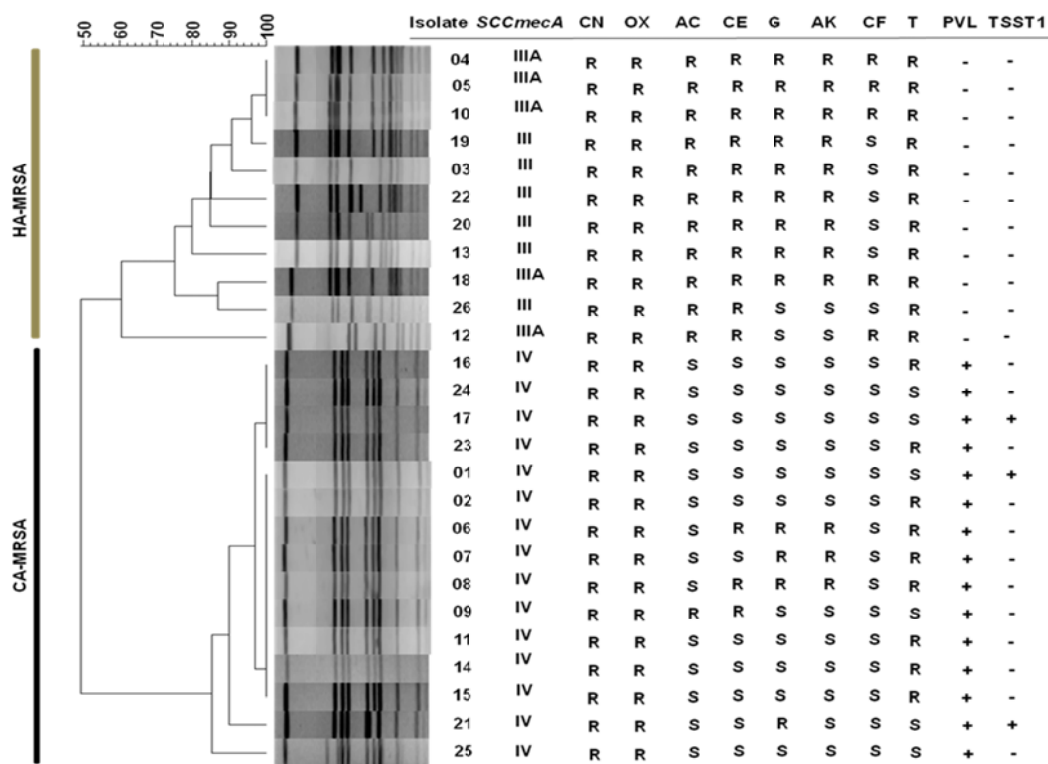


Figure 1. PFGE patterns obtained from 26 methicillin resistant *S. aureus* (MRSA) isolates cultured from students of laboratory science and laboratory staff of a hospital in north Saudi Arabia. Cluster analysis was performed using the method of DICE with UPGMA with band tolerances set to 1.0%. *SCCmecA*, *Staphylococcal* chromosomal cassette *mec* PCR; CN, ceftazidime; OX, oxacillin; AC, amoxicillin-clavulanate; CE, cefotaxime; G, gentamicin; AK, amikacin; CF, ciprofloxacin; T, tetracycline; PVL, Pantone-Valentine Leukocidin PCR; TSST1, Toxic shock syndrome 1 PCR; R, resistant; S, susceptible; HA-MRSA, healthcare-associated MRSA; CA-MRSA, community-associated MRSA.

hospital in the Eastern region of KSA increased by six-fold during a 5-year period, between 2000 and 2008 (Monecke et al., 2012). The overall estimation of MRSA prevalence in Saudi was 35.6%, whereas MRSA prevalence mean was different between regions. While, variation in MRSA proportion exists in several cities (5.97 to 94%).

In regional perspective, Saudi has a higher prevalence of MRSA than Bahrain, Kuwait and Lebanon countries. In comparison, MRSA prevalence in Egypt, Oman, Iran and Jordan was reported to be more than 50%. Considering the worldwide scenario, the mean incidence of MRSA across China was over 50%; in Shanghai over 80% and in Spain, the prevalence of MRSA was 29.2% (Yousef et al., 2013).

In a recent report, 76% of the healthcare workers were tested positive for nasal carriage of MRSA, though they were asymptomatic. This indicates a very high incidence of MRSA (Iyer et al., 2014). Both CA-MRSA and HA-MRSA are resistant to methicillin (and all β -lactam antibiotics), however main differences exist in epidemiology, microbiologic characteristics, clinical aspects of infection, and management strategies

between the two (Bukharie, 2010). Over the past decade, relatively a higher number of studies of the emergence of CA-MRSA have been published worldwide. It becomes imperative to study the distribution of MRSA among the healthcare workers, because these workers are part of community and are exposed to the hospital environment regularly (Iyer et al., 2014).

PVL-positive, community associated strains have been reported in Kuwait, Abu Dhabi, Lebanon, Egypt, Tunisia, Algeria as well as in people travelling from and to various Middle Eastern countries (Monecke et al., 2012). In our study, the percentage of CA-MRSA was found to be higher among the students (first, second and third year) with low or no exposure to hospital settings and the HA-MRSA was found to be higher among the healthcare workers (fourth year, fifth year students and hospital staff) with high exposure to hospital settings. The results of typing revealed two major lineages with one lineage (isolates 16 - 25) were being associated with PVL positivity and the carriage of *SCCmecA* Type IV (CA-MRSA). In general, this lineage is also less genetically diverse than members of lineage 2 (isolates 04 - 12). The CA-MRSA (lineage 1) isolates show a higher susceptibility

pattern to non- β -lactam antibiotics and HA-MRSA (lineage 2) isolates showed higher resistance pattern than lineage 1 isolates, which is in accordance with previously published results (Portillo et al., 2013).

As the epidemiology of MRSA disease changes, including both community- and health care-associated disease, accurate information on the scope and magnitude of the burden of MRSA disease in the Saudi population is needed to be studied in detail for infection prevention and control. As per our knowledge, this is the first study of the incidence and distribution of CA-MRSA conducted among the laboratory science students and laboratory staff of a hospital in North Saudi Arabia. Our study reports a high percentage of CA-MRSA isolates among the healthcare workers who have lesser exposure to the hospital environment as compared to those with high exposure. Also, the genetic relatedness, presence of PVL and identical antibiogram of our CA-MRSA makes this study interesting as carriage of these isolates in the healthcare workers may play a key role in the epidemiology and pathogenesis of infection in the hospitals in future. Strategies to interrupt transmission of CA-MRSA to hospitalized patients via healthcare workers like regular hand washing and use of antiseptics should be implemented. Continuing surveillance is needed more accurately to assess the prevalence, geographic distribution and epidemiology of community acquired MRSA at broader level.

Conflict of interests

The authors have not declared any conflict of interest.

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