Molecular characterization of staphylococcal cassette chromosome *mec* and virulence encoding genes in methicillin-resistant staphylococci at a medical center in Lebanon

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Abstract

Background: Methicillin-resistant staphylococci (MRS) are major human pathogens accounting for most hospital-acquired (HA) and community acquired (CA) infections worldwide. The recent increase inMRS in a medical center in Lebanon elicited the determination of SCC*mec* types, genotypes, and prevalence of Panton-Valentine leucociden (PVL) and toxic shock syndrome toxin-1 (TSST-1) among the MRS isolates.

Methods: Thirty-six MRS isolates collected between October 2010 and September 2011 at a medical center, Lebanon were typed using phenotypic and genotypic methods. Antimicrobial susceptibility was determined using the disk diffusion agar method. SCC*mec* typing was performed by multiplex PCR and sequence analysis. The prevalence of the genes encoding PVL and TSST-1 virulence factors and their transcription levels, were determined respectively by PCR and semi-quantitative real-time PCR. The genomic relatedness of the isolates was assessed by random amplified polymorphic DNA (RAPD) analysis.

Results: Antimicrobial susceptibility revealed three distinct antibiotypes. The predominant SCC*mec* type found among the MRS isolates was type IVa (51%). Twenty-nine percent harbored SCC*mec* type III and 14% harbored SCC*mec* type II. One isolate harbored SCC*mec* type IVc, and another harbored SCC*mec* type I. All methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were negative for the gene encoding for PVL, and two were positive for the gene encoding for TSST-1. RAPD analysis demonstrated high genomic diversity among the MRS isolates.

Conclusion: This study demonstrated the SCC*mec* types and the clonality of the MRS strains, allowing the differentiation between HA and CA-MRS strains. CA-MRS have increased in the hospital environment and rendered highly resistant to erythromycin and clindamycin.

Introduction

Staphylococcus aureus is considered a leading cause of serious and sometimes life-threatening infections. *S. aureus* is frequently found on the skin, nasal and other mucous membranes of humans, and in various food products. In England in 1961, the first methicillin-resistant *S. aureus* (MRSA) was reported, only one year following the introduction of methicillin. Since then, MRSA has been recognized as a major cause of health-care associated infections. Recently, MRSA has emerged as an important community pathogen, and this was followed by the recognition of methicillin-resistant coagulase negative staphylococci (MRCNS) as important nosocomial pathogens [1,2]

A study by Tokajian S, 2014, showed that the local epidemiology within the Middle East (western Asia) region is fluctuating, owing to the introduction of new strains with the global exchange of several clones [3].Another study performed by Shehabi *et al.* demonstrated the presence of 1-3 clinically important staphylococcal toxin genes more frequently colonizing Jordanian outtpatient infants[4]. Furthermore, another study by Nazeer A *et al.*, 2012 retrieved 73 studies through PubMed using the search terms "methicillin-resistant *S. aureus*" (MRSA), and Saudi Arabia. They reported MRSA incidence or prevalence in patients and/or percentage among *S. aureus* isolates and evaluated these metrics against the closest matching SHEA (Society for Healthcare Epidemiology of America) /HICPAC (Healthcare Infection Control Practices Advisory Committee metrics) [5]

Methicillin resistance occurs in *Staphylococcus* species due to the expression of an altered penicillin-binding protein termed PBP2a or PBP2', that is encoded by the mecA gene [2] The mecA gene is located on a mobile staphylococcal cassette chromosome *mec* (SCC*mec*) element. This element exists in MRSA; as well as, MRCNS isolates. To date, at least five SCC*mec* (I-V) types have been described with sizes ranging between 21 to 67 kb. The SCC*mec* types (I-V) have aided in the differentiation between hospital-acquired (HA) and community-acquired (CA) MRS strains. The larger Scc*mec* types (I-III) have been associated with HA infections; whereas, the smaller SCC*mec* types (IV and V) have been associated with CA infections[6-9].

SCC*mec* includes the *mec* gene complex which is comprised of the *mecA* gene and its regulators and the *ccr* gene complex that encodes site-specific recombinases. These recombinases are responsible for the mobility of the SCC*mec*[10].

Strains of *S. aureus* isolated from human sources can produce a variety of virulence factors. Panton-

Valentine leucociden (PVL) is a pore-forming cyto-toxin that is associated with severe skin and soft tissue infections and necrotizing pneumonia. PVL with SCCmec type IV is considered as a marker for CA-MRSA infections; however, exceptions exist[11]. Another main virulence factor includes the toxic shock syndrome toxin-1 (TSST-1), which is a superantigen having a size of 29.1 kDa. TSST-1 is encoded by the *tst* gene. TSST-1 causes toxic shock syndrome (TSS), a severe and potentially fatal manifestation when it enters the bloodstream[12].

Infections due to hospital-acquired methicillin resistant staphylococci (HA-MRS) must be controlled otherwise they may become permanently present in a hospital environment. These endemic MRS are very challenging to overcome. In most cases, they increase the number of infections, lengthen the duration of hospital stay, and raise the amount of health-care costs. Since MRS strains are generally resistant to a broader range of antimicrobial agents, the chances of treatment become limited and the spectrum of antibiotic prescriptions changes. Therefore, the adequate identification of such resistant bacteria is a crucial and necessary obligation for the selection of the proper antimicrobial treatment as well as for the implementation of appropriate infection control measures [13].

In Lebanon and the Middle East there are numerous molecular characterization and epidemiological studies on MRSA especially during the last decade, highlighting their increased prevalence in the region [4,14-19]. However, there is scarce data available concerning the antibiotic susceptibility profiles, the SCC*mec* types and the genotypes of MRS strains that could be circulating in Lebanon.

Accordingly, the objective of this study was to perform phenotypic and genotypic characterization of MRS isolates from clinical specimens at a medical center in Lebanon.

Materials and methods

Bacterial isolates, identification and confirmation of methicillin-resistance by phenotypic methods

Thirty-six non-duplicated methicillin resistant staphylococci were isolated from clinical specimens (blood, pus, urine, bone, catheter, soft tissue, wound swabs, respiratory samples, and exudates) of in and out-patients at a medical center in Lebanon from October 2010 to September 2011. These isolates were identified as staphylococci based on their Gram stain, colonial morphology, as well as their catalase properties. Rapid agglutination and coagulase tests were done to distinguish between *S. aureus* and coagulase negative staphylococci (CNS). Methicillin resistance was confirmed using an oxacillin disk on Mueller-Hinton agar (Oxoid, England) according to Clinical and Laboratory Standards Institute (CLSI)guidelines[20].

Antimicrobial susceptibility testing of staphylococcal isolates

Antimicrobial susceptibility testing was performed by disk diffusion agar method on Mueller-Hinton agar against a panel of antimicrobial agents disks (Oxoid, England) according to CLSI guidelines[20].

DNA extraction

DNA was extracted from the staphylococcal isolates using illustra bacteria genomic Prep Mini Spin kit (GE Healthcare, UK) in accordance with the manufacturer's protocol for purification of genomic DNA from Gram-positive bacteria.

Molecular characterization by multiplex PCR amplification and DNA sequencing

Three multiplex PCR assays carried out using the PCR Sprint Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) were used for characterization of the methicillin-resistant staphylococcal isolates.

Polymerase Chain Reaction

SCCmec-typing Multiplex-PCR 1for typing and sub-typing of the SCCmec element with the simultaneous detection of the mecA gene

The PCR mixture contained nine pairs of primers, specific for typing and sub-typing the SCC*mec* element; as well as, an internal control primer for confirming the presence of the *mec*A gene, were used in this multiplex polymerase chain reaction (M-PCR) assay. The various types and sub-types detected by the M-PCR included: I, II, III, IVa, IVb, IVc, IVd, and V. M-PCR was performed in a 25 µl final reaction mixture with 5 µl of the prepared DNA template having a final concentration 10 µg/ml, 2.5 µl of 10x Taq DNA polymerase buffer with KCl (Fermentas, USA), 2.5 µl of $MgCl_2$ (25mM) (Fermentas, USA), 0.2 µl of Taq DNA polymerase (5U/µl) (Fermentas, USA), 1 µl of 5 mM dNTPs (Fermentas, USA), 13.4 µl of nuclease free distilled water (Amresco, USA) and 0.2 µl of each primer (Thermo Scientific Inc., USA)[13].

Mec gene complexcharacterization M-PCR 2

Two pairs of primers were used in this M-PCR assay to characterize the *mec* gene complex as either class A or B. The M-PCR was performed as described in **2.5.1** with thefollowing modification: 13.64 µl of nuclease free distilled water (Amresco, USA) and 0.08 µl of each primer, (Thermo Scientific Inc., USA)[13].

Ccr gene complex characterization M-PCR 3

Six primers were used in this M-PCR assay to characterize the *ccr* gene complex into four allotypes (types 1, 2, 3, and 5). The M-PCR was performed as described in **2.5.2**

Amplification was performed using the same cycling conditions for all three sets of M-PCR reactions with denaturation at 94°C for 5 min; 35cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 1.5 min; and extension at 72°C for 10 min.

MRSA-PVL PCR.

Was performedto detect the PVL toxin gene. The PCR was performed as described in Amplification was performed with denaturation at 94°C for 5 min; 35 cycles of 94°C for 10 s, 52°C for 20 s, and 72°C for 40 s; and extension at 72°C for 5 min.

Gel electrophoresis

The amplicons from M-PCR1, 2, and 3 were separated on 2% agarose gels using a suitable DNA ladder; either, a 50 bp DNA ladder (Fermentas, USA) or a 123 DNA ladder (Sigma-Aldrich, USA). Gels were visualized under a UV transilluminator(Haakebuchler Instruments Inc, USA) and captured using Olympus digital camera, and the DigiDoc-ItProgram (UVP, UK). The amplicons were purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, California) and sequenced by the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA,USA).

RNA extraction& reverse transcription

RNA extraction was performed using the illustra RNAspin Mini RNA Isolation kit (GE healthcare, UK) for all MRSA isolates (n=13). Subsequently,reverse transcription was performed using the QuantiTect® Reverse Transcription Kit (QIAGEN, Germany) to synthesize cDNA from the extracted RNA.

Real Time Semi-Quantitative qRT- PCR

qRT- PCRwas carried out using the Bio-Rad CFX96 Real Time System C1000 Thermal Cycler (Germany) to determine the transcription levels of the gene encoding TSST-1.The qRT-PCR was performed in a 20 µl final reaction mixture with 10 µl of QuatiFast SYBR Green PCR master mix, 4 µl of RNase free H_2O, 2 µl of forward primer having a concentration of 5µM, 2 µl of reverse primer having a concentration of 5µM, and 2 µl of synthesized cDNA. The

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following cycling conditions were used: denaturation at 94°C for 5 min; 40 cycles of 94°C for 10 s, 56°C for 20 s, and 72°C for 40 s; and extension at 72°C for 5 min. Data analysis was performed using the Bio-Rad CFX Manager software (BioRad, USA).

Random Amplified Polymorphic DNA analysis (RAPD)

RAPD analysis was performed in order to detect the genomic polymorphisms of the thirty-six MRSA isolates. RAPD was done using the Ready-To-Go RAPD Analysis Beads kit (GE Healthcare, UK Limited) as specified by the manufacturer, using the PCR Sprint Thermal Cycler (Thermo Fisher Scientific, Waltham MA, USA).

Results

Bacterial isolates

Out of thethirty-six non-duplicated methicillin resistant staphylococci (MRS) collected from clinical specimens, thirteen were *S. aureus* and twenty-three were coagulase negative staphylococci (CNS). Of these MRS isolates 27.8% were recovered from outpatients. Twenty-five percent came from patient suites, 14% from the ICU, 11% from the NICU, 11 % from the operating theater, 5.6% from the children's wing, and 5.6% from the recovery room. Sources of the MRS isolates were pus/tissue (n = 6), wound swabs (n = 9), blood (n = 11), respiratory tract (n = 4), urine (n = 2), and miscellaneous specimens (n = 4). The majority of the MRS isolates were isolated from males (56%) and in the age group ≥ 40 years (67%) (**Tables: 1a and 1b**).

Antimicrobial susceptibility

The disk-diffusion agar technique identified all 36 staphylococcal isolates as methicillin resistant. All 36 (100%) bacterial isolates were resistant to oxacillin, ampicillin, penicillin, augmentin, and cefalotin. However, all (100%) were sensitive to vancomycin and teicoplanin. The percentage of methicillin-resistant coagulase-negative staphylococci (MRCNS)

Table 1a. Clinical data and sources of MRSA isolates

Sample #	Clinical Ward	Date of Isolation	Source	Nature of Specimen	Sex/Age of Patient (years)
2	Outpatient	20/9/10	Abdomen	Wound Swab	F/16
3	Patient suites	28/9/10	Venous	Blood	M/72
4	Outpatient	7/10/10	NA	Urine	F/56
5	Outpatient	16/10/10	Venous	Blood	NA
6	Patient suites	20/1/11	Placenta	Tissue	F/62
7	Patient suites	20/2/11	Respiratory Tract	Sputum	M/45
10	Patient suites	6/3/11	Throat	Swab	M/22
20	Outpatient	6/6/11	Arm	Wound Swab	F/59
23	Operating Theater	21/6/11	NA	Bone	M/44
26	Patient suites	30/6/11	NA	Wound Swab	M/46
27	Outpatient	27/7/11	Leg	Wound Swab	F/28
28	Outpatient	29/7/11	Back	Wound Swab	M/73
29	Recover Room	8/8/11	NA	Wound Swab	F/2 days

*MRSA: methicillin resistant *S. aureus,* NA: not available, M: male, F: female

Table 1b. Clinical data and sources of MRCNS isolates

Sample #	Clinical Ward	Date of Isolation	Source	Nature of Specimen	Sex/Age of Patient (years)
1	Outpatient	10/9/10	NA	Urine	M/97
8	Children's Wing	17/2/11	Venous	Blood	M/3 months
9	Outpatient	28/2/11	Leg	Wound Swab	M/50
11	ICU	9/3/11	Venous	Blood	M/82
12	ICU	15/3/11	Venous	Blood	F/81
13	ICU	22/3/11	Respiratory Tract	Sputum	M/57
14	Outpatient	22/3/11	Skin Ulcer	Blood	F/53
15	Patient suites	10/4/11	Venous	Pus	M/77
16	Patient suites	11/4/11	Venous	Blood	F/38
17	NICU	13/4/11	Lungs	Blood	M/1 day
18	NICU	2/6/11	Venous	DTA	M/0
19	Operating Theater	3/6/11	Left hip	Blood	F/60
21	Outpatient	9/6/11	Neck	Tissue	F/55
22	NICU	13/6/11	Left Eye	Wound Swab	M/2 days
24	Patient suites	23/6/11	NA	Exudates	M/74
25	Patient suites	27/6/11	NA	Tissue	M/76
30	NICU	4/9/11	NA	Catheter	M/1 day
31	Operating Theater	8/9/11	NA	Tissue	F/42
32	Recovery Room	6/9/11	Left Thigh	Wound Swab	F/79
33	Operating Theater	8/9/11	NA	Catheter	F/42
34	ICU	10/9/11	Venous	Blood	M/80
35	ICU	14/9/11	Venous	Blood	F/69
36	Children's Wing	17/9/11	Venous	Blood	M/1 month

* MRCNS: methicillin resistant coagulase-negative staphylococci, NA: not available, M: male, F: female, ICU: intensive care unit, NICU: neonatal intensive care unit, DTA: deep tracheal aspirate

which were resistant to clindamycin and erythromycin were 65% and 91% respectively; whereas, the percentage of methicillin-resistant *S. aureus* (MRSA) which were resistant to clindamycin and erythromycin were 31% and 38% respectively.

SCCmec typing

The SCC*mec* typing M-PCR1 assay included specific primers for the various SCC*mec* types, as well as an internal control primer for the detection of the *mec*A gene. This single M-PCR assay determined SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V with the simultaneous discrimination between MRS and methicillin sensitive staphylococci (MSS) isolates (**Figure 1**).

M-PCR1 showed that 35/36 (97.2%) of the MRS isolates were positive for the *mec*A gene confirming methicillin resistance. Isolate 1 was negative for
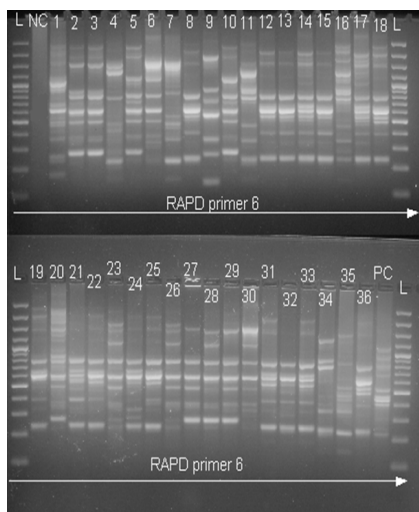
Figure 1. Gel electrophoresis of the M-PCR assay for the characterization of the SCC*mec*types in MRS isolates. Lane 1 of the upper and lower gels represents the molecular weight marker (L: 50 bp ladder); lane 2 of the upper and lower gels represents the negative control (NC)

the *mec*A gene; however, it was phenotypically regarded as a methicillin resistant strain by the disk-diffusion agar method.

SCC*mec* typing also allowed for further typing and sub-typing of the MRS isolates, revealing the following: eighteen MRS isolates (51%) contained SCC*mec* type IVa (776 bp), ten MRS isolates (29%) contained SCC*mec* type III (280 bp), five MRS isolates (14%) contained SCC*mec* type II (398 bp), one MRS isolate (3%) contained SCC*mec* type IVc (200 bp), and one MRS isolate (3%) contained SCC*mec* type I (613 bp). No SCC*mec* type V isolates was detected.

MRSA-PVL PCR

A total of 13 cultured MRSA isolates were subjected to MRSA-PVL PCR and none were PVL gene positive.

Real Time semi-quantitative qRT- PCR

Assay for detecting the gene encoding for the TSST-1 virulence factor showed that 2/13 MRSA isolates (isolates 2 and 3) were positive for the *tst* gene.

Random amplified polymorphic DNA analysis (RAPD)

In order to determine the genetic relatedness of these MRS strains, genotyping was done using Random Amplified Polymorphic DNA (RAPD) analysis. RAPD revealed 8 distinct clusters. These types were designated type A, B, C, D, E, F, G, and H; each with a prevalence of 8%, 17%, 25%, 14%, 19%, 3%, 11%, and 3% respectively.

The predominant RAPD type C included 9 methicillin resistant coagulase-negative staphylococci (MRCNS) isolates, having 58.5% genomic similarity; five of them were CA and four were HA strains. All of these isolates were resistant to erythromycin, and all except three were resistant to clindamycin. Moreover, all were sensitive to the glycopeptides class of antimicrobial agents (vancomycin and teicoplanin). Isolates 12, 13 and 15 of RAPD type C were genetically identical, all three containing SCC*mec* type IVa, indicating that they are community acquired strains. Isolates 12 and 13 were derived from the ICU and isolate 15 from patients' suites.

The RAPD type E included 7 MRS isolates (6 MRSA and 1MRCNS), having 65.1% genomic similarity; four were HA and three were CA strains. These isolates were all susceptible to the glycopeptides. All but three were sensitive to erythromycin, and only two strains were resistant to clindamycin.

The RAPD type B included 6 MRCNS isolates, having 55% genomic similarity; three were HA and three were CA strains. The vast majority of these isolates were resistant to clindamycin and erythromycin except for one isolate which was sensitive to both. All were susceptible to the glycopeptides. Isolates 21 and 22 of RAPD type B were genetically identical. Both were hospital acquired strains having SCC*mec* type II. Isolate 21 was isolated from an outpatient while isolate 22 was isolated from the NICU.

The RAPD type D included 5 methicillin resistant *S. aureus* (MRSA) isolates, having 55.3% genomic similarity; four were CA and one was a HA strain. These isolates were all sensitive to clindamycin,

erythromycin, and the glycopeptides. Isolates 2 and 3 of RAPD type D were genetically identical containing SCC*mec* type IVa; and hence, were CA strains. Isolate 2 was isolated from an out-patient whereas isolate 3 was isolated from patients' suites. These 2 MRSA isolates were positive for the TSST-1 toxin gene.

The RAPD type G included 4 MRS isolates (2 MRSA and 2 MRCNS), having 30.2% genomic similarity; three were HA and one was a CA strain. These isolates were all susceptible to the glycopeptides; however, they were resistant to erythromycin. All but one isolate were resistant to clindamycin.

The RAPD type A included 3 MRCNS isolates, having 45.6% genomic similarity; two were CA and 1 was a HA strain. All three isolates were resistant to erythromycin and sensitive to glycopeptides, and one was sensitive to clindamycin.

The RAPD type H included only 1 MRCNS isolate. This isolate was HA, for it contained SCC*mec* type III. It was resistant to clindamycin and erythromycin and sensitive to the glycopeptides.

The RAPD type F also included only 1 MRCNS isolate which did not demonstrate the presence of the *mec*A gene; it was only phenotypically regarded as a methicillin resistant strain. This isolate was sen-

sitive to clindamycin, erythromycin, and glycopeptides (**Figures 2 & 3**).

Discussion

This study highlighted the antimicrobial-resistance profiles of the collected MRS isolates, which can serve as a useful reference for assessing the antibiotic resistant trends of the MRS strains in Lebanon.

Antimicrobial susceptibility testing showed that MRCNS are inclined to be more multi-drug resistant
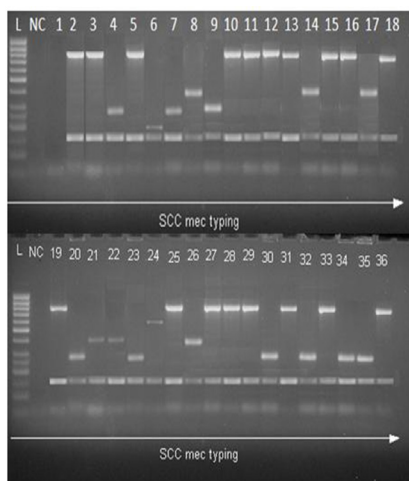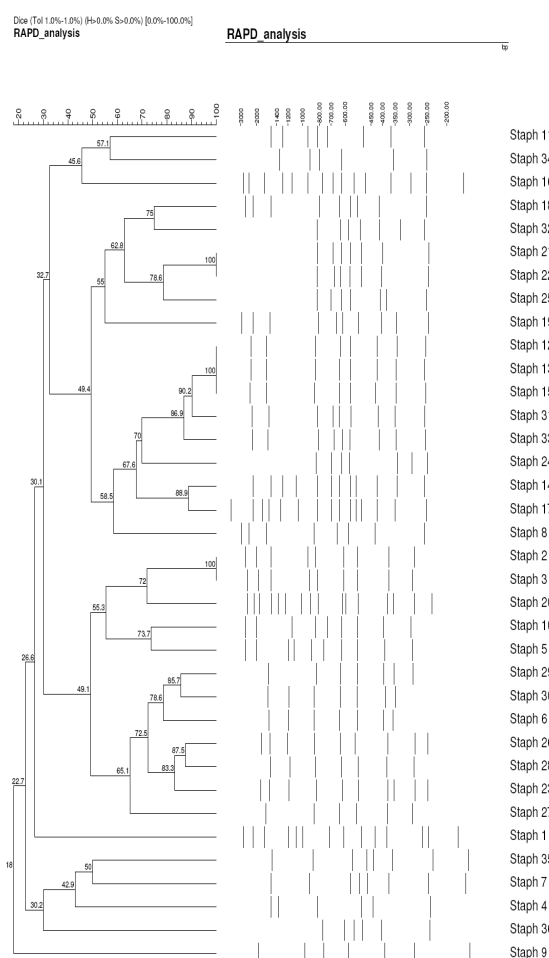

Figure 3. Dendrogram obtained from RAPD analysis, depicting clonal relationship (eight clusters: A, B, C, D, E, F, G, & H) of the 36 MRS clinical isolates obtained from a Medical Center, Lebanon.

Figure 2. RAPD gel analysis for the MRS isolates; NC: negative control; L: molecular weight marker (100 bp ladder).

Table 2. Characteristics of MRS isolates

Isolate #	Species	Antibiotic Susceptibility Type	SCC mec type	RAPD type
1	CNS	1	N/A	F
2	S.aureus	1	IVa	D
3	S.aureus	1	IVa	D
4	S.aureus	2	III	G
5	S.aureus	1	IVa	D
6	S.aureus	1	IVc	E
7	S.aureus	2	III	G
8	CNS	2	II	C
9	CNS	2	III	H
10	S.aureus	1	IVa	D
11	CNS	3	IVa	A
12	CNS	2	IVa	C
13	CNS	2	IVa	C
14	CNS	2	II	C
15	CNS	2	IVa	C
16	CNS	2	IVa	A
17	CNS	3	II	C
18	CNS	2	IVa	B
19	CNS	1	IVa	B
20	S.aureus	1	III	D
21	CNS	2	II	B
22	CNS	2	II	B
23	S.aureus	2	III	E
24	CNS	2	I	C
25	CNS	2	IVa	B
26	S.aureus	3	III	E
27	S.aureus	1	IVa	E
28	S.aureus	1	IVa	E
29	S.aureus	1	IVa	E
30	CNS	2	III	E
31	CNS	3	IVa	C
32	CNS	2	III	B
33	CNS	3	IVa	C
34	CNS	2	III	A
35	CNS	2	III	G
36	CNS	3	IVa	G

*CNS: coagulase-negative staphylococci, N/A: non-available

than MRSA. Similar results have been documented in several studies [21]. The percentage of resistance to erythromycin and clindamycin in MRCNS isolates in this study was 91% and 65% respectively; whereas, the percentage of resistance to erythromycin and clindamycin in MRSA isolates was 38% and 31% respectively. The high resistance rates to erythromycin and clindamycin renders the use of these antimicrobial agents inefficient in the treatment of MRS infections. Currently, the glycopeptides (vancomycin and teicoplanin) are considered the drugs of last resort against MRS infections; however, the emergence of vancomycin-intermediate and resistant staphylococcal strains has imposed a serious threat [22]. Moreover, all of the Staphylococcus isolates in this study were sensitive to vancomycin and teicoplanin.

Molecular characterization of the studied MRSA isolates by performing staphylococcal cassette chromosome mec (SCCmec) typing using M-PCR revealed that 53.8% of these MRSA isolates were SCCmec type IVa, 38.5% were SCCmec type III and 7.7% were SCCmec type IVc.

None of the MRSA isolates were *pvl* positive, but 15.4% of MRSA isolates were tst positive by qRT-PCR. The presence of the *tst* gene, encoding for TSST-1, in isolates 2 and 3 is alarming because it can contribute to the invasiveness and severity of the *S. aureus* infection. Therefore, routine investigation for genes encoding virulence factors is recommended in order to predict potential diseases that may be associated with these virulence factors. This may lead to the application of the appropriate measures if possible to manage such diseases.

Furthermore, SCC*mec* typing by M-PCR allowed for the differentiation between HA-MRSA and CA-MRSA[23]. Established molecular criteria classified isolates of MRSA harboring SCCmec type I-III as HA-MRSA and those harboring SCC*mec* type IV-V as CA-MRSA. The majority (61.5%) of the collected MRSA isolates were CA. The existence of these isolates carrying SCC*mec* type IV in the hospital indicates the presence of a community reservoir or possibly the entrance of these isolates into the hospital environment; hence, CA-MRSA isolates are no longer confined to the community [24-26]. This change in the epidemiology of CA-MRSA has raised speculations concerning the origin of CA and HA MRSA. The basic structure of the SCC*mec* genetic element can provide an insight about the origin of CA and HA-MRSA. As noted earlier, the various SCC*mec* types share some basic structural features, including direct repeats, the *ccr* gene complex and the *mec* gene complex. The structural differences between the community-associated SCCmec types IV-V and the hospital-associated SCCmec types I-III rely mainly on the larger size and the presence of additional resistance determinants associated with HA-MRSA strains. Therefore, a possible explanation is that CA-MRSA has entered the hospital setting, acquired additional fragments and resistance determinants rendering them HA.

CA-MRSA isolates have been known to be susceptible to non-beta-lactam antimicrobial agents. The antimicrobial susceptibility results obtained in this study confirm it; while 80% of HA-MRSA isolates showed multidrug-resistant phenotypes, all CA-MRSA isolates presented with a multi-drug susceptible pattern. Even though the CA-MRSA isolates were susceptible to multiple classes of antimicrobial agents as compared to HA-MRSA, 54% of the patients were hospitalized, indicating the severity of infections caused by CA-MRSA.

SCC*mec* typing and RAPD analysis revealed that the staphylococcal isolates 2 and 3 which were characterized as CA-MRSA belonging to cluster D, isolates 12, 13 and 15 which were characterized as MRCNS carrying SCC*mec* type IVa belonging to cluster C, and isolates 21 and 22 which were characterized as MRCNS carrying SCC*mec* type II belonging to cluster B, were genomically identical, and they were isolated within the same time period, hence, indicating possible outbreak strains[27].

The SCCmec genetic elements were highly preva-

lent among MRCNS in this study. Moreover, there were strains of MRCNS and MRSA that showed close or possible genomic relatedness by RAPD (isolates 29 & 30 and isolates 7 & 35 showed 85.7% and 50% genomic similarity respectively). This indicates that MRCNS constitute potential reservoirs of the SCCmec that provide *S. aureus* with antimicrobial-resistant determinants [28-32].

RAPD analysis revealed the presence of multiple SCCmec types within a single RAPD cluster. This indicates that staphylococcal clones having similar genomes may have developed methicillin-resistance upon the acquisition of the SCCmec genetic element by methicillin-sensitive strains of staphylococci.

The MRS isolates were diversified as defined by RAPD analysis, and there was a significant genomic heterogeneity particularly among staphylococcal isolates carrying the SCCmec type IV, which clustered into six RAPD types. This observation points out to the enhanced ability of SCCmec type IV genetic element to become mobilized, which is followed by its insertion into the chromosome of Staphylococcus species. Further, SCCmec types I, II and III were more commonly associated with HA isolates, whereas types IVa and IVc were more commonly associated with CA isolates.The small size associated with SCCmec type IV might aid in the transfer of this element on a plasmid or bacteriophage and hence, contributing to the enhanced spread of methicillin-resistance [33].

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