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Research Article

Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolated from Khartoum Hospitals

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Abstract

Background: Methicillin-Resistant *S. Aureus* (MRSA) is responsible for serious infections in humans. Resistant to Methicillin is acquired by harboring the *mec*A gene that is carried on a mobile genetic element called the Staphylococcal Cassette chromosome *mec* (SCC*mec*), SCC*mec* elements have been categorized into five types (I–V). Recently according to variation in the combinations between the J, ccr complex, and *mec*A gene regions define the different types of SCC*mec*, of which 13 (I-XIII) have been described in the literature.Majority of MRSA strains that carry SCC*mec* types I, II, or III tend to be Hospital-Acquired Mrsa (HA-MRSA) strains, whereas strains that carry SCC*mec* types IV or V are Community-Acquired MRSA (CA-MRSA). A powerful cytotoxin produced by S.aureus is encoded by Panton-Valentine Leucocidin (PVL) gene, which is mostly linked to the CA-MRSA strains. The present study was undertaken to identify and characterize MRSA bacterial isolates from Soba teaching Hospital by determining the existence two major genotypic markers; the presence of PVL gene and SCC*mec* type. The study also aimed to determine the diversity of SCC*mec* type of MRSA strains circulating in Khartoum, Sudan.

Result: A descriptive hospital based study, conducted in Soba teaching hospital in Khartoum. A total of 75 MRSA clinical isolates were analyzed using standard microbiological technique. Duplex PCR was performed on genomic DNA for MRSA isolates to detect the *mec*A and PVL genes. In addition, SCC*mec* typing was done by using multiplex PCR assay.Over a 75 MRSA clinical isolates, 62 (82.7%) isolates were harbored the *mec*A gene. Result from SCC*mec* typing had shown that 9.6% were type I, 12.8% were type III, 1.61% were type IVb, 3.22% were type IVc, 20.9% were type IVd and 16.1% were type V. SCC*mec* type II and IVa were not found among isolates. 13.3% of MRSA found to carry the PVL gene, all of them were CA-MRSA strains type IVd and V.

Conclusion: MRSA is increasing in their prevalence. CA-MRSA positive PVL strains that invade hospital represent a serious problem, so frequently investigation and surveillance of MRSA in hospital is essential in order to control their infection.

Keywords: MRSA; SCC*mec*; PVL; *mec*A

Background

Architecture of Staphylococcal Cassette Chromosome *mec* (SCC*mec*) type and existence of Panton Valentine Leukocidin (PVL) toxin are important genotypic markers that differentiate between community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains and hospital-acquired (HA)-MRSA strains [1]. According to the SCC*mec* types, HA-MRSA usually carriy large SCC*mec* element; types I, II and III (34 67kb) [2] but CA-MRSA harbour newly discovered smaller SCC*mec* element type IV (24kb) [3] or less frequently V or a variant VT [4]. In combine with SCC*mec* typing the Centers for Disease Control and Prevention (CDC) determined some criteria that define CA-MRSA according to clinical data, such as: diagnosis of MRSA within 48 hours of patient admission, absence of history of MRSA infection or colonization, and hospitalization, dialysis, or surgery in last 12 months and absence of permanent catheter or invasive medical device. Production of PVL toxin is frequently associated with CA-MRSA strain carrying SCC*mec* type IV and occasionally with SCC*mec* type V or VT. This association may be due to acquirement by Methicillin-sensitive *S. aureus* (MSSA) that acquired the lukS-PV and lukF-PV genes for PVL production and subsequent acquirement by this modified strains of the mobile SCC*mec* type types IV or V resulting in the resistant Community acquired S.aureus phenotype. The emergence of Community Associated MRSA (CA-MRSA) within hospitals represent a significant public health threat as it was reported in numerous studies [1,2,5]. Several distinguishing characteristics make the CA-MRSA distinct from HA-MRSA. Primarily CA-MRSA generally carry the SCC*mec* type IV element while the nosocomial strains tend to carry SCC*mec* types I, II, and III [6-8] While CA-MRSA strains usually carry the Panton-Valentine Leukocidin (PVL) genes [4,9]. PVL is an *S. aureus*-specific exotoxin often associated with severe skin infections

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and necrotizing pneumonia [4,6]. Community acquired strains tend to demonstrate fewer antimicrobials resistance than strains acquired within hospitals [10].

The present study was undertaken to identify and characterize MRSA bacterial isolates from Soba teaching Hospital by determining two major genotypic markers; existence of of PVL gene and SCC*mec* type, and to elucidate the diversity of SCC*mec* type of MRSA strains circulating in this region.

Methods

This a descriptive hospital based study that was conducted during the period from April to November 2017 on a total of 75 MRSA isolates. Isolates were identified as *Staphylococcus aureus* by performing a gram stain, catalase and coagulase test. The isolates were randomly collected from patients attending soba teaching hospital from different specimens including blood, pus and swabs.

Antibiotic susceptibility testing

Antibiotic susceptibility testing for S.aureus was done according to the Kirby-Bauer disc diffusion method based on Clinical and Laboratory Standards Institute (CLSI) recommendations using the following antibiotics discs (Co-trimoxazol, Clindamycin, Erythromycin, Tetracycline, Gentamaicin and Vancomycin). MRSA was determined by Oxacillin disc 1mg.

Bacterial growth for DNA extraction

Collected isolates were stored on glycerol slope at 4°C, then were cultured onto blood agar and incubated overnight at 37°C.

DNA extraction

The bacterial DNA were extracted by using Gene Matrix bacterial Genomic DNA purification kit (Eurex, Poland) according to manufacture instruction, obtained DNA were stored at -20°C until used.

Multiplex PCR assay for detection of the *Mec*A and PVL genes

The PCR reaction for the detection of *mec*A and PVL genes were carried out in Thermal cycler (Techne, England) according to the methods described by McClure et al (2006) [6].

Multiplex PCR assay for typing of SCC*mec*

The Multiplex PCR amplification was performed according to the methods described by Zhang et al (2005) [8]. Using primers sequences specific for SCC*mec* sub types I, II, III, IVa, IVb, IVc, IVd, and V (Table 1).

Result

Out of 75 the Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates, 62 (82.7%) were found positive for Staphylococcal Cassette Chromosome *mec*(SCC*mec*) using multiplex PCR (SCC*mec* typing) and 10(13.3%) were found positive for of Panton Valentine Leukocidin (PVL) toxin using duplex PCR (Table 2). Based on genotpye of SCC*mec*, 6 (9.67%) were found positive for SCC*mec* I, 8 were found positive for SCC*mec* III, 1 were found positive for SCC*mec*IVb, 2 (3.22%) were found positive for SCC*mec*IVc, 13 (20.9%) were found positive for SCC*mec*IVd , 10 (16.1%) were found positive for SCC*mec* V (Table 3).

Table 1: Primers sequences used for SCC*mec* typing.

SCC*mec* type	Primers	Oligonucleotide sequence (5-3)
SCC*mec* I	Type I-F	GCTTTAAAGAGTGTCGTTACAGG
	Type I-R	GTTCTCTCATAGTATGACGTCC
SCC*mec* II	Type II-F	CGTTGAAGATGATGAAGCG
	Type II-R	CGAAATCAATGGTTAATGGACC
SCC*mec* III	Type III-F	CCATATTGTGTACGATGCG
	Type III-R	CCTTAGTTGTCGTAACAGATCG
SCC*mec* IVa	Type IVa-F	GCCTTATTCGAAGAAACCG
	Type IVa-R	CTACTCTTCTGAAAAGCGTCG
SCC*mec* IVb	Type IVb-F	TCTGGAATTACTTCAGCTGC
	Type IVb-R	AAACAATATTGCTCTCCCTC
SCC*mec* IVc	Type IVc-F	ACAATATTTGTATTATCGGAGAGC
	Type IVc-R	TTGGTATGAGGTATTGCTGG
SCC*mec* IVd	Type IVd-F5	CTCAAAATACGGACCCCAATACA
	Type IVd-R6	TGCTCCAGTAATTGCTAAAG
SCC*mec* V	Type V-F	GAACATTGTTACTTAAATGAGCG
	Type V-R	TGAAAGTTGTACCCTTGACACC

Table 2: Staphylococcal cassette chromosome mec **(SCC*mec*)** and Panton Valentine Leukocidin **(PVL)** positive samples among MRSA isolates from Soba Hospital.

Number tested	75
SCC*mec* n (%)	62 (82.7)
Untypable n (%)	13 (17.4)
Total n(%)	75 (100)
PVL n (%)	10* (13.3)

*Detection among the 75 MRSA isolates, all of them belong to SCC*mec* IVd/V

Table 3: Frequency of SCC*mec* types and PVL among MRSA isolates.

SCC*mec* (62)		**PVL (10)**
I	6 (9.6%)	0
II	0	0
III	8 (12.8%)	0
IVa	0	0
IVb	1 (1.61%)	0
IVc	2 (3.22%)	0
IVd	13 (20.9%)	4 (40%)
V	10 (16.1%)	6 (60%)
Untypeable	22 (35.5%)	0

Discussion

The prevalence of MRSA in Africa ranges from unknown in some parts of Africa and up to 50% in other parts [11]. SCC*mec* typing is an important tool to understand the molecular epidemiology and evolution of MRSA[10], also it became a useful technique for distinguishing between HA-MRSA and CA-MRSA. Most HA-MRSA infections are SCC*mec* types I, II, and III, while CA-MRSA infections are SCC*mec* types IV and V [12].

In the present study out of 75 MRSA isolates, 62(82.7%) were carrying the SCC*mec* gene. The SCC*mec* typing for them revealed that

20.9% were SCC*mec* IVd, 16.1% SCC*mec* V, 12.9% SCC*mec* III, 9.67% SCC*mec* I,3.22% SCC*mec* IVc , 1.61% SCC*mec* IVb. No sample proved positive for SCC*mec* II. The predominant SCC type were SCC IVd and SCC V, this results reflect the invasion of CA-MRSA into hospital and may replace the classical HA-MRSA strains. Similar results were obtained in Kuwait, among 135 MRSA isolates from hospitals, where 102 (75.6%) of the isolates carried SCC*mec* type IV, 11 (8.1%) carried SCC*mec* type IVa, 10 (7.4%) carried SCC*mec* type IVc and 12 (8.9%) carried SCC*mec* type V genetic element [13]. Also, among 77 MRSA, type IV/IVA was most common (42.9%) in a University hospital in Switzerland [14]. On the other hand, a study done in Malaysia found that SCC*mec* III remained predominant among MRSA strains in their hospital over two years [15]. In a study in Makka involving206 S.aureus isolates, reported that 114 (55.3%) of the isolates were MRSA of which 100 isolates carried the *mec*A gene. Subsequent results from SCC*mec* typing showed that 3% were type I; 9% were type II; 47% were type III, and 29% were type IV [12] and 12 isolates (12%) could not be classified into any of the described SCC*mec* types.

Presence of PVL gene among Staphylococcus strains increases the disease severity [12], and the spread of PVL positive strains carrying *mec*A gene exhibiting resistance to all beta lactam antibiotics is becoming common [10]. In our study 13.3% of the SCC*mec* positive isolates were also found positive for PVL gene.

The distribution of SCC*mec* types is varied from region to region worldwide [12], and the emergence of community-associated MRSA (CA-MRSA)has led to dramatic change in the epidemiology of Methicillin-Resistant *Staphylococcus aureus* (MRSA) [14]. In the present study, only 62 of 75 MRSA isolates were confirmed to carry the *mec*A gene. Our study also indicated that a high percentage (42%) [26 out of 62 SCC*mec* positive isolates] of the isolates were CA-MRSA, however22 (35.5%) of the isolates could not be typed. This is much higher than 12 and than that reported from another study conducted in Makkah region, located on the West coast of Saudi Arabia, where CA-MRSA accounted for 15.8% of all MRSA isolates [16].

The Panton-Valentine Leukocidin (PVL) genes have been demonstrated primarily among CA-MRSA strains [4,9]. In this study all PVL positive isolates where CA-MRSA, similar study done in France showed that most CA-MRSA contain the PVL gene [17]. There is evidence that PVL-positive CA-MRSA clones have spread throughout the world [18] as seen in this study where we find a prevalence of 16.1% PVL containing CA-MRSA.

Increased incidence of virulent MRSA strains in both hospitals and the community highlights the importance of their rapid identification in order to appropriately control S.aureus dangerous infection.

Conclusion

MRSA is increasing in prevalence and in their resistant to antibiotics. This study had shown that most of detected strains are community acquired. CA-MRSA positive PVL strains that invade hospital represent a serious problem, so frequently investigation and surveillance of MRSA in hospital is essential in order to control their infection.

Abbreviations

MRSA: Methicillin Resistant *Staphylococcus aureus*; SCC*mec*: Staphylococcus Cassette Chromosome *Mec*; HA-MRSA: Hospital Acquired- Methicillin Resistant *Staphylococcus aurues*; CA-MRSA: Community Acquired-Methicillin Resistant *Staphylococcus aurues*; PVL: Panton Valentine Leukocidin; DNA: Deoxyribonucleic Acid; PCR: Polymerase Chain Reaction; MSSA: Methicillin Sensitive *Staphylococcus aureus*; CLSI: Clinical and Laboratory Standard Institute.

Declarations

Ethics approval and consent to participate

Ethics approval was obtained from ethical committee of Al-Neelain University. Collection of the samples were carried out according to Soba Teaching hospitals guidelines for sample collection and verbal consents of the patients.

Authors' contributions

ANA, T H, MOM and OMK did the sample collection, culture and PCR. We helped in the analysis of data and worked in the preparation and edition of the manuscript. AMH, IME and KAE contributed to the conception and design of the study and helped in the drafting of the manuscript. All authors read and approved the final manuscript.

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