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SCCMEC GENOTYPING OF METICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN NASAL CARRIERS OF HEALTH WORKERS

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ABSTRACT

Nasal colonization with meticillin-resistant Staphylococcus aureus in normal, asymptomatic individuals constitutes a high percentage. MRSA strains are divided into hospital-associated MRSA(HA-MRSA), which is associated with increased treatment duration and health care costs, and is associated with Sccmec types (I, II, III), and community-associated MRSA(CA-MRSA), which is commonly associated with Sccmec types IV, V. We conducted a study to determine the frequency of nasal carrying of MRSA among health care workers in the intensive care unit in our hospital and to determine the frequency of different Sccmec types in MRSA isolates. **Methods:** nasal swabs were taken from health workers in the intensive care unit at Tishreen University Hospital, Lattakia, Syria, to investigate the presence of MRSA. The presence of the MecA gene was confirmed by PCR, and the most common Sccmec type was determined by multiplexPCR. **Results:** Among 60 nasal swabs taken from health personnel worker in the intensive care unit, 24 strains of Staphylococcus aureus were collected. Meticillin-resistant Staphylococcus aureus (MRSA) was found in 20 samples (83.3%), and this was confirmed by MecA amplification. Sccmec typing of these 20 isolates was performed by multiplex PCR. Type IV was the most common type, with a frequency of 75% (15/20), followed by type I, with frequency of 15% (3/20); type V,I with a frequency of 5% for each one. **Conclusion:** Our study suggests that an effective control protocol should be adopted to prevent the transmission of MRSA strains from health care workers to hospitalized patients.

KEYWORDS: MRSA, Health care workers, Sccmec IV, MecA, HA-MRSA,CA-MRSA.

INTRODUCTION

Staphylococcus aureus is one of the most important major human pathogens and is responsible for infections from mild to life-threatening. Therefore, infections caused by Staphylococcus aureus constitute a serious public health concern worldwide.[1]

Meticillin (a beta-lactamase-resistant antibiotic) appeared for the first time in 1959 and was used to treat Staphylococcus aureus infections. During a short period in 1961, the first meticillin-resistant Staphylococcus aureus (MRSA) bacteria appeared, as a MRSA strain was reported in London.[2]

MRSA is the most important opportunistic pathogen and is characterized by the development of virulence and resistance to antibiotics.[3,4] This bacterium can colonize multiple sites in the human host, but most often it colonizes the anterior nostrils of 30-50% of normal individuals. In addition, the host may cause a wide range of infections through asymptomatic transmission of this bacterium.[5]

Resistance to meticillin is due to the acquisition of the Mec A gene. This gene is not natively present in the S. aureus genome and its expression is due to the production of a special penicillin binder called PBP2a, which has a lower susceptibility to beta-lactam antibiotics than PBPs.[6]

The Mec A gene is widely distributed in coagulase-positive staphylococci and is usually carried on a mobile gene element called S. aureus cassette chromosome mec **(**Sccmec**)**.[7] Sccmec consists of two main components: the ccr gene complex, and the mec gene complex. The ccr gene complex is the cause of Sccmec movement as it encodes site-specific recombination processes and regions surrounding open reading frames. The mec gene complex consists of: the mec gene, the mec R1-mecI regulatory gene and inclusion elements for the possible integration of some unlinked resistance determinants. According to the combination between ccr allotypes and mec gene complex, 11 types have been reported for Sccmec (I →XI).[6,7]

MRSA strains are generally divided into two main groups: hospital-associated MRSA(HA-MRSA) and

community-associated MRSA (CA- MRSA).[8] Scc mec types I,II,III are mainly associated with multidrug-resistant HA-MRSA, while Scc mec types IV and V have been most commonly reported in CA-MRSA infections.[9,10]

Molecular typing methods are essential for ongoing surveillance and infection control programs and may help prevent the spread of MRSA infection. Among the various techniques used in MRSA genotyping, Sccmec typing is a simple, effective and cost-effective method that can distinguish between HA-MRSA and CA-MRSA strains.[11,12]

In this study, we evaluated the frequency of nasal carriage of MRSA among health workers in the intensive care unit at Tishreen University Hospital in Latakia, Syria. The aim of the study was to evaluate MRSA Sccmec type in nasal carriage of healthcare workers.

MATERIALS AND METHODS

Materials

2-1 Study samples

Samples were collected from the nasal cavity of 60 health workers in the intensive care unit at Tishreen University Hospital - Lattakia, Syria, to isolate Staphylococcus aureus using a cotton swab. Samples were taken regardless of age and gender.

2-2 Identification of bacterial isolates

The clinical samples were cultured on blood agar and mannitol salt agar (MSA), and Staphylococcus aureus was identified through traditional biochemical tests, including catalase and coagulase tests, and mannitol fermentation.

2-3 Methicillin resistance test

MRSA samples were identified by incubating the strains on Muller-Hinton medium for 24 hours at 37°C using oxacillin (1 µg) and cefoxitin (30 µg) according to CLSI 2021 standards using the Kirby-Bauer method.[13] MRSA samples were identified and the sensitization results were interpreted by measuring the peridisc diameter.

The presence of MecA in MRSA isolates was assessed by amplification. Forward and reverse primers were designed at the Atomic Energy Commission in Damascus after searching through the NCBI search engine to ensure their specificity and to achieve MecA product (359pb). Sequences of primers used for amplification of the mecA gene are listed in table 1.The amplification process was performed by Techne TC -512 thermal cycler Gradient with one cycle of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing 52°C for 30 seconds, extension at 72°C for 45 seconds and a cycle of final extension at 72°C for 7 minutes. All PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

2-4PCR-based assignment of SCCmec elements

Before this work, DNA of all samples was extracted using the method used by Hanano et al. 2013, which relied on the use of Liquid nitrogen.[14] The design of this multiplex PCR was described by Boye et al.[15] An assay of multiplex PCR was performed in 50 µL reactions with 1 µ of AmpliTaq DNA polymerase, 1×PCR buffer, 1.5 mM MgCl2, 200 µM deoxyribonucleotidtriphosphate and 2 µL genomic DNA and distilled water to a final volume of 50 µL. The primer concentrations were as follows: 0.2 pmol/µL each of primers β and α3; 0.25 pmol/µL each of primers ccrCF and ccrCR; 0.08 pmol/µL each of primers 1272F1 and 1272R1; and 0.1 pmol/µL each of primers 5RmecA and 5R431. The sequences of primers used for amplification of SCCmec types are provided in Table 1. A multiplex PCR reaction was performed for 1 cycle at 94°C for 4 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 60 seconds at 72°C, with a final extension for 4 minutes at 72°C. The PCR products were visualized on a 1% agarose gel stained with ethidium bromide. For comparison, ready to use, Fermentas Gene Ruler 1kb DNA ladder, was used.[16]

Table 1: Primers used for determining SCCmec types.

Name	Sequence of Primer (5'-3')	Length (bp)	Target	Sccmec				
				I	II	III	IV	V
β	F:ATTGCCTTGATAATAGCCYTCT	937	ccrA2-B		*		*	
α3	R:TAAAGGCATCAATGCACAAACACT							
ccrF	F:CGTCTATTACAAGATGTTAAGGAA	518	ccrC			*		*
ccrR	R:CCTTTATAGACTGGATTATTCAAAA							
1272F1	F: GCCACTCATAACATATGGAA	415	IS1272	*			*	
1272R1	R: CATCCGAGTGAAACCCAAA							
5RmecA	F: TATACCAAACCCGACAACTAC	359	mecA-IS431					*
5R431	R: CGGCTACAGTGATAACATCC							

Data management

The statistical analysis was done using IBM SPSS statistics(Version25) statistical Analysis Software. The values were represented in Number (%) and Mean ± SD.

RESULTS

Among 60 nasal swabs taken from health personnel worker in the intensive care unit, 24 strains of Staphylococcus aureus were collected. Meticillin-resistant Staphylococcus aureus (MRSA) was found in

20 samples (83.3%), and this was confirmed by MecA amplification. Sccmec typing of these 20 isolates was performed by multiplex PCR. Type IV was the most common type, with a frequency of 75% (15/20), followed by type I, with frequency of 15% (3/20); type V,I with a frequency of 5% for each one. (Fig. 1)

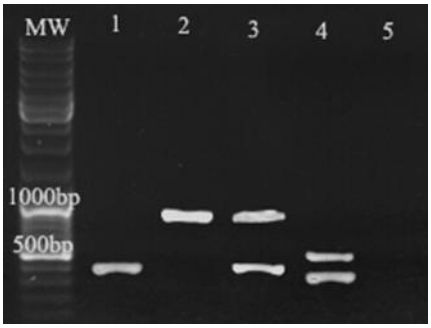

FIG. 1: Sccmec types. MW path: standard molecular marker, path1: type I, path 2: type II, path 3: type IV, path 4: type V, path 5: negative control.

DISCUSSION

MRSA infection is one of the most important causes of increased morbidity and mortality.[16] Annually, HA-MRSA infection occurs in 1,900 patients in American hospitals, a number similar to the number of deaths caused by AIDS and tuberculosis.[17]

In our study, the gene MecA was found in 20(83.3%) S. aureus isolates, and in these isolates all samples were resistant to oxacillin and cefoxitin. The prevalence of gene MecA in our study compared to studies conducted in other countries was: 36.6% Greece, 38.3% Italy, 45.76% Philippines, 40.2% Iran.[18,19]

The difference in the distribution of gene MecA between countries can be explained by the population group on which the study was conducted, as in our study it was limited to the nasal carriers of health workers only and the diversity in the types of clinical samples (in our study we took nasal swabs).

Molecular genotyping methods for MRSA are necessary to know the origin of the strains, their epidemiology, and the type of antibiotic to be used.[20] In our study, the most common Sccmec type was IV type, with a frequency of 75% (15/20), followed by type I, with frequency of 15% (3/20. That is, most of the MRSA isolates originated from CA-MRSA. Our study was the first to identify the Sccmec types in Syria. In several studies in Iran, it was reported that the dominant Sccmec type is III among MRSA strains, while in Korya and Japan it is type II.[7,19,21] That is, the MRSA isolates in these studies belong to HA-MRSA.

In Switzerland, the dominant Sccmec type was IV (76.6%), while types I and II were in the minority, and type III was absent.[22] The difference in the Sccmec types between countries may be due to differences in the study sample and sample collection methods.

CONCLUSION

We have found a significant spread of MRSA among health care workers in the intensive care unit, which poses a direct threat to the possibility of transmitting MRSA to immunocompromised patients in this unit. In addition, it was identified Sccmec type IV as the dominant type. These results suggest that an effective control protocol should be adopted to prevent the transmission of MRSA strains from health care workers to hospitalized patients.

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