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Original Article

Molecular Characterization and SCCmec in Methicillin Resistant *Staphylococcus aureus* from Healthy Worker Nasal Swab in Larestan Hospital

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ABSTRACT

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Key words
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Background and Aims: The aim of present study was to investigate the molecular characteristic of *Staphylococcus aureus* to detect mec A gene and to type SCCmec in strain isolated from healthy worker at Larestan Hospital.
Material and Methods: This study was carried out from the 250 nasal swab healthy worker at Larestan hospital. Multiplex polymerase chain reaction for mecA gene was performed in all samples. Also, agar screen plate with oxacillin was carried out using CLSI guidelines. The two methods were then compared.
Results: Of 250 samples, 37 (14.8%) samples are *Staphylococcus aureus*, 28 (75.7%) of samples were confirmed at Methicillin-resistant *Staphylococcus aureus (*MRSA) harboring mec A gene detected by Multiplex polymerase chain reaction (PCR) and 9 (24.3%) negative mec A. Sixteen of 28 (57.1%) were HA-MRSA and the remaining 12 (42.8%) were CA-MRSA. The Multiplex PCR assay for SCCmec complex of MRSA strains showed that 9 (32.1%) samples were SCCmec type I, 8 (28.6%) SCCmec type IVb, 5 (17.9%) SCCmec type II, 4 (14.3%) SCCmec type V and 2 (7.1%) SCCmec type III. Agar screen plate with oxacillin was found in all the 28 MRSA samples to harbor mecA gene and all was resistant.
Conclusion: Our results illustrated that more than 70% of staph aureus strains were positive for mec A gene and more than 50% of them were HA-MRSA. In comparison to other methods, PCR and Agar screen method more sensitivity determines MRSA isolates. However, PCR was identified as the ideal method for detecting MRSA strains.

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Introduction

Staphylococcus aureus (S. aureus) is one of the most common nosocomial pathogens which can cause a broad spectrum of infections, ranging from mild skin infections to severe abscesses, sepsis, endocarditis, osteomyelitis, urinary tract infections (UTI), and fatal necrotizing pneumonia [1]. *S. aureus* strains have the ability to become resistant to different classes of antimicrobial agents such as methicillin [2]. Resistance to methicillin in *S. aureus* was first reported in 1961, just one year after its introduction, and methicillin-resistant *S. aureus* (MRSA) has spread extensively worldwide during the last few decades [3]. Resistance to methicillin is due to the presence of the staphylococcal cassette chromosome mec (SCC*mec*) element, which is a class of mobile genetic element that carries the methicillin resistant determinant *mecA*. [4]. Methicillin is a narrow-spectrumβ-lactam antibiotic of the penicillin class. MRSA produces an additional penicillin‑binding protein (PBP) 2 (or PBP2a), which has low binding affinities for most of the penicillin as well as cephem antibiotics. PBP2' is encoded by the chromosomally located *mecA* gene which was exogenously acquired, since the methicillin‑susceptible *S. aureus* strains do not have the *mecA* gene [5]. Hospital-associated MRSA (HA-MRSA) characteristically colonizes or infects hospitalized individuals with predisposing risk factors such as surgery, presence of indwelling medical devices (IMDs), an immune compromised state or prior antibiotic exposure [6]. It is often isolated from wound infections, line-associated bacteremia and ventilator associated pneumonia [7]. The strains usually harbor SCC*mec* type I, II and III, and are multidrug-resistant (MDR) [1]. The Community-Acquired (CA-MRSA) infects healthy individuals without any healthcare contact, harbors smaller and more mobile SCC*mec* types (IV and V), is susceptible to non-β-lactam antimicrobial drugs and typically manifests as skin and soft tissue infections. Life-threatening conditions, including osteomyelitis, severe necrotizing pneumonia, and fatal sepsis have also been reported [2]. It has a superior epidemicity than HA-MRSA, Consequently, the CA-MRSA strains possess a high attack rate in outbreak settings, are more virulent than HA-MRSA and have rapidly disseminated among countries [4].

This study aimed to describe molecular typing of MRSA that could be useful for determining the appropriative treatment strategy and subsequent effective management and control of the corresponding infections.

Materials and Methods

Sample collection and identification of bacteria

During Febraury to March 2015, 250 nasal swabs were collected of healthy worked at Larestan Emam Reza and Evaz and Beyram hospital. The research was confirmed by Ethics Committee of Islamic Azad University, Larestan Branch, Iran. Of the 250 nasal swabs 186(74.4%) were female and 64 (25.6%) were male. Average age was 22-30 year. All

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specimens were collected from hospital healthy workers who were cultured on Brain Hearth Infusion broth and Manitol salt agar (Himedia, India) and then identified by using Gram's stain, Coagulase, Catalase, OF, DNase. Gram's stain: Gram positive (irregular cluster) Coagulase (tube test): positive Catalase: positive/DNase or Thermo stable Nuclease: positive (clear zone around colony in DNase agar) OF: positive (produce acid of glucose in anaerobic condition).

Screening test

An agar screen plate was supplemented with 2.5% Nacl and 6 µg/ml oxacillin (sigma,USA) for gross isolation of methicillin-resistant isolates. This test was carried out according to the CLSI guidelines (CLSI, 2006a). A McFarland/5 suspension was spotted onto Muller Hintone agar containing 2.5% Nacl and 6 µg/ml oxacillin and incubated at 35ºC for 24h [8].

DNA extraction

Pure colonies of MRSA on BHI agar were inoculated into 3 ml of Brain Hearth Infusion broth and shaken at 37ºC overnight. Cells were harvested by centrifuging at 8000 g for 5min. The pellet was suspended in 400-600µl of lysis solution containing Lysosyme (sigma, USA), 10 Mm EDTA, 10 Mm Tris hydrochloride and incubated at 37ºC for 45 min. The resulting suspension was heated at 95ºC for 10 min. The lysate extracted once with phenol-chloroform, then, precipitated in absolute ethanol at -20ºc overnight. DNA was collected by centrifugation at 12000 g for 15 min. and dried at room temperature. The dried DNA was dissolved in 50 µL of distilled water.

Amplification of SCC mec complex

The multiplex polymerase chain reaction (PCR) assay for SCC mec type used nine pairs of primers (Cinnagene, Iran), including those for subtype I, II, III, IVa, IV_b, IVc, IVd, V, as well as primers for the mecA gene (Table 1). PCR was optimized by pre-denaturation at 95 ºc for 5min, followed by 35 cycles of 95ºC for 1 min., 58-60ºC for 1min., and 72ºC for 1 min., ending with a final extension step at 72ºC for 1 min. and hold at 4ºC.

Table 1. Primer, concentration, size and specificity of the generated Amplicons Kunyan Zhang et al (2005)

Specificity of amplicon	Concn (M)	Oligonucleotide sequence	Amplicon size (bp)
SCC*mec* I	0.048	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	613
SCC*mec* II	0.032	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398
SCC*mec* III	0.04	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280
SCC*mec* IVa	0.104	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776
SCC*mec* IVb	0.092	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493
SCC*mec* IVc	0.078	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200
SCC*mec* IVd	0.28	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881
SCC*mec* V	0.06	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325
mecA	0.046	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147

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Results

Bacterial isolate

In PCR method to obtain the desired result we should have DNA quality and the quantity is evaluated with:

1-Use of gel: 5 ml extracted DNA of MRSA were isolated from nasal healthy workers on agarose gel .8% carried and for a period of 15-20 minute at voltage 90 volts electrophoresis, observe sharp bond in beginning gel and do not see any other bond on the gel which is an indication of DNA high quality. This method will be assessed for extracting product quality.

2-Nano-Drop technique. The Nano-Drop Specterophotometer devices are capable of measuring very small amounts of sample spectrum in the area of visible-UV spectrum in less than a second at the same time. With this device we can define the concentration and purity of extracted DNA according to ng/µl.

MRSA typing: A few representatives of PCR-amplified SCCmec complex gene are presented in Fig.1. Based on molecular typing, the MRSA isolates were classified into eight SCCmec type. Type I was the most prevalent (32.1%). No Type IVa and IVc, IVd were detected (Table 2). No mecA (147 bp) amplification was observed in 9 (24.3%) of the isolates. Agar screen plate: All 28 MRSA strains isolated were resistant to oxacillin and on Muller Hintone agar containing 2.5% Nacl and 6 µg/ml oxacillin grew. However, Agar screen plate is only standardized for oxacillin. MRSA isolates are characterized as resistant. Staphylococcus subsp. aureus PTCC1764 is positive control that have mecA gene.

Fig. 1. Amplification pattern of SCCmec typing with Multiplex PCR
I:600-700 bp, II:280 bp, IV_b:400-500bp, V:493 bp, mecA:147 bp

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Table 2. SCCmec type frequency in 28 strain of MRSA

Sccmec	CA-MRSA, HA-MRSA	Frequency N(%)	bp
Sccmec I	HA-MRSA	9 (32.1)	613
Sccmec II	HA-MRSA	5 (17.9)	398
Sccmec III	HA-MRSA	2 (7.1)	280
Sccmec IV_b	CA-MRSA	8 (28.7)	493
Sccmec v	CA-MRSA	4 (14.3)	325

Discussion

Detection of MRSA is important for patient care and appropriate utilization of infection control resources. The molecular assay for MRSA detection was developed using both clinical isolates and patient samples and compared with conventional methods (9).

In the present study, MRSA strains are isolated from nasal healthy worker. Methicillin resistance is identified in the routine laboratory by oxacillin agar screen which is simple and relatively cheap. This test showed 98.5%, 100%, 99.4% sensitivity, specificity, and accuracy, respectively. It is therefore highly reliable in discriminating isolates harboring the mecA gene [8]. In comparison to other methods such as Disk diffusion and PCR, agar screen method has more sensitivity to determine MRSA isolates. However, PCR was identified as the ideal method for detecting MRSA strains; Because a number of strains were found to be sensitive to oxacillin in phenotypic test due to lack of mecA expression while still containing the gene [10].

Indeed, it is necessary to detect MRSA in healthy individuals. These individuals can act as carriers and thus as a potential source of microorganisms, which are important for the epidemiology and pathogenesis of hospital infections [11].

It is notable asymptomatic colonization that can persist for month to years therefore the molecular typing of clinical MRSA isolate could be useful for determining the appropriate treatment [6]. Also, more effective disinfection procedures and hand hygiene guidelines could, in part, help to prevent the spread of MRSA in the hospital environment [12].

These results suggest that efficient control protocol should be adopted in hospitals to prevent the transfer of MRSA between healthy workers and patients. Application of this protocol along with antibacterial susceptibility pattern of MRSA strain could prevent of emergence multi drug-resistance strains [13].

In the present study, SCC mec typing showed that type I was the predominant type (32.1%), followed by type IV_b(28.7%). However, a further 39% of isolate carried SCCmec II, III, V.

Although a high prevalence of type III has been reported in the world [3], but in this study and in this region, type I was the predominant.

The prevalence of CA-MRSA is currently low worldwide, but appears to be increasing .The prevalence of CA-MRSA is <0.5%, but HA-MRSA strains are also circulating in the community [14].

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Conclusion

Our findings show that clinical isolate of MRSA in our hospital carrying various kind of staphylococcal cassette chromosome mec (SCCmec) type and more than 70% of *S. aureus* strains were positive for mec A gene and more than 50% of them were HA-MRSA. In comparison to other methods, PCR and Agar screen method more sensitivity determines MRSA isolates. However, PCR was identified as the ideal method for detecting MRSA strains.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

Acknowledgment

There is no Acknowledgment to declare.

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