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Prevalence of *mecA* and *femB* genes in Methicillin-Resistant *Staphylococcus aureus* Isolated from Iran's Military Hospitals

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Methicillin-resistant *Staphylococcus aureus* is the cause of nosocomial infections leading to high mortality. This cross-sectional study was conducted to study a total of 200 clinical specimens from Iranian military hospitals in 2013-2015. Disk agar diffusion test using cefoxitin disk (30 μg) showed methicillin resistance in 59% of our isolates. *mecA* and *femB* genes were identified in all of the MRSA isolates using PCR method. Our results demonstrated the spread of HA-MRSA isolates in the community and propagating CA-MRSA isolates in the studied hospitals.

Keywords: Methicillin- resistance *Staphylococcus aureus,* Cefoxitin, *femB*, *mecA*.

The resistance of microorganisms to antimicrobial factors is a growing problem and a global challenge. Drug resistant bacteria in hospitals and communities is a common cause of concern as it leads to treatment failure, increase in diseases and mortality. *Staphylococcus aureus* is an important cause of serious infection in the hospitals and communities. Methicillin, the first synthetic penicillin, was used in 1961 for the treatment of *Staphylococcus aureus* infections. The first methicillin-resistant *Staphylococcus aureus* (MRSA)was identified in England at the same year. Infections caused by MRSA strains leads to high mortality. These organisms acquired resistance to a broad group of beta-lactam antimicrobial factors increasing in the world[1,21,23,30].

A study in America, compared the patients who were not infected by *Staphylococcus aureus* , with those suffered from *Staphylococcal* infection. This study showed that mortality rate in *Staphylococcal* group was five times more than non-*Staphylococcal* group, and in respect, the cost of hospitalization and cost of additional treatment would be three times more in the comparison with non-*Staphylococcal* group[2,5,3]. Recently, the MRSA strains have been divided into two subgroups: the MRSA acquired from therapeutic environment (Health Care Associated-MRSA) and acquired from community (Community Associated-MRSA). HA-MRSA is the main problem of hospital infections, and it was seen in patients who were in hospitals for more than 48 hours. The high risk factors for HA-MRSA includes patients with open wound or foreign object or immunodeficiency .On the other hand, the CA-MRSA strains, in recent years, have become an increasing major concern for public health. CA-MRSA strains have been separated in patients who have no history of contact with the health care facilities, and prolonged bed rest in a hospital[6,17,9].

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The first report of CA-MRSA was indicated from Australia in 1990, and consequently more reports from countries like: France, Finland, New Zealand, and England. CA-MRSA strains are becoming one of the most common causes of skin infections in patients not referred to hospitals. Unlike HA-MRSA, these strains usually cause more infection in younger people[810]. When traditional *Staphylococcus aureus* were compared with MRSA strains at the level of molecular genetics, MRSA strains were found to have additional moving chromosomal DNA fragments called staphylococcal Casset SCCmec, Chromosome mec or genomic islands SCCmec[16,20].

SCCmec Included the genes encoding the penicillin-binding protein (PBP2a or PBP2'), regulatory genes mecR1 and mecI[13].

penicillin-binding protein did not allow bacterial survival in the lethal concentrations of beta-lactam. In clinical setting with high pressure of antibiotics, strains of HA- MRSA in comparison to CA-MRSA shows to higher spectrum of antimicrobial resistance[18,20,14]. So far, five types and four sub-types of the common SCCmec have been recognized based on the complex combination of mec[4,6,7] from 20.9 up to 66.9kb. SCCmec I (34/3 kb), SCCmec II (53 kb), SSCmec III (66/9), SCC mec IV (20/9 to 24/3 kb) and SSCmec V (28 kb). The types of I, II, III are called hospital related strains (HA-MRSA), and the types and subtypes of IV and V are called community related strains (CA-MRSA)[9,19].

I, IV and V types are only resistant to beta-lactam antibiotics, while II and III types are associated with multi-drug resistance. The strains of CA-MRSA in comparison with HA-MRSA were more capable to produce Penton Valentin Leucocidin Toxin (PVL)[29]. The spread of PVL toxin in CA-MRSA strains varies in different countries. This rate was 77% in USA and 5% in Eastern Europe. In some studies, the pneumonia related to PVL production with CA- MRSA strains have been reported with the 37% mortality rate in 48 hours and in some other studies this rate increased to 75%[5,27]. The increase of community acquired MRSA, and also increasing number of hospital acquired MRSA infections were the very current concerns. The SCCmec typing in the method of Multiplex PCR was used not only in differentiation of community and hospital strains, but also as a useful method in monitoring over multi-drug resistant HA-MRSA strains and virulent CA-MRSA strains[4] . Considering the importance of the HA-MRSA and CA-MRSA strains, and concerns about the inconsistent spread of this organism into the communities and hospitals, a SCCmec casette typing has been performed in order to understand better the epidemiology of MRSA in this area of the country[16,19,27].

MATERIALS AND METHODS

This experimental study was performed in a cross-sectional method between April 2013 – April 2015 in Bacteriology laboratory and Research Center of Infectious and Tropical Diseases in 2 military hospitals. The study population consisted of emergency and dialysis outpatients, and those who were referred to hospital wards (like patients of the ICU, surgery, infection, burns wards and etc who were admitted more than 48 hours), and they were hospitalized . From 200 patients, 189 were clinical cases. Cultures obtained from patients were inoculated in nutrient broth and incubated in 37C for 18-24 hours. Then the colonies were examined for positive heat, catalase production, coagulase, hemolysin, DNase, resistance to Bacitracin (4/0 U) and Cefoxitin (30 µg), (Mast diagnostic UK) in order to identify the isolates of MRSA[6,9,24]. These isolates were subsequently examined for genes like femB and mecA by the method of PCR[7,8,12,13].

For identifying resistance to Meticillin, the methods of Disc Agar Diffusion based on the *clinical laboratory standards institute* (CLSI) (2011), Mueller Hinton cultural and Cefoxitin disc (µg30) were used in the temperature of 35C and for a period of 18 hours. The standard strains of *Staphylococcus aureus* ATCC 25923 were used for negative control (Methicillin resistance), and *Staphylococcus aureus* ATCC 33591 as positive control (Methicillin resistance)[9,25,28].

The marker DNA (bp 100 and kb 1) was used to determine the molecular weight. The produced data gained from using descriptive statistics (frequency – percentage and Mean ±SD), and chi- square test with SPSS-18 statistical software. Primers used in this study listed in table 1.

RESULTS

Of the 200 patients, 67% were male and 33% female. The patients were 2 to 88 years of age, and the minimum and maximum admissions were between 1 to 81 days. From 200 clinical cases, 169 *Staphylococcus aureus* were isolated based on biochemical characterization and 100 MRSA *Staphylococcus aureus* were isolate based on resistance to Meticillin and Cefoxitin. The most MRSA isolates rates referred to nasal samples (24%). Of all the hospital wards, the ICU (30%) the highest and ENT (zero percent) accounted for the lowest incidence of MRSA. The method of Diffusion Disc using Cefoxitin Disc comparing with PCR showed 100% sensitivity and 100% specificity. In the Chi-square, there was no significant difference between specificity and sensitivity ($p>0.05$). In this study, 37% of all MRSA isolates carried out *femB* and 73% off all isolated MRSA carried out *mecA* genes (figures 1& 2).

DISCUSSION

Today, due to the misuse of antibiotics, we faced an increasing resistance of them. In Iran also, dissemination of resistant antibiotics to infectious bacteria has been identified as an important challenge for medical communities for treatment of infectious diseases[17,19,23,29]. As *clinical laboratory standards institute* (CLSI) suggested (2011), to confirm the methicillin resistance in the *Staphylococcus*, we could use the sensitive test to the Oxacillin and Methicillin[9,15,18].

In this study, the rate of resistance to methicillin was tested by the method of Diffusion Agar with the Cefoxitin disc (30 µg). Finally 59% of isolates were detected as resistant. Also in PCR method, 59% of strains had mecA gene. In two separate studies performed by Rao Venkatakrishna (2009) and Sangeetha (2012) comparing to PCR method, the sensitivity and specificity rate in Cefoxitin disc method were reported 100%[15,16,26]. *Staphylococcus aureus* was an important acute

Table 1. Primers have been used in this study

Primers	(5"- 3") sequence	Size	source	reference
mecA-F	GTGAAGATATACCAAGTGATT	147 bp	*mecA*	9
mecA-R	ATGCGCTATAGATTGAAAGGAT			
femB-F	CGTGAGAATGATGGCTTTGA	338 bp	*femB*	18
femB-R	TTAATACGCCCATCCATCGT			

Fig. 1. PCR analysis for prevalence of *mecA* gene. Line 1: lader 100pb, line 2: positive control, line 3: negative control, line 4: patient isolate, a 174bp PCR product

Fig. 2. PCR analysis for prevalence of *femb* gene. Line 1: lader 100pb, line 2: positive control, line 3: negative control, line 4: patient isolate, a 338bp PCR product

infectious factor in hospitals and communities, and infections associated with resistant strains to the Methicillin (MRSA) ended up to high mortality rate. Their spread in different countries were like 1% in Norway and Sweden, 5 to 50% in USA[1,5,25], 50% in Iran and more than 80% in India[14,17].

The first MRSA strains (SCCmec I) was reported from England (1960), MRSA (SCCmec II) from Japan, and several years later in 1985 from New Zealand. Several MRSA strains containing SCCmec IV were developed at the beginning of 90s. In 2004, SCCmec V strains were observed in Australia, and finally SCCmec VI and VII reported from Portugal and Taiwan[4,11,10,22].

The results of our study presented that of all the 100 MRSA isolates, 82% were from HA-MRSA groups (I and III types), 3% were CA-MRSA (IVc, Iva, and compound types of III-IVc), and 15% not classified types. The survey of the CA-MRSA types based on the duration of the patients' admission showed that all three isolates were from hospital areas, and the reasons for HA-MRSA hospital isolates in our study were: (1)The inpatients were more than outpatients. (2) MRSA colons might vary in different geographical zones and different hospitals. (3) The misuse of antibiotics in communities and Hospitals might remove the sensitive colons, but would cause the presence of colons that were produced under the pressure of high volume of antibiotics. In our study, 76% were inpatients and 24% outpatients. 90% of the MRSA were selected from inpatients and 10% from outpatients.

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