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Research Article

High Frequency of *icaAD*, clumping factors A/B, *fib* and *eno* Genes in *Staphylococcus aureus* Species Isolated From Wounds in Tehran, Iran during 2012-2013

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Background: *Staphylococcus aureus* (*S. aureus*) isolates have emerged in healthcare and community, and cause a wide spectrum of clinical signs. On the other hand, methicilin-resistant *S. aureus* (MRSA) isolates can resist a wide range of antibiotics, which can make treatment of infections much more difficult.

Objectives: The current study aimed to determine *S. aureus* characteristics isolated from wounds.

Materials and Methods: This definitive and cross-sectional study was conducted in Tehran, Iran. A total of 15 *S. aureus* isolates were collected from wound samples with sterile swabs and were identified by the conventional diagnostic tests. The antibiotic susceptibility pattern was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2012) protocol. The *mecA* gene, Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types, *agr* specific groups and biofilm related genes were detected by Polymerase Chain Reaction (PCR) assay.

Results: The majority of the isolates were resistant to amoxicillin, tetracycline and erythromycin, but all were susceptible to vancomycin and linezolid. Eight (40%) isolates were methicillin resistant and the *mecA* gene was detected in these isolates. The majority of (90%) of MRSA harbored SCC*mec* type III, and two isolates harbored type V. The majority (70%, n = 14) of the isolates belonged to *agr* I, followed by *agr* II (15%, n = 3), *agr* IV (10%, n = 2) and *agr* III (5%, n = 1). The frequencies of *clfAB*, *fnbAB*, *fib*, *eno*, *cna*, *ebps* and *bbp* genes were 100%, 100%, 65% (n = 13), 55% (n = 11), 70% (n = 14), 70% (n = 14), 55% (n = 11), 0% and 0%, respectively.

Conclusions: Half of the isolates were MRSA, the majority of which harbored SCC*mec* type III. Moreover, most of *S. aureus* isolates belonged to *agr*I. The frequency of *icaAD*, *clfAB*, *fib* and *eno* genes were high in *S. aureus* species isolated from wounds.

Keywords: Methicillin-Resistance; Biofilm; *Staphylococcus aureus*

1. Background

Staphylococcus aureus (*S. aureus*) isolates are ubiquitous pathogens that cause a wide spectrum of clinical signs, mild and status to systemic and even fatal infections (1). *Staphylococcus aureus* infections can cause by healthcare or community settings and usually occur in afflict hospitalized and/or immunocompromised individuals (2). MRSA isolates can resist a wide range of antibiotics, which make the treatment of infections much more difficult.

MRSA isolates are resistant to beta-lactam antibiotics via a new Penicillin Binding Protein2a (PBP2a) that confer significantly reduced affinity to beta-lactams (3). The *mecA* gene encoding this PBP is located in SC-C*mec* elements (4). Moreover, MRSA isolates acquired from nosocomial are named HA-MRSA with Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types I, II and III (5), while those acquired from community are called CA- MRSA that harbor SCC*mec* types IV and V. On the other hand, the accessory gene regulator (*agr*) genes play a vital role in the *S. aureus* pathogenesis and therefore, the appreciation of the relationship between these genes and clinical signs may be useful for these genes. Furthermore, *S. aureus* isolates can colonize on skin surface and epithelium of the body via a number of surface-attached and secreted proteins (6-8). Thus, colonized individuals may be at risk of endogenous infections of *S. aureus* entering into the sterile sites of the body via wounds or indwelling medical devices (9). Biofilm formation plays a vital role in chronic and persistent infections caused by *S. aureus*. Biofilm formation takes place through either the *icaAD*BC (via synthesis of a polysaccharide named PIA) genes, microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) or both (10). The *icaAD* genes encode the enzymes assembling the PIA. Enzymes named sor-

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tases are responsible for attaching these components covalently to the peptidoglycan (11) and thus these components contribute to the biofilm formation (12). MSCRAMMs play an indispensable role in triggering of endovascular, bone and joint and prosthetic-device infections (13). These molecules interact with proteins such as collagen (mostly with Cna), fibronectin (mainly via Fn, via FnbAB), fibrinogen (Fib, with ClfAB and Fib) and thus evade the immune system responses and therefore, progress the infection (10, 14, 15). Clumping Factor A (ClfA) protein, for instance, binds to fibrinogen (Fg) and is also responsible for clumping of *S. aureus* in blood plasma, through which culminating in arthritis and endocarditis (16). On this base, detection of various genes related to the pathogenesis and antibiotic resistance is an important cue to insight the capabilities of clinical isolates in different specific sites of infections.

2. Objectives

The current study aimed to characterize isolates of *S. aureus* collected from wound samples.

3. Materials and Methods

3.1. Bacterial Isolates

A total of 15 *S. aureus* wound isolates collected from hospitalized patients were evaluated, inpatients were included (n = 15) and all the outpatients (data not available) were excluded, from July 2012 to January 2013 in Tehran, Iran. Afterwards, the isolates were identified with conventional tests including catalase, coagulases, acid production from mannitol on mannitol salt agar and DNase tests.

3.2. Genomic DNA Extraction

The total genomic DNA was extracted through the preparation of a suspension of bacterial isolates in 200 µL of TE buffer and then with lysostaphin (including 200 µL and 20 µL of lysostaphin [2 µg/mL, Sigma]). The total DNA was isolated according to Straubinger method (17).

3.3. DNA Amplification

DNA was amplified with specific primers previously published to detect *mecA* gene and SCC*mec* types, *agr* genes and several biofilm related genes among the wound isolates (18).

The annealing temperature was 55°C (30 seconds) for *mecA* gene and 51°C (one minute) for SCC*mec* types, according to Zhang study (19). The specific primers for *mecA* gene and SCC*mec* types are shown in Table 1. To observe the PCR products by electrophoresis, 5 µL of each product was blended with 1 µL of each gel red and loading buffer dyes, and were run in 1% agarose gel electrophoresis and was observed by transluminator UV.

3.4. Analysis of Data

The SPSS software and Chi-square were employed to analyze the collected data.

4. Results

4.1. Bacterial Isolates

Nine *S. aureus* isolates were collected from males and eleven from females. The isolates were collected from intensive care unit (45%, n = 9), outpatients included in the study (25%, n = 5), emergency (25%, n = 5) and infectious diseases (5%, n = 1).

4.2. The Antibiotic Susceptibility Test Pattern

The majority of the isolates were resistant to amoxicillin (80%, n = 16), tetracycline (45%, n = 9) and erythromycin (35%, n = 7), but all were susceptible to vancomycin and linezolid. Resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, clindamycin and gentamicin were 30% (n = 6), 15% (n = 3), 20% (n = 4) and 20% (n = 4), respectively.

4.3. Methicillin Resistance

Eight (40%) isolates were methicillin resistant, *S. aureus* (MRSA) with oxacillin, and the *mecA* gene was detected in these isolates. Two MRSA were resistant to all the antibiotics, except to linezolid and vancomycin. Moreover, three isolates were only susceptible to SXT, in addition to vancomycin and linezolid. The AST pattern of MRSA is exhibited in Table 1.

4.4. The SCCmec types

The majority of (90%, n = 18) MRSA harbored SCC*mec* type III, and two isolates harbored type V. MRSA wound isolates with SCC*mec* type III were resistant to a wider spectrum of the antibiotics.

4.5. The agr Specific Groups

The majority (70%, n = 14) of the isolates belonged to *agr* I, followed by *agr* II (15%, n = 3), *agr* IV (10%, n = 2) and *agr* III (5%, n = 1).

4.6. The Frequency of Genes Encoding the MSCRAMMs

The frequencies of *clfAB*, *fnbAB*, *fib*, *eno*, *cna*, *ebps* and *bbp* genes were 100%, 100%, 65% (n = 13), 55% (n = 11), 70% (n = 14), 70% (n = 14), 55% (n = 11), 0% and 0%, respectively. The relationship between these genes and methicillin resistance or other characteristics of *S. aureus* from wound samples was not confirmed.

Table 1. The Antibiotic Susceptibility Pattern and the Characteristics of Methicilin-Resistant *S. aureus* Isolates in the current Study [a,b]

Isolates	**SCC*mec***	*agr*	*clfAB*	*fnbA*	*fnbB*	*eno*	*cna*	*ebps*	*bbp*	**Antibiotic Resistance (Resistance)**
1	III	III	+	+	+	+	+	-	-	CIP, E, GM, T, Amx, CD
2	V	I	+	-	+	+	-	-	-	Amx, T
3	III	I	+	+	-	+	+	-	-	CIP, E, T, Amx, CD
4	III	IV	+	+	-	+	+	-	-	CIP, E, GM, T, Amx, CD
5	III	I	+	+	+	+	+	+	-	CIP, E, GM, T, Amx, CD
6	III	I	+	+	+	-	+	-	-	CIP, E, GM, T, Amx, CD
7	III	I	+	-	-	+	-	-	-	CIP, E, GM, T, Amx, CD, SXT
8	V	II	+	+	-	+	-	-	-	CIP, E, T, Amx

[a] Abbreviations: Amx, amoxicillin; CD, clindamycin; CIP, ciprofloxacin; E, erythromycin; GM, gentamicin; T, tetracycline.

[b] The frequencies of *clfAB*, *fnbAB*, *fib*, *eno*, *cna*, *ebps* and *bbp* genes were 100%, 100%, 65% (n= 13), 55% (n = 11), 70% (n = 14), 70% (n = 14), 55% (n = 11), 0% and 0%, respectively.

5. Discussion

In the current study, the majority of the isolates (90%) were resistant to amoxicillin. This occurs because of plasmid carried beta- lactamases that are capable of transmitting to new isolates more rapidly. Moreover, MRSA isolates had a wider spectrum of antibiotic resistance. All the isolates were susceptible to vancomycin and linezolid; likewise the majority (75%) were susceptible to SXT. These results exhibit that three of the aforementioned antibiotics remain the last resorts to remove these isolates. Several previous studies indicated that vancomycin (glycopeptides) is the last drug to control MRSA. In 2012 and 2013, the prevalence of MRSA in Namazi and Faghihi hospitals of Shiraz were 146 (42.3%) and 199 (57.7%), respectively (20). In the study by Rahimi in 2009, 88% of the collected isolates were MRSA (21). In the study by Ghasemian et al., the inducible clindamycin and methicillin resistance were not high (22). Results of a survey performed by Adebayo were similar to those of the current study, in which he showed that all the isolates were susceptible to vancomycin and linezolid, and higher antibiotic resistance was detected among MRSA strains (23). Similarly, Nitishkumar observed that all the tested isolates were susceptible to vancomycin and the antibiotic resistance was significantly higher in MRSA than MSSA strains (24). These results show that vancomycin and linezolid antibiotics remain among few effective antibiotics to remedy MRSA infections.

In the present study, MRSAs with SCC*mec* type III were significantly more resistant to antibiotics, indicating that these isolates are acquired from hospital (4).Other previous studies reported results similar to those of the current study findings: The study of Japoni in South of Iran reported that the frequency of SCC*mec* type III among MRSA isolates was74.3% (25). Likewise, Fatholahzadeh reported the type III in 78% (26) of the studied isolates. In the study by Ghasemian et al., SCC*mec* type III was reported as the major SCC*mec* type 31 (22). In the study by Reiter, among patients with cystic fibrosis, all harbored SCC*mec* type III (27).

The *agr* groups can regulate the *S. aureus* pathogenesis. It was determined that the majority of the wound isolates belonged to *agr* I. In a previous study, it was determined that the majority of the isolates belonged to *agr* I (28). There was no relationship between antibiotic resistance and *agr* groups. In the survey by Mirzaee, 63 MRSA isolates were collected and twenty nine (46%) of the isolates were strong biofilm producers. Moreover, the *icaD* and *icaC* genes were detected in all isolates, and the prevalence of *icaA* and B were 63% and 51%, respectively (12).

All the isolates in the current study harbored *clfAB* gene. The clumping factors play a critical role in attachment and colonization of *S. aureus* in body surfaces, such as skin surface and epithelium. Similar to our present study, Raphael detected *clfB* in 91.8% of the isolates (29). In the previous studies, the prevalence of these genes was similarly high (30, 31). To the authors` best knowledge, there is scarce previous studies detecting *clfAB* gene prevalence in Iran. Moreover, in the survey by Atshan, all strains harbored *clfA*, *B* genes (32). A study showed that 69.7% of the isolates harbored *clfA, B* genes. However, Momtaz confirmed that nearly 20% of *S. aureus* isolates that caused mastitis contained *clfA* gene (33). These studies suggested that *S. aureus* strains from different infection sites in addition to the epidemiological differences may contain different frequencies or express the clumping factors, essential for colonization. Besides, it was observed that all the MRSA and MSSA strains harbored *clfAB* gene. In the current study the frequencies of *fnbA* and *fnbB* were 65% and 55%, respectively. The gene encoding fibrinogen binding protein (*fib*) was present in 70% of the wound isolates. A study by Bodén detected the *fib* gene in all *S. aureus* strains (34). Similarly, the authors' previous study determined that all clindamycin inducible resistant and vancomycin intermediate *S. aureus* (VISA) isolates contained this gene (22). The prevalence of *eno* and *cna* genes were 70% and 55%, respectively, exhibiting the important role of these genes to colonize *S. aureus*. Since the laminin and collagen constitute most of the proteins in the skin tissue, the protein products of these two genes have numerous receptors to attach to *S. aureus*. Aydiner reported that 78.4% of *S. aureus* isolates harbored *cna* gene (35). In contrast, Arciola showed that the *cna* gene was present in 46% of isolates (36). *ebps* and *bbp* genes were detected in none of the isolates. These two genes contribute to the colonization of *S. aureus* isolates to catheters and medical device surfaces and then culminate in systemic infections such as osteomyelitis bloodstream and other infections. In the study by Paniagua in catheter associated *S. aureus* isolates, for example, the most frequent biofilm related genes included: *clfA*, *clfB*, *cna*, *bbp*, *ebps* and *ica* (65.6 %, n = 21) (37). Similar to the current study, Tang studied various sources and then detected the *bbp* gene in only one *S. aureus* isolate (38). Therefore, several factors affect the frequency of the genes encoding pathogenesis factors and antibiotic resistance in *S. aureus* isolates, including clinical sites, epidemiological differences, the period of study, the locations in which the studies are performed and other factors that there is not no sufficient knowledge about them. The limitations of the current study was the low number of wound samples, the lack of measurement of expression capability of surface protein genes by RT-PCR and low knowledge about the degree of wound infection among patients and also MIC of isolates to vancomycin.

All the isolates and the majority of them were susceptible to vancomycin/linezolid and co-trimoxazole, respectively. The prevalence of MRSA in wound samples was 40% .The majority of MRSA harbored SCC*mec* type III. Moreover, most of the isolates belonged to *agr* I. the majority of the isolates contained *clfAB*, *fib* and *eno* genes responsible for colonization. The difference of wound isolates with other clinical isolates was not significant.

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