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Original Article

Characterization of *Staphylococcus aureus* Isolated From Wound Infectious in Diabetes Clinic of Hazrat Fatemeh Zahra (SA) Hospital

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Abstract

Background: *Staphylococcus aureus* is a gram positive pathogen which causes a wide range of infections. The present study aimed to investigate genotypic and phenotypic screening about biofilm formation in S. aureus isolated from wound infections in a diabetes clinic in Hazrat Fatemeh Zahra (SA) hospital.
Methods: A total of 267 clinical samples were collected from various types of wound infections in the diabetes clinic of Hazrat Fatemeh Zahra (SA) hospital, Isfahan, Iran. The methicillin-resistant *S. aureus* (MRSA) isolates were selected and biofilm formation and its related genes were analyzed by polymerase chain reaction (PCR).
Results: The results showed that 95 out of 132 samples were MRSA. The high resistance was seen to methicillin, erythromycin, ciprofloxacin, and penicillin. Phenotypic results showed that 48.3% of the isolates were high biofilm producers, 29.1% were average biofilm producers, and 10.6% were low biofilm producers.
Conclusions: According to the results of this study, the expression levels of biofilm-associated genes significantly increased, and high prevalence of antibiotic resistance was one of the important reasons for the development of drug resistance in patients.
Keywords: *Staphylococcus aureus*, Methicillin resistance, Biofilm, Multiplex PCR, Iran

Background

Staphylococcus aureus has long been recognized as an important pathogen of hospital acquired infections (1). Due to the expansion of the drug resistance pattern and importance of antibiotic resistance, the study of these microbial strains is one of the major challenges which should be taken into consideration (2).

Methicillin resistance in *S. aureus* is mediated by a penicillin binding protein (PBP2a) which is encoded by the *mecA* gene (2-4). According to previous studies, 50% to 90% of *S. aureus* strains isolated from hospital infections were resistant to methicillin (5,6).

Staphylococcus aureus is the most common cause of skin and soft-tissue infections like impetigo, furunculosis, superficial and surgical wounds and abscess (7-13). PIA is a polysaccharide composed of β1-6-linked N-acetyl glucosamine with partially deacetylate residues, which encloses the human cells or medical tools and protects the microorganism against both host immune system and antibiotic treatment (6). In this study, we investigated effective MSCRAMM-encoding genes, during biofilm formation on Congo Red Agar (CRA) medium and polystyrene plates, via phenotypic and genotypic screening of methicillin resistant *S. aureus* strains isolated from the patients with nosocomial infections in Hazrat Fatemeh Zahra (SA) hospital, Isfahan, Iran.

Methods and Materials

Bacterial Isolation and Culture Conditions

Sample collection and *Staphylococcus* identification were performed in diabetes clinic of Hazrat Fatemeh Zahra (SA) hospital, Isfahan, Iran. A total of 267 clinical samples were selected from various types of wound infections, from which 132 samples were *S. aureus*. The isolates were selected from infectious wounds in patients hospitalized in diabetes clinic of Hazrat Fatemeh Zahra

(SA) hospital**.** The isolates were identified by conventional microbiological methods, such as growth on blood agar, mannitol salt agar, DNase and coagulase tests (9). Finally, The API-20-Staph system kit (bioMérieux, France) was used for confirmation (14-17).

Antibiotic Susceptibility Testing

Biofilm-producing *S. aureus* isolates were selected and the antibiotic resistance pattern was performed by disk diffusion method on Mueller–Hinton agar. *S. aureus* isolates were tested for susceptibility to penicillin (10 µg/disk), imipenem (10 µg/disk), cefazolin (30 µg/disk), cefalotin (30 µg/disk), ceftriaxone (30 µg/disk), gentamicin (10 µg/disk), ciprofloxacin (5 µg/disk), clindamycin (2 µg/disk), azithromycin (15 µg/disk), erythromycin (15 µg/disk), mupirocin (30 µg/disk), rifampicin (5 µg/disk), tetracycline (30 µg/disk), trimethoprim (5 µg/disk), vancomycin (30 µg/disk), methicillin (30 µg/disk), and nitrofurantoin (300 µg/disk) by the Kirby-Bauer disk diffusion method (MAST, Merseyside, England), according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) 2015 (13).

Phenotypic Assay on Slime Production and PCR Amplification

The slime production assay was performed by cultivation of the *S. aureus* strains on CRA plates as described in different studies. No slime producing strains produced pinkish red, smooth colonies with a darkening at the center. Slime producing isolates grow as black colonies and non-slime producers constitute red colonies. These biofilm-producing isolates were selected to determine antibiotic resistance pattern (12,16). The bacterial genomic DNA of *S. aureus* strains were extracted with a plasmid Minikit (QIAGEN, Germany) according to the protocol (18-22). The biofilm genes were determined by specific primers listed in Table 1 (23-26).

Results

In this study, we investigated the *S. aureus* strains isolated from wound infections from the patients in diabetes clinic of Hazrat Fatemeh Zahra (SA) hospital. The antibiotic resistance pattern of isolates was determined by disk diffusion agar method. The results showed that 95 isolates out of 132 samples were methicillin-resistant *S. aureus* (MRSA) (27). All the 95 isolates produced characteristic black colonies on CRA, and were categorized as slime producers. All of the 95 MRSA isolates produced biofilm in different levels. High rate of resistance was observed to methicillin (85.2%), erythromycin (73.8%), ciprofloxacin (86.1%), and penicillin (86.6%). On the contrary, the low rate of resistance was seen to vancomycin (0%) and nitrofurantoin (12%). The phenotypic method showed that 48.3% of the isolates were highly able to produce biofilm, 29.1% were intermediate biofilm producers, and 10.6% of the isolates were low biofilm producers (27).

Frequency of *S. aureus* isolates were detected in different patients most notably in the age range of 61-70 years old. This study demonstrated that *icaC* (73.2%) and *icaD* (49.2%) genes had the highest frequency among

Table 1. Primer Sets of the Genes Involved in Biofilm Formation (26)

Number	Gene	Primer Sequence (5′-3′)	Amplicon Size (bp)
1	*icaA* (intercellular adhesion gene)	(F)-GAGGTAAAGCCAACGCACTC (R)-CCTGTAACCGCACCAAGTTT	151
2	*icaD* (intercellular adhesion gene)	(F)-ACCCAACGCTAAAATCATCG (R)-GCGAAAATGCCCATAGTTTC	211
3	*icaB* (intercellular adhesion gene)	(F)-ATACCGGCGACTGGGTTTAT (R)-TTGCAAATCGTGGGTATGTGT	140
4	*icaC* (intercellular adhesion gene)	(F)-CTTGGGTATTTGCACGCATT (R)-GCAATATCATGCCGACACCT	209
5	*fnbA* (fibronectin binding protein A)	(F)-AAATTGGGAGCAGCATCAGT (R)-GCAGCTGAATTCCCATTTTC	121
6	*fnbB* (fibronectin binding protein B)	(F)-ACGCTCAAGGCGACGGCAAAG (R)-ACCTTCTGCATGACCTTCTGCACCT	197
7	*clfA* (clumping factor A)	(F)-ACCCAGGTTCAGATTCTGGCAGCG (R)-TCGCTGAGTCGGAATCGCTTGCT	165
8	*clfB* (clumping factor B)	(F)-AACTCCAGGGCCGCCGGTTG (R)-CCTGAGTCGCTGTCTGAGCCTGAG	159
9	*fib* (fibrinogen binding protein)	(F)-CGTCAACAGCAGATGCGAGCG (R)-TGCATCAGTTTTCGCTGCTGGTTT	239
10	*ebps* (elastin binding protein)	(F)-GGTGCAGCTGGTGCAATGGGTGT (R)-GCTGCGCCTCCAGCCAAACCT	191
11	*eno* (laminin binding protein)	(F)-TGCCGTAGGTGACGAAGGTGGTT (R)-GCACCGTGTTCGCCTTCGAACT	195
12	*cna* (collagen binding protein)	(F)-AATAGAGGCGCCACGACCGT (R)-GTGCCTTCCCAAACCTTTTGAGCA	165

the genes involved in biofilm formation. The frequency of other genes involved in biofilm formation is shown in Figure 1.

Discussion

The mechanism of biofilm formation in *S. aureus* is weakly understood. However, in fact these genes are associated with both slime production and biofilm formation according to Ammendolia et al study in 1999 and Arciola et al study in 2001 (15,20). Several research have shown that slime production and biofilm formation in *S. aureus* and *S. epidermidis* are associated with the presence of *icaA* and *icaD* genes as described in Arciola et al study in 2001, and Cramton et al and Vasudevan et al studies in 1999 and 2003, respectively (20-22).

In this study, the relative quantification of the expression of these genes in clinical MRSA isolates were made in diabetes clinic of Hazrat Fatemeh Zahra (SA) hospital, Isfahan, Iran. In the study of Resch et al in 2005, biofilm cells were compared with planktonic cells, the results of which showed *ica* gene could be necessarily expressed to form biofilm (23). Likewise, the *icaC* expression had the highest prevalence for the *icaABCD* operon in Beenken et al study in 2004 and Vandecasteele et al study in 2003 in which 62.2% frequency for *icaC* gene was demonstrated (24,25).

However, the present study showed different expression levels of the genes found in 95 biofilm forming strains. Moreover, all of the strains harboring *icaA* (35.3%), *icaD* (49.2%), *icaB (28.1%),* and *icaC* (73.2%) produced slime on CRA, micro plate titration, and genotypic methods. Thus, no association was found between *icaA* and *icaD* genes. Slime production as detected with CRA and micro plate titration is similar to that in the study of Rohde in 2001 (28). Statistical analysis on biofilm formation and frequency of genes showed that there was a significant relationship between biofilm formation by the phenotypic methods and presence of *icaD* and *icaA* genes (*P* value <0.05).

The prevalence of *clfA*, *clfB*, *cna*, and *eno* genes in the tested strains were comparable with those found in *S. aureus* strains isolated from different niches and hosts in various studies. Although we found that the prevalence of biofilm formation was high in human infections, the prevalence of *fnbA*, *fnbB*, and other genes was much higher in the study of Vandecasteele et al in 2003 (25). According to our results, the frequency of *clfA* was 42.5%, which is comparable to Peacock et al study in 2002. They also illustrated that the expression level of the *clfA* gene in the *S. aureus* strains increased 24 hours after growth. Statistical analysis verified a significant relationship between phenotypic and genotypic frequency of the genes (*P* value= 0.0015) (29). PCR test results showed that nine MSCRAMM-encoding genes like *clfA, clfB, fib*, and *fnbA* were detected in all of the strains isolated. Moreover, it

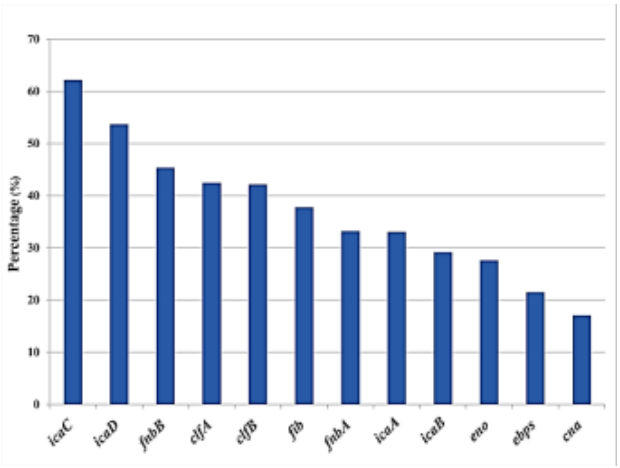

Figure 1. Expression Levels of the Genes Involved in Biofilm Formation

was demonstrated that genotypically different strains of vancomycin-intermediate *S. aureus* (VISA) have different capabilities in slime production (19).

Conclusions

This study mainly tried to investigate the expression of 12 genes involved in biofilm formation in MRSA isolated from wound infections in diabetes clinic of Hazrat Fatemeh Zahra (SA) hospital. According to the study results, the expression of these genes significantly increased. In addition, high prevalence of antibiotic resistance pattern was one of the important reasons for the development of drug resistance in patients.

Conflict of Interests

None.

Acknowledgments

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