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Biofilm formation in Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the important antibiotic resistance pathogen. Chronic hospital acquired infections by *S. aureus* have become a major problem in recent years. One of the main mechanisms for chronic hospital acquired infections is defined by biofilm formation. Therefore, the current research aimed to identify the prevalence of biofilm formation among MRSA clinical isolates in Ilam hospitals and to determine the gene responsible for biofilm formation. For this reason, 26 MRSA clinical isolates were identified and subjected to biofilm by micro-plate assay and PCR for identification of *icaa* and *icaD*. The results demonstrated that 69.2% (n = 18) of MRSA clinical isolates possess *icaD* and were interestingly were negative for *icaa*; while biofilm formation were negative in three isolates that were positive for *icaD*. The current study strongly recommended *icaD* as a main factor to produce the biofilm formation in MRSA clinical isolates and suggested this gene as a target for antibiofilm therapy. The reason for negatively in 2 isolates could be explained in the abstract by low accuracy of micro-late assay. Another reason that could be concluded was silencing of *icaD* in these three isolates.

1. Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the important antibiotic resistance pathogen. There is an increasing in the prevalence of MRSA worldwide. The reports of intensive care units (ICUs) in the United States showed an increase in MRSA from 36% in 1992 to 64.5% in 2003 (Klevens et al., 2006). In Europe, the prevalence ranges from 1 to 50%. Johnson et al. in 2005 reported the increase in the prevalence of MRSA from 2% in 1990 to 43% in 2002 in the United Kingdom (UK), while in the Netherlands the prevalence was low and remained as 5% (Johnson et al., 2005). The morbidity and mortality caused by MRSA infections in the UK increased during the period 1993 to 2002 (Crowcroft and Catchpole, 2002). Infections by MRSA lead to long term of hospitalization and higher costs (Cosgrove et al., 2005). Surveillance programs appear to be necessary, such as the European Antibiotic Resistance Surveillance System (EARSS) that monitors seven most invasive bacteria responsible for antimicrobial resistance (www.earss.rivm.nl). 1n 1993, Australia reported the prevalence of the first MRSA in the community (Udo et al., 1993). This was followed by reports of four MRSA in the community, causing pediatric deaths (Centers for Disease Control and Prevention, 1999). Now, the prevalence of MRSA in the community is reported worldwide (Borer et al., 2002; Aires de Sousa et al., 2005). Thus, there is a change in the epidemiology of community associated and hospital associated MRSA worldwide.

Chronic hospital acquired infections by *S. aureus* have become a major problem in recent years with increasing the use of prosthetic biomedical implants. Chronic infection of a prosthetic implant could serve as a septic focus that able lead to osteomyelitis, acute sepsis, and death, particularly in immunocompromised patients (Heilmann et al., 1998). Bacteria colonize prosthetic implants as a biofilm, multiple layers of sessile cells that adhere to the implant surface as well as to each other. Once a biofilm has formed, it can be very difficult to treat clinically because the bacteria on the interior of the biofilm are well protected from the host immune response as well as antibiotic agents (Hoyle and Costerton, 1991).

Polysaccharide intracellular adhesion (PIA) is regulator of the biofilm formation, which is mediated of cell to cell adhesion and is the gene product of *icaADBC* (Ammendolia et al., 1999). The intercellular

Abbreviations: S. aureus, *Staphylococcus aureus*; MRSA, Methicilin Resistant *S. aureus*; EARSS, European Antibiotic Resistance Surveillance System; ica, the intercellular adhesion.

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adhesion (ica) locus consisting of the *icaADBC* genes that encodes proteins mediating the synthesis of PIA and polysaccharide/adhesin PS/A in staphylococci species (OGara and Humphreys, 2001). Among *ica* genes, *icaC* and *icaD* have been reported to play a significant role in biofilm formation in *S. aureus* and *S. epidermidis* (Yazdani et al., 2006).

Therefore, our goal was to determine the ability of MRSA clinical isolates to produce biofim by evaluation the *icaD* and *icaa*.

2. Methods

2.1. Bacterial isolates

26 MRSA clinical isolates were identified during the period Mar. 2013 to Jan. 2014 in Ilam Hospitals, Iran. *MRSA* clinical isolates were isolated from lesion, sputum, blood stream and urine infections (Table 1).

2.2. Staphylococcus aureus identification

The isolates were cultured on blood agar and incubated for 24 h at 35 °C. Single colonies of each isolates were evaluated by gram staining. Then, Gram-positive cocci were tested for Catalase Tube Test (CTT), oxidase, growth on Monnitol Salt Agar (MSA), and DNase activity (Winn et al., 2006).

2.3. Identification of MRSA

The 0.5 McFarland of *S. aureus* was prepared in Mueller Hinton Broth (MHB). Then, disc diffusion with oxacillin (1 μg) applied to identification of MRSA as phenotypic. Resistant strain subjected for identification of *mecA* by using specific primer.

2.4. Biofilm assay

MRSA clinical isolates were grown overnight in MHB (MH; GibcoBRL) supplemented with 0.25% glucose. Cultures were then diluted 1:200 and incubated overnight in 96 micro-titer plates at 35 °C. Microtiter wells were washed twice with phosphate-buffered saline, dried in an inverted position, and stained with 0.1% crystal violet (Yazdani et al., 2006). Then, plates were incubated at room temperature for 15 min, and after 2 times washing, solubilized in 200 ul of 95% ethanol and then read with ELISA reader at 570 nm wavelength.

Identification of *icaA* and *icaD*.

Total chromosomal DNA of isolates was extracted by using boiling method. All clinical isolates of MRSA were subjected to PCR for *icaA* and *icaD* detection by using specific primers (Table 2).

3. Results

3.1. Staphylococcus aureus identification

Gram-positive, short chain or cluster cocci were studied and further analysis undertaken. Bacteria with catalase positive, oxidase negative, growth on MSA with using mannitol, coagulation positive and DNase activity were considered as *S. aureus*.

Table 1
MRSA collected from different infections in Ilam Hospitals.

Samples	Number	Percent (%)
Urine	4	15.3
Blood	15	57.7
Sputum	1	4
Lesion	6	23
Total	26	100

Table 2
Primers used for determination of biofilm formation.

Gene	Sequence (5′ → 3′)	
icaA	F	ACAGAGGTAAAGCCAACGCA
	R	ACCTGTAACCGCACCAAGTT
icaD	F	TCAAGCCCAGACAGAGGGAAT
	R	CGCGAAAATGCCCATAGTTTC

3.2. MRSA identification

Resistant isolates to oxacillin were subjected for PCR. The PCR results indicated that the *mecA* gene was in all putative MRSA strains, consistent with disc diffusion results. MRSA positive *mecA* showed a distinct band with a size of 574 bp (Fig. 1).

3.3. icaD was responsible for biofilm formation in MRSA clinical isolates from Ilam hospitals, Iran

Our results demonstrated that 69.2% (n = 18) of MRSA clinical isolates possess *icaD* and were interestingly negative for *icaa*. According to biofilm assay results, 61.5% (n = 16) of MRSA clinical isolates produce a thick layer of biofilm.

4. Discussion

Bacterial adhesion factor is considered as a virulence factor that has an important role in to infections associated with catheters and other indwelling medical devices (Francois et al., 1996). The ability of *S. aureus* to colonize in artificial material is associated with two main mechanisms. Firstly, production of polysaccharide slime and secondly, presence of adhesions for the host matrix proteins that are adsorbed onto the biomaterial surface (Montanaro et al., 1998). When the biofilm existed it will be easy to escape from immune systems and cause chronic infections (Cramaton et al., 2001). The current study demonstrated that the majority of *S. aureus* (n = 18) were positive for *icaD*. The *icaD* is considered as a major factor for biofilm formation in MRSA clinical isolates in Ilam hospitals. On the other hand, only 16 MRSA clinical isolates were positive for biofilm formation by micro-plate assay. The reasons for negatively in 2 isolates were low accuracy in the discussion of micro-plate assay. Another reason may be concluded was silencing of *icaD* in these three isolates. Also, our results showed all MRSA clinical isolates were negative for *icaa*.

Study by Arciola et al. (2001) demonstrated that all *S. aureus* biofilm positive isolates were possess *icaD* genes that required for full slime synthesis; this consistent with our results that showed the main role of *icaD* for biofilm formation. Three of biofilm negative isolates were positive for *icaD* that was not consistent with study by De Silva et al. (2002).

The current study strongly recommended *icaD* as a main factor to

Fig. 1. *MecA* gene identification in oxacillin resistant *S. aureus*. Lane M: Marker; lane 1–7, *mecA* gene = 574 bp, lane 8, *S. aureus* ATCC 43300 (MRSA, positive control); lane 9, *S. aureus* ATCC 25923 (MSSA, negative control).

biofilm formation in MRSA clinical isolates and suggested this gene as a target for prevention of biofilm formation.

CRediT authorship contribution statement

Maryam mohamadian collected the samples and done the PCR. Sobhan Ghafourian designs the primers. Nourkhoda sadeghifard done the identification. Iraj pakzad done the electherophoresis, behzad badakhsh wrote the manuscript and edited.

Declaration of competing interest

There is no conflict of interest.

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