

Detection of the intercellular adhesion gene cluster (*ica*) in clinical *Staphylococcus aureus* isolates

Nachweis des interzellulären Adhäsions-Gencluster (*ica*) in klinischen *Staphylococcus aureus*-Isolaten

Abstract

Staphylococcus aureus is a major hospital and community pathogen having the aptitude to cause a wide variety of infections in men. The ability of microorganisms to produce biofilm facilitates them to withstand the host immune response and is recognized as one factor contributing to chronic or persistent infections. It was demonstrated that the *ica*-encoded genes lead to the biosynthesis of polysaccharide intercellular adhesion (PIA) molecules, and may be involved in the accumulation phase of biofilm formation. Different studies have shown the decisive role of the *ica* gene as virulence factors in staphylococcal infections.

This study was carried out to demonstrate the relationship between *ica* gene and production of slime layer in *S. aureus* strains. Sixty *S. aureus* strains were isolated from patients. The isolates were identified morphologically and biochemically following standard laboratory methods. After identification, the staphylococcal isolates were maintained in trypticase soy broth (TSB), to which 15% glycerol was added, and stored at -20°C . Slime formation and biofilm assay was monitored. A PCR assay was developed to identify the presence of *icaD* (intercellular adhesion gene) gene in all isolates. Thirty-nine slime producing colonies with CRA plates (65%) formed black colors, the remaining 21 isolates were pink (35%). In the quantitative biofilm assay 35 (58%) produced biofilm while 25 (42%) isolates did not exhibit this property. All isolates were positive for detection of *icaD* gene by PCR method. The interaction of *icaA* and *icaD* in the investigated isolates may be important in slime layer formation and biofilm phenomena.

We propose PCR detection of the *ica* gene locus as a rapid and effective method to be used for discrimination between potentially virulent and nonvirulent isolates, with implications for therapeutic and preventive measures pertain to the management of colonized indwelling catheters.

Keywords: *Staphylococcus aureus*, biofilm, intercellular adhesion gene, PCR detection

Zusammenfassung

Staphylococcus aureus ist ein wichtiger nosokomialer und community-assoziiertes Krankheitserreger, der verschiedene humane Infektionen verursachen kann. Durch die Fähigkeit von Mikroorganismen zur Biofilmbildung wird ihre Widerstandsfähigkeit gegenüber der Immunabwehr mit der Folge chronischer oder persistierender Infektionen erhöht. Es wurde nachgewiesen, dass durch *ica*-codierende Gene die Biosynthese des interzellulären Polysaccharid-Adhäsins exprimiert wird, das eine Hauptkomponente für die Akkumulation des Biofilms darstellt. In verschiedenen Studien wurde die kritische Rolle der *ica*-Gene als Virulenzfaktor für Staphylokokken-Infektionen nachgewiesen.

Die vorliegende Untersuchung wurde durchgeführt, um den Zusammenhang zwischen dem *ica*-Gen und der Schleimbildung durch *S. aureus*

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zu analysieren. Hierzu wurden 60 *S. aureus* Patientenisolate identifiziert, morphologisch und biochemisch nach Standardmethoden charakterisiert und in Trypticase-Soja-Bouillon (TSB) mit Zusatz von 15% Glycerol bei -20°C aufbewahrt. Die Schleimbildung und Biofilmbildung wurde in einem speziellen Assay detektiert. Mittels eigens entwickelter PCR wurde alle Isolate auf das Vorkommen des *icaD* (intercellular adhesion gene) untersucht. Von den auf CRA-Platten Schleim produzierenden Kolonien waren 39 (65%) schwarz, die anderen 21 (35%) pinkfarben. Im quantitativen Biofilmassay bildeten 35 der Isolate (58%) einen Biofilm. Bei all diesen Isolaten wurde das *icaD*-Gen nachgewiesen.

Das lässt den Schluss zu, dass der PCR-Nachweis des *ica*-Locus als rasche und effektive Methode zur Unterscheidung zwischen potentiell virulenten und avirulenten Isolaten herangezogen werden kann, um die Therapie und Prävention der Biofilmbildung auf Implantaten zu verbessern. Der Synergismus zwischen *icaA* und *icaD* in den untersuchten Isolaten scheint bedeutend für die Schleimbildung und Biofilmmak-kumulation sein.

Schlüsselwörter: *Staphylococcus aureus*, Biofilm, Interzelluläres Adhäsionsgene, PCR Detektion, Adhäsionsfaktor

Introduction

Staphylococcus aureus is an important pathogen causing a wide spectrum of infections [1], [2]. A number of studies have been conducted to explain the structures and pathogenic mechanisms by which *S. aureus* is able to cause serious infections [3], [4]. The ability of *S. aureus* to produce biofilm enables this organisms to withstand the host immune response and is considered to be the cause of chronic or persistent infections, as biofilm creation protects bacteria from opsonophagocytosis and antimicrobial agents [5]. Another concern related to this pathogen is increasing resistance to oxacillin and other antibiotics, but also distribution of multiresistant isolates within the hospital setting [6]. Staphylococcal pathogenesis is multifactorial, involving a combination of adherence and biofilm formation [7]. Complex aggregations of microorganisms can form irreversible attachments to the surfaces and formation of biofilm [8]. Biofilm producing bacteria are the source for persistent or chronic infections [6]. The significance of biofilm production for the virulence of *S. aureus* was supported by a number of clinical and animal studies [9]. Cell aggregation and biofilm accumulation are mediated by the products of a gene locus composing of the genes *icaADB* and *C*, which encode the essential proteins for the production of polysaccharide intercellular adhesion (PIA) and capsular polysaccharide/adhesion (PS/A) in *Staphylococcus spp.* [10], [11]. It was demonstrated that the *ica*-encoded genes are responsible for the biosynthesis of the PIA, which contains *N*-acetylglucosamine as a main constituent and in the accumulation phase of biofilm formation, playing a crucial role in invasiveness of *S. aureus* [1]. Different studies have shown the decisive role of the *ica* gene as virulence factors in staphylococcal infections [4], [11].

In this study, we developed new primers specific for *icaD* and determined further the possible relationship between

icaD gene and producing slime layer in clinical isolates of *S. aureus* strains.

Materials and methods

1. Bacterial isolates and phenotypic identification

Sixty *S. aureus* strains were isolated from patients and used in this study. Isolates were identified morphologically and biochemically by standard laboratory methods. The coagulase and DNase tests were performed for discrimination of *S. aureus* from coagulase negative staphylococci (CoNS). After identification, the staphylococcal isolates were maintained in trypticase soy broth (TSB), to which 15% glycerol was added, and stored at -20°C .

2. Slime forming colony

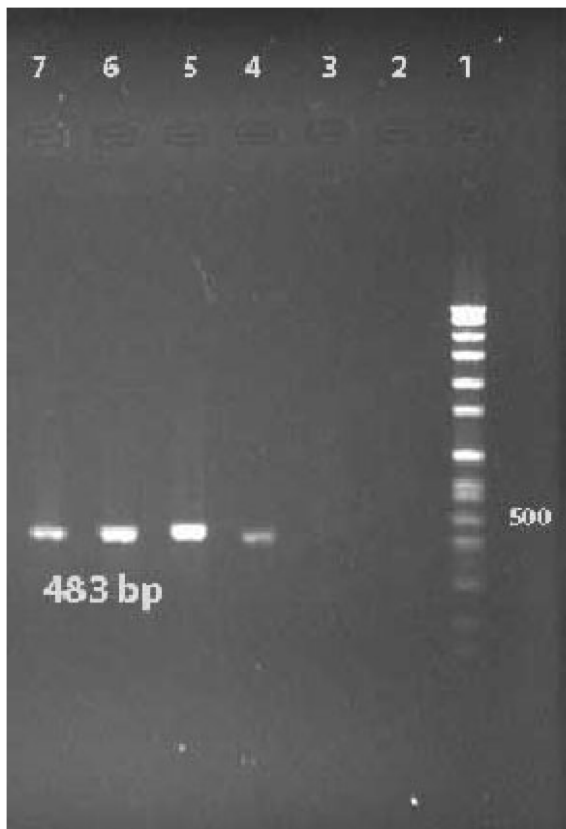
This test was used to evaluate slime formation in *S. aureus* strains on CRA plates, as previously described [10]. Pink colonies were recorded as nonbiofilm producers while the black colonies were recognized as biofilm producers.

3. Biofilm assay

S. aureus strains were incubated in (TSB) at 37°C for 24 hours; grown colonies were diluted in 1:200 and incubated in microtiter plates. After 24 hours the wells were washed with PBS buffer two up to three times and left in the room temperature for drying. In the next step 0.4% crystal violet solution was used as stain for 10 min. Finally the absorbance at 490 nm was determined; an OD of 490 nm >0.12 was regarded as a biofilm positive sample.

4. Primer design and PCR amplification

PCR assay was achieved to detect the presence of *icaD* (intercellular adhesion) gene in all isolates. For preparing DNA, colonies of bacteria were dissolved in 20 µl lysis buffer (0.25% SDS, 0.05N NaOH) and heated at 95 °C for 5 min. In the second step, the lysate was centrifuged and diluted using distilled water. A second centrifugation step for 5 min at 16,000 g was performed to remove the cell debris. Supernatants were frozen at -20 °C until further use. The primer sequences **F: 5' GAA CCG CTT GCC ATG TGT TG 3' (20 bp) & R: 5' GCT TGA CCA TGT TGC GTA ACC 3' (21 bp)** for amplifying a 483 bp of *icaD* gene was designed from the published GenBank sequences (AF086783) with AlleleID 6 Primer Analysis Software. For recognition of *icaD* gene each 25 µl PCR mixture contained 2.5 µM of MgCl₂, 100 µM of each dNTPs, 1U Taq DNA polymerase, and 1 µM of each primer with 200 ng of the DNA sample. PCR amplification was carried out with use of a DNA Thermal cycler (Eppendorf Master cycler personal) with an initial denaturation step (2 min at 95 °C) followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 57.5 °C for 30 s and elongation at 72 °C for 40 s). Steps 2–4 were repeated 29 more times. Finally, 7 µl of PCR mixture was analyzed by 1.5% agarose gel electrophoresis. After electrophoresis, gels were seen under UV light. The Gene Ruler™ 100 bp DNA Ladder Plus (Fermentas, Germany) was used as a DNA ladder.



Lane 1: DNA marker; lane 2: negative control; lane 3: distilled water; lane 4–7: positive samples

Figure 1: PCR results of *ica* gene for *S. aureus*

Results

The investigated 60 strains were isolated from samples of blood catheter (40%), urine catheter (27.5%), sputum (8.3%), wounds (10%), and tracheal tube (14.2%), during the period October 2011 to February 2012. Thirty-nine slime producing colonies with CRA plates (65%) formed black colors and, the remaining 21 were pink (35%). In the quantitative biofilm assay 35 (58%) produced biofilm, while the remaining 25 (42%) isolated did not exhibit this ability. All of these isolates were positive for detection of *icaD* gene by PCR method (Figure 1).

Discussion

S. aureus is responsible for infections in humans. It has been demonstrated that strains having an ability to form biofilm cause additionally “chronic polymer-associated” infection [9], [12], mostly seen as implant-associated infections. Biofilm support the adhesion and colonization of *S. aureus* on surfaces, frequently leading to persistent and difficult to eradicate infections [4], [7]. Indeed, an increasing number of different *S. aureus* adhesion molecules is found [13]. The *ica* operon of *S. aureus* and *S. epidermidis* contain *ica* (ADBC) that allow *Staphylococcus spp.* to form slime layers and biofilm. It was shown that the presence of *icaA* gene in microorganisms yielded from indwelling catheter samples from patients hospitalized for one week was notably frequent. Cramton et al. [14] demonstrated the presence of *icaA* gene in *S. aureus* strains, which was confirmed by Arciola et al. [1] describing 23 *S. aureus* strains isolated from 14 catheter associated infections with the ability to form slime layer based on the presence of the gene *icaA*, but also *icaD*. Indeed, while *icaA* is required to encode N-acetylglucosaminyl-transferase, coexpression of *icaD* can increase the capsular polysaccharide phenotypes [15], indicating a significant role of the *icaD* locus as a virulence factor in the pathogenesis of *S. aureus* isolated from catheters [13], [16].

Infections associated with the use of invasive medical devices, e.g., catheters, are mainly due to *S. aureus*, particularly those strains which create an extracellular slime and parts of the biofilm, making clinical treatment extremely challenging. The process of biofilm formation needs polysaccharide intercellular adhesion, which is synthesized by the enzymes encoded by the intercellular adhesion cluster (*ica*). In this study, the percentage of slime producing strains was 58%, while 65% of these had black colonies on CRA plate. However, all isolates contain *icaD* gene. Our study showed that *S. aureus* isolates had no ability to form biofilm unless they were positive for *icaD* gene. Subsequently, the PCR product was purified from the gel and sequenced (sequence accession no.: JN226155).

Since the presence of adhesion molecules is required for the establishment of an infection, the presence of *ica* adhesion genes may explain the role of the various adhe-

sion mechanisms in the pathogenesis infection associated with in-dwelling medical devices [17], [18]. It can be concluded that infections caused by *ica* locus carrying *S. aureus* strains can lead to clinically difficult to treat conditions. The detection of the *ica* locus in clinical *S. aureus* isolates may improve the clinical decision for treatment and prevention options, and could support development of strategies to interact the bacterial capacity to colonize and invade in-dwelling medical devices. PCR detection of the *ica* operon may be an effective method to differentiate between virulent and non virulent strains. Finally, the synergistic effect of *icaA* and *icaD* genes in the clinical *S. aureus* strains investigated here may be important to further understand slime layer formation and biofilm phenomena.

Notes

Competing interests

The authors declare that they have no competing interests.

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