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Research Article

Molecular detection of virulence factors (adhesion genes) in some *Staphylococcus epidermidis* locally isolated from different clinical sources

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Abstract: The *icaA* and *icaD* genes are found in bacterial cells. The *icaA* and i*caD* genes allow a bacterium to produce biofilm. Biofilms are an important virulence determinant in bacteria because biofilm formation significantly increases resistance to antibiotics and promotes host defenses. The ability of biofilm production can be determined by two phenotypic methods and genotypic methods of some adhesion factors (*icaA* and *icaD*). The phenotypic methods included the Congo-red agar (CRA) and Microtiter plate (Mtp). The results showed that 58 (69%) from 84 isolates were positive for producing biofilm on (CRA) plates and the 55 (65%) from 84 isolates were positive for adhesion and biofilm formation by (Mtp) on the O.D. 490nm. The extraction of DNA is subjected to use for polymerase chain reaction (PCR) which is used to amplify specific genes. The production of viscous layer of *Staphylococcus epidermidis* was investigated by using Molecular analysis (PCR) monoplex to amplify the virulence factor (*icaA, icaD*) genes (adhesion factors) with the amplicon sizes 188 bp and 198 bp, respectively. The results of the genes *icaA and icaD* represented as sixty for (46) 54.8% and thirty (30) 35.7%, respectively. The conclusion to these study detection to *icaA* and *icaD* genesis important virulence factor.

Keywords: *Staphylococcus epidermidis*, Virulence factor genes, Polymorphism.

1. Introduction

Staphylococcus epidermidis is known as a natural colonizer of healthy human skin and mucosa. It is also a common nosocomial pathogen along with other Coagulase Negative Staphylococci (CNS) [1]. *S. epidermidis* is the most common coagulase-negative *Staphylococcus* (CONS). The pathogenesis of these infections depends on the ability of the *S. epidermidis* strain to adhere to the surface by the production of an exopolymer that forms a multilayer composition known as biofilm [2]. Biofilm formation is organized by expression of polysaccharide intracellular adhesion (PIA) which mediates cell to cell adhesion and it is the product of the gene *icaADBC*. The biofilm protects CONS against the action of antibiotics which is managed for the treatment of these infections and against the patient's immune system as well [3]. Biofilms are a population of multilayered cells growing on a surface and being enclosed in exopolysaccharide matrix. Biofilm formation is considered to be a two-step process in which the bacteria first adhere to a surface, followed by a multiplication to form a multilayered biofilm and microbial biofilms are considered to be the major problem posed to catheterized patients because they cause chronic infections which are difficult to treat, lead to longer hospitalization time, and can result in much higher treatment costs [4]. Among *ica* genes, *icaA* and *icaD* have been reported to play a significant role in biofilm formation in *S. epidermidis* [5]. The recent findings point to an important role of the *icaA* and *icaD* due to their ability to strongly produce biofilm in a high percentage of clinical isolates collected from patients with catheters associated infection [6]. Therefore, co-expression of *icaA* with *icaD* can increase biofilm production remarkably [7]. The aim of this study was to detect the presence of *icaA* and *icaD* genes in *S. epidermidis* from clinical sources by phenotypic and genotypic amplification.

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2. Material and Methods

2.1 Sample collection

Eighty-four isolates were obtained from 125 samples taken from clinical sources which were collected from three hospitals in Baghdad include Al-Yarmouk General Teaching Hospital, Al-Karama Teaching Hospital, Ibn Al-Balady Teaching Hospital. These samples included (wound, ear swab, burn swab and urine). All samples were cultured on blood agar and incubated at 37ºC for 24 hours, and then on mannitol salt agar differential medium, *S. epidermidis* does not perform a color change. All the isolates were identified on the basis of colony morphology, biochemical test catalase and coagulase test. Then identified using a commercial biotyping system (Api STAPH, bioMerieux, Inc., Hazelwood, MO) [8, 9, 10].

The strains were stored in brain heart Infusion broth (BHIB) with 20% of glycerol at-20°C. The working cultures of the isolates were prepared in BHIB at 37°C for 18 h. Chromosomal DNA from the *S. epidermidis* strains isolated from different samples was extracted.

2.2 Detection of the bacterial ability to adhere and produce slime layer

2.2.1 Qualitative adherence assay

This method is used to detect the bacterial ability to adhere and produce the slim layer. This is performed by culturing the bacterial colony on the Congo red agar plate, the black colonies indicate highly production of a slime; the red colonies is a sign of negative result [11].

2.2.2 Quantitative adherence assay

Quantitative biofilm production is determined using a quantitative adherence assay. The optical density of each well was measured at 490nm (OD 490) using an ELISA reader and compared with the negative control [12].

2.3 DNA extraction

DNA extraction using Genomic DNA extraction Kit, concentration and purity were determined using NanoDrop 1000 spectrophotometer at 260/280nm.

2.4 Detection of *icaA & icaD* genes

Staphylococcus epidermidis isolates were investigated for the presence of virulence factor genes (*icaA* and *icaD*). PCR is used for amplifying (*icaA*) and (*icaD*) genes using specific primers. The sequence of primer used for amplification of *icaA* gen forward 5-TCTCTTGCAGGAGCAATCAA -3, Reverse 5-TCAGGCACTAACATCCAGCA -3 the band size (188pb) and the sequence of primer used for amplification of *icaD* gen forward 5-ATGGTCAAGCCCAGACAGAG -3, Reverse 5-CGTGTTTTCAACATTTAATGCAA -3 the band size (198pb). The volume of PCR reaction is 20µl reaction mixture. It has been found that the best size of the DNA template is 2µl. 1µl of each forward primer and reverse primer of (*icaA*), and 1µl of each forward primer and reverse primer of (*icaD*) with a concentration of 10pmol/µl of each of them separated in there monoplex PCR. In the steps of PCR reaction, temperatures 94°C for (*icaA*), 95°C for (*icaD*) is considered as the beginning of the interaction of (initial denaturation) for a single session. When the product was migrated electrically, the best glow under ultraviolet light is a product reaction containing the annealing temperatures 55°C for (*icaA*) and 59.5°C for (*icaD*), while other temperatures are not enough to get the required glow. Therefore, these temperatures are adopted to get the best results in several experiments. PCR products of (*icaA*) and (*icaD*) genes were analyzed by electrophoresis on a 2% agarose gel.

3. Results and Discussion

A total number that obtains of *icaA* and *icaD* were (46, 30), respectively. The isolates that were obtained from 84 samples collected from clinical sources included wound swab, burn swab, ear swab and urine. All samples were cultured on blood agar and incubated at 37ºC for 24 hours. According to the preliminary results, Table [1] showed the number of samples contains *icaA* and *icaD* genes of *S. epidermidis* from clinical sources.

Table 1. Numbers of samples contains *icaA* and *icaD* genes of *S. epidermidis* from clinical sources.

Sample source	Ratio of sample collected	*icaA*	*icaD*
Wound	42	23	18
Ear swab	30	19	8
Urine	9	3	3
Burn	3	1	1
Total	84	46	30

3.1 Demographic distribution of *icaA* and *icaD* genes in terms of Sample origin

The results reported that from (125) clinical samples, (84) isolates were positive for *Staphylococcus epidermidis.* Several biochemical tests were carried out to identify the *S. epidermidis.* All Gram-positive isolates gave positive results in the Catalase tests. The positive reaction indicated the liberation of free oxygen as gas bubbles after mixing of hydrogen peroxide solution with a little amount of bacterial growth [13]. In order to support the previous biochemical test, coagulase-negative and detect the ability produce of biofilm by growth on Congo red agar and biofilm producers form black colonies on CRA, whereas non-producers form red colonies [14]. From the total number (84) *Staphylococcus epidermidis* clinical isolates that studied had shown a high percentage of 69% isolates that show 58 of 84 *S. epidermidis* isolates to produce slime positive (Fig. 1.1); This results

compatible with the study that found 65% *S. epidermidis* are slime producers on CRA [15]. The results of Automated Multiscan reader at 490nm wavelength showed 55 of 84 (65%) of *S. epidermidis* are of adhesion and biofilm formation –positive as the (OD490 ≥ 1.2) means a highly adhesion and biofilm formation; (0.6 ≤ OD490 < 1) means a low-grade adhesion and biofilm formation and (OD490 < 0.6) were adhesion and biofilm formation –negative. This result is compatible with the study which found 66% *S. epidermidis* were adhesion and biofilm formation –positive [16]. The results of PCR to *icaA* gene presented in 46 (54.8%) of DNA *S. epidermidis* isolates with a PCR product size 188 bp (Fig. 1.2). PCR results showed that *icaD* gene was presented in 30 (35.7%) of DNA *S. epidermidis* isolates with a PCR product size 198 bp (Fig. 1.3).

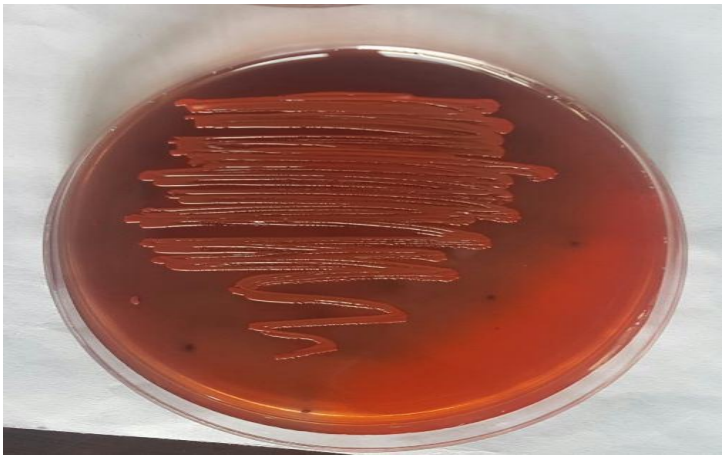

Fig. 1.1. Slime layer producing *S. epidermidis* colonies on the Congo red agar.

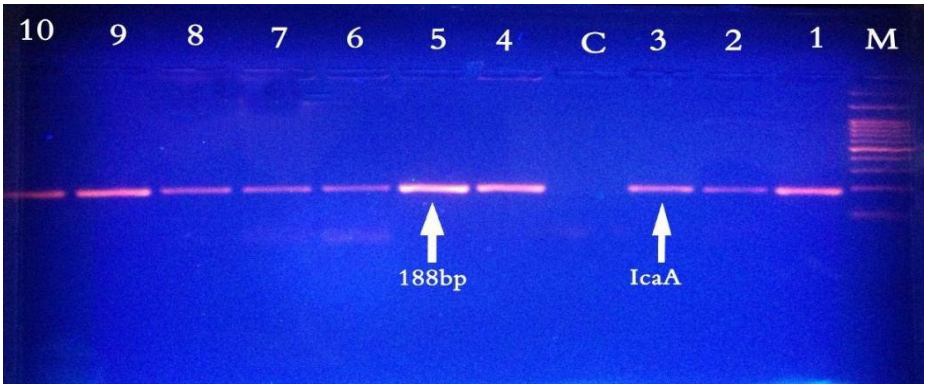

Fig. 1.2. Agarose gel electrophoresis (2% agarose, 5 V/cm^2) PCR results with primer for *icaA* gene. M: Molecular size marker; C-: control negative; lane 1-10 (188 bp) band obtained with DNA from *icaA* gene.

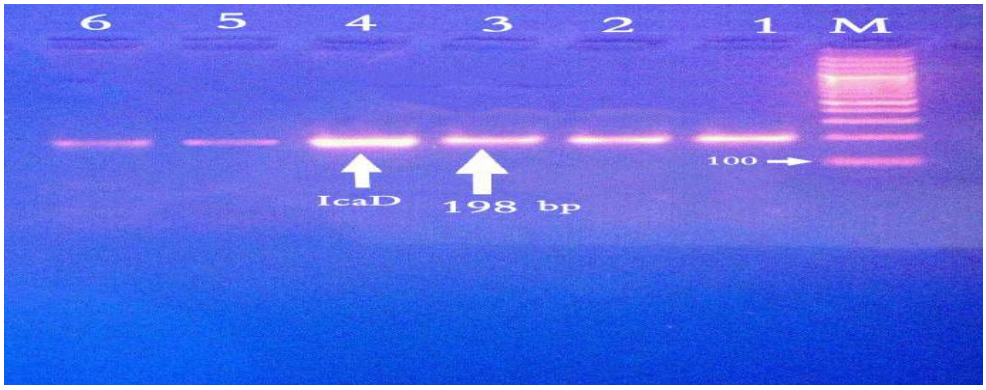

Fig. 1.3. Agarose gel electrophoresis (2% agarose, 5 V/cm^2) PCR results with primer for *icaD* gene. M: Molecular size marker; lane 1-6 (198 bp) band obtained with DNA from *icaD* gene.

The results of phenotypic methods (CRA and MTP) show that the ability of *S. epidermidis* to produce slime is not associated with the presence of *icaA* and *icaD* genes may contain other *ica* operon like *icaB* or *icaC* [17]. In *S. epidermidis*, the *ica* operon show to play an important role in biofilm formation, and also in the pathogenesis of infections associated with inhabitance or implanted medical devices [7, 18, 19, 20, 21]. The expression of the *ica* genes, and as a result the formation of biofilms, seems to be highly variable among staphylococci. Thus, biofilm formation is influenced by the environmental signals and it can be induced in response to external stress and subinhibitory concentrations of certain antibiotics [22, 23, 24, 25]. The *icaD* have been reported to play a significant role in biofilm formation in *S. epidermidis* since their expression is linked with the production of Polysaccharide intracellular adhesion (PIA) is the most characterized component of staphylococcal biofilms; there for some sample have low expression of PIA [26].

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