

Detection of Adhesion Genes and Slim Production among *Staphylococcus Aureus* and *Staphylococcus Epidermidis* Isolated from Hemodialysis Patients

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Abstract:

The presence of the ica loci and adhesins genes in clinical *Staphylococcus aureus* and *Staphylococcus epidermidis* strains were considered important factors of virulence. In this study, one isolate of *S. epidermidis* and six isolates of *S. aureus* were isolated from cases of septicemia in hemodialysis patients and were investigated for slime production using Congo red agar methods (CRA). Biofilm production of *S. epidermidis* and *S. aureus* by using (CRA) plate revealed that all seven isolates were slime producers.

In order to detect the adhesion genes (clf A, fnb A and cna) and presence of slime genes (ica A and ica D) genes polymerase chain reaction was used. All seven isolates were ica A and ica D positive. While the (clf A, fnb A and cna) were positive in *S. aureus* and negative in *S. epidermidis*. This study confirms the presence of clf A, fnb A and can and ica A/D genes in the majority studies *S. aureus* strains isolates from different sites of infection.

Keywords: *Staphylococcus aureus*, *S. epidermidis*, Biofilm production, Adhesion genes

1. Introduction

Staphylococcus aureus is prevalent human pathogen of increasing concern to public health worldwide. This pathogen is one of the leading causes of hospital-acquired infection, and additionally leads to significant level levels, of infection via community transmission (Van Belkum *et al.*, 2009).

Staphylococcus epidermidis are opportunistic pathogens that mainly depend on the presence of indwelling foreign bodies to express then pathogenic capacities (Gotz, 2002).

Both *S. epidermidis* and *S. aureus* are concerned polysaccharide intracellular adhesion (PIA, also called biofilm). PIA is one of the most important virulence determinants that facilitate to adherence and colonization of bacteria (Daran *et al.*, 2010).

Dialysis patients are at increased risk for infection with *staphylococci* that often occurs in patients with kidney disease, these bacteria are much more likely to invade the body.

Once these bacteria have access to the blood stream, they frequently spread to bones, Joints and the heart causing potentially lethal destruction of those tissues (Chigbu and Ezeronye, 2003).

The PIA is the most important component of the staphylococcal slime and is encoded by the ice ADABC operon (Mack *et al.*, 1994, Heilmann *et al.*, 1996).

PIN synthesis is closely related with the expression of ica A and ica D genes. the main function of ica A is that responsible for the synthesis of the N - acetyl -D - glucosamine polymer structure ica A expression in collaboration with ica D provides a significant increase in enzymatic activity. The capsular polysaccharide occur as result of this co-expression of ica A with ica D (Maira -Litran *et al.*, 2002).

During adhesion, nonspecific physio -chemical interaction is followed by a more specific adhesion - mediated phase (Vandecasteele *et al.*, 2003).

Clumping factor A (CIFA) is fibrinogen - binding surface protein of *S. aureus* that is important virulence factor in several infection models (Mc Deevitt *et al.*, 1997).

It is possible that CIFA acts as virulence factor in certain infections by inhibiting phagocytosis, as well as promoting adhesion to fibrin and fibrinogen (Higgins *et al.*, 2006). It was reported that some adhesions such as fnb A (fibronectin -binding proteins), CIFA (fibrinogen - binding proteins) and Can (collagen - binding protein) can affect the bacterial binding to surface of the host.

Cif A, can, fnb A proteins included in various infections have been found to play an important role in the pathogenesis of various infections. Can, fnb A protein, have been demonstrated to contribute to tissues colonization such as medical device -related infections (Duran *et al.*, 2010). Molecular methods are the most convenient techniques for the detection of the slime and adhesion producing strains (Arciole *et al.*, 2001). The determination of the slime and adhesion genes is often preferred to PCR -based molecular methods in the various studies.

2. Aims of the study:

To investigate some adhesion genes (CIF A, fnb A, can) and presence of slime genes (ica A and ica D genes) in both *S. aureus* and *S. epidermidis* strains isolated form blood samples of hemodialysis patients.

3. Material and Methods:

Samples: Blood samples were collected from (60) patients suffering from kidney disease in hemodialysis department of Murjan hospital – Hilla – Iraq, during the period (10 /2012 -1/2013). Only right is dates of *S. epidermidis* and fourteen is dates of *S. aureus* were obtained from all samples by standard bacteriological methods. All samples were collected by Brain – heart infusion broth and plated on to blood agar, and incubated aerobically at 37°C overnight. Isolates were identified to the species level based on the standards biochemical and microbiological methods (McFadden, 2000).

Slime Production: The presence of slime production of all staphylococcal isolates was evaluated by Congo red agar method phenotypically as described by Freeman et al., (1989). Congo red medium contained the following agents: 37 g/l brain heart infusion broth, 50 g/l sucrose, 10 g/l agar and 0.8 g/l Congo red .Bacterial strains (*S. aureus* and *S. epidermidis*) were cultured on to Congo red agar (CRA). The assay plates were incubated at 37°C for 24 hrs. All plates were examined in terms color changes after 24 to 48 hrs. of incubation. A black discoloration of the colony was interpret as a position test result . When non –slim producing bacteria grew in culture plate, the color of the bacterial colonies did not change CRA plate cultures were evaluated by two independent observers for the detection of slime synthesis.

DNA extraction for Gram positive bacteria: DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Geneaid).

Detection of Adhesion and Slim genes: The primers and PCR condition used to amplify genes encoding Adhesion and Slime with PCR one listed in table (1). The primers for adhesion includes (clf A, fnb A and cna), as well as the primer specific for Slime includes (ica A and ica D). Each 25 ML of PCR reaction contained 2.5ML of each upstream and downstream primer, 2.5 ML of free nuclease water, 5ML of DNA extraction and 12.5 ML master mix. The PCR amplification product was visualized by electrophoresis on 1% agarose gel for 30 min at 100 v. The size of amplicon were determined by comparison to the 100 bp allelic ladder (promega USA).

4. Results

In present study, both the presence of Slime genes (ica A and ica D) and phenotypically Slime production on Congo red agar were investigated in all staphylococcal strains. To . searched in one strain of *S. epidermidis* and six strains of *S. aureus* that give positive result in CRA determine the expected bp lengths (381 for the ica D, 1315 for the ica A bp), DNA leader with defined molecular weight in the range 100 to 1500 were used.

The ica A and ica D positively were determined in total *S. epidermidis* and *S. aureus* (100%).Which in this study the detection of adhesions genes fin A , cna , and clf revealed that these genes not present in *S. epidermidis* and present (100%) in all six strains of *S. aureus*.

5. Discussion

S. aureus and *S. epidermidis* are opportunistic pathogens that mainly depend on the presence of indwelling foreign bodies to express their pathogenic capacities. They are the most common causes of septicemia in hemodialysis patients. Among the 22 strain of staphylococci 7 strains (one *S. epidermidis* and six *S. aureus*) were ica A / ica D positive strains, these seven strains were positive to CRA method forming black colonies and 15 strain were CRA negative . These differences could be due to the fact that ica A /ica D expression is subjected to environmental conditions (Cramton *et al.*, 2001, Zmantar *et al.*, 2006). Slime production and adhesion are considered to be a crucial virulence factor among the staphylococci presence of slime and adhesions genes shown to be exacerbation of keratitis (Jett and Gilmore, 2002), endocarditis in hemodialysis patients (Robinson, *et al.*, 1997). Limited numbers of studies have been carried out on adhesion and Slime genes of both *S. epidermidis* and *S. aureus* is dated form blood samples of hemodialysis patients. Biofilm formation by both *S. epidermidis* and *S. aureus* considered one of the most virulence factors of this bacteria *S. aureus* recognized as the most frequent causes of biofilm – associated infection to large variety of matrix components to initiate colonization, (Otto, 2008). In the present study the occurrence of Slime genes and Slime production in staphylococcal of this study is Similar to that detected by Arciola *et al.*, (2003). The adherence of staphylococci is frequently mediated by MSCRAMM (microbial) surface components recognizing adhesive matrix molecules .The collagen – binding protein fibrinectin – binding proteins and fibrinogen – binding protein belong to this family (O’Neill *et al.*, 2009). The specific surface proteins (fnb A, clf A, cna) are expensed mainly by *S. aureus* strains (Foster and Mc Devitt, 1994). These specific surface proteins provide the specific interaction between bacteria and extracellular matrix proteins of the host cells. As a result they contribute to bacterial colorization (Zmantar *et al.*, 2008).

The bacterial adhesion thought to be an important step in the beginning of the infections. can, fnb A ,and clf A were the most important staphylococcal adhesions (Arciola *et al.*, 2005a). It has been reported that can gene was

found to express in more than 88% among *S. aureus* (Duran *et al.*, 2010). Fnb A has been reported in a high proportion among the staphylococcal strains is dated from various clinical infections (high than 95%) (Rice *et al.*, 2001). Clf A prevents phagocytosis during bacterial infection. (Higgins *et al.*, 2006) in conclusion presence of Adhesion and Slime gene complicates the healing for different types of infections.

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Table (1): primers sequences and PCR conditions to detect adhesion and Slime genes

Genes	Primer sequence (5' -3')
Clf A F	CCGGATCCGTCAGATGACC
Clf A R	GCTCTAGATCACTCATCAGGTTGTTTCAGG
Fnb A F	GATACAAACCCAGGTGGTGG
Fnb A R	TGTGCTTGACCATGCTCTTC
Can F	AAAGCGTTGCCTAGTGGAGA
Can R	AGTGCCTTCCCAAACCTTTT
Ica A F	CCTAACTAACGAAAGGTAG
Ica A R	AAGATATAGCGATAAGTGC
Ica D F	AAACGTAAGAGAGGTGG
Ica D R	GGCAATATGATCAAGATAC

Table (2): Company of primers is alpha (USA).

Size of product	PCR Condition	Reference
1000	94 c 5 min 1 x	14
	94 c 1min 55 c 1 min 72 c 1 min	
	72 c 10 min	
	Same condition	
191	Same condition	14
192	Same condition	14
1315	94 c 4min 1 x	14
	94 c 45 s 52 c 30 s 72 c 1 min	
	72 c 7 min	
	Same condition	
381	Same condition	14

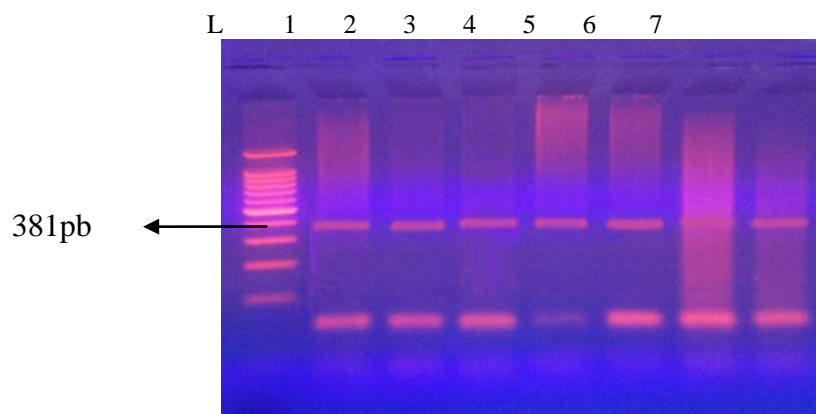


Figure (1) gel electrophoresis of PCR amplification of *icaD*, Lane 1500 pb ladder; 1, *S. epidermidis* , 2, 3,4, 5, 6, 7:No. of isolates of *S. arueus*

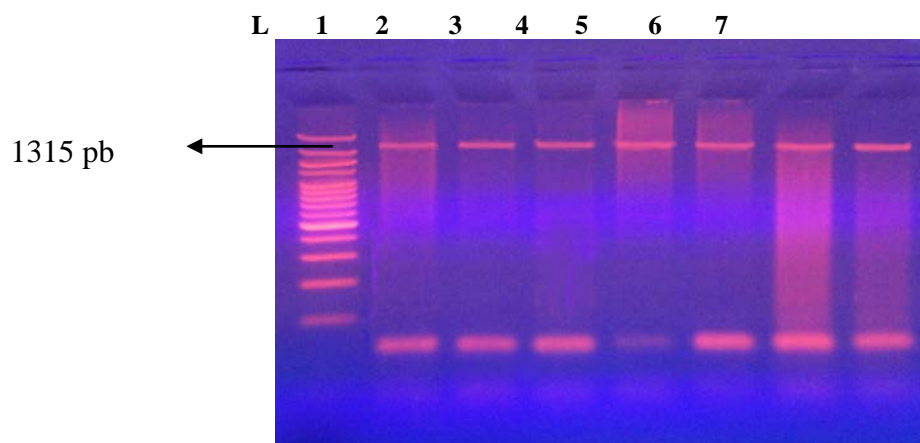


Figure (2) gel electrophoresis of PCR amplification of *icaA*, Lane 1500 pb ladder; 1, *S. epidermidis* , 2, 3,4, 5, 6, 7:No. of isolates of *S. arueus*

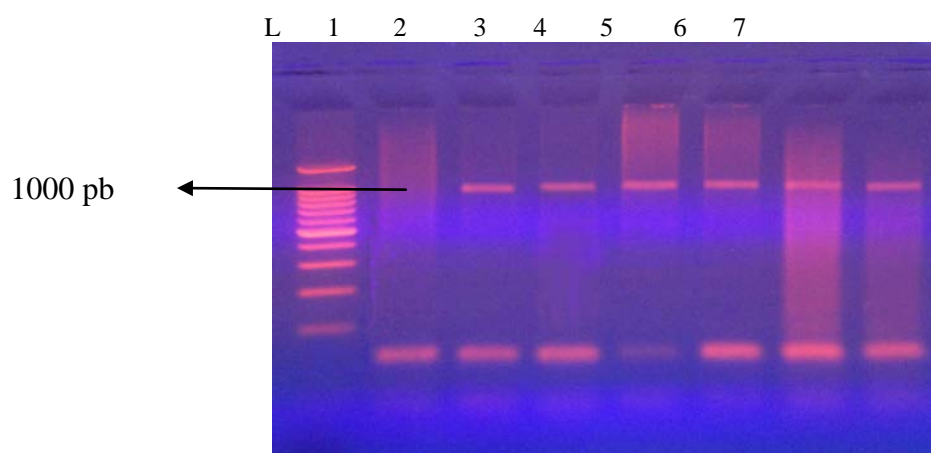


Figure (3) gel electrophoresis of PCR amplification of *clfA*, Lane 1500 pb ladder; 1, *S. epidermidis* , 2, 3,4, 5, 6, 7:No. of isolates of *S. arueus*

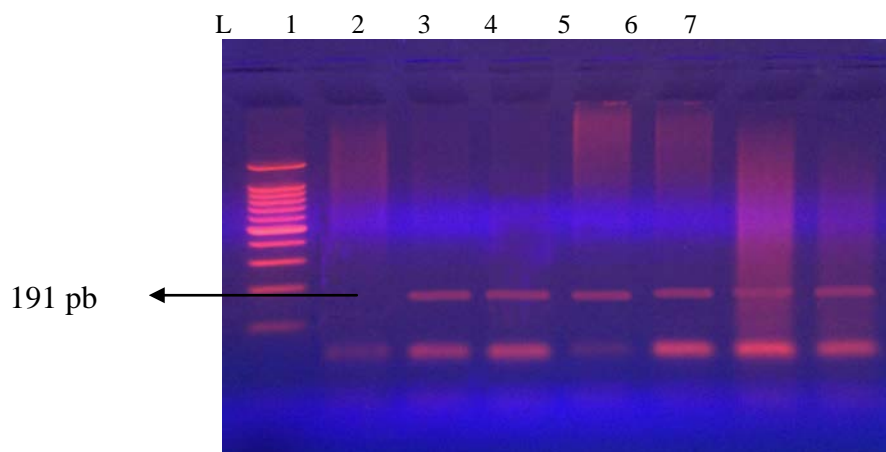


Figure (4) gel electrophoresis of PCR amplification of *fnbA*, Lane 1500 pb ladder; 1, *S. epidermidis* , 2, 3,4, 5, 6, 7:No. of isolates of *S. aureus*

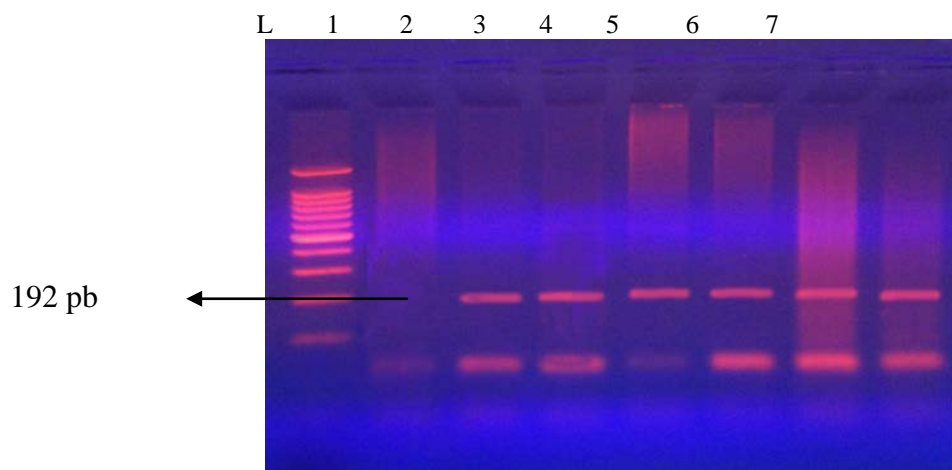


Figure (5) gel electrophoresis of PCR amplification of *cna*, Lane 1500 pb ladder; 1, *S. epidermidis* , 2, 3,4, 5, 6, 7:No. of isolates of *S. aureus*

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