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Detection of intracellular adhesion (*ica*) B gene in *Staphylococcus aureus* isolated from wound infections

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Abstract

Background:*Staphylococcus aureus* is an imperative cause of community acquired infections and nosocomial infections,The formation of biofilm on host surfaces and adherence is painstaking to be significant virulence factor in *S. aureus*.

Objective: The main objective of this study is to detect and describe the distribution of *S. aureus* and ***ica***B gene in wound infection samples taken from patients at Al-Hussein Teaching Hospital in Thi-Qar province, Iraq, during the period extended from February to September 2007.

Materials and Methods:A total number of bacterial isolatesincluded 130 sample recovered from different woundsof patients in burn unit. All isolates of the targeted bacteria were subjected for screening the biofilm allied gene (*ica*B) by Polymerase Chain Reaction (PCR) technique.

Results: The recent data indicated that 72 (55.3%) identified as *S. aureus*, and the females were likely more affected with *S. aureus* infection than males (47 > 25). The results of PCR recorded that *ica*B gene was expressedonly in 61% of all isolates.

Conclusion: The recent results recorded a high percentage of *ica*B gene which related with biofilm production may be increased the pathogenicity of this pathogen which caused different human diseases.

Keywords: *S. aureus*, PCR, *ica*B.

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Introduction:

Staphylococcus aureus is an imperative causeof community acquired infectionsand nosocomial infections,and it caused a surfeit of diseases likepneumonia, skin, soft tissue infections, and blood stream infections[1].one of the important virulence factors of *S. aureus* is a strain of staphylococci characterized by different levels of virulence. The virulence of this type of bacteria is recognized by its ability to form biofilms. Biofilm is a functional factor necessary for the attachment of these bacteria to the surfaces and their embedding in various extracellular polymeric substances. Intracellular adhesion locus (ica) is considered as a group of final biofilm product of that produced by *S. aureus* which involves complex mechanism[2].

The formation of biofilm on host surfaces and adherence is painstaking to be significant virulence factor in *S. aureus*[3] that controlled by various virulence factors expressed during *Staph. aureus* nosocomial infections and also by genes encoding antibiotic resistance that frequently presented. Cramton *et al*. [3]documented that the intracellular adhesion locus (*ica*) is required for biofilm production, also whom suggest that the *ica* locus could potentially be an important target in the therapy of implant infections[4], and approximately 60% of *S. aureus* strains were produced the biofilm[5]. While, contradictory the study performed by Rohde *et al*. [6] suggested that all *S. aureus* strains possess *ica*ADBC genes. The first identification of *ica*ADBC operon in *Staph. epidermidis* followed by showing to be current in *S. aureus*[7]. Although Arciola *et al*.[8] reported the greatest of *S. aureus*isolates appear to contain this operon, and *ica*ADBC operon is consist of the four genes including: *ica*A, *ica*B, *ica*C and *ica*D[9]. The formation of biofilm is influenced by amount of factors, and the synthesis of the polysaccharide intercellular adhesion (PIA) by the bacteria is the most important factors that increase the bacterial virulence in addition to their support to the survival of Staph. areus in a variety of environments7. Also icaADBC genes encodes for the synthesis of PIA[10]. A *ica*B is the deacetylase responsible for the de-acetylation of mature PIA and the trans-membrane protein[11]. The operon expression augmented under anaerobic internal environment of biofilm[12].The aim of this study was to investigatethe frequency of the biofilm producing related gene (*ica*B) in *S. aureus* isolates originated from wound infections.

Material and Methods:

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Ethical approval This research was approved by the Medical College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Type of study: Cross sectional descriptive study extend all over February to September 2007**.** In total, 130 bacterial isolates were taken from patients hospitalized in the burn unit at Al-Hussein Teaching Hospital in Thi-Qar from February to September 2007. All specimens were taken from wounds and the bacteria were maintained in a sterile transport medium (Amies transport medium) used for bacterial isolation.

Laboratory methods

All strains of *S. aureus* were isolated from 130 swabs which collected from burn wound infections identified depending on cultural properties on different culturing media, followed by biochemical tests[13]. The confirmed diagnosis was performed by using Analytical Profile Index system Staph (API) (BioMerieux/France).

Preparation of bacterial DNA

Genomic DNA was extracted from all isolates byusing Genomic DNA Extraction kit (Geneaid/Korea).

PCR Protocol

All isolates of *S. aureus* (72/130) were subjected to amplification of *icaB*gene.The specific primer pairs of *ica*Bas following: forward: 5'-CCC AAC GCT AAA ATC ATC GC-3' and reverse: 5'-ATT GGA GTT CGG AGT GAC TGC-3'. The PCR cycling conditions of currentgene: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min and final extension for 9 min[14].

Results

Among 130 of bacterial isolates taken from wound infections of burn patients, only 72 (55.38%) were positive for *S. aureus* recovered from all bacterial isolates. Also, it was detected that only 47 out of 71 bacterial isolates was positive for *S. aureus* in female with a percentage of was 65.3%. However, this was less in males, 25 out of 59 with a percentage of 34.7% as shown in table 1.

The PCR results of *ica* B gene were detected and recognized by agarose gel electrophoresis that measured by bands of size 1080 bp as presented in figure 1.

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Table (1): Percentage of *S. aureus* isolated recovered from bacterial isolates taken from wounds of patients of burn infection among males and females.

Gender	No	No. of S. *aureus* (+ve)
Male	**59**	**25 (34.7%)**
Female	**71**	**47 (65.3%)**
Total	**130**	**72**

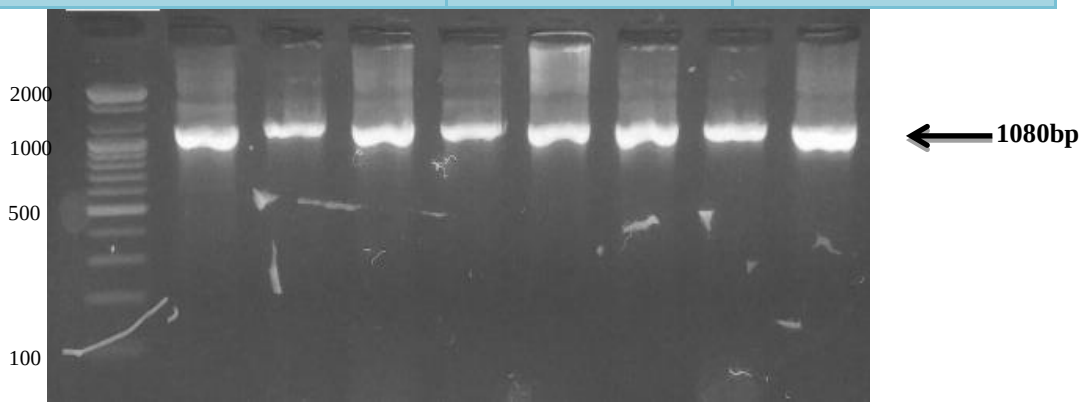

Fig. (1): Agarose gel electrophoresis of *ica* Bgene amplification ligated PCR used a 1.4% agarose gel,Lane M:DNA ladderLanes, 1-8: These bands of size 1080 bp represent the positive results of amplified*ica* B gene

Discussion:

The results of the recent study is showed that 72 of bacterial isolates (55.38 %) were characterized as *S. aureus* from all collected samples. The *S. aureus* is one of the most significant causes of wound infections. Fitzpatrick *et al.*[15] recorded that *staphylococcus* spp. which produced the biofilm had become more predominant as cause of the hospital-acquired infection.The results of current study are analogous with results of Alwashand Saleh[23], described the occurrence of *S. aureus* in burns was 33.3%.

The results of present study documented that the females were likely more affected with *S. aureus* infection than males (47 > 25). The current results were in agreement with the results of Rajput *et al.*,[25]indicated that burn infection in females was (60%). While the same results of the present work were dissimilar to study that conducted in Iraq by Abid, [24]showed that males tend to be more infected with *S. aureus* than women with 52.2%.

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The occurrence of the biofilm allied gene (*ica*B) amongst entirely a *S. aureus* isolates showed that *ica*B gene was expressed in 61% of the bacterial isolates. The adherence on surfaces and biofilm formation on wounds is associated with increased the pathogenesis of *S. aureus* according to previous studies which proved this truth[16], and the biofilms help microbes to survive hostile environments such as antibiotics and the host immune response[16,17]. Also the initial detection of biofilm formation by*Staphylococcus* must be the crucial stepsin the direction ofthe prevention of nosocomial infections[18].

S. aureus had the ability to produce the biofilm on an inert or living surface[19,20]; its capacity which supportthis pathogen for causing the infection and severe morbidity[21].

The current results incorporated with results of the study performed by Mirzaee*et al*.[22] recorded that the prevalence of *ica*Bgene in Methicillin resistant *S. aureus*(MRSA) isolates was 51%.

Conclusion:

The recent data recorded a high percentage of *ica*B gene which associated with biofilm production may be increased the pathogenicity of this pathogen which caused different human diseases.

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الكشف عن وجود جين الألتصاق الخلوي في المكورات العنقودية الذهبية المعزولة من أصابات الجروح

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الملخص:

الهدف: تعتبر بكتريا المكورات العنقودية الذهبية احد المسببات الاساسية لحالات الاصابة في المجتمع بالإضافة الى العدوى الناتجة في المستشفيات. ان تكوين biofilm على سطوح خلايا المضيف والتي تساعد على التصاق هذه الاحياء المجهرية على سطوح تلك الخلايا هي احد عوامل الضراوة المهمة للمكورات العنقودية الذهبية.

المواد وطرق العمل: من مجموع ١٣٠ عينة من المكورات العنقودية الذهبية تم جمعها الجروح في مستشفى الحسين التعليمي في محافظة ذي قار، العراق. حيث تم استخدام كل عزلات البكتريا الهدف للكشف عن الجين المسؤول لتكوين biofilm (*icaB*) بواسطة تقنية تفاعل سلسلة البلمرة الجزيئية (PCR).

النتائج: بينت النتائج الحالية ان ٧٢ (٥٥,٣%) تم تشخيصها على انها بكتريا المكورات العنقودية الذهبية. كما اوضحت النتائج الجزيئية لاختبار تفاعل سلسلة البلمرة الجزيئية للجين الهدف ان ٥١.١٥% من العزلات تحتوي على هذا الجين.

الاستنتاج: اوضحت النتائج الحالية نسبة عالية من وجود جين icaB ذات العلاقة بتكوين biofilm والذي قد يكون لع علاقة بزيادة الامراضية لهذه البكتريا المسببة لانواع متعددة من الامراض للانسان.