

Detection of Biofilm Genes (*IcaA* and *IcaD*) in *Staphylococcus* spp.

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ABSTRACT

One hundred thirty one clinical bacterial samples were collected from skin, pus, urine and blood from patients at Al-Salam General Teaching Hospital in Mosul city during March until July/ 2012, The present study included identification using API ID 32 STAPH system to the species *Staphylococcus aureus*, *Staph. lugdunensis*, *Staph. epidermidis*, *Staph. hominis*, *Staph. auricularis* and *Staph. haemolyticus*, Biofilm formation genes at *icaA* (188 bp.) were found in 35.7% of *Staph. aureus*, *Staph. hominis*, and *Staph. xylosus* but 42.8 % of *Staph. aureus*, *Staph. epidermidis*, *Staph. hominis*, and *Staph. xylosus* contained *icaD* (198 bp.) gene only.

Keywords: *Staphylococcus*, *icaA*, *icaD*.

تحديد جينات (*IcaA* و *IcaD*) المكونة للأغشية الحيوية لأفراد جنس *Staphylococcus* spp.

المخلص

جمعت (131) عزلة من الجلد، القيح، الادرار والدم من المرضى المراجعين لمستشفى السلام التعليمي العام في مدينة الموصل للفترة من آذار لغاية تموز 2012، أجري التشخيص التأكيدي اعتمادا على عدة الفحص الجاهز API ID 32 STAPH الخاصة بالمكورات العنقودية لأنواع *Staph. aureus* ، *Staph. epidermidis* ، *Staph. hominis* ، *Staph. auricularis* و *Staph. haemolyticus* ، وجد جينا الأغشية الحيوية، *icaA* (188 bp.) و *icaD* (198 bp.) في 35.7 % فقط من *Staph. aureus* ، *Staph. hominis* و *Staph. xylosus* بينما 42.8 % من *Staph. aureus* ، *Staph. epidermidis* ، *Staph. hominis* و *Staph. xylosus* تحتوي الجين *icaD* فقط.
الكلمات الدالة: *Staphylococcus*, *icaA*, *icaD*.

INTRODUCTION

Members of the genus *Staphylococcus* are gram positive cocci that occur singly, in pairs, tetrads, short chains (three or four cells), and irregular grape-like clusters. They are non-motile, non-spore-forming, and usually un-encapsulated or have limited capsule formation. Most species are facultative anaerobes and positive to catalase test (Dworkin *et al.*, 2006).

Staphylococcus spp. can form a biofilm on nearly any synthetic polymers used as prosthetic devices. In addition, they can bind to blood, matrix proteins, and human cell receptors. *Staph. epidermidis* is regarded as a leading species in causing chronic polymer-associated clinical infection (Mack *et al.*, 2013).

Colonization of tissues or implants is frequently a first step in infection; and biofilm formation plays a crucial role. One can imagine that the observed genetic variability in biofilm formation, which ranges from super to non-biofilm formers, underlies a well-directed survival and

spreading program that we are only gradually learning to understand. Once a Staphylococcal biofilm is formed, the cells are nearly invulnerable. They are then shielded from the immune system and resist antibiotic treatment. Therefore, it is very important to understand the various aspects of biofilm formation, it is suggested that to develop more specific ways of overcoming Staphylococcal resistance in chronic infections (Pitts *et al.*, 2003 and Romeo, 2008).

MATERIALS AND METHODS

Sample Collection:

One hundred thirty one samples were collected clinically from patients (skin, pus, urine and blood) of both sexes sufferings from bacterial resistant to most antibiotics at Al-Salam General Teaching Hospital, in Mosul city from March to July, 2012. Samples were collected by transport media and sterile swabs damped in normal saline. Isolation and identification were carried out using standard microbiological methods all isolates were identified by API ID 32 STAPH system (Bio Merieux).

Genes detection:

Ica A gene (188 bp.) was amplified using primers F (5'-ACA CTT GCT GGC GCA GTC AA-3'), R (5'-TCT GGA ACC AAC ATC CAA CA-3') and *Ica D* gene (198 bp.) was amplified using primers F (5'-ATG GTC AAG CCC AGA CAG AG-3'), R (5'-AGT ATT TTC AAT GTT TAA AGC AA-3') (Alpha DNA Company, Montreal, Quebec-CANADA) and the sequences were checked out depending on the Gen Bank Sequence Database (<http://www.ncbi.nlm.nih.gov/>). The PCR mixtures (12.5 μ l GoTaq Green Master Mix, 5.5 μ l Nuclease Free Water, 1 μ l Forward Primer, 1 μ l Reverse Primer and 5 μ l Extracted DNA, Promega Corporation-USA).

The program is first 2 min at 94°C, followed by twenty five cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min and followed with final extension at 94°C for 4 min and cooling 4°C for 3 min. The presence of a PCR bands were confirmed by 1% (wt/vol) agarose gel electrophoresis and visualization with ethidium bromide (Mariana *et al.*, 2009).

RESULTS AND DISCUSSION

The results obtained from API test were as follows: four isolates were *Staph. aureus*; three isolates were *Staph. lugdunensis*; two isolates were *Staph. epidermidis*; two isolates were *Staph. hominis*; one isolate was *Staph. auricularis* and one isolate was *Staph. haemolyticus*, the *ica* locus which is required for the synthesis of the polysaccharide intracellular adhesion (PIA) of Staphylococcus plays a role in cell to cell interactions during biofilm formation and is predominantly present in clinical isolates (Ando *et al.*, 2004).

icaA:

The species *Staph. hominis*, *Staph aureus* and *Staph xylosus* only contain the gene of biofilm which form 35.7% (*icaA* 188 bp.). Fig. (1).

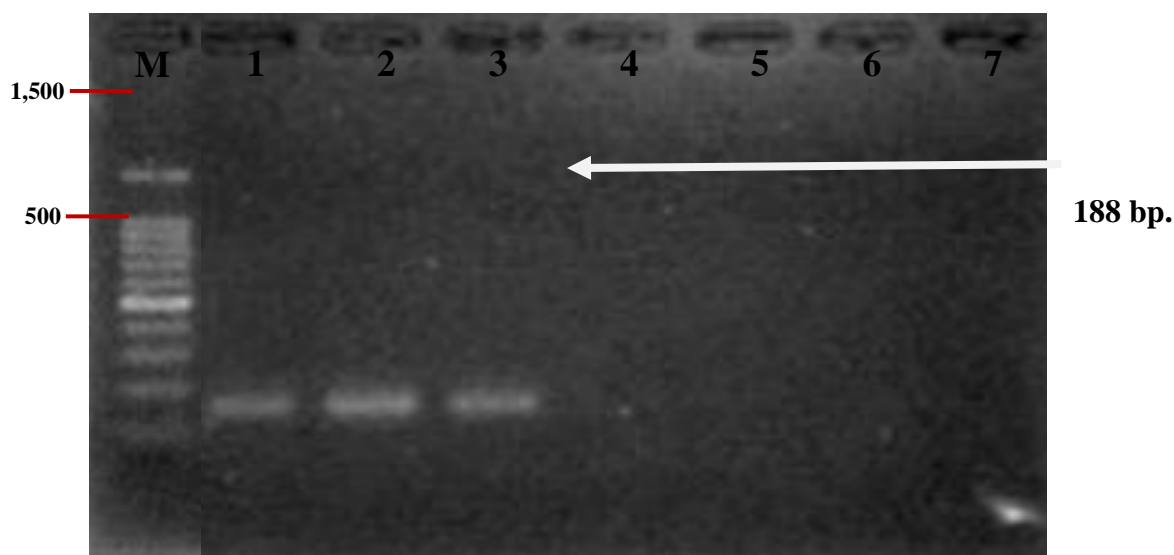


Fig. 1: *Staphylococcus* spp. *icaA* gene.

Five isolates contain *icaA* gene. Mariana and coworkers, (2009) stated that *Staph. aureus* and *Staph. epidermidis* contained this gene, and Nasr and colleagues, (2012) found *icaAD* genes in (32.0%) of Staphylococcal isolates, biofilm formation causes catheter associated and nosocomial infections with the presence of *icaA* and *icaD* genes (Arciola *et al.*, 2001).

***icaD*:**

The species *Staph. hominis*, *Staph. epidermidis*, *Staph aureus* and *Staph xylosus* contain the gene of biofilm which form 42.8% (*icaD* 198 bp.) Fig. (2).

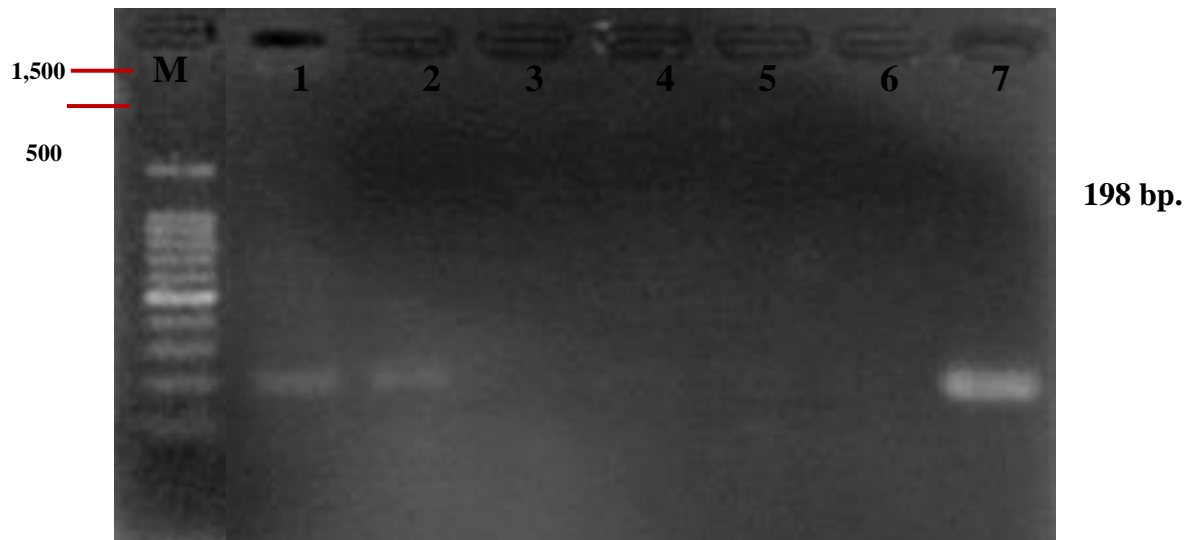


Fig. 2: *Staphylococcus* spp. *icaD* gene.

Yazdani and their colleagues (2006) and Nasr and their workers (2012) indicated a high prevalence of the *icaAD* gene among *Staph. aureus* isolates and their presence is not always associated with *in vitro* formation of slime or biofilm, Cafiso and coworkers, (2004) recorded that (35%) of *Staphylococcus* spp. contained *icaA* and *icaD* genes and some of them carried *icaD* gene only, Satorres and Alcaraz, (2007) found that (35.2%) and (48.4%) of *Staph. aureus* and *Staph. epidermidis* strains contained *icaA* and *icaD* genes respectively, Mariana and colleagues, (2009) recorded that all *Staphylococcus* spp. under test were containing this gene, Mahmoud Gad and coworkers, (2009) stated that both *icaA* and *icaD* genes were parts of one operon and so the entire operon was either present in or absent and they found that all Staphylococci isolated from catheter segments showed a higher extent of biofilm production than those isolated from urine samples.

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