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# Detection of *clfA* Gene in *Staphylococcus aureus* Isolated From Tonsillitis Patients

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**Abstract**

*Staphylococcus aureus* is an opportunistic pathogen and it was one of virulent causer of tonsillitis. The present investigation was aimed to the molecular detection of adhesion gene (*clfA*) was done by polymerase chain reaction (PCR) and DNA sequencing. Only 64 isolates (42%) were mannitol fermenter and recorded as *S. aureus*, these isolates recovered from 152 swabs were collected from tonsillitis patients in ENT department in Al–Habboby Teaching Hospital, Thi-Qar province, during the period from November 2016 to March 2017. Out of 64 isolates, 56 (87.5%) were harbored *clfA* gene. The sequencing of PCR products showed significant alignments identities (96-99%) to the *S. aureus* which are located in BLAST-NCBI Genbank. Phylogenetic analysis of *S. aureus* based upon the neighbour-joining of partial *clfA* gene sequences showed that these sequences were derived from *Staphylococcus* genes.

**Keywords:** *S. aureus*, tonsillitis, *clfA* gene, gene sequencing.

**Introduction:**

*Staphylococcus aureus* is an opportunistic pathogen with the ability to invade and persist in unprofessional phagocytes: fibroblasts, osteoblasts and different types of epithelial cells. The infectious potential of this bacterium is determined by a large number of cell-associated and extracellular virulence factors, some of which are implicated in the adhesion process and others in the bacterial invasion (Holban *et al*., 2013). *S. aureus* can produce secreted virulence factors such as enterotoxins and surface-exposed virulence factors (fibrinogen, protein A, fibronectin binding proteins) (Zhang *et al*., 2016). Fibrinogen is the most abundant host protein in endothelial lesions. Clumping factors A and B (*ClfA*, *ClfB*) are fibrinogen-binding proteins expressed by *S. aureus* on bacterial cells, promoting adherence to cell surfaces. The *ClfA* factor is expressed during the bacterial growth, whereas *ClfB* is present only during the early logarithmic phase (Peacock *et al*., 2000). The clumping factor is very important for the virulence of *S. aureus*, and is thought to be essential for colonization and establishment of infections, It participates in the infection process by facilitating bacterial binding via soluble or immobilized fibrinogen as fibrinogen plays a significant role in platelet thrombus formation and almost all *S. aureus* strains have the *clfA* gene (Josefsson *et al*., 2008; Karahan *et al*., 2011; Delfani *et al*., 2016). Microbial surface components recognizing adhesive matrix molecules on *S. aureus* surface, mediate staphylococcal adherence to components of the extracellular matrix of the host (Vazquez *et al*., 2011). These components are attached covalently to peptidoglycan by sortase enzymes (Heilmann, 2011). Furthermore, these components participate in biofilm formation, in addition to the *ica* operon that produces the polysaccharide intercellular adhesion (PIA) (Mirzaee *et al*., 2014). MSCRAMMs can bind to molecules such as collagen (mostly via Cna), fibronectin (via FnbAB), and fibrinogen (with ClfAB and Fib) and thus evade immune system, and then can develop infections (Foster *et al*., 2014). The aim of this study was to screen the *clfA* gene among the isolates of *S. aureus* from tonsillitis patients. Likewise, to comparative genomic sequencing analysis and phylogenetic tree generating, allows for an epidemiological discrimination of closely related bacterial isolates.

**Material and methods**

**Bacterial isolates**

One hundred and fifty two swabs were collected from patients infected with tonsillitis whom admitted to ENT unit in AL-Habbuby Teaching Hospital of Thi-Qar province, during the period from November 2016 to March 2017 by moistened sterile swabs with normal saline, these swabs directly inoculated on mannitol salt agar (LAB/ United Kingdom) and incubated at 37ºC for 24 h.

**Identification of *S. aureus***

*S. aureus* was identified depending on the morphological properties on culture media and biochemical tests (Catalase test, Coagulase test, DNase production test) which done according to Bergeys manual (Harley & Prescott, 2002 ; Brooks *et al*., 2007). API Staph system (BioMerieux, France) was used to identify a *Staphylococcus* and *Micrococcus*.

**Bacterial DNA extraction**

All isolates of *S. aureus* had been incubated on Brain Heart Infusion Broth (LAB/ United Kingdom) for 18–24 h at 37° C. Chromosomal DNA extraction was performed using Genomic DNA Extraction kit (Geneaid/Korea).

The specific primer pairs of *clfA* gene as following: forward: 5'- ATT GGC GTG GCT TCA GTG CT -3' and reverse: 5'- CGT TTC TTC CGT AGT TGC ATT TG -3'. The PCR cycling conditions of this gene: initial denaturation at 94°C for 5 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension for 10 min after the last cycle (Tristan *et al*., 2003). Electrophoresis of PCR product was carried out in 1% agarose gel and the presence of a 292bp band indicate a positive result for this gene.

**Results and Discussion**

The results of the present study showed that the incidence of *S. aureus* was 64 isolates (42%). *S. aureus* was one of the most common and virulent causer of tonsillitis (Jeong *et al*., 2007).

The recent data differenced from the local study in the same field which recorded higher percentages of *S. aureus* prevalence, such as Dakhil & Hamim, (2016) which revealed that the incidence of *S. aureus* was 62 (64.5 % ) from which 60 isolates (96.77%) that recovered from tonsillitis.

The molecular analysis of biofilm-associated gene (*clfA* gene) revealed that 87.5% of isolates had this gene, and the size of this gene was approximately 292 bp, Figure(1). Clumping factor A was one of important virulence factors which critical for pathogenicity of invasion *S. aureus* and colonization in infection sites. Momtaz *et al*., (2010) suggest that clinical strains of *S. aureus* may contain different frequencies of clumping factors, being essential for colonization. *S. aureus* has different mechanisms of virulence, pathogenicity and favors the development of antibiotic resistance and increases vulnerability to infection (Almeida *et al*., 2007). Moreover, Gotz, (2002) suggested that infections related with biofilm production are generally frequent, because the antimicrobial treatment predominantly eliminates planktonic forms, leaving the sessile cells free to reproduce and propagate the biofilm after treatment, so, the pathogen in biofilms are more protected against the host immune system. The biofilm-associated diseases include infections caused by heart valve implants, catheters, and contact lenses.

The results of current data disagreed with results of Atshan *et al*., (2012); Ghasemian *et al*., (2015) and Omara *et al*., (2016) documented that all *S. aureus* strains carried *clfA* gene, while Gowrishankar *et al*., (2016) confirmed through their study that only 58.7% of isolates harbored this gene.

There was didn't expression of *cflA* gene between MRSA and MSSA strains (Souza *et al*., 2014).

The occurrence of *clfA* gene may be abundant in clinical *S. aureus* isolates compared with that from animal sources. Momtaz *et al*., (2010) reported that nearly 20% of *S. aureus* isolates causing mastitis contain *clfA* gene.

The evolutionary history was inferred using the Neighbor-Joining method Saitou and Nei, (1987). The optimal tree with the sum of branch length = 0.03635857 is shown. (above the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method Tamura *et al*., (2004), and were in the units of the number of base substitutions per site, Figure (2). The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 503 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 Kumar *et al*., (2016).

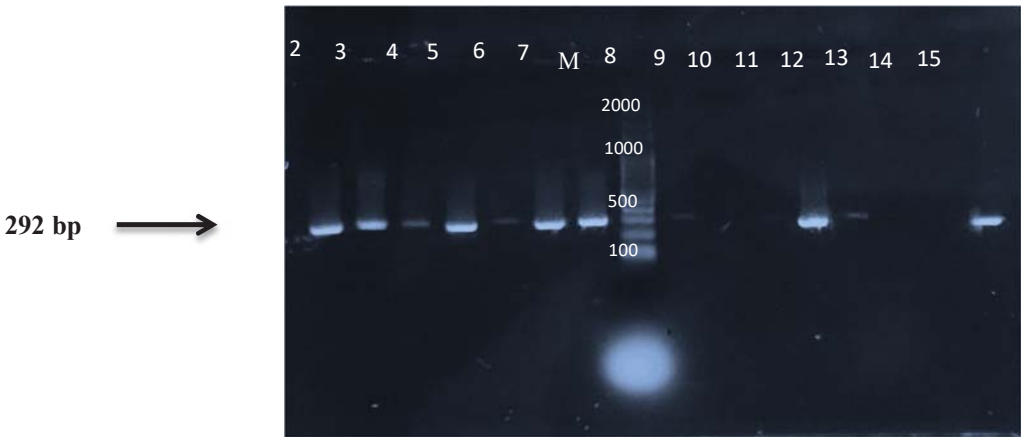


**Figure (1): Agarose gel electrophoresis of *clfA* gene amplification, M: ladder, 1-8, 10-12, 15: positive results, 9,13-14: negative result.**

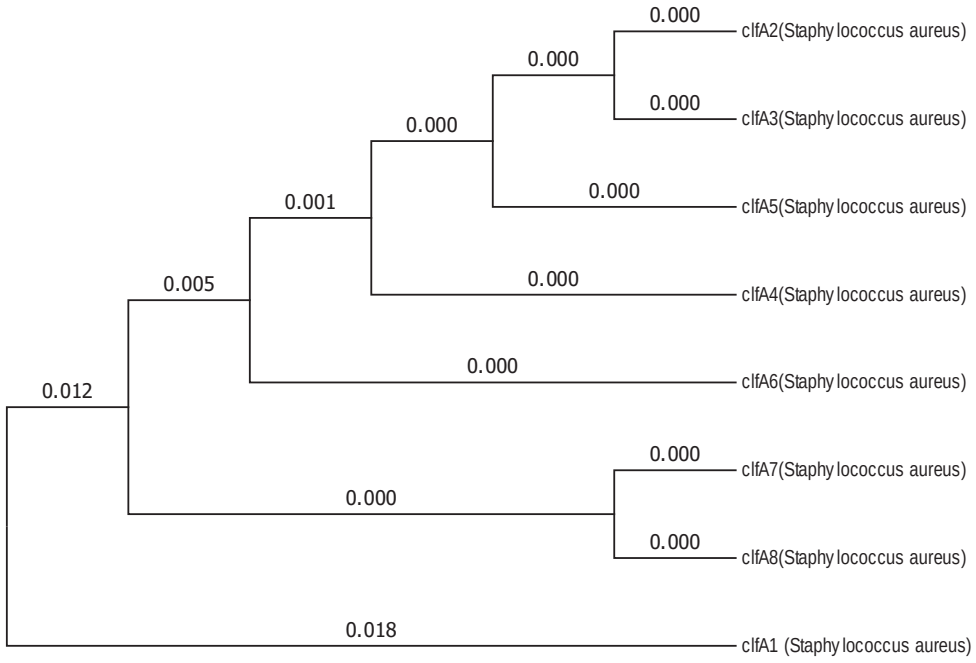


**Figure (2): Evolutionary relationships of taxa, based on Clumping factors gene partial sequence that used for *S. aureus* detection from Human tonsillitis samples.**

**Conclusion:**

The recent results recorded a high percentage of *clf*A gene which related with promoting adherence to cell surfaces and biofilm production that may be increased the pathogenicity of this bacteria which caused different human diseases.

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