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MOLECULAR DETECTION OF AAP GENE IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM TONSILLITIS

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Abstract

The study included genetic study accumulation associated-protein gene to common bacteria that causes tonsillitis, 30 isolated from surface tonsillitis. Primary identification was depended on Gram stain and biochemical tests. Finally identification with vitek 2 system was done. The results demonstrate 25 isolate of them were *Staphylococcus aureus*. Genetically study for gene encoding accumulation associated-protein gene that associated with pathogencity of *S. aureus* by using PCR technique. The result appear *Staphylococcus aureus* contain on 12(26.6%) *Aap* gene that encoding to accumulation associated-protein gene.

Keyword: *S. aureus*, *Aap* gene

Introduction

Tonsillitis is an inflammation of tonsils tissue that affects both gender, and all age groups particularly children. It is mainly attributed to microorganisms represented by bacteria and viruses and it is acute, recurrent acute or chronic tonsillitis (Babita *et al*., 2014). Some studies have reported that bacteria causing tonsillitis inhabit not only the tonsillar surface but also spreading to the tonsillar deep tissue (Panga *et al*., 2016).

Many studies revealed the bacterial associated with tonsils infection produce or have sever virulence factors facilitated invasion tl mammalian cells membrane and proteolytic enzymes such as coagulase, catalase, hyaluronidase and fibrinolysis, that are controlled by system of global transcriptional regulators called staphylococcal accessory regulators and accessory gene regulator (Robinson *et al*., 2005). The simplest and most common molecular method that has been used to detect biofilm formation genes are detected in the polymerase chain reaction (PCR) via specific oligonucleotide primers which can be chosen from the sequences available in the public databases such as gene bank. *S. aureus* contain intracellular adhesion *(Ica)* especially *IcaA, IcaD* that responsible for slime layer formation and biofilm accumulation associated protein (*Aap*)gene encode for many protein on surface and has important role in biofilm formation (Gad *et al.,*2004).

Materials and Methods

Specimens Collection and Bacterial Identification

One hundred and thirty clinical specimens were collected from patients suffering from tonsillitis. Those specimens were collected from patients (male and female, adults and children) suffering from tonsils infections by taken swab from infection area and also after tonsillectomy, after the sample is taken after the surgery, the sample surface is sterilized and opened with a sterile scalpel and by using swab taken from the fibrosis found in the tissue . Specimens were inoculated on three types from culture media which included blood agar, mannitol salt agar and MacConkey agar which considered as predominant enrich media, selective and differential media for the isolation, purification and identification of many types from bacteria. The plates were incubated at 37˚C for 24 hours then a single pure isolated colony was transferred to trypticase soya agar for the preservation and to carry out other biochemical tests and viteck system that confirmed the identification of isolates.

DNA Extraction

Genomic DNA was extracted by using a commercial extraction system (Favorgen/Taiwan).

Molecular Identification

Gel electrophoresis was used for detection of DNA by UV transilluminator. The PCR assay was performed to detect the (*Aap)* genefor *S. aureus* shown in table (2). This primer was designed by Alpha DNA company, Canada as in table (1). Amplified products were confirmed using 0.8% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 70v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products (Levy *et al*., 2008).

Table 1 : Primer used in this study

Primer Type	Primer Target	Primer sequence (5'-3')	Amplicon size (bp)	Reference
Aap	Aap	F: ATA CAA CTG GTG CAG ATG GTT G R: GTA GCC GTC CAA GTT TTA CCA G	399	Mohammad *et al.*, (2011)

Table 2: PCR program of *Aap* primer that apply in the thermocycler

Gene	Initial denaturation	No.of cycles	Denaturation	Annealing	Extension	Final extension
Aap	97˚C for 6min	35	92˚C for 30sec	55˚C for 30sec	72˚C for 45sec	72˚C for 10min

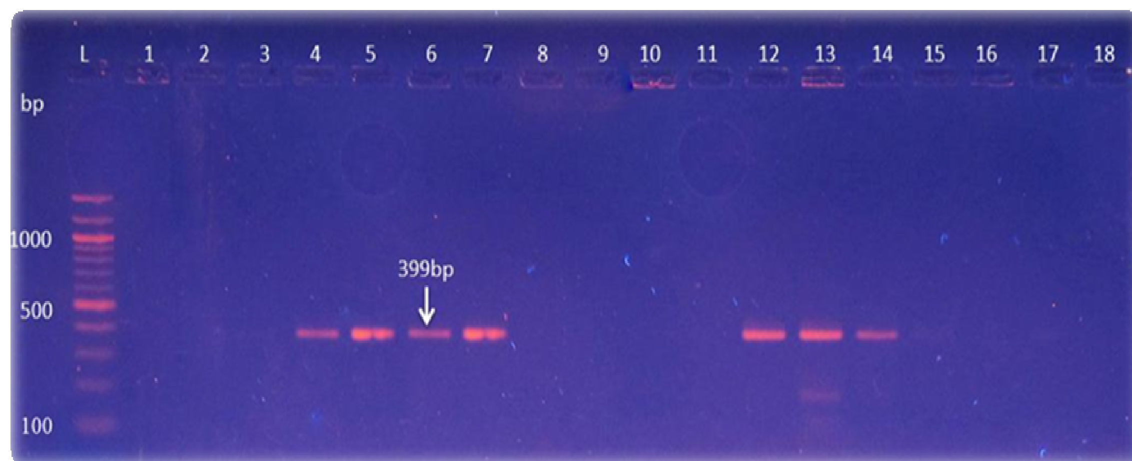
Results and Discussion

Detection of (*Aap* gene)

The Polymerase chain reaction technique has been used to amplify accumulation associated-protein gene *Aap*(399bp) from genomic DNA of *Staphylococcus aureus* specimens. The positive results of *Aap*(399bp) gene were recorded in 12 (26.6%) bacterial isolate as show figure (1-1A)(1-2B)(1-3C). This results were disagree with study of (Mohammad *et al.*, 2011)They showed that 94.8% of specimens *staphylococcus epidermidis* had *Aap* genes only. The *SasG* proteins of *S. aureus*, which has sequence similarity with *Aap* of *staphylococcus epidermidis* (Corrigan *et al.*, 2007). *Aap* is a 220 kD protein that needs to be proteolytically cleaved to a smaller 140 kD form to induce biofilm formation agree with Almayali *et al.* (2018) *Staphylococcus aureus* gave positive for *IcaA* and *IcaD* gene that involved biofilm production.

Aap can promote either the primary attachment or accumulation phase of biofilm formation depending on the strain being studied. Primary attachment is mediated by the N-terminal A domain, while the B regions mediate biofilm accumulation (Conlon *et al.,* 2014). The proteins have 5-17 B repeats, each comprising nearly identical 78 residue G5 subdomains followed by an E spacer region of 50 residues (Gruszka *et al.*, 2012). This is consistent with the finding that biofilm formation by *SasG* require sat least five repeats to be expressed on the cell surface. *S.aureus* strain requires *Aap* for primary attachment to surfaces (Conrady *et al.,* 2013).

Aap is capable of promoting either the primary attachment or accumulation phase of biofilm formation depending on whether the A domain (Conlon *et al.*, 2014). Expression of this genes masked the ability exponentially grown *S. aureus* cells expressing protein A, clumping factor B, the fibronectin binding protein A, B and has ability to binding MSCRAMMs to ligands and to promote formation of biofilm (Corrigan *et al.*, 2007).



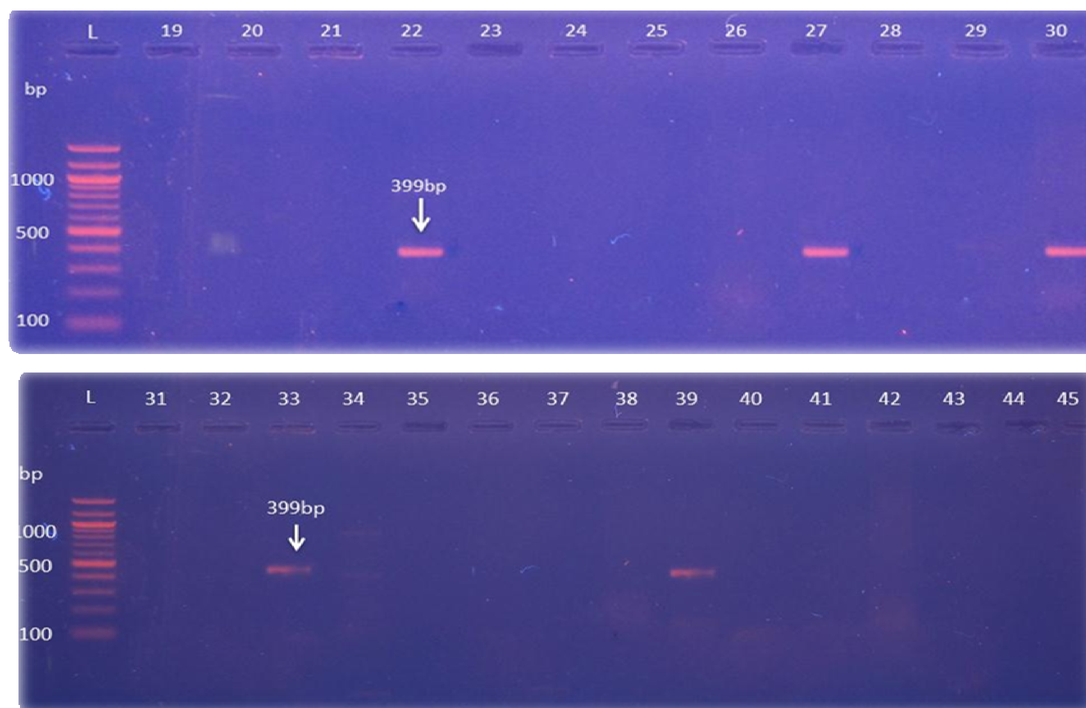


Fig. (1-1A)(1-2B)(1-3C): Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *Aap* with product 399bp. The electrophoresis was performed at 70 volt for 1.5-2hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (4,5,6,7,12,13,14,22,27,30,33,39 show positive results with gene *Aap*).

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