

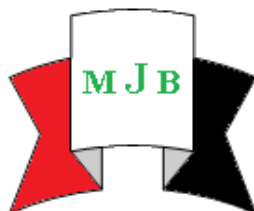
Molecular Detection of Enterotoxin A and B and Biofilm Gene of *Staphylococcus aureus* Isolated from Nasal Carriers

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Abstract

In this study, (100) clinical samples were obtained from nasal passage from healthy individuals in Hilla city who work in restaurants in Hilla province, during a period of three months (from November 2013 to January 2014).

A total of (100) samples only twenty – six of *S.aureus* were isolated from nasal passage.

Molecular detection of Enterotoxin A and B was also studied by using specific markers. It was seen that Enterotoxin A was positive in (9) isolates where Enterotoxin B was detected in (5) isolates.

On the other hand, molecular detection of *bap* gene by PCR technique showed that (4) *S.aureus* isolates (50 %) gave positive results for this marker only in the plasmid but none was seen in the chromosomal DNA.

Key words: *Staphylococcus aureus*, enterotoxins A & B, Biofilm gene, PCR

الخلاصة :

تم في هذه الدراسة الحصول على (١٠٠) عينة سريرية من ممر الأنف من الأفراد الذين يعملون في المطاعم في محافظة الحلة، خلال فترة ثلاثة أشهر من نوفمبر ٢٠١٣ إلى يناير ٢٠١٤. من مجموع ١٠٠ عينة، وجد أن ستة وعشرين منها كانت من البكتريا *S.aureus* المعزولة من ممر الأنف.

وتم الكشف الجزيئي عن Enterotoxins A, B باستخدام علامات محددة حيث وجد أن Enterotoxins A كان إيجابياً في (٩) عزلات ووجد Enterotoxins B في (٥) عزلات.

من ناحية أخرى، أظهر الكشف الجزيئي للجين BAP بواسطة تقنية PCR أن (٤) البكتريا المعزولة أعطت نتائج إيجابية لهذه الصفة فقط في البلازميد ولكن غير موجود في DNA الكروموسومي.

Introduction

Staphylococcus aureus produces a wide variety of toxins including staphylococcal enterotoxins (SEs; SEA to SEE, SEG to SEI, SER to SET) with demonstrated emetic activity, and staphylococcal-like (SEI) proteins, which are not emetic in a primate model (SEIL and SEIQ). SEs and SEIs have been traditionally subdivided into classical

(SEA to SEE) and new (SEG to SEIU2) types [1].

SEs are a major cause of food poisoning, which typically occurs after ingestion of different foods, particularly processed meat and dairy products, contaminated with *S.aureus* by improper handling and subsequent storage at elevated temperatures. Symptoms are of rapid onset and include nausea and violent vomiting, with or without diarrhea [1]

The *S.aureus* enterotoxins (SEs) are potent gastrointestinal exotoxins synthesized by *S. aureus* throughout the logarithmic phase of growth or during the transition from the exponential to the stationary phase. They are active in high nanogram to low microgram quantities [2].

SEA is the most common toxin associated with food poisoning concerning to staphylococcal enterotoxin and is a protein with a mass of 27.2 kDa. *S. aureus* strains produce a group of extracellular protein toxins, including the so-called superantigens [3]. Most SEA gene location on phage [4]. This enterotoxin alone or together with other SEs/ SELs is the most commonly reported in staphylococcal food poisoning [5]. The small intestine is a site of emetic action by SEA and appears to involve the 5-hydroxytryptamine (5-HT) or serotonin pathway. Serotonin is an important signaling mediator in the gastrointestinal tract and can activate enteric neurons, stimulate muscle responses, and enhance secretion [6].

SEA has two distinct binding sites on both sides of the peptide binding groove of class II MHC [7]. SEA molecules must be bound to both sites for optimal activity, which allows for class II MHC crosslinking, and stable interactions with T cells [6].

SEB has one distinct binding sites on both sides of the peptide binding groove of class II MHC [7]. Most SEB location on chromosome, plasmid, transposon [8]. SEB can survive in boiling water for several minutes, whereas ricin is all but inactivated after some 15 minutes at +80°C.

Staphylococcal enterotoxin B could pose a great risk to consumer health and can be classified as a low molecular protein (24.2kDa).

A biofilm is a microbially-derived sessile community, typified by cells that are attached to a substratum, interface, or to each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression and protein production [8]. Biofilm formation in a way represents the natural stationary phase of bacterial growth.

Different pathogenic mechanisms of the biofilms have been proposed. These include: Allow attachment to a solid surface, "Division of labor" increases metabolic efficiency of the Community, Evade host defenses such as phagocytosis, Obtain a high density of microorganisms, Exchange genes that can result in more virulent strains of microorganisms, Produce a large concentration of toxins, Protect from antimicrobial agents and Detachment of microbial aggregates transmits microorganisms to other sites [9].

Bacterial biofilms are therefore to be dependent on icaADBC dependent and independent. One of the major factors identified in bacterial biofilm formation is the SarA protein. This protein is found to play an important role in biofilm development. All *staphylococcus* stains have Bap protein, having capacity to formed high adhesion power for formation of biofilm process. Bap protein is involved in biofilm formation on artificial medium providing a role of attachment [10].

fnbA and fnbB are actually the extracellular matrix proteins which participated in intercellular accumulation and biofilm formation in *S. aureus*. Various other protein factors were also reported to be participating in ica independent *S. aureus* biofilm formation pathway like atl protein [11].

In *S. aureus* protein A (SpA) production was found essential for biofilm formation [12].

Table 1 : Primers of phylogenetic groups used in PCR.

Gene	Primer sequence	Size of product bp	Ref.
<i>ent – A F</i> <i>ent – A R</i>	GGTATCAATGTGCGGGTGG CGCCACTTTTTTCTCTTCGG	102	[13]
<i>ent – B F</i> <i>ent – B R</i>	GTATGGTGGTGTAAGTACGAGC CCAAATAGTGACGAGTTAGG	164	[13]
<i>bap F</i> <i>bap R</i>	CCCTATATCGAAGGTGTAGAATTGCAC GCTGTTGAAGTTAATACTGTACCTGC	971	[10]

Materials and Methods

A total 100 specimens which were collected from nasal passages in Hilla city who work in restaurants ,during a period of three months (from November 2013 to January 2014) .The patient's age ranged from (17 years - 54 years).

The samples were processed on Blood agar medium , luria broth , Nutrient agar and Mannitol salt agar agar and were incubated at 37°C

overnight. The identification of Gram positive bacteria, performed by standard biochemical methods (catalase test, oxidase test, Coagulase,

A- B hemolysis, Motility, Mannitol fermentation, and Milk agar for pigment).

DNA and plasmid extraction for Gram positive bacteria:

This method was performed according to the genomic DNA and plasmid purification kit supplemented by a manufacturing company (Viogene/ Taiwan).

Detection of phylogeny groups by PCR:

PCR was conducted to determine the phylogenetic grouping of the isolates by targeting two genes *ent – A* , *ent – B* and *bap* . Each 30 µl of PCR reaction mixture for PCR contained 3 µl of upstream primer, 3 µl of downstream primer, 4 µl of free nuclease water, 5 µl of DNA extraction and 15 µl of master mix. Thermal cycler conditions were as

follows: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s. A final extension of 72°C for 7 min was performed at the end of PCR.

Successful PCR amplification was confirmed by agarose gel electrophoresis [14]. Agarose gel was prepared by dissolving 0.6gm of agarose powder in 5ml of TBE buffer (pH:8) then the volume completed to 50 ml deionized water, in boiling water bath, allowed to cool to 50°C and ethidium bromide at the concentration of 5µl/ml was added.

The comb was fixed at one end of the tray for making wells used for loading DNA sample. The agarose was powered gently into the tray, and allowed to solidify at room temperature for 30 min. The comb was then removed gently from the tray.

The tray was fixed in an electrophoresis chamber which was filled with TBE buffer covering the surface of the gel, 5µl of DNA sample was transferred into the signed wells in agarose gel, and in one well we put the 5µl DNA ladder mixed with 1µl of loading buffer.

The electric current was allowed at 70 volt for 30 min. UV transilluminater was used for the observation of DNA bands, and gel was photographed using a digital camera.

Results and Discussion

Molecular detection of *Staphylococcus* enterotoxin A *eta* was done by using

specific PCR primer, As show in figure (1).

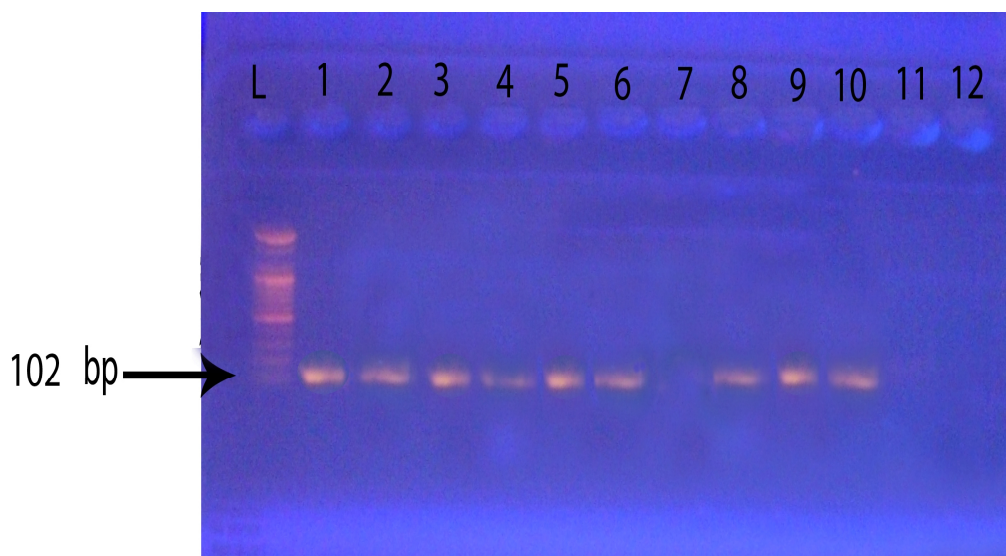


Figure (1): Gel electrophoresis of PCR product of *eta* gene

(1,2,3,4,5,6,8,9,10) isolates with positive result for *eta* gene, (7,11,12) isolates with negative result for *eta* gene. L= ladder (100 -1500). The electric current was allowed at 70 volt for 30 min

It was found that *eta* was observed in (9) isolates. This result agreement with [15] and [16] who are detected *eta* gene by PCR and found ability *S. aureus* to produce *eta* gene are (19.2 %), (41%) respectively.

Molecular detection of enterotoxin B was done by using specific primer. Since

etb is present in some isolates under studying, with the long length in (164 bp) as shown in figure (2). These result is in agreement with result obtained by [15] and [16] who are detected *eta* gene by PCR and found ability *S. aureus* to produce *eta* gene are (9.2 %), (7.7%) respectively.

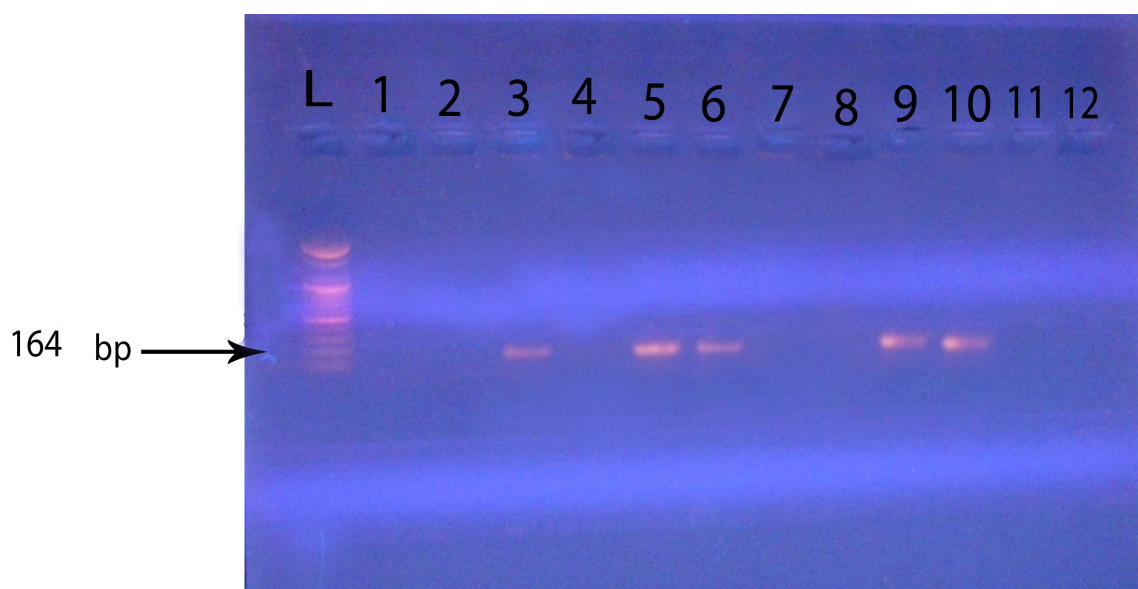


Figure (2): Gel electrophoresis of PCR product of *etb* gene

(3,5,6,9,10) isolates with positive result for *etb* gene, (1,2,4,7,8,11,12) isolates with negative result for *etb* gene. L= ladder (100 -1500). The electric current was allowed at 70 volt for 30 min.

Some *S. aureus* isolates causes staphylococcal food poisoning (SFP) by producing enterotoxins (SEs). Prevalence rate is higher than reported in German clinical (43%) and nasal (39.5%) isolates (18), in clinical isolates (23%) in Jordan, and in isolates (55.8%) from Taiwanese patients with food poisoning, but lower than that in isolates collected from Japanese (76%) patients with food poisoning. Recently, [2] reported a higher prevalence of SE genes (86%) in methicillin resistant *S. aureus* (MRSA) strains isolated from the blood cultures of patients in Ankara, Turkey [15].

Factors affecting *S. aureus* enterotoxinogenesis include: Temperature (34 – 40°C), PH (7–8) and Atmosphere (Aerobic) [17].

Enterotoxins A and B both are present in 5 isolates .Enterotoxin A alone is present in 9 isolates. Enterotoxin B alone is present in 5 isolates.

However, the presence of one gene or two genes may confer the ability of bacteria to cause food poisoning.

Staphylococcal enterotoxin B can be destroyed by pepsin digestion at pH 2 but it is pepsin resistant at higher pHs, which are normal conditions in the stomach after food ingestion. Pepsin destroys the activity of SEB at a pH of about 2, but the pH of the stomach would be this low only after starvation [18].

The molecular basis of biofilm production is also investigated through using specific primer. Both DNA of chromosome and plasmid is used.

It was shown that *bap* gene is not detected in chromosomal DNA for all isolates but is detected on the plasmid only.

However, specific PCR primers were used for detection of *bap* gene. It was found that *bap* gene was present in 4 isolates (50%) of *S. aureus* isolates as shown in figure (3).

The presence of *bap* on the plasmid, does not mean that *pab* gene is not encoded by chromosome because most *S.aureus* isolates produce biofilm ordinarily. But it may be attributed that *bap* gene may has another DNA sequence or may be

invaginated within other genes that are not detected easily by PCR.

However the presence of *bap* gene on the plasmid may confer the ability of this strain to transfer through different strain of *S. aureus*.

This means that gene is present in some isolates from nasal passages. This result is

in agreement with the result obtained by [19] who found that 42% of the isolated *S. aureus* expressed of *bap* gene. The ability of *S. aureus* to form biofilms is an important virulence factor in many persistent infections.

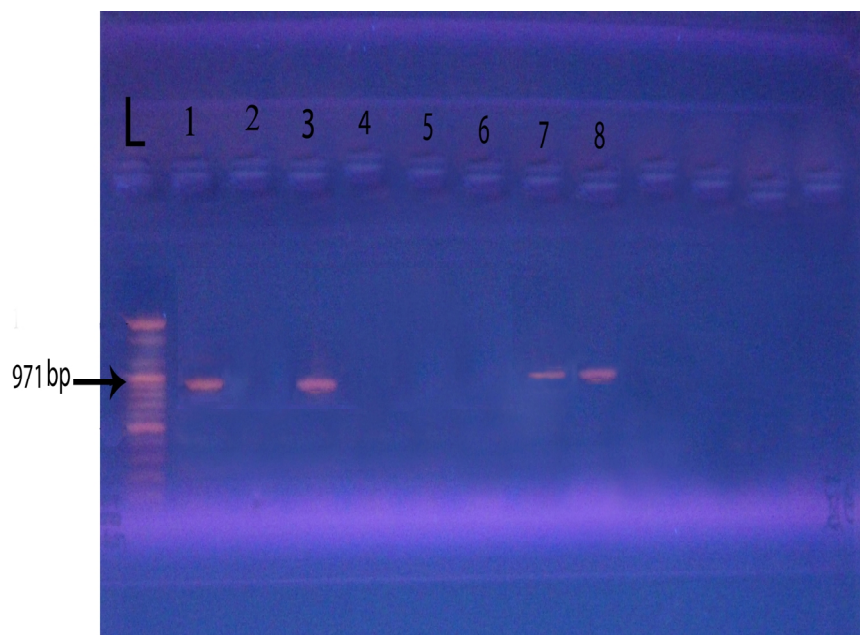


Figure (3): Gel electrophoresis of PCR product of *Bap*
(1,3,7,8) isolates with positive result for *Bap* from nasal passage. L= ladder (100-1500). The electric current was allowed at 70 volt for 30 min.

In this case, when the *Bap* have high molecular weights, and present genetically on the plasmid, this means that the plasmid is mega plasmid with high molecular weights which can carry genes with high molecular weights.

Moreover, the presence of tandem repeats in *Bap* genome may give a picture these protein may have a site on jumping gene such as transposons.

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