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Detection of Fibronectin Binding Protein (*fnb* A) gene in *Staphylococcus aureus* isolates

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Abstract:

This study designed to molecular evaluation of the fibronectin-binding proteins gene (*fnb* A) in clinical isolates of *S. aureus* which recovered from 120 swabs from pharyngitis and tonsillitis patients during the period from February, 2017 to November, 2017. Among all bacterial isolates, only 32 (26.66%) were positive on mannitol salt agar and biochemical methods, which identified as *S. aureus*. The molecular analysis of *fnb* A gene was done by employing the polymerase chain reaction (PCR). The results revealed that 59% of isolates were found positive for *fnb* A gene.

الخلاصة

صممت هذه الدراسة للتقيم الجزيئي لجين (البروتينات المرتبطة بالفايبرونكتين) في عينات بكتريا المكورات العنقودية الذهبية السريرية المعزولة من 120 مسحة قطنية من مرضى التهاب البلعوم والتهاب اللوزتين خلال الفترة من شباط، 2017 الى تشرين الثاني، 2017. اثنان وثلاثون عزلة (26.66%)من كل البكتريا المعزولة شخصت على انها بكتريا المكرات العنقودية الذهبية بالاعتماد على النمو على وسط اكار المنتول الصلب والاختبارات البايوكيميائية، اوضحت نتائج التحليل الجزيئي باستخدام تقنية تفاعل البلمرة الجزيئي ان 59% من عينات المكورات العنقودية الذهبية تحتوي على جين.

Keyword: *S. aureus*, PCR, *fnb* A gene.

Introduction:

Staphylococcus aureus is a successful commensal bacteria that efficiently colonizes both human and hosts (Lowy, 2011). *S. aureus* is an opportunistic bacteria with its capability to occupy and persist in

unethical phagocytes: osteoblasts, fibroblasts and diverse types of epithelial cells. The infectious potential of this bacteria detected by a huge number of cell related and extracellular virulence factors, some of which are concerned in the adhesion process and others in the bacterial invasion (Holban *et al*., 2013). The intracellular presence of *S. aureus* in host tissues has been recently highlighted, advising the ability of the pathogen to dodge the host defense mechanisms and to persist in host cells (Zautner *et al*., 2010).

The adherence of *S. aureus* to human tissue was mediated by a number of genes encoding of factors related to cell wall microbial surface components, such as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), this molecules play an significant role in *S. aureus* pathogenesis, allowing its transition from the bacteremia stage to infections such as endocarditis or osteomyelitis (Bur *et al*., 2013). The MSCRAMMs group includes protein A, the fibronectin-binding proteins (*fnb* A and B), collagen-binding protein (*cna*), elastin-binding protein, clumping factors A and B (*ClfA* and *ClfB*), those virulence elements are significant for the invasiveness and development of infections by invasive *S. aureus* (Sabat *et al*., 2006; Sivaraman *et al*., 2009).

The invasive infections caused by *S. aureus* isolated strains, which harbor the *fnb*A and *fnb*B adhesins genes (Peacock *et al*., 2000), and those virulence factor used to prevent the phagocytosis process by leucocytes (Higgings *et al*., 2006). The present study aimed to investigate the prevalence of *S. aureus* in pharyngitis and tonsillitis patients, PCR-based technique was utilized to detect the gene encoding for the fibronectin-binding protein (*fnb* A).

Material and Methods:

Ethical approval

This research was approved by the Veterinary Medicine College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Laboratory methods

Totally, *S. aureus* isolates were isolated from 120 swabs which collected from pharyngitis and tonsillitis patients whom admitted to ENT

unit in AL-Habbuby Teaching Hospital of Thi-Qar province through the period from February, 2017 to November, 2017 and identified depending on cultural properties on mannitol salt agar and blood agar (LAB/ United Kingdom), followed by biochemical tests (Harley and Prescott, 2002). The confirmed diagnosis was performed by using API system (BioMerieux/France). Staphylo Monotec test kit Plus (Mast/United Kingdom) used to identify all *S. aureus* isolates as serological diagnosis of these bacteria.

Extraction of bacterial DNA

All isolates of *S. aureus* were inoculated on Nutrient broth (NB) (LAB/United Kingdom) and incubated for 24h at 37°C. Bacterial DNA was extracted from fresh overnight culture in NB broth by using DNA Bacteria plus kit (Geneaid/Korea) according to the manufacturer's instructions.

PCR diagnosis of *fnb* A gene

The specific primer pairs of *fnb* A gene as following: forward: 5'-ATC AGC AGA TGT AGC GGA AG -3' and reverse: 5'- TTT AGT ACC GCT CGT TGT CC -3'. The PCR cycling conditions of *fnb* A gene: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min and final extension for 5min after the last cycle (Abraham, and Jefferson, 2010) with modification. Electrophoresis of PCR product was carried out in 1.4% agarose gel and the presence of a 198 bp band indicate a positive result for *fnb* A gene.

Results and Discussion:

From of 120 collected swabs, only 32 (26.66%) was identified as *S. aureus*. The *S. aureus* was noticeable causer of pharyngitis and tonsillitis infection but create less often when compared to the *Streptococcus pyogenes* pharyngitis (Jeong *et al*., 2007; Gowrishankar *et al*., 2013).

The current results were disagreement with results of local study performed by Dakhil and Hamim, (2016) showed that the occurrence of *S. aureus* was 64.58%. The percentage of *S. aureus* was higher than the results of study done by Sadoh *et al*., (2008) indicated that the occurrence of this agent was 12.83%. The present data were differenced from results

of studies performed by Al-Ahmary *et al*., (2012) and Thomas, (2012) demonstrated that a percentage of *S. aureus* was 44% and 52% respectively.

Among the examined *S. aureus*, 19 amplified of the targeted gene, the percentage was 59% with the molecular weight of approximately 198 bp (Fig.1). The present study disagreed with results of Duran *et al*., (2010) revealed nearly all of the strains were positive for *fnb* A gene (97.7%). While, Nashev *et al*., (2004) noted that all *S. aureus* isolates harbored the goal gene.

On other hand, the prevalence of *fnb* A gene in recent study was slightly with the results of other studies, like Mirzaee*a et al*., (2015) and Gowrishankar *et al*., (2016) showed that high percentage of methicillin resistant *S. aureus* had *fnb*A gene, were 77.8 and 82.2%, respectively.

While, the present results were incorporated with results of study performed by Kiavari *et al*., (2012) recorded that *fnb* was identified in 65.29% of clinical and 54.67% nasal *S. aureus* strains and had higher prevalence in isolates from endocarditis, osteomyelitis and burn associated wounds.

Interestingly, some studies recorded that high percentages of *S. aureus* isolates harbored *fnb*A gene whereas no detectable in MRSA isolates like Tangchaisuriya *et al*., (2014) documented 93.5% of *S. aureus* had the current gene and lacked in all MRSA isolates. Also Taneike *et al* (2006) described that completely isolates of MRSA which remote from nosocomial eruptions in Japan lacked *fnbB.* While, the results of Wisniewska *et al*., (2008) showed that *fnbA* and *cna* established in 90% and 63%, respectively of all MRSA strains. O'Neill *et al*., (2007) showed that a higher percentage of methicillin sensitive *S. aureus* (14%) than MRSA (0%) harbored the goal gene.

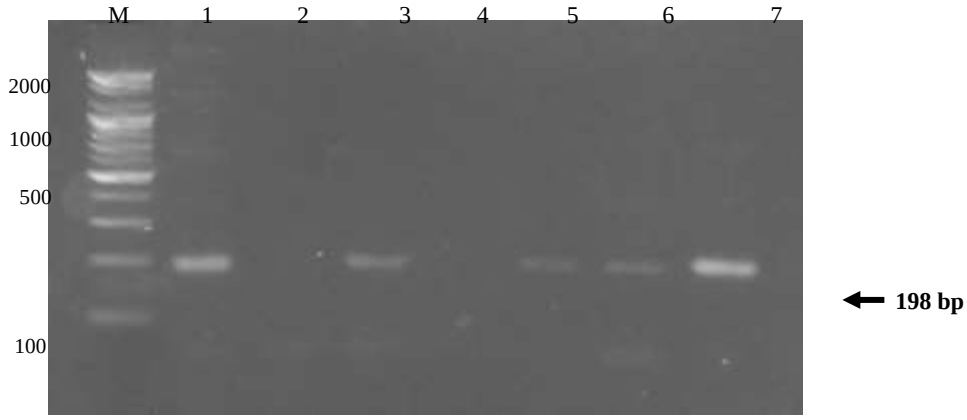

Fig.(1): Agarose gel electrophoresis of *fnb* A gene amplification, M: ladder, 1,3, 5-7 : positive results, 2,4,Negative result.

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