Relationship between Alpha-toxin production and biofilm formation by *Staphylococcus aureus* isolated from Eye's infections

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Abstract

Staphylococcus aureus is a common pathogen associated with eye's infections. *S. aureus* is capable of biofilm formation, which increases its persistence and boots its levels of antimicrobial resistance . A total of 50 *S. aureus* isolated from eyes of patients with eye's infection : 41(82%) isolates were positive – alpha toxin production and 37 (74 %) isolates were positive – biofilm formation .Where as 32 (64%) isolates were positive – alpha toxin production and biofilm formation, 11(22%) isolates were negative - alpha toxin production and biofilm formation and 7(14%) isolates were showed different production. Resulets showed, high significant association between alpha toxin production and biofilm formation in *S. aureus* isolates.

Introduction

S. aureus is one of the most important pathogens in humans and animals. The pathogenesis of a particular *S. aureus* strain is attributed to the combined effect of extracellular factors and toxin (1).

Alpha-toxin is a major virulence factor in *S. aureus* eye's infections, that may result in loss of visual acuity and blindness (2,3). Biofilm (slime layers) are surface – associated, sessile bacterial communities. A mature biofilm is formed when planktonic cells initially colonize a surface, aggregate and / or grow in to multicellular colonies and embed themselves in an exopoly saccharide matrix (4). *S. aureus* is capable of biofilm formation, which increases it's persistence and boosts its level of antimicrobial resistance (5).

The purpose of this study was to describe the relationship between alpha-toxin production and biofilm formation by *S. aureus* isolated from eye's infection.

Methods and Materials

- Bacterial isolates: 50 isolates of *S. aureus* from patients, who attended Ibn Al-Haietham Eye hospital, Baghdad. All isolates, were identified by api- system (Api- staph) (Bio Mereiux).
- Slime production test: isolates were tested for slime (biofilm) production with the use of technique described by (6). In brief, a loop of organisms from a pure growth on blood agar plate was inoculated on to 5 ml of Trypton soya broth (.....) and incubated at 37°c for 48hr. the contents of the tubes were aspirated and the tubes were stained with 1% crystal violate (BDH) for 7 min.

* A crystal violate stained film lining the wall of the tubes indicated a positive test.

- Alpha- toxin detection: the supernatant of *S. aureus* isolates were tested for hemolysin activity by procedure described by (7). The supernatant of isolates were diluted in PBS (Phosphate Buffer Saline) in micro titer plate. An equal amount of solution of washed 2% rabbit erythrocytes was added. The presence of hemolysin (Alpha-toxin) expressed as complete lysis of erythrocytes after 30 min. at 37° c.
- Statistical analysis: Chi square test (=3.457) was used to determine the differences between isolates in Alpha-toxin production and biofilm formation.

Results and Discussion

A total of 50 *S. aureus* isolates were studied: 41 (82%) isolates were positive- Alpha- toxin production and 37(74%) isolates were positive biofilm formation Fig. (1).

Fig. (2) shows, 32(64%) isolates were positive, Alpha-toxin production and biofilm formation, whereas the 11(22%) isolates were negative-Alpha-toxin production and biofilm formation and 7(14%) isolates were showed different productions.

These results suggest that Alpha-toxin and biofilm are a major virulence factors in eye infections caused by *S. aureus*, mediating the destruction of tissue in eye infected with bacterial pathogen. Biofilm not only helps the organism in adhesion to host cells, but also protect it from phagocytosis and the action of antibiotics (8,9) .

We found a high significant association ($p=0.0032<0.05$) between positive Alpha-toxin production and positive biofilm formation. Results showed a role for Alpha-toxin in *S. aureus* biofilm formation.

This is supported by observation mode presently in some reports. Nicky and O'toole, 2003 , (4) had formed that Alpha-toxin, secreted multimeric , hemolytic toxin encoded by the *hal* gene (this gene, regulating the production of the Alpha-toxin in *S. aureus*) (10), plays an integral role in biofilm formation and in particular, this toxin appears to be required for cell- to- cell interactions. They had found that *hal* mutant was unable to fully colonize plastic surface under both static and flow condition. Therefore, they had found that alpha-toxin is required for cell-to-cell interaction during biofilm formation (4,11).

Alpha-toxin is, in part, controlled by agr system. It has been shown, that an agr mutant produces less alpha-toxin but, is a hyper-biofilm forming strain (12).

However , the agr system regulates a wide array of virulence factors, including those involved in surface binding and surface associated virulence. Thus, even though alpha-toxin production is reduced in an agr mutant, other surface associated virulence factors, may be over expressed, functionally compensating for the lock off alpha-toxin (4). Furthermore, *in vivo* studies of device related infections have shown that alpha-toxin is not regulated by *agr* that is expression is predominately controlled by the two component regulator *Sae* (13) Therefore, alpha-toxin may be produced in an agr independent fusion when *S. aureus* colonize in dwelling devices in the biofilm made of growth.

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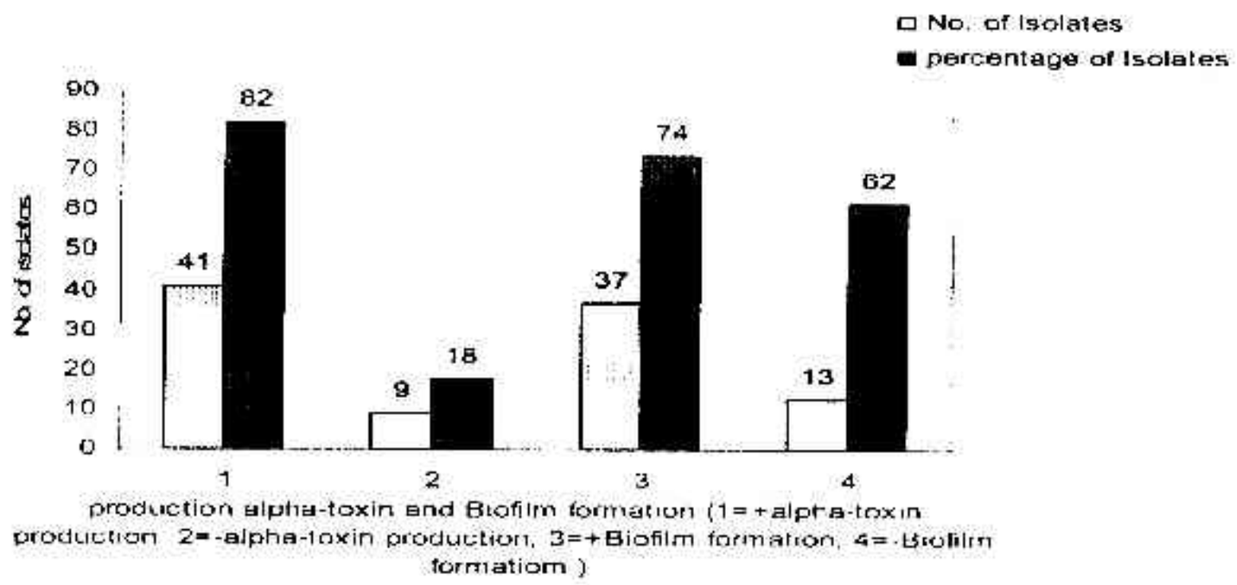

Fig.(1) percentage of *S.aureus* isolates positive alpha-toxin production and Biofilm formation

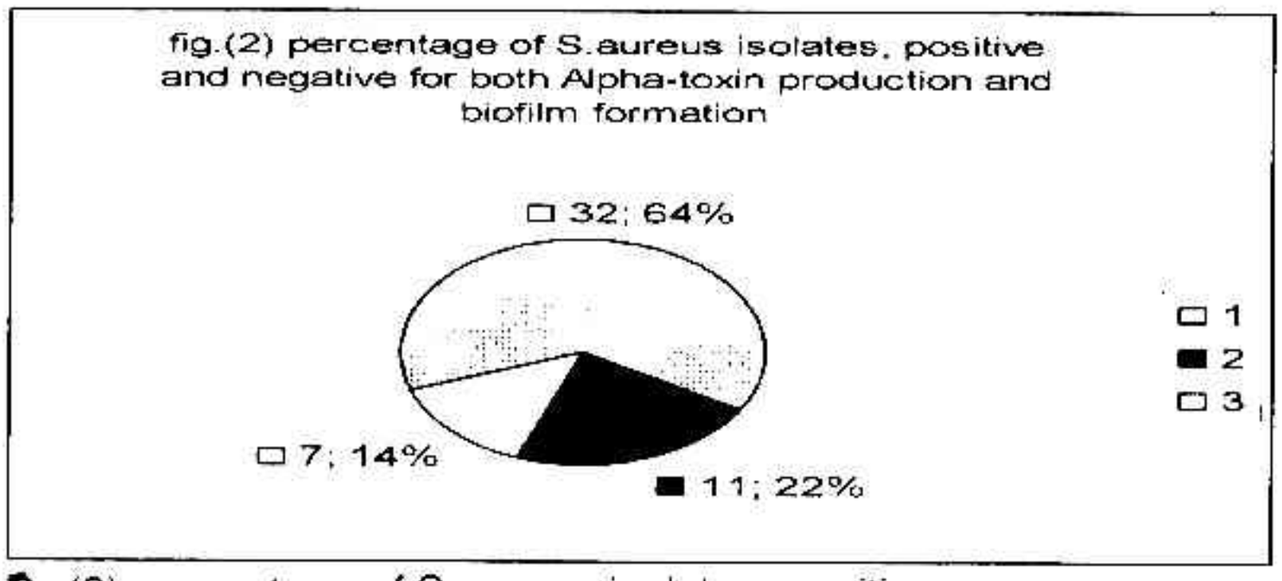

fig.(2) percentage of S.aureus isolates, positive and negative for both Alpha-toxin production and biofilm formation

* 1= +alpha-toxin production and biofilm formation
* 2= - alpha-toxin production and biofilm formation
*3=differences in production

العلاقة ما بين إنتاج سم ألفا وتكوين الغشاء الحيوي من بكتريا المكورات العنقودية الذهبية المعزولة من إلتهابات العين

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الخلاصة

تعد العنقوديات الذهبية من الممرضات الشائعة والمرتبطة بالتهابات العين التي لها القابلية على انتاج طبقة الغشاء الحيوي الذي يفعل ويعزز من مستويات المقاومة للمضادات الحيوية من المجموع الكلي لـ 50 عزلة لبكتريا *Staphylococcus aureus* التي عزلت من اصابات مختلفة للعين ظهر 41 (82%) عزلة منتجة لسم الفا و 37(74%) عزلة منتجة لطبقة الغشاء الحيوي . في حين ان 32 (64%) عزلة كانت منتجة لسم الفا وطبقة الغشاء الحيوي وما و 11 (22%) عزلة غير منتجة لكل من السم الفا والغشاء الحيوي و 7(14%) عزلة اظهرت انتاجية مختلفة لهذين العاملين . تدل هذه النتائج على وجود علاقة معنوية عالية ما بين انتاج سم الفا وتكوين طبقة الغشاء الحيوي في العنقوديات الذهبية .