

## Partial purification of Exfoliative toxin of *Staphylococcus aureus* Isolated from skin Infections

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### الملخص

يُميز الذيفان المحدث للتقشر المسبب لمتلازمة الجلد المسموط الناجمة عن الإصابة بالعنقوديات الذهبية بتساقط وانفصال طبقة الأدمة الداخلية إحدى طبقات الجلد . تم في هذه الدراسة استخلاص هذا الذيفان من سلالات العنقوديات الذهبية المعزولة من إصابات جلدية حادة . وأظهرت النتائج وجود علاقة بين الإصابات الجلدية المختلفة ونتاج هذا التوكسين من قبل السلالات المختلفة المسببة لهذه الإصابات وكانت نسبة السلالات المنتجة له إلى السلالات الكلية المعزولة 12 % . وتبين أيضاً أن الذيفان المنقى جزئياً سبب تحطماً وتساقطاً في طبقة الأدمة العليا في الجلد عند حقنه في الفئران حديثة الولادة .

### Abstract

Exfoliative toxins causes staphylococcal scalded skin syndrome which was characterized by a specific Intraepidermal separation of the skin layers. We describe here the extraction of the ETs from staphylococcus aureus strains isolated from hospitalized patients suffering from severe epidermal lesions , and we found that their was an association between skin lesions and ETs producing strains. The incidence of the exfoliative toxin producing strains of *S. aureus* from hospitalized patients was 12 % , Further more it appeared that partially purified ETs injected in to neonatal mice revealed a clefting and destruction of skin which was demonstrated in the upper epidermis.

### Introduction

Staphylococcal Infections are world wide increasing in all age groups, and its showing an increasing resistance to conventional antibiotics despite the availability of a wide range of these antibiotics. These infections are still carrying a significant morbidity and mortality particularly among adults (1). *Staphylococcus aureus* is a frequent bacterial pathogen in human disease, skin infections are particularly

prevalent, impetigo caused by *staphylococcus aureus* is one of the most common infectious diseases in children (2). Approximately 30% of the impetigo patients develop bullous impetigo which is caused by *S. aureus* strains that produce exfoliative toxins (ETs) (3), three forms of staphylococcal skin disease have been described, neonatal staphylococcal – scalded skin syndrome (SSSS). Bullous impetigo (B1) and generalized scarlatini form eruption with exfoliation .

(SSSS) Ritter disease and (B1) have many clinical feature in commons and the lesions of (B1) are actually considered to represent a localized form of (SSSS) (4).

Staphylococcal dermatitis characterized by the separation of the epidermis at the stratum granulosum, this disruption is mediated by one of two *staphylococcus aureus* exotoxin, exfoliative toxin A and B (ETA and ETB) (5). Exfoliative toxin (ETs) or exfoliatin which is a proteolytic toxin splits the epidermis of humans and new born mice between the stratum granulosum and stratum spinosum with out cell lesions (6).

The toxins act specifically at the zona granulosa of the epidermis to produce the characteristic exfoliation, although the mechanism by which it achieved is still poorly understood (1).

It has been shown that a large proportion of *S. aureus* strains isolated from patients with atopic dermatitis release a super antigen, including enterotoxin, and ETs (7,8) several investigators have isolated two serotypes of ETs (ETA and ETB) from *S. aureus* strains derived from patients with staphylococcal scalded skin. Syndrome (9,10) . ETA is a heat – stable toxin and its production is genetically controlled by chromosomal DNA (11,12) While ETB is a heat – labile toxin and its production is controlled by a 42 kb plasmid (13) However , both toxins have the same molecular mass 27k Da the same susceptible animal species (human and suckling mice) and the same toxic activities.

## Material and Methods

### *Staphylococcus aureus* strains : -

25 strains of *Staphylococcus aureus* had been taken from Al-Zahrawii hospital in Mosul which was isolated from patients suffering from severe skin disease.

### Exfoliative toxin – producing *S. aureus* : -

Bacteria were grown from a starter culture incubated at 37°C with aeration for 24 h in brain heart infusion broth. Cultures were centrifuged (10000 xg , 30 min) and filtered using ( 0.2 µm Millipore filter). 0.1 ml of cell free culture filterate was injected subcutaneously in new born mice according to (14) Injected mice were observed after 24 hours for the appearance of nikolsky sign at the site of injection .

### Partial purification of Exfoliative toxins :-

ETs had been prepared and partially purified according to (15) In which growth culture at stationary phase were treated with 4 volumes of absolute ethanol for two days to precipitate the toxins. The precipitate was collected suspended in 6 M urea, centrifuged to remove insoluble material (10000 xg ; 30 min) and dialyzed for 48 h at 4°C against D.W. 0.1 ml of the dialysate was inoculated subcutaneously in newborn mice also and the injected mice were observed after 24 h. for detection of proteolytic activity of exfoliative toxins.

### Results and Discussion :-

This study improved the ability of only 3 of 25 strains of *Staphylococcus aureus* isolated from severe dermonecrotic lesions to produce significantly greater inflammatory skin response in neonatal mice, epidermal positive result (Nikolsky sign as shown in Fig (1 A,B) which agreed with (16) who indicated that neonatal mice injected with ETs produced by *S. aureus* caused blister formation in skin similar to that seen in patients with bulbous impetigo and SSSS. It remained unclear exactly how ETs causes this blister until it was discovered that it had structural and sequence homologies to serine proteases and suggested that they act as proteolytic enzymes.

(6) indicated that a number of authors found the incidence of ETs producing strains among all strains of *Staphylococcus aureus* isolated from hospitalized patients were few. [ They studied whether there was an epidemiological association between skin lesions (SSSS) and colonization of skin with ETs producing staphylococci. The incidence of exfoliative toxin – producing strains of *S. aureus* from hospitalized patients of all ages was found to be 6.2%. Which agreed with the incidence of the present study which was 12% ] Similar findings have been reported by several investigators the survey of (17) revealed that 164 (33%) of 500 pregnant women attending an antenatal clinic carried *S. aureus* in the nose , axilla and perineum, strains isolated 5 of them only produced ETs as determined by the neonatal mouse assay. Other studies reported toxin production in 6 % of 2632 *S. aureus* strains isolated from hospitalized patients of all ages (18). Another study of (19) found the incidence to be 4.4 % for *S. aureus* isolated from human diarrhea and wounds produced ETs. The study of (20) reported the incidence of ETs producing staphylococci isolated from children with dental disease to be 19 %. The study also revealed that injecting 0.1 ml of partially purified toxins into newborn mice, causes an intra epidermal cleavage as shown in fig (1 C,D).

This result was in agreement with the results of others such as (21) who indicated that at least two serologically distinct toxins ETA and

ETB can cause disease in human and are produced by a small but significant proportion of *S. aureus* strains although they differ in some physicochemical properties, and both toxins produce the dermatological effect described above in neonatal mice. The toxins are species specific affecting human, monkeys and mice but not rats, rabbits, dogs, guinea pigs or frog (22,23) most of the work on ETs has been done with ETA and the neonatal mouse model.

The toxins are produced during the post exponential phase of bacterial growth (22) and excreted from colonizing staphylococci before being absorbed into the systemic circulation (16).

The toxins reach the zona granulosa of the epidermis by diffusing through dermal capillaries this was confirmed by the <sup>125</sup>I – labeled ETs injected into neonatal mice. Apart from the skin there is no significant binding of these toxins to any other body organ (24).

Histological studies have shown that addition of ETs to confluent keratinocyte cultures isolated from pieces of human skin obtained from plastic surgery or mouse organotypic skin cultures results in the disappearance of small vesicles that are usually present between the cells (25,26).

This is followed by formation of intra – cellular fluid – filled gaps in the granular – spinosum. Which eventually leads to the characteristic mid epidermal splitting seen in SSSS. Cytolysis or necrolysis does not occur and inflammatory response or cell degeneration has not been observed in neighboring region (27,28).

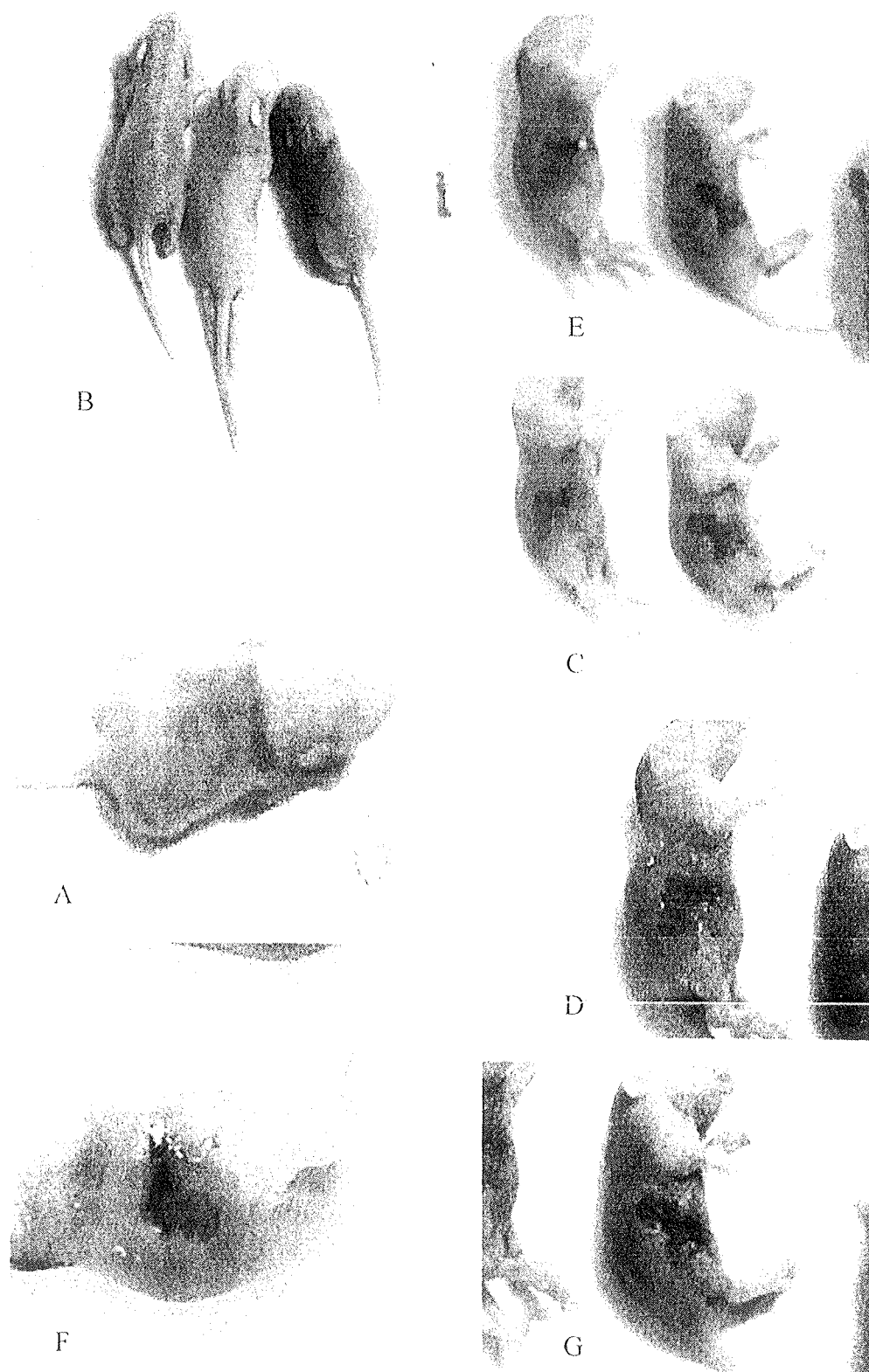


Fig (1) : A,B, Nikolsky sign at site of injection in new born mice with 0.1 ml of cell free extraction of *Staphylococcus aureus* ETs.  
C.....G , Intraepidermal destruction and cleavages caused by injection of 0.1 ml of partially purified ETs isolated from *Staphylococcus aureus* strains.

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