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DETECTION OF SOME EXFOLIATIVE TOXIN AND EDIN B GENE FOR *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SKIN LESIONS

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ABSTRACT : Polymerase chain reaction technique (PCR) is used for detection of some virulence genes particularly exfoliative toxins named *eta*, *etb* and *etd* genes. The results showed that 46% of the isolates gave positive amplicon for *eta* gene and 8.3% of the isolates gave positive result for the presence of *etb* gene and 66.6% of them gave positive result for *etd* gene. However, none of the isolates have the three genes at the same time. Also epidermal differentiation factor b (Edin b) was also investigated and the results showed that 17 isolates of *S. aureus* have this factor at rate (70.8%). Isolation and identification of *S. aureus* from skin lesions, Molecular study of exfoliative genes such as *eta, etb* and *etd* genes by PCR technique and investigation of *edinb* gene among *Staphylococcus aureas* isolates.

Key words : *S. aureus*, exfoliative toxin, ET genes, edin b gene.

INTRODUCTION

Staphylococcus aureus is considered as both a human commensal of the skin and a frequent cause of clinically important infections in hospital. It colonizes about one third of healthy humans and is most often found in the nose (Tong *et al*, 2015). However, as pathogenic bacteria, *S. aureus* causes a wide range of infections (*e.g.*, skin and soft tissue infections, sepsis pneumonia, Osteomyelitis, endocarditis) (Ouedraogo *et al*, 2016).

Exfoliated toxins are so important in strains which cause skin disease in human but these toxins are encoded by exfoliatve genes, which are present in *S. aureus* genome separately or adjacently (Botka *et al*, 2015) and confer the ability of this bacteria to cause a disease. However, some of them is found in non-pathogenic *S. aureus* and may be non-functional (Yamaguchi *et al*, 2002).

Besides *S. aureus* considered one of commensal bacteria have to balance the lifestyle between efficient surface adherence, to avoid removal by mechanical forces, and not being recognized and destroyed by the host's innate and /or adaptive immune systems. For *S. aureus* this is challenged by their own ability to express an arsenal of virulence factors that induce host cell and tissue damage which in turn would stimulate an immediate immune response (Vandenesch *et al*, 2012).

MATERIALS AND METHODS

Ethical approval

The necessary ethical approval from ethical committee in Marjan Hospital in AL-Hilla city and out patients clinics was obtained moreover agreement from the family and patients for sampling and carrying out this work was obtained.

Patient's specimens

This study includes 100 specimens, which were collected from skin infection in Marjan and AL-Emam AL-Sadeq hospitals in Hilla city, during a period of four months (from November 2017 to February 2018). The patient's age ranged from (6 years-70 years).

Laboratory diagnosis and identification of bacterial isolate

Skin swabs collection immediately inoculation into transport media tube for preserved from dryness until transport to laboratory, then the swabs were culture on growth media such as brain heart infusion and manitol salt agar and incubation at 37^0C for 24 hours. By depended on MaCfadden (2000) diagnosis procedure recommended take single colony from each positive culture and noting the morphology properties (color production, colony shape texture and edge). Also using Gram stain procedure for detection bacterial belong to Gram positive or Gram negative (Winn *et al*, 2006).

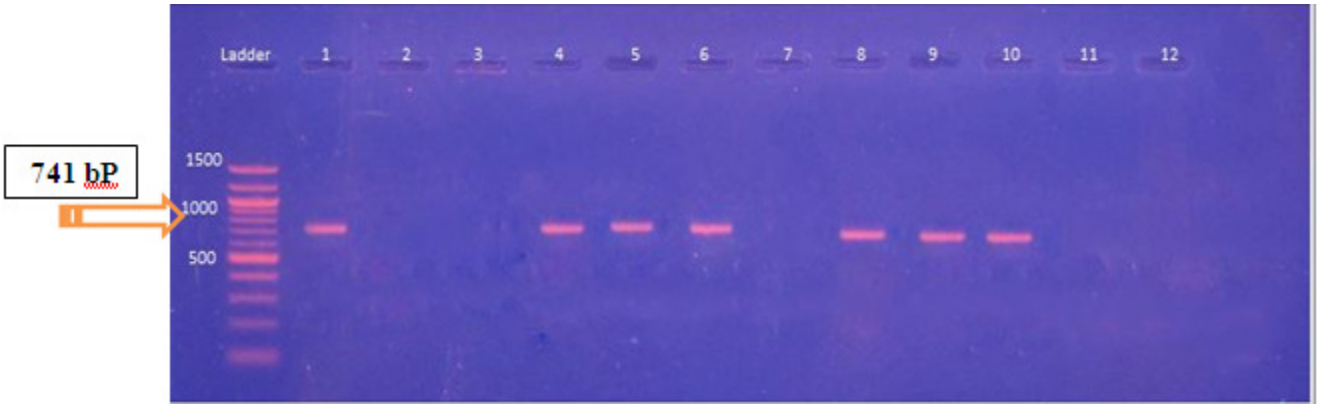

Fig. 1A : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *etA* with product 741bp. The electrophoresis was performed at 80 volt for 1 hr.vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *etA*.

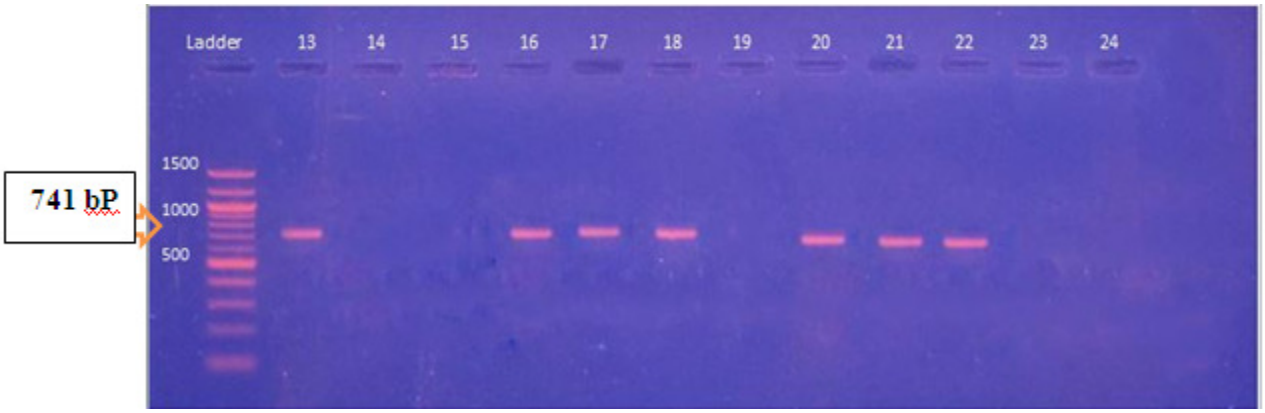

Fig. 1B : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *etA* with product 741bp. The electrophoresis was performed at 80 volt for 1 hr.vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *etA*.

Molecular identification

DNA was extracted by using geneaid kit specific for DNA extraction from Gram negative bacteria as *E. coli* belong to gram negative bacterial type and accordance with geneaid protocol.

Detection of some virulence factors for *Staphylococcus aureus* by PCR

The major Exfoliative gene detected were *eta, etb* and *etd*.

RESULTS AND DISCUSSION

Molecular detection of exofolative toxin gene was done for 24 isolates of *S. aureus* only (11) 46% of the isolates showed positive result for exfoliative toxin A. The positive results were detected by the presence of 741 bp bands when compared with allelic ladder as shown in Fig. 1.

The present results agree with that obtained by Yamaguchi *et al* (2002), who found that 47.7% of the *S. aureus* strains gave positive for this gene, whereas it is not correlated with Dunyach-Remy *et al* (2016), who showed that only (10%) of *S. aureus* were exfoliatve toxin A positive. And also disagree with Djahmi *et al* (2013) who found that this gene is absent among *S. aureus* isolates. Also, Sotto *et al* (2012) noticed that only (0.5%) have this gene.

It's known that *eta* gene is encoding ETA toxin, which is located on a *S. aureus* prophage (Yamaguchi *et al*, 2000) while other ET genes were localized on other accessory elements (as a plasmid) such as *etb* gene and/or a pathogenicity islands (PAIS) such as *etd* gene, which give an interpretation on why this gene is absent in some *S.aureus* strains.

ETA have been identified as one of the major causative agents of the blistering skin disease pemphigus neonatorum and/or generalised staphylococcal scalded skin syndrome (SSSS) (Yamaguchi *et al*, 2002).

Some data have confirmed that *ETA*-converting phages are crucial in the pathogenesis of staphylococcal skin blistering infections because of the extensive prevalence of *ETA*-producing impetigo strains of S. aureus. The study done by Botka *et al* (2015) have suggested that *S. aureus* has more than *eta* gene loci due to the presence of large group of diverse temperate phages.

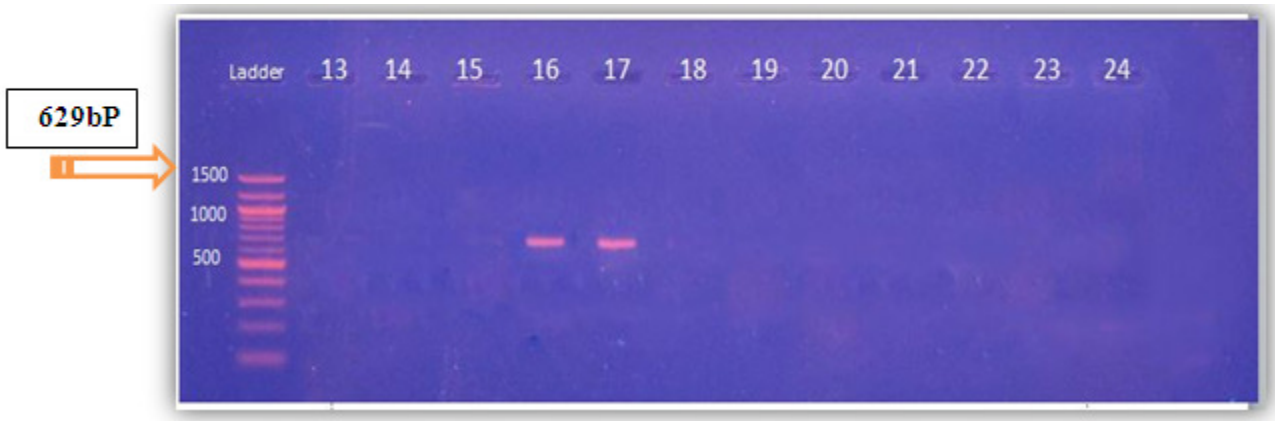

Fig. 2 : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *etb* with product 629bp. The electrophoresis was performed at 80 volt for 1 hr. vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *etb*.

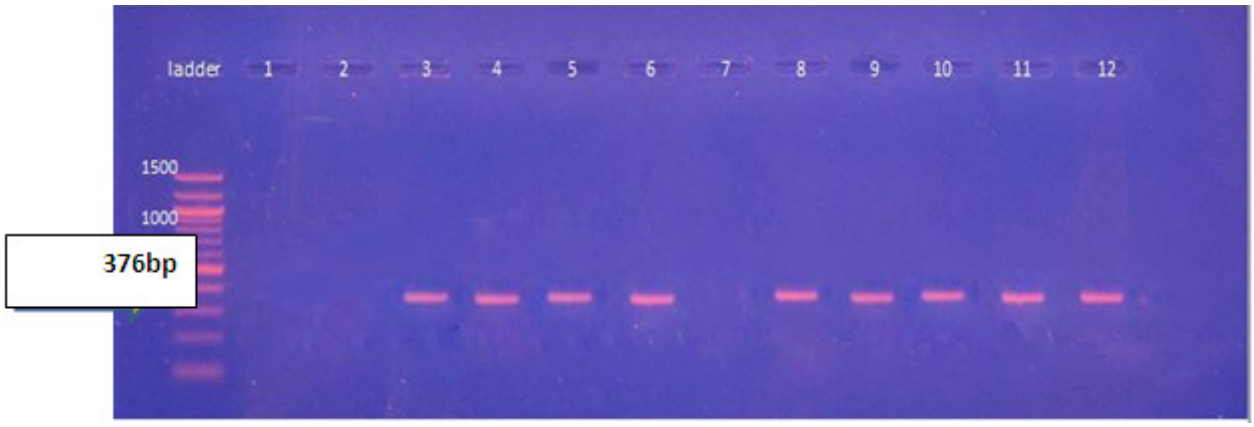

Fig. 3A : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *etd* with product 376bp. The electrophoresis was performed at 80 volt for 1 hr. vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *etd*.

Detection of Exfoliative toxin B *(etB)*

Molecular studies of Exfoliative toxin B gene were done for all *S. aurus* samples by using specific PCR markers. When PCR was performed, results clearly indicate that only 2 isolates (8.3%) contained Exfoliative toxin B gene and the amplified products produced a band at the level of 629 bp as shown in Fig. 2.

This result is agree with Yamaguchi *et al* (2002) who showed that 11.4% of the isolates carried *etb* gene and disagree with Djahmi *et al* (2013) and Sotto *et al* (2012) who found that exfoliatve toxin B (etb) genes were absent from *S. areus* isolates. And also the same result found by Sila *et al* (2009), who noticed that *etb* gene was absent among *S. aureus* isolates.

Exfoliative poison B (ETB), likewise to exfoliative poison An (ETA), is the dynamic serine protease delivered by a few strains of *Staphylococcus aureus*. They are nearly connected with poison intervened epidermolytic skin issue in people (Botkaa *et al*, 2017).

ETB is cadherin-like transmembrane glycoprotein desmoglein 1 (Hanakawa *et al*, 2002). In late investigations worried about the ETB-creating strains which have portrayed an expansive *etb* quality positive plasmid (Botkaa *et al*, 2017). In this way, the non attendance of plasmid containing this quality may decipher why this quality is missing among most *S. aureus* confines.

However, the absence of *etb* gene for *S.aureus* is mostly due to the absence of plasmids encoding such gene.

Detection of exfoliative toxin D *(etd)*

Molecular detection of this gene was carried out by using a specific PCR primer were done by comparison with allelic ladder, which gave a 376bp. It was found that exofolative toxin gene present in 16(66.6%) of the positive samples as shown in Fig. 3.

Our results disagree with Sotto *et al* (2012) and Djahmi *et al* (2013), which demonstrated that the presence of this gene among *S. aureus* isolates reach to 6% and 11.8%, respectively.

The presence of *etd* gene in *S. aureus* at high rate may be attributed to the presence of pathogenicity islands among these isolates and these PATI may include many virulence factors inside them.

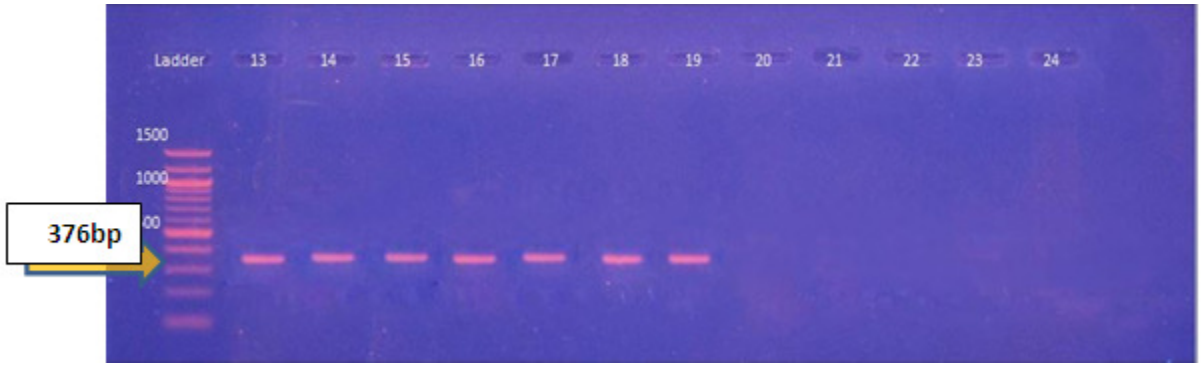

Fig. 3B: Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *etd* with product 376bp. The electrophoresis was performed at 80 volt for 1 hr.vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *etd.*

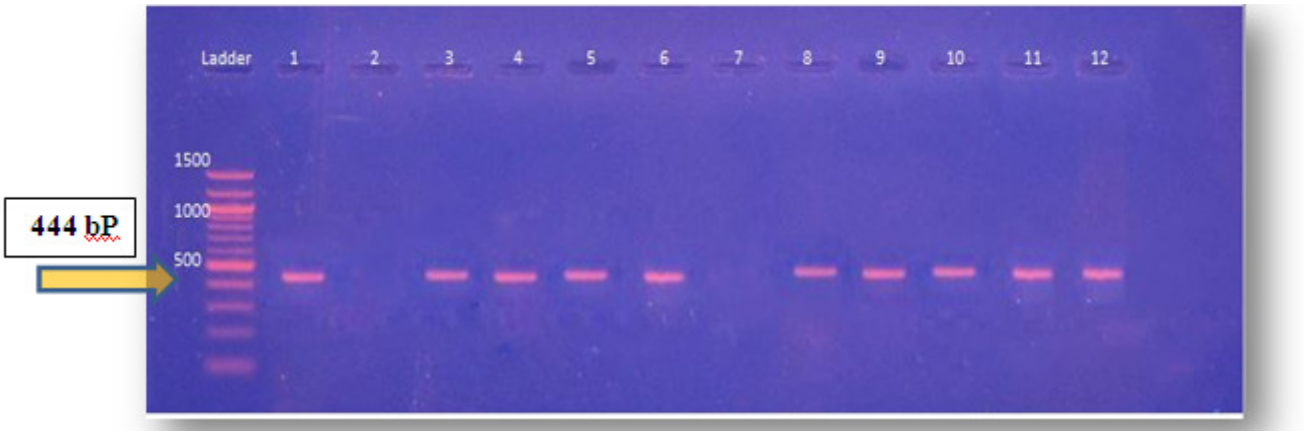

Fig. 4 A : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *edin b* with product 444bp. The electrophoresis was performed at 80 volt for 1 hr. vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *edin b.*

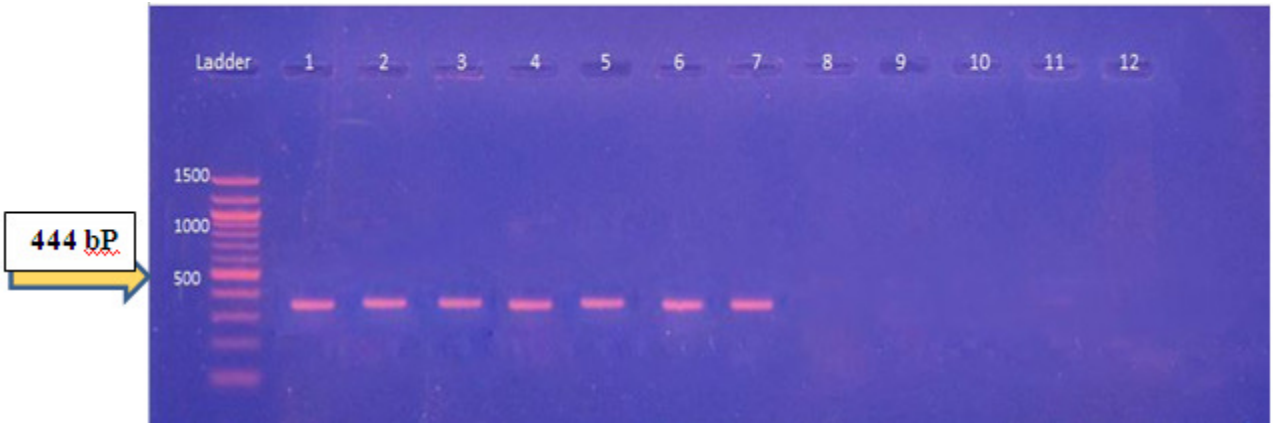

Fig. 4 B : Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *S. aureus* using primer *edin b* with product 444bp. The electrophoresis was performed at 80 volt for 1 hr. vertical Lane DNA molecular size marker (100 bp ladder). Horizontal Lanes show positive results with gene *edin b.*

Exfoliative poison D (ETD) was distinguished as of late as another exfoliative poison serotype. Like other exfoliative poisons, ETD incites intra-epidermal cleavage through the granular layer of the epidermis of neonatal mice (Yamasaki *et al*, 2006).

ETD is by all accounts related with the development of cutaneous abscesses and furuncles described by broad tissue harm, which may be a consequence of the limited activity of ETs (Johnson and Stell, 2000).

However, it was demonstrated that *etd* gene quality is more overwhelming among *S.aureus* segregates, though the others exfoliative poisons (ETA and ETB) qualities are less basic among those disconnects as shown in Table 2.

Table 1 : Contents of the reaction mixture.

No.	Contents of reaction mixture	Volume
1.	Green master mix	5ì l
2.	forward primer	2.5ì l
3.	reverse primer	2.5ìl
4.	DNA template	6ì l
5.	Nuclease free water	4ì l
	Total volume	20ì l

Table 2 : Distribution of exfoliative toxins genes among *S.aureus* isolates.

No.	Exfoliative toxin A	Exfoliative toxin B	Exfoliative toxin D
***S.aureus* 24**	**11 isolates (46%)**	**2 isolates (8.3%)**	**16 isolates (66.6)**

According to the data above, only two isolates was found to have the three genes at the same time, this will increase the virulence of bacteria.

Detection of epidermal cell differentiation inhibition toxin B gene (*edin b gene*)

Edin b gene was also detected in *S.aureus* samples and found that 17(70.8%) gave positive results to this gene, which gave molecular length (444) bp as shown in Fig. 4.

However, Ouedraogo *et al* (2016) found that only (31.9%) of *S. aureus*, where *edin b* positive. However, Jianga *et al* (2018) found that *edin b* gene where entirely absent among *S. aureus* isolates.

The prevalence of this gene may confer the translocation of bacteria from its normal habitat in the skin to the blood stream. Also, this gene may be present on specific phage which can infect *S. aureus* and increase its pathogenicity. On the other hand, most isolates positive for *Etd* protein and this will increase their ability to cause skin infection and other disease. However, Yamaguci *et al* (2002) have mentioned that *Etd* and *Edin b* genes are located on the same pathogenicity islands in *S.aureus*.

EDIN protein may support bacterial dispersal in tissues by a hematogenous course, through the enlistment of substantial trans cell burrows in endothelial cells named full scale openings (Lemichez *et al*, 2010) and furthermore EDIN poisons advance the arrangement of contamination foci in a mouse model of bacteremia (Munro *et al*, 2010).

The epidermal differentiation factor (EDIN) of *S. aureus* have a place with such factors of very much decided method of activity, despite the fact that their suggestion in staphylococcal disease stays poorly characterized EDIN has a place with a family of bacterial exotoxins focusing on the little host protein RhoA, which they repress (Aktories, 2011).

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