PREVALENCE OF EXFOLIATIVE TOXIN GENES AMONG CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN IRAQ

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ABSTRACT

The aim of this study is to determine the prevalence of *eta, etb* and *etd* genes among clinical isolates of *S. aureus*. 91 isolates of the bacterium were isolated from different clinical sites during the period from 2019 to 2020 from Baghdad hospitals, all the isolates were diagnosed by different biochemical tests and molecular method (PCR) using *nuc* gene. the PCR technique was used to detect *eta, etb* and *etd* genes among the isolates, the results showed that 91(100%) of the isolates were have *nuc* gene. while, 83 (91.2%) of the isolates at least carrying one of the *ET* genes; 20 (22%) , 0 (0%) and 63 (69.2%) of the 91 isolates expressed eta, *etb* and *etd* genes, respectively. While, 8 (8.8%) of isolates were lacking of these genes. In addition, 14 (15.4%) of isolates were carrying both *eta* and *etd* genes. Although the *etd* gene was found in all sample types, but *eta* gene was found only in wound, ear, throat and nose while, *etb* gene was not found in all types of clinical samples.

Keywords: **PCR, scalded skin syndrome, *nuc gene*, molecular method.**

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مدى انتشار مورثات سموم تقشر الجلد بين عزلات المكورات العنقودية الذهبية السريرية في العراق

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المستخلص

الهدف من هذه الدراسة هو تحديد مدى انتشار مورثات سموم تقشر الجلد (*eta,etb, etd*) بين العزلات السريرية للمكورات العنقودية الذهبية ، تم عزل 91 عزلة من البكتيريا من مواقع مختلفة خلال الفترة من 2019 إلى 2020 من مستشفيات بغداد ، تم تشخيص جميع العزلات باختبارات كيميائية حيوية مختلفة وبواسطة تفاعل البلمرة المتسلسل بواسطة مورث *nuc*, كذلك تفاعل البلمرة المتسلسل تم استخدامه لتشخيص مورثات تقشر الجلد (*eta,etd, etd*) بين العزلات. في هذه الدراسة وجدنا91 (100%) من العزلات كانت تمتلك المورث *nuc*.بينما, 83 (91.2%) من العزلات على الاقل كانت تحمل واحدا من مورثات سموم تقشر الجلد. 20 (22%),0 (0%) و63 (69.2%) من 91 عزلة امتلكت مورثات(*etd,etb,eta*) على التوالي. بينما, 8 (8.8%) من العزلات كانت تفتقد لهذه المورثات. بالاضافة ,14(15.4%) من العزلات كانت تحمل كلا المورثين (*eta, etb*). بالرغم من ان المورث (*etd*) قد وجد في جميع انواع العينات, لكن المورث(*eta*) وجد فقط في عينات الجروح, الاذن, البلعوم والانف. مورث ال(*etb*) لم يكن متواجدا في اي نوع من العينات.

كلمات مفتاحية: تفاعل البلمرة المتعدد, جين *nuc*, الطريقة الجزئية, متلازمة الجلد المنسلخ

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INTRODUCTION

Staphylococcus aureus is a bacterial species resident in the skin and nasal membranes with a dreadful pathogenic potential to cause a variety of community and hospital-acquired infections (16) and (1). For decades, *S. aureus* has been predominantly as a nosocomial pathogen and is a leading cause of mortality in hospitals. However, the community *S. aureus* infections are increased. important clinical *S. aureus* infections include skin (20) and soft tissue infections, bacteremia, infective endocarditis, osteoarticular infections, and pleuropulmonary infections. Some clinical diseases include epidural abscess, meningitis, toxic shock syndrome and urinary tract infections (23) and Otitis media (6). The pathogenicity of *S. aureus* is due to toxins. Invasiveness and antibiotic resistance (2). Accordingly, one of the significant characteristics of *S. aureus*, its ability to secrete toxins which involving in severity diseases that including: exfoliative toxins, toxic shock syndrome toxin-1 and staphylococcal enterotoxins which implicated in staphylococcal scalded skin syndrome, toxic shock syndrome and staphylococcal food poisoning (SFP), respectively (19). Exfoliative toxins Which is directly responsible for the clinical manifestation of staphylococcal scalded skin syndrome (SSSS) (3). This type of blistering skin disease is divided into two clinical forms, the localized and generalized forms. The generalized form, which is called Ritters disease, frequently occurs in infants and children (12). While, the localized form are epidermal infections such as bullous impetigo (11). Three isoforms of ETs which are ETA, ETB and ETD, The *eta* gene encoding ETA has a prophage origin which is located on a chromosome, and the *etd* gene encoding ETD is chromosomally located in a 14.8 kb pathogenicity island. However, the etb gene encoding ETB is located on a 42 kb plasmid (14). These toxins are capable of cleaving desmogelin 1, a cadherin protein, which mediates cell—cell adhesion in keratinocytes in the skin (10). The ETA and ETB toxins are associated with the occurrence of staphylococcal scaled skin syndrome (SSSS) while the ETD toxin is causing bullous impetigo (27). The introduction of PCR method will help provide the information required for appropriate infection control during outbreaks of S. aureus because that technique only will identify S. aureus strains harboring the toxins genes (21). This study was aimed to investigate the presence and prevalence of genes encoding exfoliative toxin among different clinical isolates of *Staphylococcus aureus*.

MATERIALS AND METHODS

Isolation and identification of *S. aureus*

A total of 91 *S. aureus* isolates were isolated from different clinical sites from patients who admitted different hospitals in Baghdad city from 19th September 2019 to 20th January 2020. These isolates were diagnosed by using biochemical tests and molecular methods using *nuc* gene. Antibiotic susceptibility.

Molecular study

This study included molecular detection of three bacterial toxin genes (*eta*, *etb* and *etd*).So, All isolates were subjected to DNA extracted by using geneaid corporation genomic DNA kit (Presto mini gDNA Bacteria Kit, Korea) . The genes were amplify using specific primer (table 1), and the PCR mixture reaction contained 2 µl of DNA template (40 ng/µl), 1 µl of forward primer and 1 µl of reverse primer(10 pM/µl) and 12.5 µl of master mix (GoTaq Green Master Mix,Promega. USA). The PCR reaction was performed as following: initial denaturation at 94°C for 5 mins with 1 cycle, (denaturation at 94°C for 45 sec, annealing at 57°C, 53°C, 54°C and 55°C for *nuc*, *eta*, *etb* and *etd* respectively for 45 sec, extension at 72°C for 1 min within 35 cycle and final extension at 72°C for 5 mins within 1 cycle.

Table 1. primers used for detection of genes in this study

Primers	Sequence (5′→3′)	Product size	Reference
nuc-F	GCGATTGATGGTGATACGGTT	279bp	(17)
nuc-R	AGCCAAGCCTTGACGAACTAAAGC		
eta-F	TATCGCCAGCAAAAATAGGG	165bp	This study
eta-R	TTCCCGGAACTGTAAATCCA		
etb-F	TACCACGTTGCAAGAGAAGC	195bp	This study
etb-R	TGATTCCCCTTTTTCGTTTG		
etd-F	CGGAAAGTCTGCAGGTGATT	193bp	This study
etd-R	TCCAGAATTTCCCGACTCAG		

The PCR products were determined by electrophoresis using 2% agarose gel stained with ethidium bromide and migrated with electrophoresis apparatus (Labnet, USA) at 70 volt for 85 minutes, Then, visualized with gel documentation system with UV illumination. The 50-1500 bp DNA ladder (Bioland, USA) was used as a DNA size marker.

RESULTS AND DISCUSSION

In this study, we determined the prevalence of exfoliative toxin genes *eta*, *etb* and *etd* among 91 clinical isolates of *S. aureus*, the results showed that Of the 91 isolates, 91(100%),20 (22%) , 0 (0%) and 63 (69.2%) carrying *nuc*, *eta*, *etb* and *etd* genes respectively (figure 1). While, 8 (8.8%) of isolates was lacking of *eta*, *etb* and *etd* genes. In addition,14 (15.4%) of isolates carrying both *eta* and *etd* genes. The distribution of *nuc*, *eta*, *etb* and *etd* genes according to the type of isolates show in Figure 2.

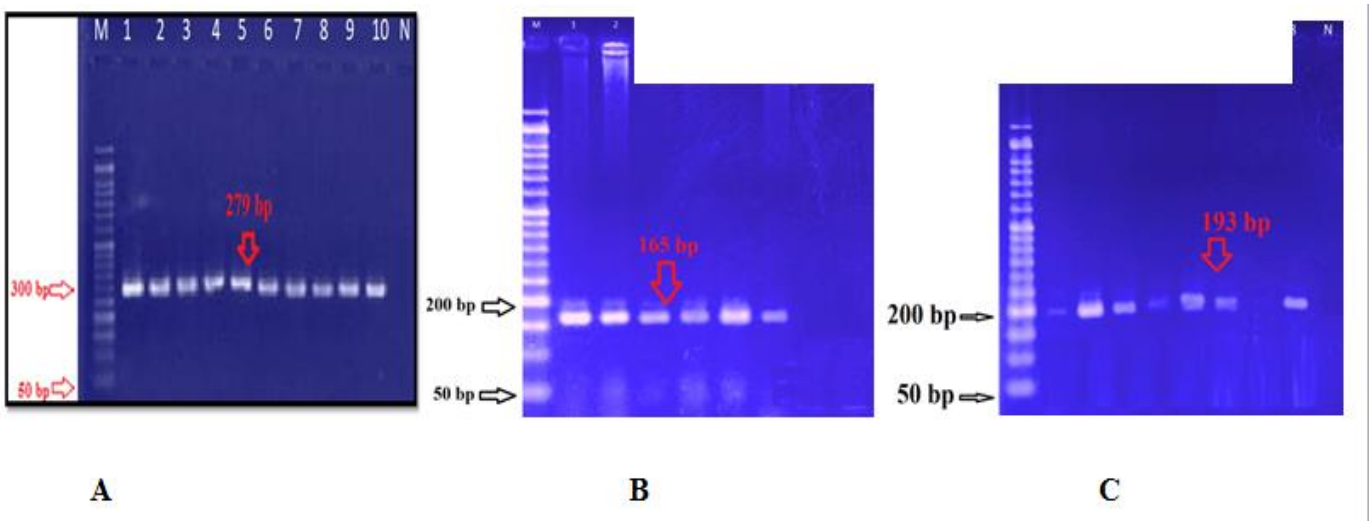

Figure 1. Agarose gel electrophoresis of PCR amplified in *S. aureus* of the (A) *nuc* gene with a product size of 279 bp, (B) *eta* gene with a product size of 165 bp, (C) *etd* gene with a product size of 193 bp . Lane M, 50-1500 bp DNA ladder; lane N, negative control; 2% agarose, TBE buffer (1x) and 70 volt for 85 minutes

The distribution of *nuc*, *eta*, *etb* and *etd* genes according to the type of isolates shown in Figure 2

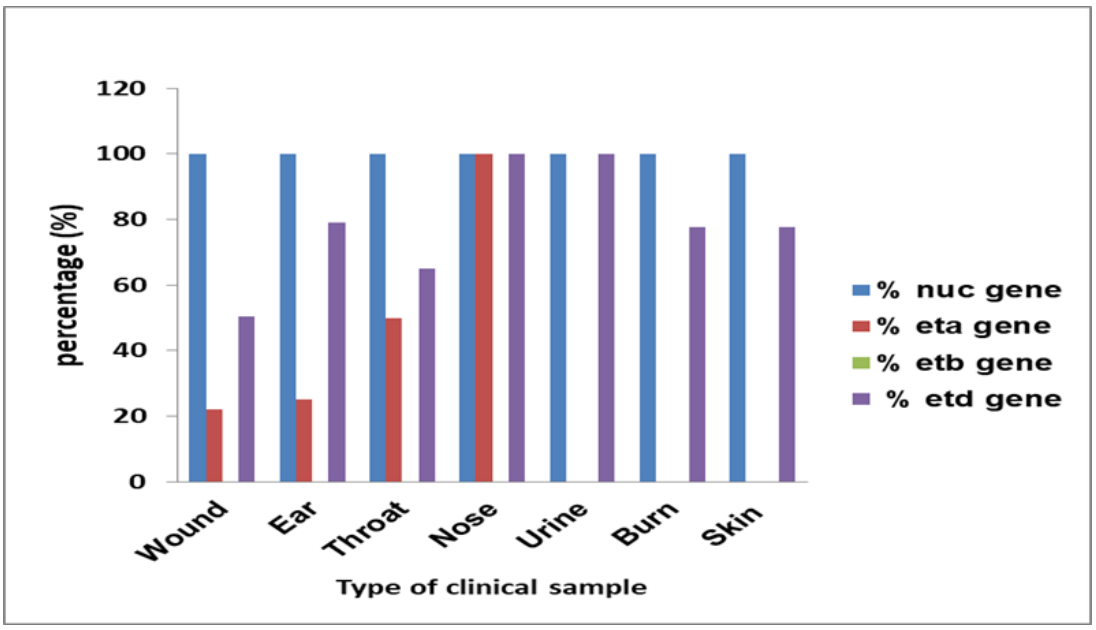

Figure 2.The frequency of ETs and *nuc* genes in isolated *S. aureus* based on different specimens

The largest numbers of *eta*-positive isolates were isolated from the nose (100%) and throat (50%) samples. Moreover, no *eta* gene was detected in urine, burns and skin samples. none of all sample type (0%) contained *etb* gene. The largest numbers of *etd*-positive strains and were found in nose (100%), urine (100%), burn (77.7%) and skin (77.7%) samples. In this study, we found that the prevalence of *eta*, *etb* and *etd* genes was in different ratios. The *etd* was more frequent than *eta* and *etb*. The variance in the distribution of genes may be caused by the origin of isolate, occurrence it in a given geographic region and infection sites (8), (5) and (21). For *eta* gene our result was identical to that found in Turkey (4) and in United Kingdom (18) that they found in these two countries the percentage of 19.2% and 22% respectively. Unlike these countries the prevalence of eta gene was different in Iraq,Anbar (21) and in China (13), (26), our result was higher than those 3%,1.6% and 1.8% respectively. While the *etb* gene in Iraq, Anbar (21), China (25) and Columbia (7) was the same as our result which were 3%, 0% and 0% respectively. But different results were higher than our ratios in Iran (14) , (10) that were 16.7% and 7.6% respecrively, detection of *etb* gene in larger samples is important to get better results related to their prevalence in different societies. The *etd* gene was found in 69.2% of our clinical isolate which is higher than any result found yet, in Iran (14) found the etd in 54% while in Nigeria (9) found that the *etd* in percentage 1.6% of their samples .in addition , 4.5% found in Netherlands (24), High prevalence of *etd* gene in this study may be due to the fact that exfoliative toxin is a serine protease that cleaves and colonize the skin of mammalians and mucosa thus, facilitate bacterial invasion through cleavage of adhesion molecules between adjacent keratinocytes (16) therefore the higher rate of this gene could be also due to high frequency of wounds (14) and burns samples. In spite of geographical diversity, data from different parts of the world suggest a higher distribution of *eta* than *etb* because of its high immunogenicity (22) and (10). From this study we concluded that the prevalence of *etd* gene was higher than eta and etb among MRSA strains of *S. aureus* in different clinical isolates in Iraq.

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