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.
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, osteoarticular infections, and *pleuropulmonary infections*. Some clinical diseases include epidural abscess, meningitis, 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 localized form, which is called *Ritter's disease*, frequently occurs in infants and children (12). While, the localized form is 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 desmoglein 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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5′→3′)</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuc-F</td>
<td>GCGATTGATGGTGATACCGTT</td>
<td>279bp</td>
<td>(17)</td>
</tr>
<tr>
<td>nuc-R</td>
<td>AGCCAGCCTTGACGAATAAGGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eta-F</td>
<td>TATCGCCAGCAAAAAATAGGG</td>
<td>165bp</td>
<td>This study</td>
</tr>
<tr>
<td>eta-R</td>
<td>TTCCCGGAACTGTAATCCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>etb-F</td>
<td>TACCACTTGCAAGAGAAGGC</td>
<td>195bp</td>
<td>This study</td>
</tr>
<tr>
<td>etb-R</td>
<td>TGATTCCCCTTTTCGTGTTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>etd-F</td>
<td>CGGAAGTGCTGAGGCAGTGATT</td>
<td>193bp</td>
<td>This study</td>
</tr>
<tr>
<td>etd-R</td>
<td>TCCAGAATTTCGCCGACTCAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 \(\eta\a\), \(\eta\b\) and \(\eta\d\) 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 \(nuc\), \(\eta\a\), \(\eta\b\) and \(\eta\d\) genes respectively (figure 1). While, 8\%(8.8\%) of isolates was lacking of \(\eta\a\), \(\eta\b\) and \(\eta\d\) genes. In addition, 14\%(15.4\%) of isolates carrying both \(\eta\a\) and \(\eta\d\) genes. The distribution of \(nuc\), \(\eta\a\), \(\eta\b\) and \(\eta\d\) genes according to the type of isolates show in Figure 2.

![Figure 1](image1.png)

**Figure 1.** Agarose gel electrophoresis of PCR amplified in \(S.\ aureus\) of the (A) \(nuc\) gene with a product size of 279 bp, (B) \(\eta\a\) gene with a product size of 165 bp, (C) \(\eta\d\) 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 \(nuc\), \(\eta\a\), \(\eta\b\) and \(\eta\d\) genes according to the type of isolates shown in Figure 2.
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 *etb* 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% respectively, 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 colonizes the skin of mammals 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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