Frequency of exofoliative toxine genes among staphylococcus aureus isolated from burn infection patients in the Specialized Centre for burns of Al - Diwaniyah city

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Abstract

Background: Exfoliative toxins (ETs) of bacterium Staphylococcus aureus are the main cause of infections in skin burns. This research sought to determine the frequency of the eta(exofoliative toxine A) gene, etb(exofoliative toxine B) gene and etd(exofpliative toxine D) gene in the S. aureus..

Methods: Between January 2021 and December 2021, 155 S. aureus isolates were gathered from burn sample patients at the Al-Diwaniyah Specialized Burns Center. The polymerase chain reaction was utilized to identify the eta gene, etb gene and etd gene found in the isolates after the species had been established using conventional diagnostic techniques.

Results: Overall, Of the 155 isolates, 25(16.1%), 19(12.2 %) and 44(28.3 %) stated the eta gene, etb gene and etd gene, correspondingly. adding, 20(12.9%) isolates expressed both the eta gene and etd gene, and 16(10.3 %) isolates carrying the etb gene and etd gene, were the eta gene, etb gene and etd gene detected in 33(21.2) .

Conclusion. Because it is likely that these genes will spread and move between strains, the discovery of the extraordinary prevalence of Exofoliative toxines genes in the present study is regarded as a thoughtful issue. Additionally, these community-wide spread isolates are crucial for maintaining good health.

Keywords: Staphylococcus aureus, Exfoliative toxin genes, Antimicrobial agents.

INTRODUCTION

The skin’s healthy condition serves as the body’s first line of protection against external microbes, but scratches, wounds, and burns to the epithelial layer can expose the skin and subcutaneous tissues to infections like staphylococcus aureus[1] Because the skin covers both the internal and external surfaces of the body, it provides a favorable environment for the growth and reproduction of these bacteria, leaving the body vulnerable to a variety of clinical illnesses that can range in severity from mild to fatal.

This is because S. aureus bacteria can produce a number of components, including the capsule and extracellular substances like a variety of enzymes like plasma coagulase and fibrinolysin, which make the body vulnerable to many clinical diseases. [2]
S. aureus bacteria are moreover characterized by their production of exotoxins such as enterotoxins that cause food poisoning. Toxins that cause septicemia, endocarditis and exfoliative toxins (ETs) that recognize and hydrolyze desmosome proteins in the skin[3].

One of the health issues patients who are hospitalized due to the illnesses they generate must deal with is the bacterial contamination of burns. The majority of patients that catch this type of illness are those receiving intensive care around the world, particularly in underdeveloped nations. It is known as hospital infection (Nosocomial infection) [4]. The occurrence and progression of infection are influenced by a number of variables, including pathogenicity, the quantity of infecting bacteria, and the severity of the infection. The pathogen that the bacterium transmits, the host's susceptibility to infection, the host's exposure to those pathogens, the type of wound and the level of burn length of hospital stay, age, sex, and antibiotic use[5]. Currently, S. aureus is the leading cause of infections in hospitalized patients. The fact that the bacterium affects a variety of organs and causes a wide range of illnesses suggests that it has a variety of unique molecular mechanisms for boosting bacterial survival in various in vivo environments[6].

S. aureus in turn is the most common germ to infect hospitalized patients. The bacterium appears to have a variety of distinct molecular strategies for enhancing bacterial survival in varied in vivo conditions given that it affects a variety of organs and causes a wide range of disorders[7]. The most common and serious S. aureus clinical infections are bacterial endocarditis, bacteremia, pleuropulmonary infections, and osteoarticular infections. Other major and significant infections include skin and soft tissue infections. Also, meningitis, epidural abscess, UTIs, and toxic shock syndrome are a few of the illnesses[8].

Soft tissue and skin infections are the greatest well-known S. aureus infections globally. The severity of these conditions ranges from small (superficial infections) to life-threatening conditions, needing all available medical incomes for treatment[9]. Burns and surgical site infections are examples of conditions where infections can develop after the skin's protective barrier has been compromised. Without a significant breach in the skin barrier, infections can still take place. For instance, folliculitis can develop in hair follicles, and furuncles and carbuncles can form in pores. Cellulitis is caused by the impact of deep layers, such as the dermis and subcutaneous tissue, while fasciitis is caused by the penetration of even deeper tissues, such as underlying muscle[10].

Exfoliative toxins can be produced by about 5% of all S. aureus strains. The ETA is most frequently found in America, Europe, and Africa, but the ETB is more prevalent in Japan. Several years later, it was discovered that there are two exfoliative toxins, ETA and ETB, that can cause epidermal blisters [11]. The specific chemical process by which ETs generate an exfoliation is still unknown. However, the stratum granulosum has since become the site of epidermal detachment, according to electron microscopy[12].

According to our knowledge, few data on the available of S. aureus isolates carrying the eta gene, etb gene and etd gene in isolations acquired from burns specimens in AL-Diwaneyah City. In this work, directed to evaluate the incidence of S. aureus strains embracing the ETs genes in burns samples strains. adding, the antibiotics susceptibility pattern of each isolate and the association of this form with the occurrence of these genes are also investigate.

**Methodology**

**Bacterial isolate**

The sensitivity testing and culturing was conducted on all clinical isolates. In the Specialized Burn Center of Al-Diwaneyah City, of the First, between January 2021 and December 2021 were included in this study. All of 225 specimens gathered from burns patients, 155 different S. aureus isolates were detected. Gram staining, catalase and coagulase tests, mannitol fermentation tests, and the presence of hemolysis on a blood agar plate after a 24-hour growth period at 37°C were used to identify the isolated S. aureus strains. Staph. aureus strains were then recognized by the VITEK-2 compact (BioMérieux, France) GP colorimetric card.

**Antibacterial resistance testing**

AST was performed using the VITEK-2 compact card for Gram-positive bacteria sensitivity, which included antibiotics indicated for routine reporting of S. aureus: Cefoxitin (fox), Chloramphenicol (C), Ciprofloxacin (CIP), Clindamycin (CD), Erythromycin (E), Gentamicin (GM), Linezolid (L), Nitrofurantoin (NI), Penicillin (P), Rifampin (R). The values for the MIC interpretative typical or division were well-known in accord with the recommendations of Clinical and Laboratory Standard Institute (CLSI) M100-S23. A value check for MIC finding was done by S. aureus ATCC25923 [13].

**polymerase chain reaction(PCR) for the detection of ET genes**

According to the manufacturer's recommendations, DNA of genome was derived by a DNA extraction kit (Geneaid, Korea) and lysostaphin. Using particular primers, PCR was used to identify the exfoliative toxin A(eta), B(etb), and D(etd) encoding genes (eta gene, etb gene and etd gene respectively) (Table 1).
**RESULTS**

**Bacterial isolates**

In the present investigational study, from a total of 225 burn samples, 155 staph. aureus isolates were identified and we assessed the frequency of S. aureus, which was present in these bacterial isolates and had the three exfoliative toxin genes.

Table (1): Sequence of the primers was used with the name and size of the product

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer</th>
<th>Nucleotide sequences 5’-3’</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>TTCCCGGAAACTGT AAATCCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGATTTCCCTTTTT CGTTTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TCCAGAATTCCC GACTCAG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The PCR mixture was complete in a full volume of 25 μl comprising: the DNA extracted, primers and PCR premix was provided by the supplier company (Promega) that contains Nuclease-Free Water and PCR Master Mix (2X). The PCR Master Mix is a pre-mixed solution to be used with Taq DNA polymerase, dNTPs from bacteria, MgCl2, and reaction buffers at the right amounts for effective PCR amplification of DNA templates. Two dyes—blue and yellow—in Master Mix allow for the monitoring of electrophoresis progress. Reactions acquired with the Master Mix are dense enough to be loaded directly onto agarose gels. The constituents of a mixture for PCR premix and the accompaniments of materials were dependent according to the procedure of the manufacturing company (Promega, USA). Each reaction included a negative control without DNA to highlight potentially dangerous PCR component cross-contamination [14].

**Statistical analysis**

The statistical analysis was carried out utilizing SPSS software, version 20, which is a statistical tool for social sciences. The association of sample type in the existence of the eta gene, etb gene and etd gene were examined using the Chi-square test and the Fischer’s test for a lower number series.

The incidence of the eta gene, etb gene and etd gene was recognized by conventional polymerase chain reaction. A illustrative pattern of a PCR reaction for the documentation of these three genes is presented in Table(2). Of the 155 isolates, 25(16.1%), 19(12.2 %) and 44(28.3 %) stated the eta gene, etb gene and etd gene, correspondingly. A absence in both the eta gene and etb gene were noticed in 15(9.67 %) strains. adding, 20(12.9%) isolates explained that both eta gene and etd gene, also 16(10.3 %) isolates carrying the etb gene and etd gene, were the eta gene, etb gene and etd gene detected at the 33(21.2) .

Table (2): Frequency of the eta gene, etb gene and etd gene positive groups in the clinical isolate

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pos. isolates(%)</th>
<th>Neg. isolates(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eta</td>
<td>25(16.1)</td>
<td>130(83.8)</td>
</tr>
<tr>
<td>etb</td>
<td>19(12.2)</td>
<td>136(87.7)</td>
</tr>
<tr>
<td>etd</td>
<td>44(28.3)</td>
<td>111(71.6)</td>
</tr>
<tr>
<td>eta, etb</td>
<td>15(9.67)</td>
<td>140(90.3)</td>
</tr>
<tr>
<td>eta, etd</td>
<td>20(12.9)</td>
<td>135(87.0)</td>
</tr>
<tr>
<td>etb, etd</td>
<td>16(10.3)</td>
<td>139(89.6)</td>
</tr>
<tr>
<td>eta, etb, etd</td>
<td>33(21.2)</td>
<td>122(78.7)</td>
</tr>
</tbody>
</table>

**Exfoliative toxins distribution**

Antimicrobial susceptibility test in combination with presence of exfoliative toxin genes

Antimicrobial susceptibility testing was used to identify each isolate's antibiotic resistance traits as shown in table(2). The highest resistance of S. aureus isolates to Penicillin G in 88(56.7%), Erythromycin (54.8%) , Cefoxitin (50.3%) and Tetracycline(42.5%) . In contrast, Ciprofloxacin, Trimethoprim and Nitrofurantoin were showed a low resistance percentage of (4.5%), (3.22 %) and (1.9%) respectively. While, some antibiotics such as Gentamicin, Rifampin, Clindamycin, Chloramphenicol and Linezolid did not face any resistance by the S. aureus so showed 100% susceptibility for each antibiotic.
Dependent on the P-Value ≤ 0.05 in table (2) results using SPSS statistical package for social sciences for data analysis (version 20 for Windows, SPSS, Chicago, USA), The presence of the eta gene dramatically boosted resistance to other drugs., the existence of eta gene with the resistance to Erythromycin, Cefoxitin, Penicillin G and Tetracycline antibiotics in the same population or patient represent a protective factor for the patient or population while for the rest of antibiotics is unaffected.

Except for Chloramphenicol, Clindamycin, Gentamicin, Linezolid and Rifampin, resistance to other antibiotics tested in the isolates bearing the etb and etd genes was pointedly greater in comparison with resistance in the isolates lacking the etb and etd genes (p < 0.05) (p = 0.031, chi value = 13.54).

In the present study, we establish that the occurrence of eta gene, etb and etd genes as figures(2,3,4)was dispersed in altered percentages. The etd was more recurrent than eta and etb. The presence of toxin genes among burns isolates of S. aureus in comparison with resistance to antibiotics can be illustrated in the table(3).

Table (3) Evaluation of the antibiotics resistance in the existence or nonexistence of the eta, etb and etd genes.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>N0 of Resistance isolates N (%)</th>
<th>eta (+)no (%)</th>
<th>(-)no (%)</th>
<th>Pvalue</th>
<th>etb (+)no (%)</th>
<th>(-)no (%)</th>
<th>Pvalue</th>
<th>etd (+)no (%)</th>
<th>(-)no (%)</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin-(fox)</td>
<td>78(50.3)</td>
<td>25(32.0)</td>
<td>53(67.0)</td>
<td>&gt;0.005</td>
<td>19(24.1)</td>
<td>79(74.7)</td>
<td>&gt;0.005</td>
<td>44(56.4)</td>
<td>34(35.3)</td>
<td>&gt;0.005</td>
</tr>
<tr>
<td>Chloramphenicol-(C)</td>
<td>0(0)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin-(CIP)</td>
<td>7(4.5)</td>
<td>4(57.1)</td>
<td>3(42.8)</td>
<td>&gt;0.005</td>
<td>3(42.8)</td>
<td>4(57.1)</td>
<td>&gt;0.005</td>
<td>2(28.5)</td>
<td>5(71.4)</td>
<td>&gt;0.005</td>
</tr>
<tr>
<td>Clindamycin-(CD)</td>
<td>0(0)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
</tr>
<tr>
<td>Erythromycin-(E)</td>
<td>85(54.8)</td>
<td>15(17.6)</td>
<td>70(82.3)</td>
<td>&gt;0.005</td>
<td>17(20.0)</td>
<td>68(80.0)</td>
<td>&gt;0.005</td>
<td>35(41.1)</td>
<td>50(58.9)</td>
<td>&gt;0.005</td>
</tr>
<tr>
<td>Gentamicin-(GM)</td>
<td>0(0)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
</tr>
<tr>
<td>Linezolid-(L)</td>
<td>0(0)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin-(NI)</td>
<td>3(1.9)</td>
<td>1(33.3)</td>
<td>2(66.6)</td>
<td>1</td>
<td>1(33.3)</td>
<td>2(66.6)</td>
<td>1</td>
<td>1(33.3)</td>
<td>2(66.6)</td>
<td>1</td>
</tr>
<tr>
<td>Penicillin-(P)</td>
<td>88(56.7)</td>
<td>17(19.3)</td>
<td>71(80.6)</td>
<td>&gt;0.005</td>
<td>13(14.7)</td>
<td>75(85.2)</td>
<td>&gt;0.005</td>
<td>44(50)</td>
<td>44(50)</td>
<td>&gt;0.005</td>
</tr>
<tr>
<td>Rifampin-(RP)</td>
<td>0(0)</td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
<td>0(0.00)</td>
<td>0(0.00)</td>
<td></td>
</tr>
<tr>
<td>Tetracycline-TE</td>
<td>66(42.5)</td>
<td>13(19.6)</td>
<td>53(80.3)</td>
<td>&gt;0.005</td>
<td>17(25.7)</td>
<td>49(74.2)</td>
<td>&gt;0.005</td>
<td>37(56.2)</td>
<td>29(43.8)</td>
<td>&gt;0.005</td>
</tr>
<tr>
<td>Trimethoprim-(TMP)</td>
<td>5(3.22)</td>
<td>2(40.0)</td>
<td>3(60.0)</td>
<td>1</td>
<td>1(20.0)</td>
<td>4(80.0)</td>
<td>1</td>
<td>2(40.0)</td>
<td>3(60.0)</td>
<td>1</td>
</tr>
</tbody>
</table>

(+): positive isolates; (-): negative isolates; no.: number; %: percentage; pv ,chi
Due to the production of a wide variety of virulence factors, including extracellular protein toxins, S. aureus is recognized as the primary nosocomial pathogen. Additionally, S. aureus is a major cause of morbidity and mortality as a result of serious nosocomial infections[15]. The spread of germs, host colonization, and tissue attack are all influenced by a number of variables that S. aureus produces, such as ETs. [16].

The genotyping results showed that the S. aureus isolated from burn patient tissues had extremely high outbreaks of the eta gene, etb gene and etd gene (16.1%, 12.2%, and 28.3%, respectively). In comparison to the prevalence rates discovered by Becker et al. in Germany, Liu et al. in China, and Kolawole et al. in Nigeria, this rate is higher. [17-19]. In the mentioned studies, eta and etb genes were frequently detected at ranges of 1.2%-4.0% and 0.5%-1.7%, separately, and no etd gene was detected in Nigeria. It’s notable that in all these cases, the number of wound and burns samples was near zero and they used diverse types of samples to conduct their research. Wu et al. (2011) in China and van Trijp et al. (2010) in the Netherlands found the eta gene in 1% and 2% of the isolates, correspondingly. Van Trijp et al. similarly discovered the etd gene in 4.5% of strains, but Wu et al. did not discover etd gene in the isolates [20,21]. So. The combination of eta-etd (12.9%) genes is more communal amongst strains than eta-etb (9.67%) or etb-etd (10.3%) genes. Furthermore, high percentage of eta, etb and etd genes are (21.2%).

Using the PCR technique, ET genes were found in clinical S. aureus isolates in present research, both individually and in diverse combinations. It has been suggested that antibiotic resistance can change how genes involved in disease are expressed. 155 (68.8%) of the isolates were isolated from burn samples, which is remarkably identical to several findings from China (77.6%) [22]. Our findings indicated a high incidence of resistance to numerous medications commonly used to treat staphylococcal infections, comparable to most places in the world. In the population we studied, penicillin resistance was high (56.7%). According to this finding, some isolates might not be detected utilizing the screening test based on the erythromycin (54.8%) resistance assay. Contrary to the findings of various Asian and African nations, where the rate of erythromycin resistance was less than 30% [23] or was (0%), in the current investigation, cefoxitin-resistant isolates were more prevalent (50.3%). However, Australia (68%) and the United Kingdom (90%) have seen larger prevalences of Cefoxitin-resistant isolates. In comparison to earlier data from Lebanon (44%) [24], we also noticed a higher incidence of tetracycline-resistant isolates (42.5%). Nevertheless, Nimmo and Zhang et al. [25] showed that 80% of Australian resistance isolates were present. Additionally, the prevalence of isolates resistant to ciprofloxacin, trimethoprim, and nitrofurantoin was lower than reported rates from Nigeria (14.7%), (10.5%), and (5.5%), respectively. Koosha et al. (2014) observed similar outcomes in Tehran’s Baqiyatallah Hospital. 126 (64.4%) and 15 (7.6%) of the 197 strains harbored the eta and etb genes, respectively. The bulk of the isolates in the cited study were obtained from samples of burns and wounds; the occurrence frequency of the eta gene is greater than our findings. Though,
among 133 strains isolated from various locations in a Tehran-based Iranian referral children's hospital, the frequency rates of the eta gene and etb gene were 11.3% and 9%, correspondingly [26].

The great incidence of ET genes might be attributed to the information that Staphylococcal ETs are particular serine proteases that function for example "molecular scissors" to enable establishment and bacterial attack through the skin and soft tissues of mammals through cleaving linkage fragments amongst neighboring keratinocytes [27]. Consequently, the current study’s extraordinary frequencies of ETs bacterial genes may be related to the frequent use of burn specimens [28]. This suggests that S. aureus had a supporting role in the emergence of these burns based on the high number of carrier isolates found in burn cases.

Conclusion

In conclusion, our findings showed that S. aureus isolates obtained from burn specimens had a high prevalence of ET genes. It is thought to be a severe issue that the Specialized Burns Center of Al-Diwaniyah city has a high breakout of ET genes in burn specimens. It is probable that they propagate plus transmit these genes between isolates because the ETs genes were encoded by specific MGEs as prophage, plasmids, and pathogenicity islands. These community-circulating isolates are crucial for public health, especially for persons with significant underlying illnesses like burns. Given the widespread occupation of this bacterium in natural healthy people, this issue has greater importance. For those under risk control, the investigation of these isolates in hospitals can be useful.

REFERENCES