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Research Article

Prevalence of Exfoliative Toxin Genes (*eta and etb*) in *Staphylococcus aureus* Isolates from Tonsillitis, Wound, and Urinary Tract Infections in Iraq

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Abstract

This study investigated the prevalence of genes coded for exfoliative toxins (*eta* and *etb*) in *Staphylococcus aureus* isolated from Iraqi patients with tonsillitis, wound infections, and urinary tract infections (UTIs). A total of 65 *S. aureus* strains were analysed using polymerase chain reaction (PCR) to detect the presence of these genes.

The results revealed that *eta* and *etb* genes were distributed similarly among the tested strains, with etb being slightly more prevalent (29% vs. 23%). Notably, the *etb* gene was particularly common in wound infections (65%). Co-occurrence of multiple toxin genes within a single strain was uncommon.

These findings suggest distinct patterns of toxin gene distribution among different types of infections, implying potential associations between specific genes and particular disease states. These results contribute to our understanding of the epidemiology of *S. aureus* in Iraq and may inform the development of targeted prevention and treatment strategies. However, further research is necessary to elucidate the clinical significance of these toxin genes and their potential as therapeutic targets.

Keywords: exfoliative toxin genes, *Staphylococcus aureus*, tonsillitis, wound infections, urinary tract infections

Introduction:

S. aureus is a major pathogen responsible for a variety of infections, including tonsillitis, wound infections, and UTIs [1]. Some isolates of these bacteria are carrying genes of exfoliative toxins (ETs) that play a significant role in skin exfoliations [2]. Among many genes, *eta* and *etb* are considered as important markers for pathogenicity of staphylococci [2]. Studying the prevalence of exfoliative toxin genes is commonly conducted [2,3]. Understanding the prevalence of these genes in clinical isolates is crucial for public health. Therefore, studying the prevalence of the *eta* and *etb* genes in *S. aureus* isolates from cases of tonsillitis, wound infections, and UTIs in Iraq will provide valuable insights into the potential risks associated with these toxins.

Exfoliative toxins are proteases that specifically target desmoglein 1, a protein in the skin, leading to the detachment of epidermal layers [4]. *S. aureus* that are carried ET toxins, encoded by the *eta* and *etb* genes, are often associated with severe clinical implications. In fact, the presence of these genes has been linked to the severe condition, staphylococcal scalded skin syndrome (SSSS), which specifically affects infants and immunocompromised individuals [5]. However, these genes are variably distributed in different clinical isolates in several types of infections from various geographical regions [6]. For instance, conducting regional studies is crucial for obtaining valuable data. Although there is several previous research that were questioning the prevalence rates of these genes worldwide, such research from Iraq seems limited.

Tonsillitis, wound infections, and UTIs are three common clinical implications caused by *S. aureus* [7]. Tonsillitis caused by *S. aureus* can cause severe throat pain, fever, and some complications [8]. Wound infections especially post-surgical operations, can lead to serious complications such as sepsis and prolonged staying at hospital [9] and might be developed into UTIs [10]. The presence of exfoliative toxin genes in *S. aureus* strains isolated from these infections could indicate a higher risk of severe disease progression.

Previous literatures indicated that staphylococcal isolates are variable in terms of carrying the *eta* and *etb* genes depends on regions and infection types. For instance, a study in Japan found a constant detection of the aforementioned genes in clinical isolates from skin infections [11], while a study in Germany reported a low detection-rate of these genes in blood isolates [12]. These differences highlight the need for more investigations in various regions which could enhance the public health strategies.

Along with the increased global focus on understanding the virulence of *S. aureus*, there is a continuous need for conducting research focuses on particular virulence factors. Indeed, understanding the distribution of these genes can help the efforts of predict potential outbreaks and build appropriate responses. Therefore, the purpose of this study is to investigate the prevalence of *eta*, and *etb* genes in *S. aureus* isolates from tonsillitis, wound, and urinary tract infections in Iraq.

Materials and methods:

Bacterial isolates:

A total of 65 *S. aureus* clinical isolates were isolated from three clinical infections, including urinary tract infections (n=20 isolates), wound infections (n=20 isolates), and tonsilitis samples (n=25 isolates). Collections of isolates occurred during the years 2020 and early 2022 from multiple healthcare centers in Iraq. Additional two reference *S. aureus* strains, MN8 and ATCC 25923, were used as controls for the PCR.

Primer design:

Primers used in this research were specifically designed to detect the genes of *eta* and *etb* using the Primer3 software. The specificity of the designed primers was evaluated using the "Primer BLAST" server of NCBI. All primers were ordered to be synthesized at Macrogen, Korea. For *eta*, forward 5'-CTGTAGGAGCTAGTGCATTTGT-3' and reverse 5'-CCAGAATTTCCCGGAACTGTAA-3'; and for *etb*, forward 5'-GAATACAGCGCAGAAGAAATCAG-3' and reverse 5'-GCTAAATCGAGTCCTTGTCCATA-3'. For further confirmations, the PCR products were sequenced by Sanger sequencing method (Macrogen, Korea).

DNA isolation and PCR amplification:

A conventional PCR assay was applied to detect the *eta* and *etb* genes among the tested isolates. Each single-isolate was cultured in about 5 ml of LB broth and incubated at 37°C overnight. Bacterial pellets were then harvested by centrifugation at 12,000 rpm for 2 min. Bacterial DNA was extracted using the protocol of the "AddPrep Bacterial Genomic DNA Extraction Kit" (addbio, Korea). Extracted DNA were evaluated using gel electrophoresis and a NanoDrop® spectrophotometer (NanoDrop-1000,

Wilmington, DE), respectively. Subsequently, 2 µl of DNA sample was amplified in a 20 µl reaction mixture using the GoTaq® G2 Green Master Mix (Promega, USA). PCR was performed using the Optimus 96G thermal Cycler (ALS, UK) with an initial denaturation at 94°C for about 5 min, followed by 35 cycles of 30 sec at 94°C, 30 sec at 56°C, and 1 min at 72°C, with a 5 min final extension at 72°C. Each amplicon was run through an electrophoresis system using 2% agarose gel stained with RedSafe (Intron, Korea). Finally, a UV transilluminator was utilized for visualization of the sizes of products (for gene *eta* was 649 bp and for gene *etb* was 344 bp).

Sensitivity and specificity of primers:

DNA from three independent strains (*Staphylococcus epidermidis, Staphylococcus saprophyticus, and Enterococcus faecalis*) were extracted to be used in the evaluation the specificity of primers. Additionally, a serial dilution of purified genomic DNA from *S. aureus*, ranging from 1 to 35 ng, was used to assess the sensitivity of the PCR reactions, following the previously described protocol [13].

Results:

ETs detection:

In this study, we assessed the prevalence of two genes of exfoliative toxins, *eta* and *etb* in 65 clinical isolates of *S. aureus*. Out of the 65 isolates, 25 (38.5%) were obtained from cases of tonsillitis, 20 (30.8%) from urinary tract infections (UTIs), and 20 (30.8%) from wound infections. Among the two toxin genes, *etb* was the most commonly detected gene (n=19, 29%) and the *eta* gene, which was detected in 15 isolates (23%). Interestingly, both tested genes were detected in isolates from all three types of infections. Indeed, in staphylococcal tonsil infections, *eta* and *etb* appeared to be less prevalent (Table 1). *Etb* was highly detected in staphylococcal wound infections, with 13 out of 20 isolates showing positive results, while only 7 or 6 cases tested positive for *eta*. Finally, *eta* or *etb* were detected in very few cases of UTIs, with only 6 or 3 isolates testing positive, respectively.

Table 1: Distribution of ET-genes among the examined *S. aureus*

Type of infection	Number of specimens	*eta*	*etb*
Tonsillitis	25	2 (8%)	3 (12%)
Wound infection	20	7 (35%)	13 (65%)
Urinary tract infection	20	6 (30%)	3 (15%)

Co- and Double Existence of ETs in *S. aureus* isolates

Based on our models, we analysed the potential coexistence of ET genes in *Staphylococcus* isolates responsible for tonsil, wound, or urinary tract infections. Consequently, we classified the isolates into three subtypes: type A (only *eta* is detected in a single isolate), B (only *etb* is detected in a single isolate), AB (both *eta* and *etb* are detected in a single isolate).

The results revealed that type A was detected in samples form wound and UTI infections only but not from samples of tonsillitis. Similar pattern was detected for type B was also notified. Finally, type AB was not detected in any of tonsillitis samples. However, a combination of *eta* and *etb* was detected in both wound and UTI infections (Table 2).

Table 2: Co-existence of ETs in tested isolates

Type of isolate	Tonsillitis n=25	Wound infections n=20	UTIs n=20
Type A	0 (0%)	3 (15%)	4 (20%)
Type B	0 (0%)	8 (40%)	1 (5%)
Type AB	0 (0%)	2 (10%)	2 (10%)

Discussion:

This study aimed to examine the occurrence of two exfoliative toxin coded genes (*eta* & *etb*) in *S. aureus* isolates from various types of infections in Iraq (tonsillitis, wound infections, and urinary tract infections). Our findings provide valuable insights into the distribution of these two genes in various isolates of *S. aureus* in Iraq. Additionally, the study reveals notable variations in the co-existence of these genes across different isolates from common infection types.

Among the two exfoliative toxin genes investigated, *etb* was the most prevalent that was occurring in approximately 29% of the isolates, followed by *eta* at 23%. This distribution pattern contrasts a previous manifestations that was reported a higher prevalence of *eta* in clinical isolates from Iran [2]. This highlights the significance of conducting research specific to a particular region to better understand the virulence behavior of clinical staphylococcal isolates.

The *etb* was found to be highly existed in wound infections, occurring in approximately 65% of cases. Similar rate is also reported in previous research linking exfoliative toxins to skin and internal organs infections [14]. Consequently, the high prevalence of *etb* in wound infections suggests that this toxin might be required by *S. aureus* for wound infections. Interestingly, our study observed low occurrences of etA and etB in UTI isolates, approximately 30% and 15%, respectively. This result suggests that exfoliative toxins may play less role in the staphylococcal UTIs compared to other types of infections. However, although at lower rates, carrying the toxin-associated genes in UTI isolates may indicate severe symptoms.

Importantly, our study also succeeded in reporting a co-existence of multiple ET genes in a single isolate [3]. The prevalence of the *etb* gene in wound infections highlights the importance of meticulous wound care and appropriate antibiotic selection in managing these infections. However, this study has certain limitations. Although informative, but conducting further research on the expression levels of these toxin genes and their correlation with clinical outcomes would offer valuable insights into their role in pathogenesis.

Conclusion:

In conclusion, our study provides important data on the prevalence and distribution of some exfoliative toxin genes in *S. aureus* isolates from different infection types in Iraq. These findings enhance our understanding of the local prevalence of exfoliative toxin-coded genes in different clinical isolates of *S. aureus*. However, further studies are needed to determine the clinical significance of these toxin genes and their potential as targets for therapeutic interventions.

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