PREVALENCE OF EXFOLIATIVE AND SUPERANTIGEN TOXIN GENES IN STAPHYLOCOCCUS AUREUS ISOLATED FROM CLINICAL SPECIMENS IN AL-SAMAWAH CITY

[1]Mojtaba Mohseni, [2]Thara Abdulkhdhr, [3]Hussein Thaer Abdualabbas

[1]Department of Microbiology, University of Mazandaran, Babolsar, Iran; Email: m.mohseni@umz.ac.ir

[2] The Independent High Electoral Commission, Iraq; Email: tharaabdulrdha@gmail.com

[3]Microbiology Department, College of Medicine, Al-Muthanna University, Iraq; Email: hussbiooo@gmail.com

Corresponding Author: Mojtaba Mohseni, **Email Id:** m.mohseni@umz.ac.ir

Abstract

Background and Objectives: The success of *S. aureus* as a pathogen is largely attributed to its ability to produce a diverse array of toxins, including exfoliative toxins and superantigens The aim of this study was to investigate the prevalence of *eta, etb, etd* and *sed* genes in *S. aureus.*

Materials and Methods: In this study, we investigated the prevalence of *eta, etb, etd* and *sed* genes using PCR in 100 clinical (8 burns, 68 wounds,11 skin sores,13 urine) isolatesobtained from clinical samples during the year 2023. The samples wereobtained from an educational hospital in southern Iraq.

Results: Overall, 41(59%) suspected *S. aureus* isolates were identified from a total of 100 samples which includes 29 (11.89%) from wounds, 5 (2.05%) from burn, 5 (2.05%)from skins sore and 2 (0.82%) from urine . 19 (46.34%), 30 (73%), 22 (53.8%) and 38 (92.7%) of the isolates carried the *eta, etb* and *etd* genes, respectively. The most common genotype among isolates obtained from clinical samples was *seb* and the least common was *eta* .

Conclusion: Through our current study, we have noticed that the rates of *eta, etb, etd* and *sed* genes are very high, and this portends in the coming years the possibility of an increase in the number of infections caused by the aforementioned toxins.

Keywords: *Staphylococcus aureus*, Exfoliative toxin genes, superantigens

INTRODUCTION

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations . *S. aureus* is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals. *S. aureus* does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections (Lowy, 1998).

The success of *S. aureus* as a pathogen result from its exceptional genetic plasticity, which allows the acquisition of new resistance and virulence mechanisms (Chambers & DeLeo, 2009). This ability is related to generate a wide range of virulence factors, such as extracellular protein toxins, a large number of anti-microbial resistance compounds and harmful super-antigenic toxins (Ladhani et al., 1999). The most common toxins in this bacterium are exfoliative toxins (ETs) and pyrogenic toxin superantigens (PTSAgs) comprising toxic shock syndrome toxin -1 (TSST-1) and the staphylococcal enterotoxins (SEs), Ets cause the staphylococcal scalded-skin syndrome (SSSS), while PTSAgs cause the staphylococcal toxic shock syndrome (TSS), and staphylococcal food poisoning (SFP) (Becker et al., 2003). Regarding SSSS, 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. While, the localized form are epidermal infections such as bullous impetigo. However, the Bullous impetigocan afflict at all ages. Moreover, in the Bullous impetigo form, the exfoliative toxin, which produces localized strains, can locally affect the skin. In contrast, in Ritters syndrome, the infecting strains are located at distant sites, and the target tissue receives the toxin via the bloodstream(Koosha et al., 2014) . There are three isoforms of ETs: ETA, ETB and ETD, which are encoded by the eta, etb and etd genes, respectively, are capable of cleaving desmogelin 1, a cadherin protein, which mediates cell–cell adhesion in keratinocytes. From these three isoforms, the ETA and ETB toxins are associated with the occurrence of staphylococcal scaled skin syndrome (SSSS). The eta gene has a chromosomal origin that is integrated by prophages, whereas the etb gene is a plasmidic gene. In addition, theetd gene encoding ETD is chromosomally located in a 14.8 kb pathogenicity island (Koosha et al., 2014) (Yamasaki et al., 2005) . In regard to PTSAgs, these toxins have specific toxic features and share several structuraland biological characteristics, such as pyrogenicity, superantigenicity, and the ability to enhance the susceptibility to endotoxin shock (Kotb, 1995) , SEs have several antigenic types, the most common types are SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI and SEJ (Becker et al., 2003) , The toxic shock syndrome toxin-1 (TSST-1), encoded by *tst* gene, is a significant member of PTSAgs (Zhao et al., 2019) .

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 gene (Sultan & Al Meani, 2019) . . Because of the reporting of the exfoliative toxin and superantigens producing *S. aureus* strains isolated from patients in different regions of Iraq over the past years , Therefore, we sought to determine the overall prevalence of exfoliative toxins and superantigens among clinical *S. aureus* strains in Iraq . This study was aimed to investigate the presence and prevalence of genes encoding exfoliative toxin and superantigens among different clinical isolates of *S. aureus.*

MATERIALS AND METHODS

Isolation of *S. aureus* from clinical specimens In order to isolate *S. aureus*, a total of 100 specimens (wounds, burns, skin sores and urine) .All samples were then cultured in nutrient broth medium and incubated at 37 ºC for 24 hours. After incubation, serial dilutions for each sample were carried out, then 100 µl from the appropriate dilution was spread on blood agar and mannitol agar medium and incubated at 37 ºC for 24 hours (Lemaire et al., 2008) . From this study, it was observed that 100% *S. aureus* isolates showed positive results in Gram staining, Catalase Test, Coagulase Test, Oxidase test, Mannitol fermentation test, Blood hemolysis test.

DNA extraction and preparation

Detection of genes by polymerase chain reaction The bacterial isolates which were found to be *Staphylococcus aureus* by specific phenotypic features were further tested by PCR for confirmation using specific primer pairs of 16S rRNA (Table-1) . Molecular identification of *S. aureus* was done according to (Alves et al., 2018) , PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). PCR mixtures and conditions of this assay was summarized in (Table-2).

(Table -1): Primer list

Gene	Oligo Name	5` - Oligo Seq - 3`	PCR product (bp)
16SrRNA	16S_Staph_F	GGTCTTGCTGTCACTTATAGATGG	164
	16S_Staph_R	CGGAAGATTCCCTACTGCTG	
ETA	ETA-F	CTAGTGCATTTGTTATTCAAGACG	119
	ETA-R	TGCATTGACACCATAGTACTTATTC	
ETB	ETB-F	ACGGCTATATACATTCAATTCAATG	262
	ETB-R	AAAGTTATTCATTTAATGCACTGTCTC	
ETD	ETD-F	AACTATCATGTATCAAGG	376
	ETD-R	CAGAATTTCCCGACTCAG	
SEB	SEB-F	CCAGATCCTAAACCAGATGAGTT	325
	SEB-R	GTTTTTCGTTTGTCAGTTTGATG	

(Table -2): Uniplex PCR mixtures and conditions for identification of *16S rRNA* gene.

PCR mixtures		**PCR conditions**		
Contents	**Volume**	**Type of cycle**	**Condition**	**No. of cycles**
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	35
Reverse Primer	2.5 µl	Annealing	56 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

PCR Amplification of ETA, ETB, ETD, SEB genes by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). Primers were listed in (table -1). PCR mixtures and conditions of this assay was summarized in (Table-3).

(Table -3): Uniplex PCR mixtures and conditions for identification of *ETA, ETB, ETD, SEB* gene.

PCR mixtures		**PCR conditions**		
Contents	**Volume**	**Type of cycle**	**Condition**	**No. of cycles**
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	30
Reverse Primer	2.5 µl	Annealing	53 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

Gel electrophoresis is applied to ensure the validity of DNA extraction PCR results. 2% agarose concentration for the quality of the extracted DNA stained with 500µl of a 10mg/ml stock solution per 100 ml Green star dye with 100 volts for 36 minutes. The results were confirmed by using Gel Documentation system (DUALED Blue / White Transilluminator, Bioneer, Korea). The sizes of PCR products for the 16S rRNA *eta, etb, etd seb* fragments were 164 bp, 119bp and 262 bp, 376 bp, 325 bp respectively.

RESULTS

PCR was applied to all samples to detect presence of *S. aureus* using the specific primers (Table-1) ,) Amplification by PCR with these primers resulted in a 164 bp product fragment , The reaction products were separated by agarose gel electrophoresis and the presence or absence of bands was identified by examination of the gels under UV illumination Fig. 1 . PCR Amplification of 16S rRNA Gene , 41 suspected *S. aureus* isolates were identified from a total of 100 samples which includes 68 wounds , 13 urine , 11 Skin sores and 8 burns . The prevalence of Staphylococcal exfoliative toxin genes (*eta, etb* and *etd*) and superantigens (*seb*) in the clinical isolates of *S. aureus* was investigated using PCR method. The presence and occurrence of the amplicons using agarose gel electrophoresis is shown in figure 1-5. In this research, the number of 100 specimens, were collected from patient . Our findings revealed that 41 *S.aureus* isolates obtained from 100 clinical specimen as following 29(11.89%) from wounds ,5 (2.05%)from burns , 5 (2.05%)from skins sore and 2 (0.82%) from urine.

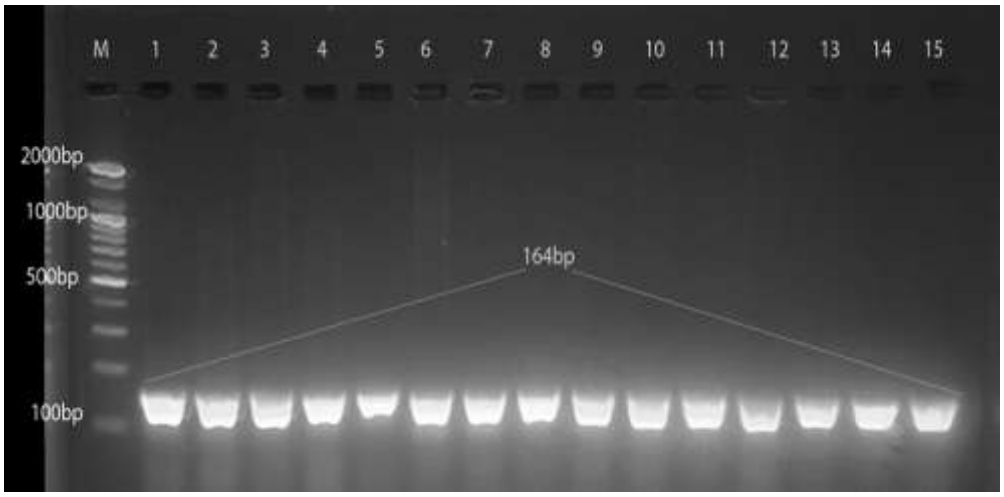

Figure (1): Identification of *S. aureus* isolates by 16S-rRNA gene. Lanes 1-15 represent the identified 164bp gene products, Lane M represent 100bp DNA ladder.

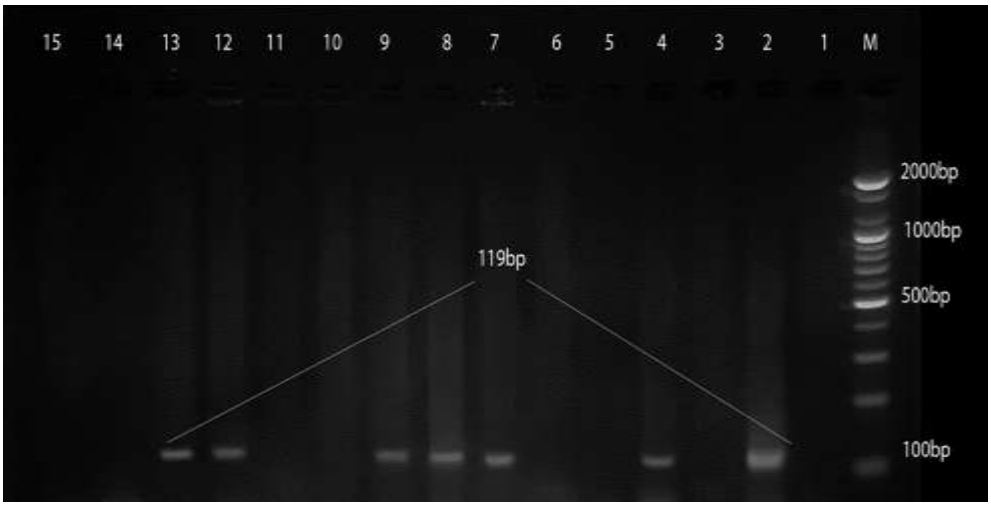

Figure (2): PCR products for identification of ETA gene among *S. aureus* isolates. Lanes (2,4, 7-9, 12 and 13) represent the identified ETA gene with 119bp amplicon, Lane M represent 100bp DNA ladder.

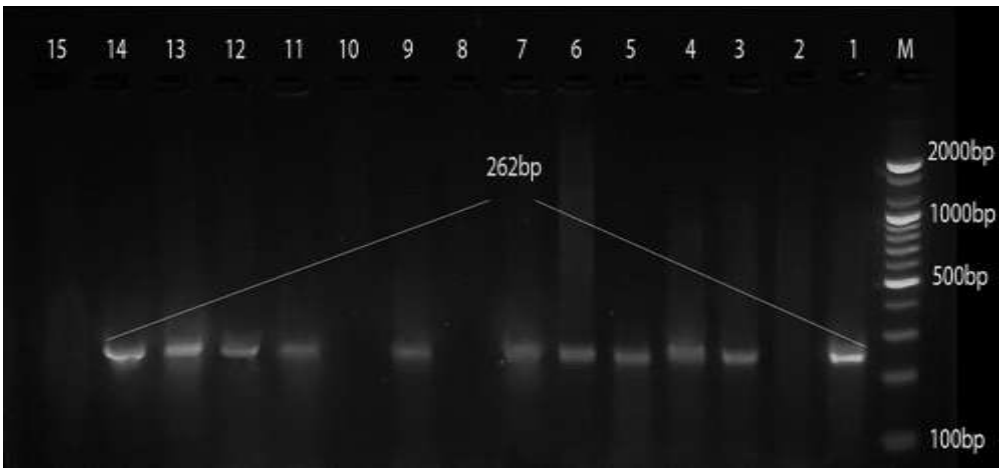

Figure (3): PCR products for identification of ETB gene among *S. aureus* isolates. Lanes (1, 3-7, 9, 11-14) represent the identified ETB gene with 262bp amplicon, Lane M represent 100bp DNA ladder.

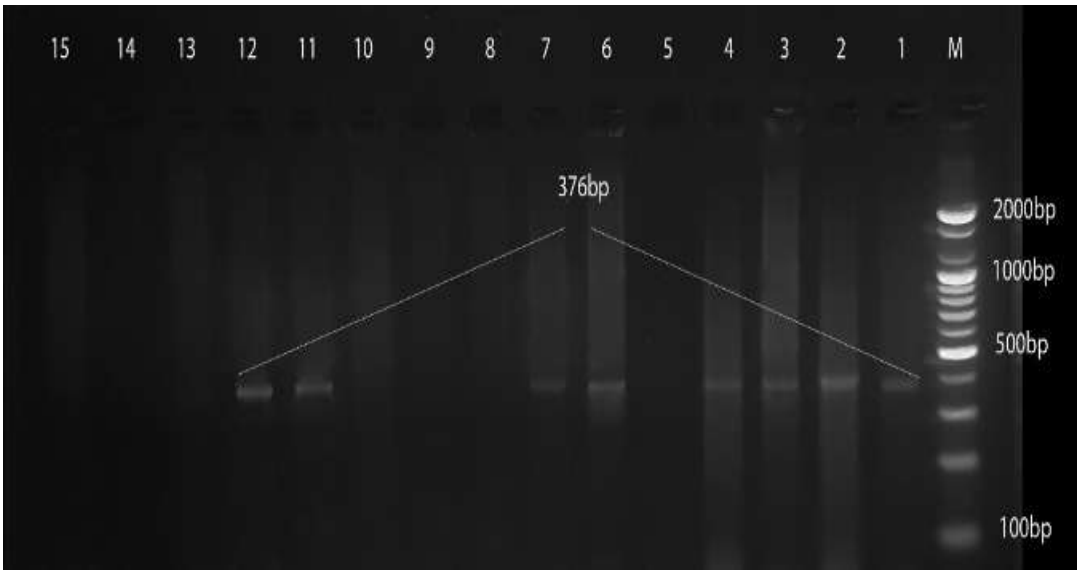

Figure (4): PCR products for identification of ETD gene among S. aureus isolates. Lanes (1-4, 6-7, 11-12) represent the identified ETA gene with 376 bp amplicon, Lane M represent 100bp DNA ladder.

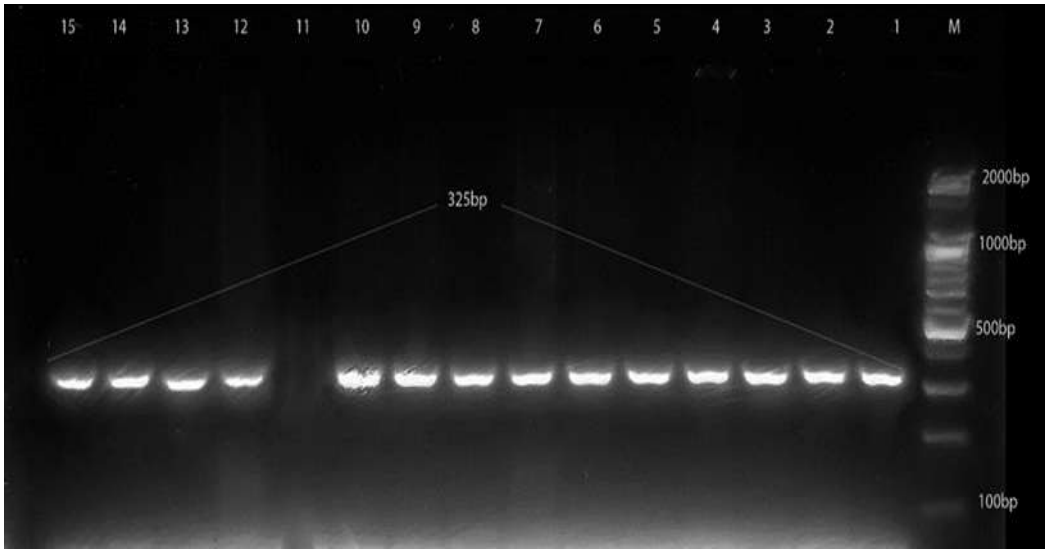

Figure (5): Agarose gel electrophoresis of PCR products obtained by using SEB-specific primer. Lanes 1-15 (exception of lane 11) represent the identified SEB gene products with 325bp, Lane M represent 100bp DNA ladder.

The prevalence of toxin gene coexistence in the strains isolated from different clinical specimens. Overall,4 toxin genotypes were observed, among which the genotypes eta, etb, etd and sed predominated at rates of 46.34%, 73%, 53.8% and 92.7%, respectively from 41 suspected *S. aureus* isolates (table – 4). the most common genotype among isolates obtained from clinical samples was *seb* and the least common was *eta* as in figure (6).

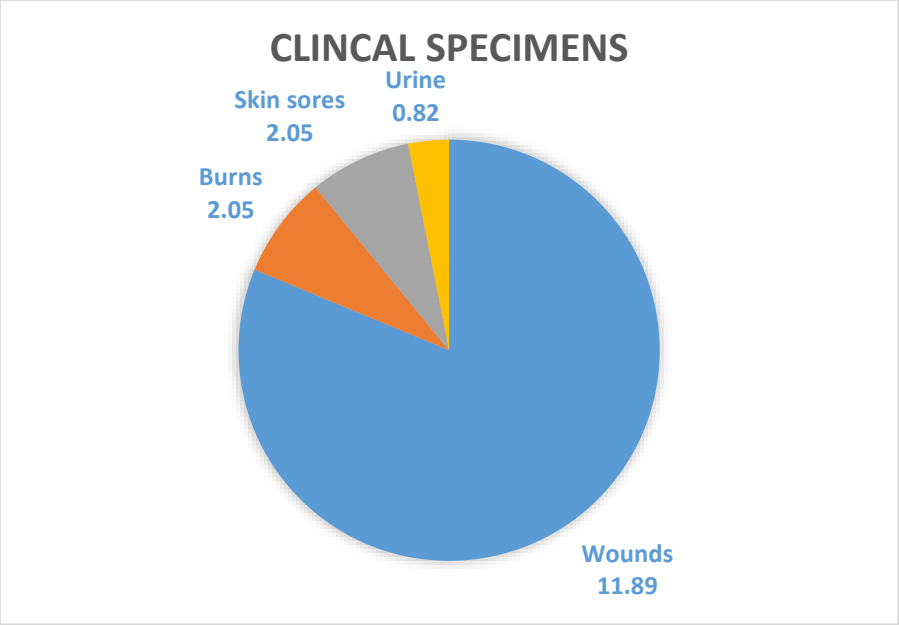

Figure (6): The isolation of *S.aureus* percentage %

Table (4) presents the distribution of exfoliative toxins (ETA, ETB, ETD) and the superantigen toxin (SEB) among the 41 *Staphylococcus aureus* isolates. The results show that the SEB gene was the most frequently detected, present in 38 isolates (92.7%), with only 3 isolates being negative. This high prevalence was statistically significant ($p < 0.001$), indicating a strong association between *S. aureus* isolates and the presence of the SEB gene. Similarly, the ETB gene was detected in 30 isolates (73%), which also showed a statistically significant difference ($p < 0.001$), reflecting its important role as a virulence factor among the studied isolates.In contrast, the ETA and **ETD** genes were detected in 19 (46.34%) and 22 (53.8%) isolates, respectively. Although these genes were present in nearly half of the isolates, their distribution did not show a statistically significant difference ($p < 0.424$).

Table (4): Distribution of exfoliative and superantigen toxins (ETA, ETB, ETD and SEB) among *S. aureus* isolates**.**

Bacteria	Total	Positive	Negative	(%)	P value
ETA	41	19	22	46.34	<0.424
ETB	41	30	21	73	<0.001*
ETD	41	22	19	53.8	<0.424
SEB	41	38	3	92.7	<0.001*

represent a significant difference at p<0.05*

The table (5) shows the results of examining 100 clinical samples, from which ***S.aureus*** was isolated in 41 samples with an overall isolation rate of (41%). The isolation rate was slightly higher in females, with 22 isolates (53.8%), compared to males, with 19 isolates (46.34%). Regarding the type of clinical specimens, the highest number of isolates was obtained from wound samples (29 isolates), followed by burns and skin sore samples (5 isolates each), while urine samples showed the lowest isolation rate with only 2 isolates. This indicates that wounds represent the most common source for the isolation of *S. aureus* in this study. Concerning the distribution of toxin genes, the SEB gene was the most

prevalent, being detected in 38 isolates (92.7%), followed by ETB in 30 isolates (73.0%), ETD in 22 isolates (53.8%), and ETA in 19 isolates (46.34%). Moreover, wound isolates exhibited the highest frequency of all investigated genes compared to other specimen types

Table (5): Test results of 100 clinical samples for isolate *S.aureus* and distribution the genes in the 41 *S.aureus* isolates

Clinical specimen	Female frequency	Male frequency	frequency	ETA	ETB	ETD	SEB
Burns	2	3	5	4	2	3	5
Wound	17	12	29	10	13	15	27
Skin sore	1	4	5	5	5	4	5
urine	2	0	2	0	0	0	1
Total	22 (53.8%)	19 (46.34%)	41 (41%)	19 (46.34%)	30 (73.00 %)	22 (53.8%)	38 (92.7 %)

Discussion

Several strains of Staphylococci express exfoliative toxins (ETs), a group of proteins that function as virulence factors and facilitate host invasion**.** Strains of *S. aureus*, a major human pathogen, produce at least four ET variants, termed ETA , ETB , ETD and ETE , the first three being relevant to human health . ETA-producing S. aureus strains can cause Staphylococcal scalded skin syndrome (SSSS) (Gismene et al., 2022). also Staphylococcal enterotoxin B (SEB) and related superantigenic toxins produced by Staphylococcus aureus are potent activators of the immune system. These protein toxins bind to major histocompatibility complex (MHC) class II, which leads to formation “cytokine storm” . Staphylococcal enterotoxin B has historically been the most intensively studied superantigen, and is listed as a category B select agent by the U.S. Centers for Disease Control and Prevention, as it can be used as an air-borne, food-borne, and water-borne toxin . Depending on the dose and route of exposure, SEB and other SEs can cause food poisoning, acute and fatal respiratory distress, and toxic shock. Staphylococcal superantigens also enhance the proinflammatory response and lethality by synergizing with other bacterial products, such as lipopolysaccharide (LPS), lipoproteins, and viruses (Krakauer, 2019) . Due to the importance of these toxins in causing *S. aureus* pathogenicity and its connection in real life, we presented this study to determine the extent of the spread of these toxin genes in Iraq.

On the basis of conventional methods (Colony morphology, Gram staining and Biochemical tests, PCR Amplification of 16S rRNA Gene) , 41 suspected S. aureus isolates were identified from a total of 100 samples which includes 68 wounds , 13 urine , 11 Skin sores and 8 burns (Table-5). The overall prevalence rate of S. aureus in sample that were collected from Al-Muthanna (South Iraq) based on conventional techniques and molecular was 41 per cent (41/ 100) was almost similar to other reports in Indonesia where it found Salasia et al.,2011 all 41 isolates examined in their studies were as S. aureus.

The finding were agree with (Das et al.,2019) who reported that among 40 isolates positive for S. aureus were subjected to catalase and coagulase test where all of the isolates found positive for catalase test and 30 of them for coagulase test. Bubble formation in catalase and curd like clot formation in coagulase test indicated that the samples were positive for S. aureus indicating their ability to breakdown the hydrogen-peroxide to release free oxygen and plasma coagulation by activation of prothrombin (Das et al., 2019). Our study also agree with (Islam et., al 2021) who reported that prevalence of S. aureusin animals and humans were 54% and 40%, respectively (Islam et al., 2021). The overall prevalence of *S. aureus* in human specimens (40%) is similar with the findings of

previous studies (Shibabaw et al., 2014) , and are considered as quite high.

Of the isolates evaluated in this study, individual resistances of S. aureus to methciliin was high (70%). High resistance to these β-lactam antibiotics was not surprising, as methicillin is one of the most commonly used antibiotics for treatment of infections in humans and animals (Gundogan et al., 2005) , The high rate of vancomycine resistance (80%) was of concern, as this antibiotic is historically regarded as the antibiotic of final resort and the highest quality level antimicrobial for the treatment of genuine MRSA diseases (Tenover et al., 2006) , On the other hand, the isolates showed more sensitive to some antibiotics as following G (40%), CIP(50%) AZM (30%), T (30%), our findings were agreed with previous study (Gurung et al., 2020) .

Overall, our results revealed high antibiotic resistante rates, which were higher than those reported in Romania (Somayeh et al., 2023) , United States (Rao et al., 2022) , These variations in the prevalence rates may be due in part to the different regional antimicrobials prescription, infection prevention and control activities, and source of isolates (Cižman & Srovin, 2018) . DNA was isolated from all the *S. aureus* suspected presumptive culture colonies by Presto Mini gDNA Bacteria Kit method and the presence of the ETA gene was detected by polymerase chain reaction. The ETA gene amplicon of 119 bp obtained from 19 samples out of 41 positive culture isolates . S. aureus was isolated by species specific ETA gene targeted PCR from with overall prevalence rate of S. aureus in samples based on PCR was 46.34% (19/41) Tab(4). This is similar to a study Nour Amirmozafaria et al., 2019 in northern Iran, Where it was total of 63 S. aureus isolates from patients and 63 isolates of 262 nasal swabs from healthy individuals were collected. The prevalence of eta gene in isolates from patients and healthy individuals were 22 (34.9%) and 18 (28.6%), respectively , They found a lower rate compared to other parts of the country(Amirmozafari et al., 2019).

In another study conducted by almiyah et al., 2022 in the period between January 2021 and December 2021 of Al – Diwaniyah city in Iraq where it was found out of 155 S. aureus isolates were gathered from burn sample patients was 25(16.1%) isolates carrying the eta gene(ALmiyah, 2022) . the presence of eta gene in most types of clinical samples must be considered as a serious health problem and demand effective

Desmoglein (Dsg1) is one type of protein belonging to desmogleins which recognized and hydrolyzed by exfoliative toxins (ETs). These toxins are serine proteases synthesized via *S. aureus* that cause the loss of cohesion between adjacent keratinocytes in the superficial epidermis and to a less extent in the mucous membranes (Imanishi et al., 2019) , In this study, eta gene was observed in isolates from patients (73.00%) , our detection rate for etb gene were considerably higher than those of other investigators. For example, in a study carried out in Lebanonon 130 isolates of S. aureus, the frequency of eta was reported to be (4.61%) (Tokajian et al., 2011) .

The findings were disagreed completly with Hamidreza Houri et al., 2020 who reported that among 80 clinical isolates of *S. aureus* were collected from adult patients with various S. aureus infections in Tehran, Iran, None of the isolates possessed etb(0%) (Houri et al., 2020). in other studies, they showed a very large variation of the recurrence of this gene, these studies were by (Roohollah Zarei et al., 2014) 15 (7.6%); (Imanishi et al., 2019) (53%); (Abbasi Montazeri et al., 2021) (23.7%). The differences in the occurrence of etb genes in these researches suggest a geographic variation in the distribution of etb strains of Staphylococcus aureus. This variability may be reflect distinct ecological reservoirs present in different countries or may originate from differences in the sensitivity and specificity of techniques used to detect ETB toxins gene.

Although, the substrate is common to all ETs, human-infecting strains of *S. aureus* produce mainly ETA and ETB, and ETD is less frequently encountered than the other two toxins, ETD-producing strains are mainly isolated from furuncles or cutaneous abscesses and not from the same tissues as the two other toxins , The differences identified at the structural level here might also somehow reflect an adaptation to ruminant hosts (Mariutti et al., 2015) . The etd gene was found in(22/41) 53.8% of our clinical isolates which agreed with (Yassin et al., 2022) who reported that among 91 sample of S.

aureus isolated from different clinical sites, the etd ratio was 63 (69.2%) (Yassin et al., 2022) . The presence of etd gene in *S. aureus* at high rate may be attributed to the presence of pathogenicity islands among these isolates and may include many virulence factors inside them. 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) .

Superantigens directly interact with the variable region of the beta chain (Vβ) of the T cell receptor (TCR). To date, more than 60 different Vβ fragments of human TCRs have been recognized. Superantigens have differed in the activation of human T cells expressing diverse Vβ fragments,SEB are more promiscuous in their Vβ-targets and activates T cells bearing Vβ3, Vβ12, Vβ14, Vβ15, Vβ17, and Vβ20 (Xu & McCormick, 2012).Molecular detection of SEB gene was carried out by using a specific PCR primer were done by comparison with allelic ladder, which gave a 325bp. It was found that toxin gene present in (38/41) 92.7% of the positive samples this percentage is considered higher than the rest of the percentages of other genes in our study.

The findings were agreed with Dejing Wu1et al., 2011 who reportted that Among the 99 CA-MRSA samples, 88.9% (88/99) harboured SAg genes, The SEB toxin was (60.6%) (Wu et al., 2011) .

Another study conducted in iraq by alwash and aburesha, (2021) on One hundred Burn, wound, and environmental samples,who reported among 19 *S.aureus* , no SEB gene was identified this results disagreed with our reslts (Alwash & Aburesha, 2021) . Nevertheless, 3% of the isolates harbor seb according to a study in a review (Sergelidis & Angelidis, 2017) .This may be due to seb being infrequently identified in S. aureus strains(Dunyach-Remy et al., 2016) .

Addittionally, mozafarianari et al., 2019 screened S. aureus strains from patients who were in the hospital over 5 months and investigated the detection of enterotoxins using the real-time fluorescence PCR and reversed passive latex agglutination (SET-RPLA) methods, A total of 6% of the samples contained SEB (Mozafarianari et al., 2019) .

prevalence of enterotoxin genes varies greatly, according to geographical distribution and the structure of the population (Kolawole et al., 2013) . Here, all the strains examined possessed at least nineteen staphylococcal enterotoxin gene with SEB being the most prevalent gene. This is a high occurrence with public health implications as staphylococcal enterotoxins can induce T-cell stimulation, resulting in systemic illness such as toxic shock syndrome and food poisoning.

Staphylococcal infections remain one of the most common infections Of these, the staphylococcal scalded skin syndrome, toxic shock syndrome, Food poisoning. most cases are easily treated, SSSS remains a potentially fatal condition, particularly in adults with underlying disease is caused by the staphylococcal exfoliative toxins, which have an exquisite ability to target and destroy a specific protein in the epidermis. Identification of desmoglein-1 as the epidermal target has explained many of the unusual features of the toxins compared to other serine proteases and opened a new area for basic and clinical research, including development of new diagnostic tests and specific antitoxin therapies. Furthermore, understanding the mechanism of action of the exfoliative toxins will not only allow us to understand the pathogenesis of SSSS, but also provide useful information on normal skin physiology and other toxin-mediated diseases. The toxins are also likely to have other useful benefits in dermatology and therapeutics in the near future(Ladhani, 2003).

Through our current study, we have noticed that the rates of these toxins are very high, and this portends in the coming years the possibility of an increase in the number of infections caused by the aforementioned toxins. Therefore, we recommend making some necessary recommendations to limit the spread of these infections or at least reduce them.

Conclusion

The present study demonstrates that *Staphylococcus aureus* isolates from clinical samples in southern Iraq possess a high prevalence of exfoliative toxin (ETA, ETB, ETD) and superantigen (SEB) genes,

indicating a strong virulence potential of the circulating strains. The SEB gene was the most predominant, highlighting the major role of superantigen-mediated pathogenicity in *S. aureus* infections, whereas ETA was the least frequent. The high distribution of ETB and ETD further emphasizes the importance of exfoliative toxins in the development of skin and soft tissue infections, particularly those associated with wounds and burns. These findings suggest an alarming spread of toxigenic *S. aureus* strains, which may contribute to increased severity and incidence of toxin-mediated infections in the future, underscoring the need for continuous molecular surveillance, effective infection control strategies, and appropriate antimicrobial management

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