Co-extensive of *sea*, *sec* and *tst* enterotoxin genes in *Staphylococcus aureus* isolates from clinical sources

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Abstract

Staphylococcus aureus is a Gram-positive bacterium that can cause various diseases in specific conditions by secreting various toxins. Enterotoxins and toxins toxic shock syndrome toxins play a high role in pathogenesis. Enterotoxins and toxic shock syndrome toxin (TSST) are pyrogenic super antigens that react with the MHC II molecule. The aim of this study was to investigate the frequency of *sea*, *sec*, and *tst* genes in *S. aureus* isolated from clinical sources. This study was performed on 100 *S. aureus* isolates from hospitals in Karaj, which were finally identified by biochemical methods. Antibiotics susceptibility test was performed by the disk diffusion agar, and the multiplex polymerase chain reaction (PCR) method was used to identify *sea*, *sec*, and *tst* genes. The highest resistance was observed to penicillin (92%), while the lowest resistance was observed to vancomycin (0%) and 48 (48%) isolates were identified as multi-drug resistant (MDR). Although 86 (86%) isolates had at least one of the analyzed genes, only 1 (1%) isolate showed the presence of co-extensive *sea*, *sec*, and *tst* enterotoxin genes and 36% isolates had the *sea* and *tst* genes. Among the 86 isolates, 79% contained the *sea* gene, 5% contained the *sec* gene, and 43% had the *tst* gene. Statistical analysis revealed a significant correlation between the presence of the *tst* gene and MDR isolates. The presence of relevant genes in clinical isolates should be considered in disease control management due to the importance of *S. aureus* enterotoxins and toxic shock syndrome genes and their role in the development and exacerbation of staphylococcal diseases. Additionally, the high prevalence of resistant isolates limits antibiotic treatment.

Keywords: *S. aureus*, Antibiotic resistance, Staphylococcal enterotoxins, Toxic shock syndrome toxin

1. Introduction

Staphylococcus aureus is a common pathogen that can inhabit various parts of the body and cause a variety of infections such as skin and tissue infections, food poisoning, hospital-acquired infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign body infections, and sepsis (1, 2). *S. aureus* has multiple virulence factors that contribute to its pathogenicity and bacterial colonization (3). These virulence factors include drug resistance, enterotoxins, and Toxic Shock Syndrome Toxin (TSST). Enterotoxins and TSST are pyrogenic superantigens that react with the MHC II molecule, causing T lymphocytes to proliferate extensively and leading to damage from the release of high levels of cytokines. *S. aureus* has been identified as having more than 23 types of enterotoxins, which contribute to gastrointestinal poisoning and gastroenteritis (4-6). The majority of *S. aureus* strains found in patients with toxic shock syndrome produce a harmful toxin called TSST-1, which can cause vital organs to fail and is often fatal (7-9). In addition, antibiotic resistance is a significant problem in dealing with various hospital infections. It not only causes treatment failure in some cases but also increases hospitalization time and treatment costs (10-14). Infections caused by *S. aureus* are becoming increasingly difficult to treat due to the widespread circulation and emergence of drug-resistant strains. Toxic shock syndrome is often treated with clindamycin and vancomycin. However, the excessive use and inappropriate prescription of antibiotics have led to an increase in antibiotic resistance, making it more difficult to treat toxic shock syndrome infections (7, 15). Furthermore, there is a lack of research studies on human samples in Iran, as most of the existing studies have focused on food and animal sources. Therefore, research is needed to investigate the frequency of *sea*, *sec*, and *tst* genes of *S. aureus* isolated from clinical sources to better understand the health risks that patients face.

2. Materials and methods

2.1.Bacterial samples and identification

The study was conducted in 2021 on 100 *S. aureus* isolates collected from different samples of patients and outpatients from Karaj city hospitals, including wound, blood, urine, sputum,

nose, and pharynx. The samples were identified using specific culture mediums and various biochemical tests in a microbiology laboratory. All identified isolates were inoculated in nutrient broth containing 20% glycerol and stored at -20°C for further experiments (16, 17).

2.2.Antibiotics susceptibility test

To determine antibiotic sensitivity, all isolates were tested using the agar disk diffusion method on Muller Hinton agar medium. The testing was performed with 12 antibiotic disks obtained from Padtan Teb co, including: Oxacillin (1 µg), Vancomycin (30 µg), Cefoxitin (30 µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg), Ciprofloxacin (5µg), Erythromycin (15µg), Clindamycin (2µg), Ceftazidime (30µg), Gentamicin (10µg), Tetracycline (30µg), Penicillin (10U), and Chloramphenicol (30µg). The results were reported as sensitive, semi-sensitive, and resistant based on the inhibitory zone. *S. aureus* strain ATCC25923 was used as a positive control. Isolates were classified as multidrug-resistant (MDR) if they were resistant to at least one antibiotic from three different antibiotic families, based on the results of the antibiotic sensitivity test (18, 19).

2.3.DNA extraction

Initially, we isolated *S. aureus* strains and extracted DNA using the BetaPrep Genomic DNA extraction kit from BETA BAYERN, Germany. The quantity and quality of the extracted DNA were assessed using OD 260/280 nm and agarose gel (1.5%). The extracted DNA was then preserved at -20°C for future use.

2.4.Triplex PCR reaction

The Triplex PCR reaction was used to confirm the presence of the analyzed genes in the studied isolates. Specific primers (Table 1) were used to determine if the isolates had the sea, sec, and tst genes. The reaction mixture contained a final volume of 30 microliters, consisting of 15 microliters of Amplicon's master mix (which includes Maxer Mix 1X, Tris-HCl 0.5 M, MgCl2 2 mM, dNTPs 1.6 mM, Taq 0.04 Units/µl, and 0.5 µl), 1 microliter (0.2 µM) of Forward and Reverse primer for each gene, 2 microliters (20 ng) of template DNA, and 7 microliters of double-distilled sterile distilled water. The genes were amplified using a thermal cycler (Applied Biosystem) under the following conditions: the temperature was set to 96°C for 5 minutes to start the process, followed by 35 thermal cycles consisting of denaturation at 94°C for 1 minute, annealing at 57°C for 1 minute, and amplification at 72°C for 1 minute. After the final amplification, the temperature was kept at 72°C for 10 minutes

(20). A negative control was used, where all materials were used except for the template DNA. For the positive control, the standard strains of *S. aureus* ATTC 13565, *S. aureus* ATTC 19095, and *S. aureus* ATTC 25923 were used for *sea*, *sec*, and *tst* genes, respectively. The Triplex PCR reaction product was run on a 2% agarose gel with a Ladder100 bp and checked under ultraviolet light with a Gel document device (21, 22).

Table 1: Sequence of primers related to *sea*, *sec* and *tst* genes in *S. aureus* isolates

Gene primer	sequence (5′to3′)	Primer length	Fragment size (bp)	Reference
sea	F:GGTTATCAATGTGCGGGTGG	20	102	(23)
	R:CGGCACTTTTTTCTCTTCGG	20		
sec	F:AGATGAAGTAGTTGATGTGTATGG	24	451	(23)
	R:CACACTTTAGAATCAACCG	20		
tst	F:ACCCCTGTTCCCTTATCATC	20	326	(23)
	R:TTTTCAGTATTTGTAACGCC	20		

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2.5.Statistical analyses

After that, we analyzed the results using Microsoft Excel 2010 software and SPSS software (2020). We used Cramer's V and chi-square test, and set the significance level at $p \leq 0.05$.

3. Results

3.1.Bacterial samples

A total of 100 *S. aureus* isolates were collected from clinical sources in Karaj, with 49 from women and 51 from men. The average age of the patients was 46.51 years. The isolates were obtained from various sample types, with the highest percentage from blood (70%) and the lowest from wounds (2%). Other samples were taken from urine, sputum, nose, and pharynx. The frequency of *S. aureus* isolates in various clinical samples is presented in Figure 1. Our study found a significant relationship between the sample type and the presence of the *sea* gene in the *S. aureus* isolates ($p<0.05$).

Figure 1: The frequency of *S. aureus* isolates in various clinical samples

3.2.**Antibiotics susceptibility**

The following sentences refers to Table 2, which contains details of the results related to the pattern of resistance and sensitivity to antibiotics. The results of the disk agar diffusion method for antibiotic sensitivity testing showed that the 92 (92%) isolates were resistant to penicillin, while 82 (82%) isolates were resistant to ceftazidime. Additionally, 47 (47%)

isolates were resistant to tetracycline, 43 (43%) to erythromycin, and 38 (38%) to cefoxitin. Other antibiotics with less resistance included ciprofloxacin (36%), oxacillin (34%), and clindamycin (33%). Moreover, 23 (23%) isolates were resistant to gentamicin, 16 (16%) isolates to trimethoprim-sulfamethoxazole, and only 4 (4%) isolates to chloramphenicol. Notably, no vancomycin-resistant isolates were observed in the study. The results of the antibiotic sensitivity test showed that 48 (48%) out of the total isolates were identified as MDR.

Table 2: The results of antibiotic sensitivity in the tested isolates

Sensitivity / Antibiotic	Total isolates (N=100)		
	Sensitive	**Resistance**	**Intermediate**
Oxacillin	66%	34%	-
Cefoxitin	62%	38%	-
Trimethoprim-Sulfamethoxazole	83%	16%	1%
Clindamycin	67%	33%	-
Ciprofloxacin	58%	36%	6%
Chloramphenicol	91%	4%	5%
Erythromycin	49%	43%	8%
Gentamicin	75%	23%	2%
Ceftazidime	1%	82%	17%
Penicillin	8%	92%	-
Tetracycline	53%	47%	-
Vancomycin	100%	-	-

3.3. Presence of enterotoxin genes

Out of 100 isolates studied, 14 did not have the *sea*, *sec*, and *tst* genes, while the remaining 86 (86%) isolates had at least one of these genes. Among the 86 isolates, 79% had the *sea* gene, 5% had the *sec* gene, and 43% had the *tst* gene. Moreover, 4% had both the *sea* and *sec* genes, 36% had the *sea* and *tst* genes, and 2% had both *sec* and *tst* genes (Figure 2). Only 1 (1%) isolate showed the presence of co-extensive *sea*, *sec*, and *tst* enterotoxin genes. Statistical analysis revealed a significant correlation between the presence of the *tst* gene and MDR isolates ($p<0.05$). Moreover, the frequency of this gene was higher in MDR isolates. The gender of patients had a significant association with the presence of the *sec* and *tst* genes ($p<0.05$). The *tst* gene was more prevalent in female patients, while the *sec* gene was more prevalent in male patients.

Figure 2: Comparison of the frequency of different enterotoxin genes in 100 *S. aureus* isolates

4. Discussion

S. aureus is a significant pathogen for humans and has been a leading cause of both community-acquired and hospital-acquired infections for several decades. Despite antibiotic

treatment, this microorganism frequently causes severe complications in hospitalized patients, and its increasing drug resistance has made treatment challenging. Genetically, this bacterium possesses genes that contribute to virulence, antibiotic resistance, and enterotoxin production, which can have dangerous effects on the host (7, 24). Our study analyzed 100 *S. aureus* isolates and found that the highest resistance rate was observed for penicillin (92%), while the lowest resistance rate was shown for vancomycin (0.0%). The resistance pattern to other antibiotics was as follows: ceftazidime (82%), tetracycline (47%), erythromycin (43%), cefoxitin (38%), ciprofloxacin (36%), oxacillin (34%), clindamycin (33%), gentamicin (23%), and trimethoprim-sulfamethoxazole (16%). In this regards, Jafari-Sales et al. reported a penicillin resistance rate of 100% in *S. aureus*, which is consistent with the findings of another study (25). In 2016, a study showed that no *S. aureus* isolates were resistant to vancomycin, which is consistent with the present study, but the highest percentage of antibiotic resistance was found for clindamycin, oxacillin, and trimethoprim-sulfamethoxazole (26). Reisi et al. reported that the highest percentage of antibiotic resistance was to penicillin and cefotaxime (100%), while the lowest was to vancomycin (0.5%), which is in accordance with our results (27). Wu et al. also found that 100 % of *S. aureus* isolates were resistant to penicillin, but no vancomycin-resistant isolates were found among the samples (28). Another study found that the level of antibiotic resistance related to penicillin among *S. aureus* isolates was 68.3%, which is similar to our results. However, this level of resistance was lower than what we reported (2). In another study, 92.5% of *S. aureus* isolates were resistant to penicillin, and 10.5% were confirmed as vancomycin-intermediate *S. aureus* (29). The results of these studies and our own demonstrate differences and similarities in the level of resistance to different antibiotics, which can be attributed to various factors such as geographical region and the type and number of collected samples. However, what is certain is the increasing rate of resistance in these bacteria.

The molecular results of the presence of enterotoxin genes in *S. aureus* isolates showed that 79%, 5%, and 43% of the isolates carried *sea, sec*, and *tst* genes, respectively. It was found that 36% of the isolates had both *sea* and *tst* genes, 4% had *sea* and *sec* genes, and 2% had *sec* and *tst* genes. Additionally, 1% of the isolates showed a positive presence of all three genes. In this regards, Goli et al. conducted a study on 49 *S. aureus* isolates, 34.7% were positive for the *sea* gene (30). Various researchers from different parts of the world have reported different frequencies of the *sea* gene in *S. aureus* isolates. Some studies similar to our results, such as those conducted by Katayoon et al. (31) and Rahimi et al. (32), have reported a high frequency of the *sea* gene in *S. aureus* isolates, with 86.2% and 100% of the

isolates carrying the gene, respectively. On the other hand, other studies, including those by Asgarpoor et al. (1), and Nashev et al. (33), have reported a lower level of the *sea* gene in *S. aureus* isolates, with carrier rates ranging from 16% to 47.4%. The frequency of the *sec* gene has been investigated in various studies, revealing a range of *S. aureus* isolates that harbor the gene. For instance, Goli et al. reported that 10% of *S. aureus* isolates carried the gene (30). Other studies, such as those conducted by Eshraghi et al. (3), and Saadati et al. (34), reported frequencies of the *sec* gene in *S. aureus* isolates of 1.6%, and 9.5%, respectively. The frequency of the tst gene, which is one of the important toxins in the virulence of *S. aureus*, has been investigated in different studies. Mohammad Jani et al. reported that the amount of *tst* gene in *S. aureus* isolates was 43%, which is in agreement with our results (35). However, other studies have reported different frequencies of this gene in *S. aureus* isolates. For example, Ramazanzadeh et al. reported 81% (36), Parsonnet et al. reported 9% (37), and Becker et al. reported 18.2% (38). After comparing the findings of various research studies with the outcomes of the current study, it is evident that some results are consistent while others vary. The discrepancies in the frequency of the genes analyzed can be attributed to diverse factors such as: geographical location, sample nature, sample size, strain's natural habitat, the overall health of the population being studied, the pattern of health behavior in clinical and community settings, and differences in the investigation methods and primers used in molecular studies.

Our study revealed high levels of enterotoxins and antibiotic resistance in *S. aureus* isolates, which can exacerbate hospital infections and contribute to the spread of antibiotic resistance. It is crucial to take action against the rise of *S. aureus* genes that produce enterotoxins and toxic shock syndrome toxins in clinical sources. Treating infections caused by this bacterium can be challenging and lead to severe consequences. Therefore, prioritizing disease control and avoiding unnecessary antibiotic use is essential to prevent resistance from spreading.

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Author contributions

Investigation, formal analysis, writing original draft: A.S.S., Conceptualization, methodology, writing & editing: M.E.B., Conceptualization, Project administration, review & editing: R.M., Review & editing: M.T.M.

Conflict of Interest

There is no conflict of interest among the authors of this article.

Ethics

It is stated that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Data availability

All data available are reported in the article.

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