Prevalence and Distribution of Resistance and Enterotoxins/Enterotoxin-likes Genes in Different Clinical Isolates of Coagulase-negative Staphylococcus

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Research

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Abstract

Background

CoNS serve as a major reservoir for genes facilitating the evolution of S. aureus as a successful pathogen. The present study aimed to determine the occurrence of genes conferring resistance to fluoroquinolone, determining of the prevalence of insertion sequence elements IS256 and IS257 and different superantigens (SAgs) among CoNS isolates obtained from various clinical sources.

Materials and Methods

The current study conducted on a total of 91 CoNS species isolated from clinical specimens in Hamadan hospitals in western Iran in 2017-19. The Antimicrobial susceptibility testing was performed using disk diffusion method and the presence of the IS256 and IS257, genes conferring resistance to fluoroquinolone and enterotoxins/enterotoxin-likes encoding genes were investigated by PCR method.

Results

Among genes encoding classic enterotoxins, sec was the most frequent which was carried by 48.4% of isolates, followed by seb in 27.5%. None of the CoNS isolates were found to be positive to enterotoxin-like encoding genes. Among the 11 CoNS isolates that have shown phenotypically resistant to levofloxacin, 9 isolates (81.8%) with gyrB, 8 isolates (72.7%) with gyrA, 8 isolates (72.7%) with grlB and 7 isolates (63.6%) with grlA were identified. The IS256 and IS257 were identified in 31.8% and 74.7% of CoNS isolates. The results of statistical analysis showed a significant association between the occurrence of SEs encoding genes and antimicrobial resistance.

Conclusion

Antimicrobial resistant determinants and staphylococcus enterotoxins (SEs) are co-present in clinical CoNS isolates that confer selective advantage for colonization and survival in hospital settings. The coexistence of insertion elements and antibiotic resistance indicate their role in pathogenesis and infectious disease.

Background

Coagulase-negative staphylococci (CoNS) serves as a important reservoir of antimicrobial resistance genes which can transmit between staphylococcal species or even other bacterial genera, but they have been implicated in rare cases of food poisoning(1, 2). Among CoNS species, S. epidermidis, S. hominis and S. haemolyticus are often developed to be resistant to multiple antibiotics(3). Staphylococcal exotoxins have been divided into three groups of Staphylococcal heat-stable enterotoxins (SEs), responsible for the pathogenesis of Staphylococcal food poisoning (SFP), exfoliative toxins (ETs) and toxic shock syndrome toxin 1 (TSST-1), causative agents of scalded skin syndrome and toxic shock syndrome, respectively(4). Besides coagulase-positive staphylococci (CoPS), It is recognized that CoNS
species are also capable of producing enterotoxins and associated with food poisoning outbreaks(5). Several SEs are classified as SE-like (SEl) toxins due to they still have not been tested for emetic activity or lack the emetic properties(6). Various types of classical SEs including SEA, SEB, SEC1, SEC2, SEC3, SED, SEE and SEH on the basis of their antigenicities are currently described(7, 8). In addition to classical SEs, 16 new types of SEs (SEG, SEH, SEI, SER, SES, SET) and SEls (SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SEIU and SEIV) have been reported(6). SEs are extracellular protein toxins with low molecular weight (26.900–29.600 KD)(8, 9). They are heat resistant and retain their biological activity after treatment with majority of proteolytic enzymes(10). SEs as well as toxic shock syndrome toxin-1 (TSST-1) are belong to the pyrogenic toxin superantigen family of exotoxins (PTSAgs)(11). Antimicrobial drug resistance in CoNS is increasing which led to serious problems for therapeutic options(12). CoNS acts as large reservoir of mobile genetic elements, which confer resistance to B-lactams, aminoglycosides, quinolones, macrolides, and tetracyclines(12). Fluoroquinolones are potent, broad-spectrum agents that were largely developed for treat of a wide range of infections due to Gram-positive and Gram-negative pathogenic bacteria(13). Efficacy of further fluoroquinolones were developed including levofloxacin, trovafloxacin, sparfloxacin, moxifloxacin, grepafloxacin and gatifloxacin have confirmed in treatment of patients with various infections with Gram-positive cocci(14). The fluoroquinolones block activity of essential bacterial enzymes DNA gyrase and DNA topoisomerase IV, which involved in DNA replication(13). GyrA and GyrB are the corresponding subunits of DNA gyrase, encoded by the gyrA and gyrB genes(15). Topoisomerase IV composed of ParC and ParE subunits (often reffered as GrlA and GrlB in Staphylococcus aureus), encoded by grlA and grlB genes(13, 16). Mechanisms of microbial resistance to fluoroquinolone include one or combination of four main bacterial mechanisms: alterations in the drug target, alteration in the cell permeability, quinolone-modifying enzymes, drug efflux pumps or gyrase protecting proteins(13, 14). Evaluation of the occurrence rate of SAgs genes among human CoNS isolates had never been done before. Regarding to the impacting of antibiotic resistance and toxins in pathogenicity of CoNS isolates as well as due to association of the staphylococcal insertion sequence elements IS256 and IS257 with antimicrobial drug resistance and pathogenesis the current study aimed at assessing the prevalence of SEs and TSST-1 toxins, ISs, antibiotic resistance trends and the quinolone resistance determining regions (QRDRs) of gyrA, gyrB, grlA and grlB genes in CoNS thriving in various types of clinical samples.

**Materials And Methods**

**Identification of CoNS isolates**

A total of the 91 CoNS isolates were collected from various clinical specimens submitted to three teaching hospitals (including Beheshti, Besat, and Farshchian Hospitals) located in Hamedan, Iran, from September 2017 to November 2018. The origins of the isolates were as follows: blood, urine, catheters, and wounds. This study was approved by the ethics committee of the Hamadan University of Medical Sciences (Code No: IR.UMSHA.REC.1396.827).

**DNA extraction from isolates**
The plasmid DNA Extraction Mini Kit (Favorgen, Taiwan) and Plasmid Extraction Kit (Sinaclon, Iran) were used for plasmid DNA extraction according to the manufacturer's recommendations. CoNS Chromosomal DNA was extracted by boiling method. Quality of extracted DNA was assessed by the Nanodrop ND1000 (Nanodrop Technologies, Inc., Wilmington, DE, USA).

**Antibiotic susceptibility testing**

The phenotypic antimicrobial susceptibility response of 91 isolated CoNS was evaluated using a panel of 11 commercial antibiotic discs which belonged to various classes of antimicrobial agents. Disk agar diffusion (DAD) method was conducted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines(17). The antibiotics used were: chloramphenicol (30 µg), cefoxitin (30 µg), clindamycin (2 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), levofloxacin (5 µg), novobiocin (5 µg), rifampicin (5 µg), trimethoprim-sulfamethoxazole (25 µg) and vancomycin (30 µg) (MAST, Merseyside, UK). S. aureus ATCC33591 was plated as a quality control strain for performing antimicrobial tests.

**Detection of SEs and TSST\textsubscript{1} encoding genes**

Multiplex PCR was used to analyze the following genes: sea, seb, sec, sed, see, seg, seh, sei, selj, selm, seln, selo, selk, sell, selp, selq, sler, selu and tsst. Multiplex PCR was performed with 5 different sets of primer mixtures (Set 1: sea, seb, sec; Set 2: sed, see, seg, seh; Set 3: sei, selj, selm, seln; Set 4: selk, sell, selq, tsst; Set 5: selo, selp, sler, selu). PCR amplifications were carried out in 25 µL volumes, containing 2 µL template DNA, 1 µL of each forward primer, 1 µL of each reverse primer, 7 µL (set 2, 3, 4, and 5) and 9 µL (Set 1) of sterile double distilled water and 8 µL of 2x Taq Premix-Master mix (Parstous Biotech Co, Iran). The primers used to amplify SEs and TSST-1 genes are listed in Table 1(18–20). Each PCR amplification reaction was performed using a Bio-Rad thermocycler (Bio-Rad, USA) with the following cycles: initial denaturation for 5 min at 95ºC and then 35 cycles at 95 ºC for 1 min (denaturation), 52 ºC for 1 min (annealing) and 72 ºC for 2 min (extension) and final extension was performed at 72 ºC for 7 min. All PCR products were analyzed by electrophoresis for 50 min at 100V through 1% agarose gel (Invitrogen). S. aureus reference strains were included in all reactions as positive control; ATCC 13565 (sea), ATCC 14458 (seb), ATCC 19095 (sec), ATCC 27664 (see), ATCC 51811 (seh), FRI 472 (sed, seg, sei, selj, selm, seln, selo, selr, selu).

**Identification of genes conferring resistance to fluoroquinolone**

The Quinolone resistance determining regions (QRDRs) of gyrA, gyrB, grlA, grlB genes were investigated by PCR amplification using specific primer sequences (21, 22). Each 20 µL PCR reaction mixture contained: 10 µL of 2x Taq Premix-Master mix, 6 µL of sterile distilled water, 1 µL of forward primer, 1 µL of reverse primer and 2 µL of template DNA. PCR conditions for amplification of grlA and grlB genes: 95 ºC, 4 min; 95 ºC, 1 min; 48 ºC (52 ºC for grlB), 1 min; 72 ºC, 60 s; 72 ºC, 4 min; for gyrA: 95 ºC, 1 min; 95
°C, 45 s; 54 °C, 60 s; 72 °C, 1 min; for gyrB: 94 °C, 2 min; 94 °C, 1 min; 48 °C, 60 s; 72 °C, 1 min; 72 °C, 5 min.

Detection of insertion sequence elements IS256 and IS257 among CoNS isolates

Multiplex PCR assay was performed for the identification of insertion sequences IS256 and IS257, the appropriate oligonucleotide primers were selected as follows; for IS256 (762 bp), the 5' primer AGTCCCTTTACGGTACATG and the 3' primer TGTGCGCATCAGAAAATACG; for IS257 (576 bp), the 5' primer CTATCTAAGATATGCATTGAG and the 3' primer TTAACTTGCTAGCATGATGC(16). 25 µl of PCR mixture contained 2 µl of template DNA, 1 µl of each primer for IS256 and IS257, 10 µl of Master Mix, and 9 µl of sterile distilled water. The PCR conditions included an initial denaturation at 94 °C for 3 min, followed by amplification; 35 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 2 min and 72 °C for 5 min.

Statistical analysis

Cramer’s V, Phi and Chi-Square test were performed to assess of variables correlation. Phi and Cramer’s V have ranges from 0 to 1, where 1 indicates a significant association and 0 indicates no relationship. Interpretation of the Phi and Cramer’s V results; > 0; No or very weak, > 0.05 weak; > 0.10 moderate; > 0.15 strong; > 0.25 very strong. The Chi-Square test was done by SPSS software version 20. P value < 0.05 was considered as statistically significant.

Results

Isolation and prevalence of CoNS isolates

Of the 91 clinical isolates of CoNS, 49 (53.8%), 37 (40.7%) and 5 (5.5%) isolates were recognized as S. epidermidis, S. haemolyticus, and S. saprophyticus, respectively. Isolates were recovered from blood 39 (42.9%), urine 33 (36.3%), catheter 13 (14.3%) and wound 6 (6.6%).

Antimicrobial susceptibility testing

The results of antibiotic susceptibility testing of 91 CoNS isolates and the distribution rate of SEs and TSST-1 toxins among antibiotic resistant strains are demonstrated in Table 2. Among CoNS species, the highest resistance ratio was 53.8% for cefoxitin, followed by trimethoprim-sulfamethoxazole (46.2%, n = 42) and none of the CoNS species was identified as being resistant to vancomycin. The highest occurrence rate genes involved in production of SEA, SEB, SEC, SEH, SEM, and TSST-1 toxins were significantly occurred among strains resistant to cefoxitin and trimethoprim-sulfamethoxazole with the frequencies of 100%, 100%; 52%, 48%; 63.6%, 50%; 50%, 50%; 80%, 60%; and 56.5%, 47.8%, respectively. Also among strains that have shown phenotypically resistance toward cefoxitin and doxycycline antibiotics, the distribution of SEE was significantly higher compared to strains resistant to other antibiotics (45.5% and 27.3% respectively).
Prevalence of Staphylococcal superantigen (SAg) genes among CoNS isolates

The distribution of SEs and TSST-1 encoding genes among S. epidermidis, S. saprophyticus, and S. haemolyticus strains and in various clinical samples are shown in Table 3. Our results demonstrated the high frequency of SEs encoding genes among CoNS isolated from blood, followed by urine, catheter, and wound, respectively. None of CoNS isolates was found to be positive to seg, sei, selj, selm, seln, selo, selk, sell, selp, selq, selr, selu genes. 44 isolates (48.4%) with sec, 25 isolates (27.5%) with seb, 23 isolates (25.3) with ttst, 11 isolates (%12.1) with see, 5 isolates (5.5%) with sem, 4 isolates (4.4%) with seh, 3 isolates (3.3%) with sed and 2 isolates (2.2%) with sea. Among 17(46%), 18 (61%) and 4(80%) of the toxigenic S. epidermidis, S. haemolyticus and S. saprophyticus, 17(53%) of strains shown the simultaneous presence of two SAg that seb + sec were detected as the most frequent (18.7%), followed by sec + tsst (12.5%), sea + seb, see + sem, seb + see, seb + tst, and sed + tsst = 3%. 6 (15.6%) of the strains were detected to harbor three SAg genes; seb + see + ttst = 5 (15.6%), sec + see + tsst, seb + sec + seh, seb + see + seh, sea + seb + tsst and sea + sec + sem = 1(3%). The four types SAg genes of seb + sec + see + tsst and seb + sec + sem + tsst, seb + sed + see + tsst were found in 3 (9.3) and 1 (3%) of toxigenic CoNS isolates, respectively. Only 2 of 17 toxigenic S. epidermidis strains had five types of SAg genes included; seb + sed + see + sem + tsst, seb + sec + see + she + tsst = 1(3%).

The distribution of fluoroquinolone resistance genes among CoNS isolates

The distribution of resistance genes among CoNS isolates and in various clinical samples are presented in Table 4. The blood sample had the highest number of strains carrying genes responsible for inducing resistance to levofloxacin, followed by catheter and urine. The genes conferring resistance to levofloxacin were found in any of the strains isolated from wound. Among 11 of the CoNS isolates that have shown phenotypically resistance to levofloxacin, 9 isolates (81.8%) with gyrB, 8 isolates (72.7%) with gyrA, 8 isolates (72.7%) with grlB and 7 isolates (63.6%) with grlA were identified. The gyrA and gyrB genes were discovered as the most dominant genes inducing resistance to levofloxacin in the S. saprophyticus strains and the gyrB and grlB genes were characterized with the highest frequency among S. haemolyticus strains.

Prevalence of insertion sequence elements IS256 and IS257 among CoNS isolates

The distribution of IS256 and IS257 among S. epidermidis, S. saprophyticus, and S. haemolyticus strains isolated from various clinical samples are demonstrated in Table 5.

Statistical analysis
Considering the Antimicrobial susceptibility patterns, it was found a significant association between novobiocin resistance and the occurrence of *sed* and *see* genes (P = 0.03, φ = 0.22 and P = 0.04, φ = 0.21, respectively), gentamicin resistance and the presence of *sec* gene (P = 0.01, φ = 0.32) as well as between rifampicin and trimethoprim-sulfamethoxazole resistance and the presence of *seh* and *see* genes responsible for production the SEH and SEE enterotoxins was observed in current study (P = 0.01, φ = 0.26 and P = 0.04, φ = 0.21, respectively). Among strains carrying insertion sequence elements found a significant association between the presence of IS256, IS257 and gentamicin and cefoxitin resistance respectively (P = 0.04, φ = 0.25 and P = 0.004, φ = 0.30, respectively). As well as meaningful relationship between the occurrence of IS256 and production of SEA found in the current survey (P = 0.04, φ = 0.22).

**Discussion**

This study provides the first report of the frequency of SEs and TSST-1 toxins among human CoNS isolates. Some investigators have reported lack of enterotoxin genes associated with human and veterinary CoNS isolates(23, 24). This study demonstrated that the most frequent classical enterotoxin-encoding genes were *sec* and *seb* among strains obtained from wound samples (48.4% and 27.5%, respectively). While the *sea*, *sed*, *seh* and *sem* genes were rarely found (2.2%, 3.2%, 4.4% and 5.4%, respectively). This is in agreement with other findings (Lyra et al., 2013; Carfora et al., 2015) that *sec* gene was described as the most common classical SAg among CoNS isolates regardless of the origin of isolates(5, 25). This was in contrast with other similar researchs which have been demonstrated the occurrence of *sea* gene in the higher frequency compared to other enterotoxin genes(8, 26, 27). In this survey the *sea* gene was only detected in a small percentage of two *S. haemolyticus* strains recovered from blood and urine samples(2.2%). According to findings of Mello et al., the *sea* gene was the most common staphylococcal SAg among isolates derived from cows with bovine subclinical mastitis with frequency of 18.2%, followed by *sec* in 14.9% of the isolates, *see* in 8.2% and *seb* in 7.7%, and the *sej*, *ses*, *set*, and *tsst* genes were not identified in any of the isolates(27). In a survey carried out by versa et al., 70% of CoNS isolates were found to be positive to the presence of enterotoxin genes. The *sea* gene amplified in 38% of isolates, *seb* in 29%, *sea* and *seb* amplified both in 24% and *sec* and *sed* were found rarely(28). In a similar survey conducted by other investigators in accordance with our study the *gene sec* found to be the most frequent among the classical genes and *sea* was only detected in one isolate(5). According to results of Andrade et al., in agreement with our findings among detected enterotoxin-encoding genes the highest prevalence was to *seh* gene (53.2%) in coagulase-positive strains (CoPS) and *sec* (46.8%) in CoNS(29). In the investigation of Nunes et al., the *sec* and *seb* genes have found as the most predominant (57%), followed by intermediate incidence of *sed/seh/selm* (33%), while *see* and *seg* genes have carried by 13% and 1% of strains, respectively(29). These discrepancies between investigations may be related to bias in various methods used and also number, nature and geographic origin of the isolates. Our findings demonstrated a high percentage of toxigenic strains among *S. epidermidis* (75.5%, n = 37), followed by *S. haemolyticus* (48.6%, n = 18). This is in accordance with other findings (Cunha et al., and Pinheiro et al.), which *S. epidermidis* is described as the CONS with the highest potential to produce of enterotoxins among identified isolates(26, 30). 17(46%) and 11 (61%) of toxigenic *S. epidermidis* and *S. haemolyticus* strains have shown two or more the SEs-encoding genes in
association that seb + sec were detected as the most frequent. The current study indicated a higher incidence of enterotoxin genes among isolates recovered from blood (42.3%), which 55% and %38 of S. haemolyticus and S. epidermidis strains were harboring at least one enterotoxin gene, predominating followed by urine (37.2%), catheter (13.5%) and wound (6.7%) sources. This was in agreement with a similar survey carried out by Pinheiro et al., who exhibited a high prevalence of enterotoxin genes among S. epidermidis and S. haemolyticus strains isolated from blood culture with frequencies of 95.3% and 79.8%, respectively(26). Strains carrying the seb, sed, see, seh and tsst genes were detected with a higher prevalence as the CoNS in urine isolates compared to blood (48% and 32%, 66.7% and 33.3%, 72.7% and 18.2%, 100% and 0%, 52.2% and 43%, respectively). Considering the antimicrobial susceptibility patterns, the highest resistance ratio was determined for cefoxitin in 53.8% and trimethoprim-sulfamethoxazole in 46.2% of isolates. The highest frequency of sea gene involved in the synthesis of SEA was characterized among strains that exhibited phenotypic resistance to chloramphenicol, erythromycin, cefoxitin and trimethoprim-sulfamethoxazole antibiotics. The sec and sed genes were the most common among strains exhibiting resistance to gentamicin and doxycycline, levofloxacin, novobiocin antibiotics, respectively. Clindamycin and rifampicin-resistant strains showed a higher frequency of CONS isolates containing the seh gene in association with other SEs-encoding genes. Regarding the results of statistical analysis in CoNS isolates observed a significant association between the incidence of SEs and resistance to antimicrobial agents. Schroeder et al., and Motamedi et al., described the role of the occurrence infective determinants genes in the development of resistance to the antimicrobial agents and fatal Staphylococcal infections(31, 32). Out of 91 CoNS isolates, 12% (n = 11) of the strains were found to be resistant to levofloxacin, which the most dominant detected CoNS species was S. epidermidis (46%), followed by S. haemolyticus (36%) then S. saprophyticus (18%). The most remarkable percentage of the resistant isolates were discovered from blood (64%), followed by urine and catheter (18%). 45% of isolates that exhibited phenotypic resistance to levofloxacin were carrying the SEs and TSST1 encoding genes that seb + sec were the most frequent, which the majority of CoNS toxigenic strains were belonged to S. haemolyticus. The results of this study confirmed that levofloxacin is highly active against CoNS isolates, showing the low percentage of resistance (12%), which is in agree with the results of cafiso et al., who reported the ratio of resistance to levofloxacin 7.7% in methicillin-resistant S. epidermidis (MRSE) and 0% in S. haemolyticus (MRSH) strains(33). Among isolates resistant to levofloxacin, 5 of 11 isolates (45.4%) were determined to be positive for carrying of the gyrA, gyrB, grlA and grlB genes. Three types of resistance genes were identified in 3 (27%) of isolates; gyrA + gyrB + grlB = 2 (18%), gyrA + gyrB + grlA = 1 (9%). In levofloxacin-resistant S. haemolyticus strains the grlA + grlB and gyrB genes were responsible for inducing resistance to levofloxacin. In a survey carried out by Osman et al., gyrA, gyrB and grlA genes in S. haemolyticus isolates were absent and 66.6% and 33.33% of MSSA isolates carried gyrA and gyrB genes, which in contrary to our results the prevalence percentage of these genes were reported with high incidence, 50%, 75% and 50%, respectively(1). According to published results by Osman et al., the prevalence of gyrA and grlA genes among various staphylococcus species in accordance with our results were with high incidence (63% and 70.4%, respectively) and gyrB gene in contrary with our findings was identified with incidence low, 26%(34). Regarding to the prevalence rate of insertion sequences, IS256 and IS257 were identified with the highest frequency among S. haemolyticus and S. epidermidis strains with
the frequencies of 58.6%, 41.4% and 38.2%, 54.4%, respectively. Studies analyzing the prevalence rate of IS256 among S. epidermidis strains have reported diverging results from 46.7–81%(35–37). Previous reports have demonstrated that IS256 is significantly associated with multi-resistant, biofilm-forming S. epidermidis isolates resident in the hospital setting(38). It was also indicated association of the IS256 with the genomes of aminoglycoside-resistant staphylococci and enterococci isolates(39, 40). Also in this study statistically found significant association between the incidence of the IS256 and resistant toward gentamicin as well as between the presence of IS257 and cefoxitin resistance (p-value 0.033 and 0.004, respectively). The IS257 is a mobile genetic element, which associated with genes mediating biofilm formation and genes conferring resistance to beta-lactamase, aminoglycosides and tetracycline antibiotics(41, 42). Montanaro et al., demonstrated a dramatic high level correlation between the presence of IS256 and gentamicin resistance(43). Considering the antimicrobial susceptibility patterns, strains harboring IS256 and IS257 were the most frequent among those with resistance to cefoxitin (53% and 76%, respectively). It was also found that IS256 may influence the expression of certain genes that are associated with pathogenesis(37). In current research IS256 was the most common among isolates harboring sea also statistically found significant relationship between production of SEA enterotoxin and the occurrence of IS256.

**Conclusion**

The high prevalence of SEs-encoding genes indicates a potential risk for causing human-originated food poisoning also its increasing antibiotic resistance is a serious problem for public health. Due to the correlation between the incidence of SAgs and the patterns of antibiotic resistance in CoNS isolates, therefore, detection of isolates harboring toxin-encoding genes associated with antibiotic resistance has become a significant issue of concern. The high degree of coexistence of cefoxitin resistance and the presence of IS257 among different species of CoNS indicates their role in infectious disease. Also correlation between the incidence of insertion element IS 256 and SEA enterotoxin as well as resistance toward gentamicin conferring selective advantage for pathogenesis and survival of invasive CoNS isolates in hospital settings.

**List Of Abbreviations**

Coagulase-negative *staphylococci* (CoNS), superantigens (SAgs), *Staphylococcal* food poisoning (SFP), exfoliative toxins (ETs) and toxic shock syndrome toxin 1 (TSST-1), pyrogenic toxin superantigen family of exotoxins (PTSAgs), quinolone resistance determining regions (QRDRs), disk agar diffusion (DAD), Clinical and Laboratory Standards Institute (CLSI)

**Declarations**

**Ethical Approval and Consent to participate**
This study was approved by the ethics committee of the Hamadan University of Medical Sciences (Code No: IR.UMSHA.REC.1396.827) and about the consent to participate is not applicable.

Consent for publication

It's not applicable

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

MN and ZS performed the tests, collected and analyzed the data, performed the analysis of the data. M. A designed the project and contributes in the whole steps of the projects. All authors read and approved the final manuscript.

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**Tables**

**Table 1.** Primers used in this study
| Gene targets | Primer sequences (5' to 3') | amplicon size (bp) | References |
|--------------|-----------------------------|-------------------|------------|
| sea          | F: TTGGAAACGGTTAAAAACGAA    | 120               | (18)       |
|              | R: GAACCTCCCATCAAAAAACA    |                   |            |
| seb          | F: TCGCATCAAACGTGACAAACG   | 478               | (18)       |
|              | R: GCAGGTACTCTATAAGTGCC    |                   |            |
| sec          | F: GACATAAAAAGCTAGGAATTT   | 257               | (18)       |
|              | R: AAATCGGATTAACATTATCC    |                   |            |
| sed          | CTAGTTTGGTAATATCTCTCTF:    | 317               | (18)       |
|              | TAATGCTATATCTTATAGGGR:     |                   |            |
| see          | F: TAGATAAAGTTAAAAACAAGC   | 170               | (18)       |
|              | R: TAACTTACCGTGGAACCTTC    |                   |            |
| seg          | F: TGCTATCGACACACTACAACC   | 704               | (18)       |
|              | R: CCAGATTCAATGCGAAGACC    |                   |            |
| seh          | F: CGAAAGCAGAGATTACACG     | 495               | (18)       |
|              | R: GACCTTTACCTTATTCGGTC    |                   |            |
| sei          | F: GACAACAAAAACTGCGAAACTG  | 630               | (18)       |
|              | R: CCATATTCCTGGCTTTACCAG   |                   |            |
| selj         | F: CAGCGATAAGCAAAAAATCAAAC | 426               | (18)       |
|              | R: TCTAGCGGAACACAGTTCAGA   |                   |            |
| selm         | F: CCAATTGAAGACCCAAAG      | 517               | (18)       |
|              | R: CTTGCTCTGTCCAGTATCA     |                   |            |
| seln         | F: ATTTGTCCTACATAGCTCGAA   | 682               | (18)       |
|              | R: TTGAAAAACTCGTCTCCA      |                   |            |
| selo         | F: AGTCAAGTGAGACCTTATT     | 534               | (18)       |
|              | R: TATGCTCCGAATGAGATGA     |                   |            |
| selk         | F: TAGGTGTCTCTAAATAATGCCA  | 293               | (19)       |
|              | R: TAGATATCGTTAGTAGCTG     |                   |            |
| sell         | F: TAACGCAGATGTAGCTCCAGG   | 383               | (19)       |
|              | R: CATCTATTTCTGCGGTAAC     |                   |            |
| selp         | F: TGATTTATTTAGTAGACCTTG   | 396               | (19)       |
|              | R: ATAACCAACCCGAATCACCA    |                   |            |
| selr         | F: GGATAAAGCGGTAATAGCAG    | 166               | (19)       |
|              | R: GTATTCGACACACTCAA       |                   |            |
| selu         | F: AATTGCTCTAAAAATTGATGG   | 215               | (20)       |
|              | R: ATTTGATTTCATCATGCTC     |                   |            |
| selq         | F: GAAAATAACACTTTATATTCAGTTC | 539           | (20)       |
|              | R: ATTTATCCAGTTTCATATGAAAT |               |            |
| tsst         | F: AAGCCCTTTGTGCTTGGCG     | 447               | (19)       |
|              | R: ATCGAATTTGGCCACATCTTT   |                   |            |
Table 2. The prevalence of superantigenes among isolates with antibiotic resistance

| Types of SAgs | Antimicrobial susceptibility patterns | I  | R  | Fox | cefoxitin | Sxt | trimethoprim-sulfamethoxazole | Ery | erythromycin | Cli | clindamycin | Chl | chloramphenicol | Riph | rifampicin | Levo | levofloxacin | Van | vancomycin | Gen | gentamicin | Dox | doxycycline | Novo | novobiocin |
|---------------|-------------------------------------|----|----|-----|-----------|-----|-----------------------------|-----|--------------|-----|-------------|-----|----------------|-----|-------------|-----|-------------|-----|-------------|-----|-------------|-----|-------------|
| TST<sub>1</sub> | (n=23)                              |    |    | 49(53.8) | 42(46.2) | 26(28.6) | 18(19.8) | 23(25.3) | 3(3.3) | 11(12.1) | 6(6.6) | 22(24.2) | 5(5.5) |                              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 2) |    |  2 |  13 |  28 |  0 |  5 |  2 |  4 | 13 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 8) |    |  2 |  12 |  22 |  1 |  2 |  2 |  3 | 11 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 1) |    |  1 |  8 |  13 |  1 |  1 |  0 |  2 |  5 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 2) |    |  0 |  4 |  7 |  0 |  2 |  1 |  0 |  2 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 3) |    |  1 |  5 | 10 |  1 |  1 |  1 |  1 |  4 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 7) |    |  0 |  3 |  1 |  0 |  1 |  1 |  0 |  0 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 9) |    |  0 |  4 |  3 |  1 |  2 |  0 |  0 |  2 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 10)|   |  0 |  0 |  0 |  0 |  0 |  0 |  0 |  0 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 4) |    |  0 |  5 | 12 |  0 |  1 |  1 |  1 |  5 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 8) |    |  0 |  4 | 10 |  1 |  3 |  0 |  1 |  3 |              |     |              |     |              |     |              |     |              |     |              |     |              |
|               |                                     | 5) |    |  0 |  3 |  2 |  1 |  2 |  0 |  1 |  1 |              |     |              |     |              |     |              |     |              |     |              |     |              |

Fox; cefoxitin, Sxt; trimethoprim-sulfamethoxazole, Ery; erythromycin, Cli; clindamycin, Chl; chloramphenicol, Riph; rifampicin, Levo; levofloxacin, Van; vancomycin, Gen; gentamicin, Dox; doxycycline, Novo; novobiocin.

Table 3. Prevalence of SEs and TSST<sub>1</sub> encoding genes among various CoNS species and various clinical samples.
| Source | Wound n(%) | Urine n(%) | Blood n(%) | superantigens | CoNS(n=91) |
|--------|------------|------------|------------|---------------|------------|
|        |            |            |            | SEA           | S. epidermidis (n=49) | S. haemolyticus (n=37) | S. saprophyticus (n=5) | Total n(%) |
| 0(0)   | 1(3)       | 1(2.6)     |            |               | 0(0)       | 2(5.4)       | 0(0)       | 2(2.2)     |
| 1(16.7)| 12(36.4)   | 8(20.5)    |            | SEB           | 14(28.6)   | 8(21.6)      | 3(60)      | 25(27.5)   |
| 3(50)  | 15(45.5)   | 20(51.3)   |            | SEC           | 29(59.2)   | 13(35.1)     | 2(40)      | 44(48.4)   |
| 0(0)   | 2(6.1)     | 1(2.6)     |            | SED           | 2(4.1)     | 0(0)         | 1(20)      | 3(3.3)     |
| 0(0)   | 8(24.2)    | 2(5.1)     |            | SEE           | 7(14.3)    | 2(5.4)       | 2(40)      | 11(12.1)   |
| 0(0)   | 4(12.1)    | 0(0)       |            | SEH           | 2(4.1)     | 2(5.4)       | 0(0)       | 4(4.4)     |
| 0(0)   | 2(6.1)     | 3(7.7)     |            | SEM           | 2(4.1)     | 2(5.4)       | 1(20)      | 5(5.5)     |
| 0(0)   | 12(36.4)   | 9(23.1)    |            | TST₁          | 14(28.6)   | 8(21.6)      | 1(20)      | 23(25.3)   |

Table 4. Prevalence of fluoroquinolone resistance genes among various CoNS species and various clinical samples

| Source | CoNS(n=91) |
|--------|------------|
|        | S. epidermidis (n=5) | S. haemolyticus (n=4) | S. saprophyticus (n=2) | Total n(%) |
| heter | gyrA       | 4 (80)       | 2(50)       | 2(100)      | 8(72.7)   |
| n(%)  | gyrB       | 4(80)        | 3(75)       | 2(100)      | 9(81.8)   |
| 100   | grlA       | 4(80)        | 2(50)       | 1(50)       | 7(63.6)   |
| 100   | grlB       | 4(80)        | 3(75)       | 1(50)       | 8(72.7)   |

Table 5. Prevalence of insertion sequences IS256 and IS257 among CoNS isolates and various clinical samples.
| Source | CoNS (n=91) |
|--------|-------------|
|        | S. \textit{epidermidis} (n=49) | S. \textit{haemolyticus} (n=37) | S. \textit{saprophyticus} (n=5) |
| Und. % | 12 (41.4) | 17 (58.6) | 0 (0) |
| Catheter n(%) | 13 (44.8) | 29 (31.8) |
| Urine n(%) | 12 (41.4) | 17 (58.6) |
| Blood n(%) | 11 (37.9) | 24 (35.3) | 68 (74.7) |

| Types of IS | IS256 | IS257 |
|-------------|-------|-------|
|              | 29 (31.8) | 68 (74.7) |