Detection of enterotoxins and genotyping of *Staphylococcus aureus* strains isolated from Isfahan Educational Hospital, Iran

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**Summary**

**Background and aims:** *Staphylococcus aureus* is known as one of the most important nosocomial pathogens, which may lead to several infections. The aim of this study was determining the enterotoxins A, C, and TSST-1 and molecular characterization of *S. aureus* strains with PFGE and MLST typing methods.

**Materials and methods:** In the present study during the six-months sampling, fifty *S. aureus* strains were isolated from patients admitted to Al-Zahra university hospital. Antimicrobial susceptibility testing, Multiplex PCR for detection of enterotoxin A, C and TSST-1, pulse field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were used for molecular typing.

**Results:** In antibiogram the highest and lowest percentage of resistance was belonged to tetracycline and rifampin respectively. Multiplex PCR indicated that 30% of the strains harbored *sea* and 34% harbored *sec* genes. However, only 4% of our collected isolates had *tsst* gene. In PFGE method analysis on all *S. aureus* strains, a total of 19 different patterns were identified. Nine various sequence types in 27 selected *S. aureus* isolates were identified by MLST.

**Conclusions:** Present study indicates a possible higher variability among our *S. aureus* strains by two different molecular typing methods; nevertheless four main common types (CT1, CT7, CT9, and CT11) with at least one toxin genes were determined.

**Introduction**

*Staphylococcus aureus* is known as one of the most important nosocomial pathogens that can be colonized on the skin of human without any noticeable symptoms and may lead to several infections such as folliculitis, impetigo, cellulitis and food poisoning (9). It is noteworthy that, the staphylococcal infections are created by following the skin barrier failure through surgery and trauma especially in immunocompromised patients. On the other hand the emergence of antibiotic resistant strains, particularly to beta-lactam, aminoglycoside and fluoroquinolone has been considered as a major concern in health care units. The methicillin-resistant *S. aureus* (MRSA) strains are the majority of the mentioned groups due to the rapid spread in health care centres (5). Producing toxins, capsule, various enzymes and enterotoxins are the common virulence factors which have been identified in *S. aureus*. A large number of *S. aureus* strains are able to produce a family of heat-stable enterotoxins such as staphylococcal enterotoxin serotypes A–E (SEA–SEE) (16).

These toxins are established as superantigens with effect on epithelial cells that subsequently proliferate and stimulate T cells (12). In comparison to other enterotoxins, enterotoxin A, B and C are responsible for food poisoning (1,3). Moreover, toxic shock syndrome toxin-1 (TSST-1), another secreted toxin by *S. aureus*, which is regarded as a toxic shock syndrome and superantigen, which can bind to MHC-II molecules and stimulating both T cells and Monocytes (8).

According to the spread of *S. aureus* related infections in different parts of hospital and distribution among patients, pulse field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) have been proved as reliable typing methods for *S. aureus* molecular typing.

The aim of this study was determining the enterotoxins A, C,
and TSST-1 and molecular characterization of S. aureus strains with PFGE and MLST typing methods.

Materials and Methods

Bacterial isolates and culture conditions

During the six month sampling period, fifty S. aureus strains were isolated from patients admitted to Al-Zahra university hospital. The strains were collected from the bloodstream, wound, urine and sputum, thereafter referred to the microbiology laboratory. Each sample was plated onto blood agar with 5% sheep blood and also nutrient agar and incubated at 37°C for 24 h. Standard microbiological tests, including Gram staining, catalase, coagulase and DNase were used to identify the isolates, then stored in trypticase soy broth (TSB) with 15% glycerol.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar, using Kirby-Bauer disc diffusion method with Ciprofloxacin, Gentamicin, Rifampin, Ofloxacin, Tetracycline, Cefoxitin and Cotrimoxazol (Himedia, Mumbai, India) according to CLSI (2017) guidelines.

DNA extraction

For this purpose, 1.5 mL of overnight cultured bacteria in TSB was added to 1.5 mL micro-centrifuge tube and centrifuged at 12000 rpm for 2 min. The supernatant was removed and the pellet resuspended in TE buffer plus lysozyme (25 mg/mL) (Sigma). The tube was gently shaken and then incubated at 37°C for 1 h. 400 µL of lysis buffer (Tris-HCL 0.01 M, EDTA 0.01 M), plus 10 µL of proteinase K (Sigma) were added and incubated at 50°C for 1 h. Finally the bacterial genome was purified with a commercial kit (K0512 Fermentas) according to the manufacturer’s instructions.

Multiplex PCR for detection of enterotoxin A, C and TSST-1

The presence of genes that encode the enterotoxins A (sea), C (sec) and TSST-1 (tst) were detected by Multiplex PCR using specific primers, as previously described (13).

PFGE

All isolates were genotyped by Pulsed-field gel electrophoresis. Genomic DNA was prepared in agarose plugs and digested with 30 U of Smal (Takara, Japan) as described previously (14). PFGE was carried out in a CHEF-DR III apparatus; (Bio-Rad, USA) at 6 V/cm for 22 h at 12°C, with pulse times of 5 s to 50 s. The gels were stained with ethidium bromide and visualized under UV illumination and then photographed. PFGE patterns were analysed by GelCompar II (Applied Maths, Belgium). Salmonella enterica serovar Braenderup strain H9812 was used as a marker.

MLST

MLST is the powerful molecular typing method which is based on the bacterial housekeeping genes sequence analysis. In the present study MLST was conducted by the method described by Enright et al. (7) The seven housekeeping genes which were used in MLST are as follows: Carbamate kinase (arc C), Shikimate dehydrogenase (aroE), Glycerol kinase (glp), Guanylate kinase (gmk), Phosphate acetyltransferase (pta), Triosephosphate isomerase (tpi) and Acetyl coenzyme A acetyltransferase (yqIL). Allele numbers and sequence types (STs) were assigned according to the S. aureus MLST website (http://saureus.mlst.net). S. aureus strains with positive toxin genes were selected for MLST.

Results

During the six-month study, various samples were collected from patients admitted to Al-Zahra university hospital. Bloodstream and wound samples had the highest ratio in comparison to other specimens (28%). Sputum, urine and body fluids were listed in the next stage. Antibiotic susceptibility pattern showed that the highest and lowest percentage of resistance belonged to tetracycline (42%) and rifampin (6%) respectively. Multiplex PCR indicated that 30% of the strains harbored sea and 34% harbored sec genes. However, only 4% of our collected isolates had tst gene (Figure 1).

In PFGE method by GelCompar analysis on all S. aureus strains, a total of 19 different patterns were identified. Clinical isolates with the same profiles were grouped in common type (CT) and other clinical isolates with different patterns were grouped in single type. Hence, in PFGE dendrogram 11 CTs (CT1-CT11) and 24 single types were recognized with 90% similarity cut-off point (Figure 2).

MLST was performed in 27 selected S. aureus isolates. Due to the MLST results, nine various sequence types (ST8, ST6, ST22, ST30, ST239, ST1936, ST1937, ST1938, and ST1940) were distinguished (Figure 3). The prevalent types were ST8 with 29.6% and ST22 (14.8%).

Discussion and Conclusions

S. aureus is an eminent bacterial pathogen which can cause severe infections. The emergence of resistant strains and the variety of virulence factors such as various toxins and enterotoxins...
have increased the importance of clinical infections (2,9). Main reasons for these infections are referred to numerous mobile genetic elements such as pathogenicity islands, plasmids and phages (11). Recent study has investigated the prevalence of sea, sec and tsst genes in different clinical samples and also molecular characterization by PFGE and MLST. In our study by Multiplex PCR 30% and 34% of strains harboured sea and sec genes respectively. In a study by Hu et al. the sec gene in addition to others was found to be the most prominent in comparison to other detected toxin genotypes (10). Also in Wang et al. study the sea gene was found with 33%, and followed by sec with 15%. Our results were similar to other investigators, however the percentage of sea and sec genes in some studies are different (20).

The prevalence of the tsst gene in our studied isolates was found to be 4%, although this ratio in other studies was different. As such, in a study by El-Ghodban et al. in Libya 7.5% of clinical strains and none of the food strains harboured the tsst gene (6). Likewise the percentage of this gene in the study conducted by Tsen et al. (1998) in Taiwan was reported to be 4.8%. The positive strains were also sec producers (17). The apparent issue in this regard is that the most clinical S. aureus strains contained at least one staphylococcal enterotoxin genes (19). The various reported percentages of toxin genes in different studies may be dependent on the frequency of these genes in different sources and special strains such as epidemic and multidrug resistant strains or the horizontal transferring of mobile genetic elements between these.

![Figure 2. Pulsed-field gel electrophoresis dendrogram of 50 S. aureus strains, with a 90% similarity cut-off point clustered by unweighted pair grouping by mathematical averaging.](image-url)
strains. Peck and colleagues reported that the 71.4% of *S. aureus* strains harboured the enterotoxin genes (15).

In antimicrobial susceptibility test, the majority of our studied strains showed susceptibility to all antibiotics except tetracycline (42%). Among molecular typing techniques, PFGE is used as a primary and the gold standard method for *S. aureus* surveillance and epidemiological studies. However, MLST is used for more detailed analysis and outbreak investigations (4,18). Using pulsed field gel electrophoresis, nineteen different patterns were identified with analysis and outbreak investigations (4,18). Using pulsed field gel epidemiological studies. However, MLST is used for more detailed

MLST typing from the nine recognized STs, ST8 was the widespread type (detected in eight of 27 strains), and thereafter ST22. In MLST, the ST8 and ST22 strains were classified as CT1, CT6, CT7, CT9, and CT11 in PFGE dendrogram.

In conclusion the present study indicates a possible higher variability among our *S. aureus* strains by two different molecular typing methods; nevertheless four main common types (CT1, CT7, CT9, and CT11) with at least one toxin genes were determined.

### References