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ORIGINAL ARTICLE

Virulence Factors of *Staphylococcus aureus* Isolates in an Iranian Referral Children's Hospital

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Abstract
Objectives: The clinical importance of *Staphylococcus aureus* (*S. aureus*) is attributed to notable virulence factors, surface proteins, toxins, and enzymes as well as the rapid development of drug resistance. The aim of this study was to compare the occurrence of virulence factors produced by *S. aureus* strains isolated from children in an Iranian referral children's hospital.
Methods: The presence of genes encoding for the enterotoxins A (*sea*), B (*seb*), C (*sec*), D (*sed*), TSST-1 (*tsst*), exfoliative toxin A (*eta*), and exfoliative toxin B (*etb*) were detected by Multiplex polymerase chain reaction (PCR) using specific primers. In addition, the standardized Kirby-Bauer disc-diffusion method was performed on Mueller-Hinton agar.
Results: In total, 133 *S. aureus* isolates were obtained from different patients. Of these *S. aureus* isolates, 64 (48%) were methicillin-resistant *S. aureus* (MRSA), and all of these tested positive for the *mecA* gene. Regarding the classical enterotoxin genes, *sea* gene (40.6%) was the most prevalent followed by *seb* (19.6%), *tsst* (12.8%), *eta* (11.3%), *etb* (9%), *sed* (4.5%), and *sec* (3%). Among methicillin-susceptible *S. aureus* (MSSA) isolates, *seb* and *tsst* were the more prevalent toxins in comparison with MRSA isolates ($p < 0.05$), while the frequency of *sea*, *sed*, *eta*, and *etb* genes were higher among MRSA isolates ($p > 0.05$).
Conclusion: In our study enterotoxin A was produced by 40.6% of the isolates (48% from MRSA and 33% from MSSA isolates) which was higher than in previous reports. According to our results, strict hygiene and preventative measures during food processing are highly recommended.

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1. Introduction

Staphylococcus aureus (*S. aureus*) is the most frequently isolated bacterium among both community-acquired and nosocomial infections [1]. *S. aureus* is the causal pathogen of a wide range of infectious diseases ranging from skin and soft tissue infections to toxin-mediated diseases such as pneumonia and bacteremia [2].

The clinical importance of *S. aureus* is attributed to notable virulence factors, surface proteins, toxins, and enzymes as well as the rapid development of drug resistance [3]. The most associated virulence factors with this microorganism are large numbers of toxins including hemolysins (α, β, γ, δ), leukocidins (Panton-Valentine leukocidin; PVL, LukE/D) [4,5], heat-stable staphylococcal enterotoxins (SEs), which cause the sporadic food-poisoning syndrome or food borne outbreaks, exfoliative toxins (ETA and ETB), and the toxin of toxic shock syndrome-1 (TSST-1) [6,7], which causes food poisoning, enterocolitis, scalded skin syndrome, and toxic shock [8].

The aim of this study was to compare the occurrence of virulence factors produced by *S. aureus* strains isolated from children in an Iranian referral children's hospital.

2. Material and methods

2.1. Sample collection

Clinical *S. aureus* samples were collected from hospitalized patients at an Iranian referral children's hospital in 2012.

2.2. Identification of *S. aureus*

Standard microbiological methods for the identification of microorganisms were applied. All specimens were inoculated onto mannitol salt agar and incubated at 37°C. The identification of *S. aureus* was performed by subsequent Gram staining, the catalase test, and the coagulase test with rabbit plasma [9].

2.3. Antimicrobial susceptibility test

The standardized Kirby-Bauer disc-diffusion method was performed on Mueller-Hinton agar using the following antibiotics: oxacillin (5 μg); vancomycin (30 μg); clindamycin (2 μg); rifampicin (30 μg); amikacin (30 μg); amoxicillin/clavulanic acid (30/15 μg); penicillin (10 μg); chloramphenicol (30 μg); trimethoprim sulfamethoxazole (1.25/23.75 μg); cefazolin (30 μg); and cephalothin (30 μg). Methicillin-susceptible *S. aureus* (MSSA) strains were differentiated from methicillin-resistant *S. aureus* (MRSA) using Mueller-Hinton agar containing 2 mg/mL oxacillin with 4% NaCl [10].

2.4. DNA extraction

DNA was extracted from *S. aureus* isolates using lysostaphin digestion [11]. The pellet of a 1-mL overnight culture was resuspended with 350 μL of lysis buffer (Tris-HCL 0.01 M, EDTA 0.01 M), to which 10 μL of lysostaphin were added. The sample was incubated at 37°C overnight. Equal volumes of phenol/chloroform/isoamylalcohol (25:24:1 by volume) were added, and nucleic acid was precipitated by ethanol using a standard protocol.

2.5. Polymerase chain reaction of the *mecA*

Confirmation of *S. aureus* and methicillin-resistance was achieved by polymerase chain reaction (PCR) targeting the *mecA* gene [10].

2.6. Multiplex PCR for the detection of toxin genes

The presence of genes encoding for enterotoxins A (*sea*), B (*seb*), C (*sec*), D (*sed*),TSST-1 (*tsst*), exfoliative toxin A (*eta*), and exfoliative toxin B (*etb*) was detected by Multiplex PCR using specific primers, as previously described [12]. Multiplex PCR was performed in a total volume of 50 μL containing 25 pmol of each primer, 50 ng of total DNA, 1.5 mM $MgCl_2$, 200 μM dNTP mixture, 1× PCR reaction buffer, and 5 units of Taq DNA polymerase. The thermal cycling conditions included an initial denaturation step at 94°C for 5 minutes followed by 35 cycles of amplification comprising three steps: 2 minutes denaturation at 94°C; 1 minute annealing at 57°C; and 1 minute extension at 72°C, ending with a final extension at 72°C for 7 minutes.

2.7. Statistical analysis

A Microsoft Excel spreadsheet was used for data processing. For comparison tests between the positive isolates from each group, we used the Chi-square test and the Fischer's test for a lower number series. A p value < 0.05 was taken to be significant.

3. Results

In total, 133 *S. aureus* isolates were obtained from different patients. Fifty-seven patients (42.8%) were female (female to male ratio, 0.75), and the mean duration of a hospital stay was 13.25 ± 8.14 days.

Forty-three patients (32.3%) were from the infection unit, with the remainder from the surgical ward ($n = 27$, 20.3%), NICU ($n = 19$, 14.3%), emergency ward ($n = 10$, 7.5%), ICU ($n = 10$, 7.5%), immunology ward ($n = 10$, 7.5%), gastroenterology ward ($n = 7$, 5.2%), and others ($n = 7$, 5.2%).

Of the *S. aureus* isolates, 64 (48%) were MRSA and all of these tested positive for the *mecA* gene.

The presence of toxin genes in the strains isolated from skin and soft tissue, blood, urinary, respiratory, and eye infections is shown in Table 1. The majority of toxin-producing *S. aureus* isolates were isolated from skin and soft tissue infections. There were no significant differences between toxin genes and the type of specimen.