Study the Enterotoxigenicity of *Staphylococcus aureus* Isolated from the Urine Samples of Pediatrics with UTIs

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**ABSTRACT**

*Staphylococcus aureus* is an important pathogen associated with urinary tract infections in a variety of hosts including humans. It produces several toxins and virulence factors that contribute to its pathogenic potential such as staphylococcal enterotoxins. This study was conducted to determine enterotoxigenicity of *S. aureus* associated with UTIs in pediatrics patients. One-hundred and seventy two urine samples were collected from pediatrics suffered from UTIs. Samples were cultured immediately and those that were *S. aureus*-positive were analyzed for the presence of sea, seb, sec, sed and see enterotoxins using PCR. Fifty three out of 172 urine samples were positive for *S. aureus* (30.81%). The prevalence of *S. aureus* in boy and girl patients were 21.25% and 39.13%, respectively (*P*<0.05). The most commonly detected enterotoxigenic genes in the *S. aureus* isolates of pediatric patients were sec (41.50%), sea (18.86%), see (15.09%) and sed (13.20%). There was significant difference between the prevalence of enterotoxigenic genes and sex of pediatric patients (*P*<0.035). The role of enterotoxin genes in the pathogenesis of UTIs is still unknown. Other newly detected genes may play a role in pathogenesis of diseases. Therefore, further studies should be conducted to demonstrate the role of enterotoxins of *S. aureus* in the cases of UTIs.

**Key words:** *Staphylococcus aureus*, Enterotoxins, Urinary Tract infections, Pediatric patients, Iran.

**INTRODUCTION**

Urinary tract infections (UTIs) are among the most common infectious diseases diagnosed especially in pediatric patients¹⁻⁵. UTIs are liable for more than 1.5 million hospitalization and 300,000 cases of severe disease in the United States annually⁶.

Among all infectious agents causing UTIs, the *Staphylococcus aureus* (*S. aureus*) is one of those who have recently received considerable attention because its high abilities to resistance against commonly used antibiotics⁴⁻⁵. In spite of such bacteria like *Escherichia coli* (*E. coli*) which has the highest prevalence rate in the cases of UTIs⁶, *S. aureus* has a lower prevalence but its high levels of resistance to antimicrobial agents causes many problems in its treatment⁷. Totally, 0.2-1% of the urine samples in developed countries, mainly contains *S. aureus* but the prevalence rate is higher in developing countries⁶⁻⁹.

There are documented data concerning the mechanisms by which *S. aureus* induces infections, particularly the role and mode of action of enterotoxigenic genes involved in its pathogenicity¹⁰. The staphylococcal enterotoxins
(SEs) are a group of low-molecular-mass and single-chain proteins that are similar in composition and biological activity but differ in antigenicity (sea to sej)\textsuperscript{11,12}. High prevalence of sea gene in the \textit{S. aureus} strains of various types of hospital infections was reported previously\textsuperscript{13}.

Adwan et al. (2006)\textsuperscript{13} reported that more than 50\% of the \textit{S. aureus} isolates of the cases of UTIs were positive for various types of enterotoxigenic genes. Unfortunately, there were scarce data about the status of enterotoxigenic genes of \textit{S. aureus} in the cases of UTIs in Iranian pediatrics. Therefore, the present study was carried out to investigate the distribution of enterotoxigenic genes of the \textit{S. aureus} isolated from the urine samples of Iranian pediatric patients suffered from UTIs.

**MATERIALS AND METHODS**

**Samples and \textit{Staphylococcus aureus} identification**

From July 2014 to October 2014, a total of 172 urine samples were collected from hospitalized boy (n=80) and girl (n=92) patients of educational hospitals and health centers of Tehran, Iran. The ultrasound technique was used to confirm the presence of UTIs\textsuperscript{14}. Urine samples were collected from the midstream using the Suprapubic Aspiration (SPA)\textsuperscript{15}.

The urine samples were transferred to the Microbiology and Infectious Diseases Research Center in a cooler with ice-packs. All samples were directly cultured into 7\% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37\(^\circ\)C for 48 h. After incubation, suspicious colonies were examined by the use of morphologies compatible with \textit{Staphylococcus} spp. (microscopical morphology, catalase and coagulase production). Studied colonies were cultured on Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany) and Tryptic Soy Agar (TSA) (Merck, Darmstadt, Germany). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol Salt Agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation test\textsuperscript{16}.

**DNA extraction and PCR confirmation**

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5ml of brain heart infusion broth and incubated over night at 37\(^\circ\)C. Then 1.5 ml of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm. The cell pellet was resuspended and lysed in 200\µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1\% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µl of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10min. at 4\(^\circ\)C. After transferring the clear supernatant into a new eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for 5min., the supernatant is then removed to another eppendorf tube and double volume of 100\% ethanol was added. The tubes were inverted 5 to 6 times gently, then centrifuged at 10,000rpm for 5minutes. The supernatant was added and mixed well, then the viscous mixture was centrifuged at 12,000 rpm for 10min. at 4\(^\circ\)C. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer\textsuperscript{17}.

Presence of \textit{S. aureus} in each DNA samples was confirmed using the Banada et al. (2012) method\textsuperscript{18}. The PCR reaction mix consist of 1 X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 0.001\% (w/v) gelatin) with 4 mM MgCl2, 250 mM of each nucleotide (deoxyribonucleoside triphosphate), 0.5 mM of each primer (forward and reverse), 4 ng of the molecular beacon and 4 U of Jumpstart Taq DNA polymerase (Fermentas, Germany).
PCR amplification for enterotoxigenic genes

The PCR method was used in order to study the distribution of sea, seb, sec, sed, see, seg, seh, sei and sej enterotoxins of the S. aureus (19-22). Oligonucleotide primers, annealing temperature, PCR programs and size of products is shown in table 1. A programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device was used in all PCR reactions. All runs included a negative DNA control consisting of PCR grade water and strains of S. aureus ATCC 13565 (sea), ATCC 14458 (seb), ATCC 19095 (sec), FRI 361 (sed, seg, sei and sej), ATCC 27664 (see) and FRI 137 (seh) were used as positive controls.

Statistical analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/16.0 software (SPSS Inc., Chicago, IL) for significant relationship between incidences enterotoxigenic genes of S. aureus isolated from the boy and girl patients. The chi-square test and Fisher’s exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a P value < 0.05.

Ethical considerations

The present study was accepted by the ethical committees of the educational Hospitals. Written informed consent was obtained from all of the study patients or their parents.

RESULTS

Total distribution of S. aureus in the urine samples of pediatric patients is shown in table 1. Of 172 urine samples studied, 53 samples were found to be positive for S. aureus (30.81%). In addition, the total prevalence of S. aureus in boy and girls patients of our study were 21.25% and 39.13%, respectively. Significant difference was seen for the prevalence of S. aureus between boy and girl patients (P <0.039). Distribution of enterotoxigenic genes in the S. aureus isolates of boys and girls is shown in table 3. Results of the gel electrophoresis of PCR amplifications are shown in figure 1-3. We found that girl patients had the highest prevalence of enterotoxigenic genes. There was significant
Table 1. The oligonucleotide primers and the PCR programs used for amplification of enterotoxins of *Staphylococcus aureus* isolates of the urine samples of boy and girl patients

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer sequence (5’-3’)</th>
<th>PCR product (bp)</th>
<th>Annealing temperature (°C)</th>
<th>PCR programs</th>
<th>PCR Volume (50µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>F: TTGGAAACGGTTAAAACGAA</td>
<td>120</td>
<td>50</td>
<td>1 cycle:</td>
<td>5 µL PCR buffer 10X</td>
</tr>
<tr>
<td></td>
<td>R: GAACCTTCCCATAAAAACA</td>
<td></td>
<td>94 °C -5 min.</td>
<td>1.5 mM MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>F: TCGCATCAAACGTGCAAAACG</td>
<td>478</td>
<td>50</td>
<td>30 cycle:</td>
<td>200 µM dNTP (Fermentas)</td>
</tr>
<tr>
<td></td>
<td>R: GCAGGTACTCTATAAGTGC</td>
<td></td>
<td>94°C -2 min</td>
<td>0.5 µM of each primers F &amp; R</td>
<td></td>
</tr>
<tr>
<td>sec</td>
<td>F: GACATAAAAGCTAGGAATTT</td>
<td>257</td>
<td>50</td>
<td>72°C -1 min</td>
<td>1.25 U Taq DNA polymerase (Fermentas)</td>
</tr>
<tr>
<td></td>
<td>R: AAATCGGATTAACATTACC</td>
<td></td>
<td>72°C -5 min</td>
<td>2.5 µL DNA template</td>
<td></td>
</tr>
<tr>
<td>sed</td>
<td>F: CTAGTTGGTAATATCTCCT</td>
<td>317</td>
<td>50</td>
<td>1 cycle:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: TAATGCTATATCTTATAGGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>see</td>
<td>F: AGGTTTTTTCAACAGTCC</td>
<td>209</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: CTGTTTTTCCTCGGCATCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seg</td>
<td>F: AAGTACGACATTTGCGTCC</td>
<td>287</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: AGAACCATCAGACTGTAGCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seh</td>
<td>F: GCTATATGGAAGGTAACACT</td>
<td>213</td>
<td>46.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: GACCTTTACTTATTTGCTGTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sei</td>
<td>F: GGTGATATGGTGAGGTAAC</td>
<td>454</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: ATCCATATTTGCGCTTTACCAG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sej</td>
<td>F: CATGAAACTGTTGTTCCGCTAG</td>
<td>142</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R: CTGAATTTCACCATAAAAGGTAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Distribution of *Staphylococcus aureus* in the urine samples of boy and girl patients

<table>
<thead>
<tr>
<th>Studied groups of patients</th>
<th>No samples collected</th>
<th>No. positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boy</td>
<td>80</td>
<td>17 (21.25)</td>
</tr>
<tr>
<td>Girl</td>
<td>92</td>
<td>36 (39.13)</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>53 (30.81)</td>
</tr>
</tbody>
</table>

difference between the prevalence of enterotoxigenic genes and sex of pediatric patients ($P < 0.035$). The most commonly detected enterotoxigenic genes in the *S. aureus* isolates of pediatric patients were sec (41.50%), sea (18.86%), see (15.09%) and sed (13.20%). There were no positive results for seg, sei and sej enterotoxigenic genes. Statistically significant differences were seen between the prevalence of sec and seh ($P < 0.016$),

Table 3: Distribution of enterotoxigenic genes of *Staphylococcus aureus* isolates of the urine samples of boy and girl patients

<table>
<thead>
<tr>
<th>Studied groups of patients (No. positive)</th>
<th>Distribution of enterotoxigenic genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>seb</td>
</tr>
<tr>
<td>Boy (17)</td>
<td>3</td>
</tr>
</tbody>
</table>
| (17.64) | (41.17) | (11.76) | (11.76) | &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &nbsp; &n

sec and sed ($P < 0.036$), sec and seb ($P < 0.021$) and sea and seb ($P < 0.047$) enterotoxigenic genes.

**DISCUSSION**

The results of the present study showed the high prevalence of *S. aureus* and its enterotoxigenic genes in the urine samples of pediatrics suffered from UTIs. Totally, 30.81% of urine samples of our investigation were infected with *S. aureus*. In the other hand, the prevalence of *S. aureus* in the urine samples of boy and girl pediatric patients were 21.25% and 39.13%, respectively.

One possible explanation for the high prevalence of *S. aureus* in the urine samples of pediatrics is the fact that maybe the hospital environment is contaminated and used antimicrobial agents were not efficient for treatment of diseases. A study which has been conducted in Nigeria showed that 50% of all clinical samples of human hospital's infections were positive for *S. aureus* which was entirely high. In keeping with the high prevalence of *S. aureus* in Iranian hospitals and health care units, increase the growing prevalence of *S. aureus* from other countries have also been reported.

Possible explanations for the high prevalence of *S. aureus* in this study are the low levels of health care in hospitals, excessive application of urine catheter, lack of sanitary conditions in hospitals, increasing the age of circumcision in boys, improper use of effective drugs and occurrence of antibiotic resistance in *S. aureus*. One possible explanation for the high prevalence of *S. aureus* in girls is that they have relatively short and wide urethra. Also, host factors such as changes in normal vaginal flora may put girls at higher risk for UTIs. Therefore, girls are more prone to get UTIs. Furthermore, management of micturition in girls is very essential. Management faults made by girls or they parents include cleaning perineum forward from the anus to the vulva that can cause urinary tract infection.
Another part of the current study focused on the distribution of enterotoxigenic genes in the *S. aureus* isolates of boys and girls suffered from UTIs. Results showed that sec (41.50%), *sea* (18.86%), *see* (15.09%) and *sed* (13.20%) were the most commonly detected enterotoxigenic genes in the *S. aureus* isolates of pediatrics patients. Higher prevalence of *sec* and *sea* enterotoxigenic genes in the *S. aureus* strains of clinical samples has been reported previously. High levels of differences in the prevalence of *S. aureus* and also enterotoxigenic genes which have been seen in the results of various studies are probably due to the differences among the origin of clinical samples, number of test samples, sensitivity of methods, and types of enterotoxins or enterotoxin genes that were detected.

The *S. aureus* strains of UTIs which carried the enterotoxigenic genes may induce releasing of specific cytokines that may inhibit the efficiency of human immune response by their expressions; this may contribute to the persistence of *S. aureus* in urogenital tract and promote inflammation in these tissues or enhance the chronicity of this disease.

In conclusion, the role of *sea*, *seb*, *sec*, *sed* and *see* genes in the pathogenesis of UTIs is still unknown. However it is possible that UTIs can be caused by *S. aureus* strains at least lack these genes. The current study showed that the *S. aureus* and its enterotoxigenic genes especially *sec*, *sea*, *see* and *sed* had the highest prevalence in pediatrics patients with UTIs. With respect to this condition in Iran, we recommended the initially manage of children affected with a community acquired UTIs with effective drugs to reduce the times of hospitalization.

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