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Distribution of SCC*mec* types in *Staphylococcus aureus* Strains Isolated from the Pediatrics Suffered From Severe UTIs

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Staphylococcal cassette chromosome *mec* (*SCCmec*) is a mobile genetic element of *Staphylococcus aureus* which includes the *mecA* gene coding for resistance to the methicillin and other types of β-lactam antibiotics. The present study was carried out to investigate the distribution of SCC*mec* types in *S. aureus* strains isolated from the urine samples of pediatrics suffered from UTIs. Totally, 162 urine samples were collected from hospitalized pediatrics suffered from UTIs. Samples were cultured and those that were *S. aureus*-positive were analyzed for the presence of *SCCmec* types I, II, III, IVa, IVb, IVc, IVd and V. Of 162 studied samples, 56 samples (34.56%) were *S. aureus* positive. Total prevalence of *S. aureus* in boy and girl patients were 29.16% and 38.88%, respectively (*P*= 0.048). the prevalence of *S. aureus* in the cases of pyelonephritis and cystitis were 38.82% and 29.87%, respectively (*P*= 0.045). Total prevalence of *SCCmec* types I, II, III, Iva, IVb, IVc, IVd and V in the *S. aureus* isolates were 26.78%, 17.85%, 35.71%, 19.64%, 30.35%, 14.28%, 10.71% and 46.42%, respectively. Higher prevalence of *SCCmec* types was reported in the cases of pyelonephritis and also in girl patients. As far as we know, the present study was the first prevalence report of *SCCmec* types of *S. aureus* isolated from Iranian pediatric patients. Our data showed that researchers should tried to find a proper replacement for methicillin.

Key words: *Staphylococcus aureus*, *SCCmec* types, Urinary tract infections, Pediatrics, Iran.

Urinary tract infections (UTIs) are one of the most routine hospital infections in pediatrics all-around the world[1, 2]. UTIs are responsible for more than 300,000 severe clinical complications, morbidity and mortality in pediatrics in the United States[3, 4].

Staphylococcus aureus (*S. aureus*) is a significant human pathogen that causes a number of diseases, ranging from skin and soft tissue infections to UTIs, life-threatening endocarditis, pneumonia and osteomyelitis[5]. *S. aureus* has developed resistance to multiple classes of antibiotics, especially methicillin and other types of beta-lactams. It has been documented that about 50% of strains of this bacterium in hospital infections were methicillin-resistant *S. aureus* (MRSA)[6-8].

The gene for methicillin resistance, *mecA*, is carried on a 21- to 67-kb element, the staphylococcal chromosomal cassette mec (*SCCmec*), which integrates at a conserved location in the *S. aureus* genome[5, 9]. SCC*mec* genetic element characterized by the presence of two essential

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genetic markers (the *mec* and the *ccr* gene complexes). SCC*mec* elements are classically classified into types I, II, III, IV and V according to the nature of the *mec* and *ccr* genes[9, 10]. Type IV of the *SCCmec* genetic element is divided to IVa, IVb, IVc and IVd alleles[9, 10].

Study the distribution of the SCC*mec* element of *S. aureus* is very important for molecular typing of MRSA strains, also it's very essential for understanding of the molecular epidemiology of MRSA in various types of infections. There were scarce published data on the prevalence of *S. aureus* and *SCCmec* types in the pediatrics suffered from UTIs in Iran. Therefore, the present study was carried out to investigate the distribution of *SCCmec* types of *S. aureus* isolated from the urine samples of Iranian pediatrics suffered from UTIs.

MATERIALS AND METHODS

Samples and *Staphylococcus aureus* identification

From March 2014 to October 2014, a total of 162 urine samples were collected from hospitalized boy (n=72) and girl (n=90) patients of educational hospitals and health centers of Tehran, Iran. The ultrasound technique was used to confirm the presence of UTIs[11]. Urine samples were collected from the midstream using the Suprapubic Aspiration (SPA)[12].

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°C for 48 h. After incubation, suspicious colonies were examined by the use of morphologies compatible with *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[13].

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 37ºC. 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 200µl 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 4ºC. 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 discarded and 1ml of ethanol (70%) was added to the pellet, and tubes centrifuged at 10,000 rpm for 5 minutes. Finally the supernatant discarded and the pellet was dried for 10 min at room temperature, the pellet was resuspended by 100µl H2O. The stock was kept at -20ºC until use. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer (14). Presence of *S. aureus* in each DNA samples was confirmed using the Banada *et al.* (2012) (15) method. 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 (deoxynucleoside 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).

Detection of SCCmec types of *Staphylococcus aureus*

Table 1 shows the lit of primers used for detection of *SCCmec* types of *S. aureus* isolated

from the urine samples of boy and girls patients suffered from UTIs (16). The multiplex PCR reactions were performed in a total volume of 50 µL, including 5 µl of 10× PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM MgCl2, 50 mM KCl, 20 mM NH4)2SO4), 1 µl dNTPs (40 mM), 1 µl (50 pmol) from the forward and reverse primers, 5 µl of the extracted DNA template and 1 µl (1U Taq DNA polymerase). The DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) were used in all PCR reactions for DNA amplifications. The thermal cycler was adjusted as follows: 94°C for 5 min, followed by 10 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 1.5 min, and 25 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1.5 min, followed by final extension at 72°C for 10 min and followed by a hold at 4°C. Fifteen microliters of PCR products in all reactions were resolved on a 2% agarose gel containing 0.5 mg/ml of ethidium bromide in Tris–borate–EDTA buffer at 90 V for 1 h, also using suitable molecular weight markers. The products were examined under ultraviolet illumination.

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 the prevalence of bacteria and *SCCmec* types of *S. aureus* isolated from the urine samples of pediatric 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

A total of 162 urine samples were analyzed for presence of *S. aureus*. Table 2 shows the total distribution of *S. aureus* in the urine samples of boy and girl patients suffered from pyelonephritis and cystitis. Results showed that 56 out of 162 urine samples (34.56%) were positive for *S. aureus*. We found that the total prevalence of *S. aureus* in boy and girl patients were 29.16% and 38.88%, respectively. Significant differences were seen for the prevalence of *S. aureus* between boys and girls (P= 0.048). Total prevalence of *S. aureus* in the cases of pyelonephritis and cystitis were 38.82% and 29.87%, respectively. Significant differences were also seen for the prevalence of *S. aureus* between pyelonephritis and cystitis (P= 0.045).

Total distribution of *SCCmec* types in the *S. aureus* of pediatric patients is shown in table 3.

Table 1. Oligonucleotide primers used for detection of *SCCmec* types of *Staphylococcus aureus* strains isolated from pediatrics suffered from UTIs[16]

Target genes	Primer sequence (5'-3')	Size of product (bp)
SCCmec I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613
SCCmec II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280
SCCmec IVa	F: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493
SCCmec IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200
SCCmec IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325

Table 2. Total distribution of *Staphylococcus aureus* in various types of studied samples

Types of samples		No. samples	No. positive results (%)
Boy	Pyelonephritis	40	13 (32.5)
	Cystitis	35	8 (22.85)
	Total	72	21 (29.16)
Girl	Pyelonephritis	45	20 (44.44)
	Cystitis	42	15 (35.71)
	Total	90	35 (38.88)
Total	Pyelonephritis	85	33 (38.82)
	Cystitis	77	23 (29.87)
	Total	162	56 (34.56)

Table 3. Total distribution of *SCCmec* types of *Staphylococcus aureus* in various types of studied samples

Type of samples	Distribution of *SCCmec* types (%)							
(No. positive)	I	II	III	IVa	IVb	IVc	IVd	V
Boy (21)	5 (23.80)	3 (14.28)	6 (28.57)	4 (19.04)	6 (28.57)	3 (14.28)	2 (9.52)	10 (47.61)
Girl (35)	10 (28.57)	7 (20)	14 (40)	7 (20)	11 (31.42)	5 (14.28)	4 (11.42)	16 (45.71)
Total (56)	15 (26.78)	10 (17.85)	20 (35.71)	11 (19.64)	17 (30.35)	8 (14.28)	6 (10.71)	26 (46.42)

Table 4. Total distribution of SCCmec types of *Staphylococcus aureus* in various types of studied samples

Type of samples	Distribution of *SCCmec* types (%)							
(No. positive)	I	II	III	IVa	IVb	IVc	IVd	V
Pyelonephritis (33)	9 (27.27)	6 (18.18)	12 (36.36)	7 (21.21)	10 (30.30)	5 (15.15)	4 (12.12)	18 (54.54)
Cystitis (23)	6 (26.08)	4 (17.39)	8 (34.78)	4 (17.39)	7 (30.43)	3 (13.04)	2 (8.69)	8 (34.78)
Total (56)	15 (26.78)	10 (17.85)	20 (35.71)	11 (19.64)	17 (30.35)	8 (14.28)	6 (10.71)	26 (46.42)

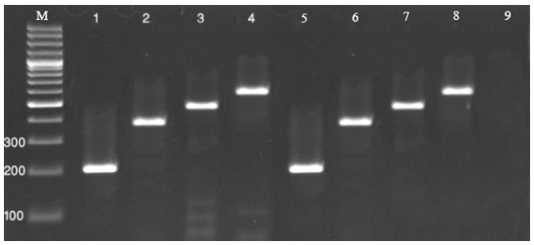

Fig. 1. Results of the gel electrophoresis for identification of *SCCmec* types in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1-4: Positive samples for *SCCmec* IVc (493 bp), *SCCmec* II (398 bp), *SCCmec* IVb (493 bp) and *SCCmec* I (613 bp), Lines 5-8: Positive controls and Line 9: Negative control

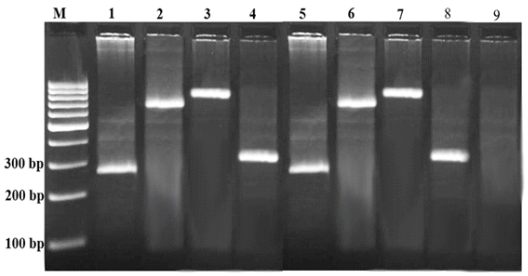

Fig. 2. Results of the gel electrophoresis for identification of *SCCmec* types in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1-4: Positive samples for *SCCmec* III (280 bp), *SCCmec* IVa (776 bp), *SCCmec* IVd (881 bp) and *SCCmec* V (325 bp), Lines 5-8: Positive controls and Line 9: Negative control

The most commonly detected *SCCmec* types in the urine samples were V (46.42%), followed by III (35.71%) and IVb (30.35%). Significant differences were seen for the prevalence of *SCCmec* types between boys and girls (*P*= 0.029). Table 4 shows the distribution of *SCCmec* types in the *S. aureus* isolates of pyelonephritis and cystitis. We found that *SCCmec* types of V, IVb and III had the highest prevalence in both types of infections. Significant differences were seen for the prevalence of *SCCmec* types between pyelonephritis and cystitis (*P*= 0.025). Statistically significant differences were also seen between the prevalence of *SCCmec* type V and IVd (*P*= 0.019), V and IVc (*P*= 0.021), I and II (*P*= 0.027), IVb and IVa (*P*= 0.031) and IVb and IVc (*P*= 0.034).

DISCUSSION

UTIs have been described to be majorly caused by Gram-negative enterobacteria with *E. coli* being the most predominant (17, 18). However, there is an increasing prevalence of *S. aureus* as a UTIs' etiologic agent in different studies including Iran (34.56%) (This study), Nigeria (33.6%)[19], Brazil (45%)[20] and India (20.5%)[21]. Another Iranian investigation which was conducted by Momtaz and Hafezi (2014)[22] reported that of 132 clinical samples, 66 were positive for *S. aureus* (50%) which was entirely high. One possible explanation for the high prevalence of *S. aureus* in the urine samples of pediatrics suffered from UTIs is the fact that the hospital environment is so contaminated and antimicrobial agents are prescribed in an irregular and impermissible manner.

Sexual distribution of *S. aureus* in our study showed a higher prevalence among the girls (38.88%) than the boy patients which support the results of previous reports (23, 24). This opinion can be elucidated by the anatomical nature of the girl urethra and its proximity to the anus which favor the fecal and skin flora easy access to the urethra. In addition, girls have relatively short and wide urethra which facilitates entrance of microbial pathogens. Also, host factors such as changes in normal vaginal flora may put girls at higher risk for UTIs. The effects of genetic factors including expression of Lewis blood group Le (a+b-) and Le (a-b-) and HLA-A3 should not be overlooked.

The results of our investigation showed that the prevalence of *S. aureus* in the cases of pyelonephritis and cystitis were 38.82% and 29.87%, respectively. Pyelonephritis is classically classified into the upper UTIs, while the cystitis is a type of infection of lower urinary tracts. It can be concluded that the *S. aureus* has a high ability to penetration into the upper parts of urinary tracts. It is also clear that the *S. aureus* that reached to the higher parts of urinary tracts and causes pyelonephritis is mainly resistant to antimicrobial agents. The high frequency of *SCCmec* types in the isolates of *S. aureus* mainly in the cases of pyelonephritis is good evidence for this claim.

Our results showed the higher pre valence of all types of studied *SCCmec* types in the *S. aureus* strains of girls than boys and also in the *S. aureus* isolates of pyelonephritis than cystitis. Total prevalence of I, II, III, Iva, IVb, IVc, IVd and V types of *SCCmec* in the *S. aureus* isolates of pediatrics in our study were 26.78%, 17.85%, 35.71%, 19.64%, 30.35%, 14.28%, 10.71% and 46.42%, respectively which was similar to the results of Momtaz and Hafezi (2014)[22], Reiter *et al*. (2010)[20], Valsesia *et al*. (2010)[25] and Chu *et al*. (2013)[26]. *SCCmec* types I, IV and V only encode resistance against β-lactam antibiotics, while *SCCmec* types II and III encode resistance against tobramycin, kanamycin and bleomycin, penicillin, macrolide, lincosamide and streptogramin antibiotics[27]. The most widely used β-lactam antibiotics in the cases of UTIs in Iranian hospitals and health care units are ampicillin, methicillin, mezlocillin, oxacillin and piperacillin. Therefore, the high prevalence of *SCCmec* types I, IVb and V indirectly showed that the *S. aureus* strains of our investigation were resistant to above antibiotics. High prevalence of *SCCmec* type III indirectly showed that the *S. aureus* isolates of our study maybe were resistant to tobramycin, kanamycin and bleomycin, penicillin, macrolide, lincosamide and streptogramin antibiotics.

In conclusion, we identified the large number of *S. aureus* with high prevalence of *SCCmec* types in the urine samples of pediatrics suffered from UTIs in Iran. The high prevalence of bacterium and its *SCCmec* types represent that infections with these strains requires the higher levels of hospital cares with high demand for novel antibiotics instead of methicillin and other types of ²-lactam antibiotics. This approach maybe can

reduce the prevalence of pyelonephritis and cystitis among Iranian pediatrics.

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