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Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Isolated from Pediatrics with UTIs

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Staphylococcus aureus **causes a variety of severe infections such as urinary tract infections. At present, 30–50% of *S. aureus* isolates from Iran are methicillin resistant (MRSA). The goals of this study were to identify the distribution antibiotic resistance properties in *S. aureus* isolates of pediatric patients. One- hundred and fifty five urine samples were collected from pediatric patients suffered from UTIs. Samples were cultured immediately and those that were *S. aureus*-positive were analyzed for the presence of antibiotic resistance genes. We found that 66 out of 155 samples (42.58%) were positive for *S. aureus*. The highest prevalence of bacterium was seen in the cases of pyelonephritis (43.8%). *S. aureus* isolates had the highest antibiotic resistance against ampicillin (90.9%), tetracycline (90.9%), erythromycin (87.8%), methicillin (83.3%), oxacillin (78.7%) and *trimethoprim-sulfamethoxazole* (77.2%). *MecA* (75.7%) and *aacA-D* (75.7%) were the most commonly detected antibiotic resistance genes. All of the MRSA strains were also resistant against ampicillin, tetracycline and erythromycin. Totally, 54.5% of strains were PVL positive and 86.1% of PVL positive strains were resistant to methicillin. Although the frequency of MRSA isolates in the current study was intermediate, resistance to other antibiotics was high and most of the isolates were found to be MDR. Regular surveillance of hospital-associated infections and monitoring of their antibiotic sensitivity patterns are required to reduce the prevalence of MRSA.**

Key words: *Staphylococcus aureus*, methicillin resistant, Pediatric, Urinary tract infections, Iran.

Urinary tract infections (UTIs) are one of the most common hospital acquired infections in pediatrics[1,2]. It has been documented that UTIs are responsible for more than 8 million referrals to hospitals, 1.5 million hospitalization, and 300,000 severe clinical syndromes in the United States annually[3]. UTIs is an important cause of mortality and morbidity in pediatrics[4].

Among all bacterial agents causing UTIs, *Staphylococcus aureus* (*S. aureus*) is one of the most prevalent cause of diseases in pediatrics[5, 6]. *S. aureus* are extremely versatile pathogenic bacteria in hospitals that cause a wide range of infections including wound, burn, minor skin and soft tissue infections, UTIs and pneumonia[7,8]. The ability of *S. aureus* to resistance against wide range of antibiotics causing severe problems in treatment of hospital infections. Infections caused by *S. aureus* are treated mainly with methicillin but in recent years, increasing numbers of methicillin resistant *S. aureus* (MRSA) have been reported worldwide from patients with community-acquired infections[9-12]. According to the available data near fifteen percent of *S. aureus* of hospital infections were MRSA[12-14].

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The pathogenicity of *S. aureus* depends on the presence of various surface antigen and extracellular proteins. The frequent recovery of staphylococcal isolates that produce leukocidal toxins from patients with UTIs, pneumonia, soft tissue infections and skin infections, suggests that the Panton-Valentine leukocidin (PVL) is a virulence marker with significant role in the pathogenicity of diseases[15,16]. Studies showed that antibiotic resistance genes are the most effective factors for causing antibiotic resistance in *S. aureus*. The most commonly detected antibiotic resistance genes in the *S. aureus* strains of human infections are *mecA* (methicillin), *linA* (lincosamides), *msrA* and *msrB* (macrolides), *vatA*, *vatB* and *vatC* (acetyl transferase genes and streptogramin A), *ermA*, ermB and *ermC* (macrolide– lincosamide–streptogramin B), *tetK* and *tetM* (tetracycline) and *aacA-D* (aminoglycosides)[17,18].

The epidemiology and prevalence of *S. aureus* in Iranian pediatrics suffered from the UTIS is unknown. Also, antibiotic agents have been prescribed in an indiscriminate and excessive manner. Therefore, the present study was carried out to investigate the antibiotic resistance properties of MRSA isolated from Iranian children suffered from UTIs.

MATERIALS AND METHODS

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.

Samples and *Staphylococcus aureus* identification

From May 2014 to September 2014, a total of 155 urine samples were collected from hospitalized pediatrics of educational hospitals and health centers of Tehran, Iran. The ultrasound technique was used to confirm the presence of UTIs [19]. Urine samples were collected from the midstream using the Suprapubic Aspiration (SPA)[20].

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[21].

Antimicrobial susceptibility test

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of *S. aureus* strains against 15 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines[16]. Susceptibility of *S. aureus* isolates were tested against ampicillin (10 µ/disk), gentamycin (10 µg/disk), amikacin (30 µ/disk), imipenem (30 u/disk), methicillin (30 µg/disk), tetracycline (30 µg/disk), vancomycine (5 µg/disk), ciprofloxacin (5 µg/disk), norfloxacin (30 µg/disk), cotrimoxazole (30 µg/disk), clindamycin (2 µg/disk), *trimethoprim-sulfamethoxazole* (25 *µg/disk*), penicillin G (10 µ/disk), oxacillin (1µg/disk), erythromycin (15µg/disk) and azithromycin (15 µg/disk) antibiotic agents (Oxoid, UK). The plates containing the discs were allowed to stand for at least 30 min before incubated at 35°C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the CLSI zone diameter interpretative standards (CLSI 2012)[22]. *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control organism in antimicrobial susceptibility determination.

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 H_2O. 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[23]. Presence of *S. aureus* in each DNA samples was confirmed using the Banada *et al*. (2012) [24] 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 antibiotic resistance genes

Presence of antibiotic resistance genes in the *S. aureus* isolates were analyzed using the method described previously[25,26]. Oligonucleotide primers used from amplification of antibiotic resistance genes is shown in table 1.

PCR detection of mecA and PVL genes

MecA and *PVL* genes were detected using the two pairs of primers introduced previously[27,28]. The PCR reactions were performed in a total volume of 25 µL, including 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 µM dNTPs each (Fermentas, Germany), 2.5 µL PCR buffer (10X), 25 pmoL of each primer, 1.5 U of Taq DNA polymerase (Fermentas, Germany) and 5 µL (40-260 ng/µL) of the extracted DNA template of the Staphylococcus isolates. The DNA thermocycler (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 10 min, followed by 10 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, and 25 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1.5 min, followed by final extension at 72°C for 7 min; the PCR products were stored in the thermal cycler at 4°C until they were collected. 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 incidences virulence genes and antibiotic resistance properties 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.

RESULTS

Table 2 shows the total distribution of *S. aureus* in various types of urine samples. Of 155 urine samples studied, 66 (42.5%) were positive for *S. aureus*. The *S. aureus* had the highest prevalence in the urine samples of pediatrics with pyelonephritis (43.8%). Significant differences were seen for the prevalence of *S. aureus* between pyelonephritis and cystitis (P <0.05).

Pattern of antibiotic resistance in the *S. aureus* isolates of various types of samples is shown in table 3. We found that the *S. aureus* isolates had the highest antibiotic resistance against ampicillin (90.9%), tetracycline (90.9%),

Table 1. Oligonucleotide primers and PCR conditions for amplification of antibiotic resistance genes in *Staphylococcus aureus* strains isolated from pediatrics suffered from UTIs [25,26].

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 °C ——— 5 min. 25 cycle: 94 °C —— 60 s 55 °C ———70 s 72 °C —— 60 s 1 cycle: 72 °C ——— 10 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
ermC	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299		
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
vatC	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	467		
tetM	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 °C ——— 6 min. 34 cycle: 94 °C —— 50 s 55 °C ———70 s 72 °C —— 60 s 1 cycle: 72 °C ——— 8 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
vatA	F: TGGTCCCGGAACAACATTTATR: TCCACCGACAATAGAATAGGG	268		
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 °C ——— 6 min. 34 cycle: 95 °C ——— 60 s 50 °C ——70 s 72 °C —— 70 s 1 cycle: 72 °C ——— 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
msrB	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGTCCTGTT	595		
vatB	F: GCTGCGAATTCAGTTGTTACA R: CTGACCAATCCCACCATTTTA	136	1 cycle: 94 °C ——— 6 min. 35 cycle: 95 °C ——— 50 s 55 °C ——70 s 72 °C —— 80 s 1 cycle: 72 °C ——— 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
linA	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323	1 cycle: 94 °C ——— 6 min. 30 cycle: 95 °C ——— 60 s 57 °C ——60 s 72 °C —— 60 s 1 cycle: 72 °C ——— 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

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

Type of samples	No samples collected	No. positive results (%)
Cystitis	45	16 (35.5)
Urethritis	53	25 (47.1)
Pyelonephritis	57	25 (43.8)
Total	155	66 (42.5)

erythromycin (87.8%), methicillin (83.3%), oxacillin (78.7%), *trimethoprim-sulfamethoxazole* (77.2%) and cotrimoxazole (72.7%). The highest levels of antibiotic resistance was seen in the cases of pyelonephritis. There were significant differences between the types of urinary tract infections and resistance to antibiotics (P <0.05).

Pattern of antibiotic resistance in the MRSA isolates is shown in table 4. All of the 55 MRSA isolates were also resistant to ampicillin, tetracycline and erythromycin. Prevalence of resistance in the MRSA isolates against gentamycin, vancomycin, clindamycin and azithromycin were 72.7%, 70.9%, 41.8% and 43.6%, respectively.

Figure 1-5 show the results of gel electrophoresis for amplification of antibiotic resistance genes. The prevalence of *mecA*, *aacA-D*, *ermA*, *ermC*, *tetK*, *tetM*, *vatA*, *vatB*, *vatC*, *msrA*, *msrB* and *linA* antibiotic resistance genes in the *S. aureus* isolates of pediatric patients is shown in table 5. The most commonly detected antibiotic resistance genes in the *S. aureus* isolates of pediatric patients were *mecA* (75.7%) and *aacA-D* (75.7%). Statistically significant differences were seen for the prevalence of antibiotic resistance genes and types of infections (P <0.05) and also between the prevalence of *mecA* and *vatB* (P <0.01), *mecA* and *msrB* (P <0.01), *aacA-D* and *ermC* (P <0.01), *aacA-D* and *vatA* (P <0.05) and *tetK* and *tetM* (P <0.05). MRSA isolates showed the highest levels of antibiotic resistance genes (Table 6). MRSA had the highest prevalence of *mecA* (90.9%), *aacA-D* (81.8%) and *ermA* (36.3%).

Of 66 *S. aureus* isolates, 36 (54.5%) strains were PVL positive. 31 out of 36 (86.1%) PVL positive strains of *S. aureus* were resistant to methicillin (Table 7). Statistically significant differences were seen between the types of infections and prevalence of PVL gene (P <0.05). The highest

Table 3. Antibiotic resistance pattern of *Staphylococcus aureus* isolated of studied samples

Type of samples (No. positive)	Pattern of antibiotic resistance (%)															
	AM10*	Gen10	Amk30	Imp30	Met30	Tet30	VAN	Cip5	Nor	Cotr	Clin	TM/Sul	Pen10	Ox	Em15	Az15
Cystitis (16)	14 (87.5)	10 (62.5)	8 (50)	3 (18.7)	12 (75)	14 (87.5)	9 (56.2)	5 (31.2)	4 (25)	11 (68.7)	6 (37.5)	12 (75)	7 (43.7)	11 (68.7)	13 (81.2.)	6 (37.5)
Urethritis (25)	21 (84)	13 (52)	11 (44)	1 (4)	19 (76)	22 (88)	11 (44)	5 (20)	4 (16)	15 (60)	5 (20)	17 (68)	10 (40)	18 (72)	21 (84)	7 (25)
Pyelonephritis (25)	25 (100)	19 (76)	18 (72)	6 (24)	24 (96)	24 (96)	19 (76)	12 (48)	10 (40)	22 (88)	13 (52)	22 (88)	17 (68)	23 (92)	24 (96)	13 (52)
Total (66)	60 (90.9)	42 (63.6)	37 (56)	10 (15.1)	55 (83.3)	60 (90.9)	39 (59)	22 (33.3)	18 (27.2)	48 (72.7)	24 (36.3)	51 (77.2)	34 (51.5)	52 (78.7)	58 (87.8)	26 (39.3)

*In this table AM10=ampicillin (10 u/disk), Gen10=gentamycin (10 µg/disk), Amk30=amikacin (30 u/disk), Imp30=imipenem (30 u/disk), Met30=methicillin (30 µg/disk), Tet30=tetracycline (30 µg/disk), VAN=vancomycine (5 µg/disk), Cip5=ciprofloxacin (5 µg/disk), Nor=norfloxacin (30 µg/disk), Cotr=cotrimoxazole (30 µg/disk), Clin=clindamycin (2 µg/disk), TM/Sul=*trimethoprim-sulfamethoxazole* (25 *ìg/disk*), Pen10=penicillin G (10 u/disk), Ox=oxacillin (1µg/disk), Em15=erythromycin (15µg/disk) and Az15=azithromycin (15 µg/disk).

Table 4. Antibiotic resistance pattern in the methicillin resistant strains of *Staphylococcus aureus* isolated from studied samples

Type of samples	Pattern of antibiotic resistance (%)														
(No. positive)	AM10*	Gen10	Amk30	Imp30	Tet30	VAN	Cip5	Nor	Cotr	Clin	TM/Sul	Pen10	Ox	Em15	Az15
Methicillin resistant strains (55)	55 (100)	40 (72.7)	35 (63.6)	9 (16.3)	55 (100)	39 (70.9)	21 (38.1)	16 (29)	46 (83.6)	23 (41.8)	51 (92.7)	33 (60)	50 (90.9)	55 (100)	24 (43.6)

Table 5. Distribution of antibiotic resistance genes in the *Staphylococcus aureus* isolates of studied samples

Type of samples	Distribution of antibiotic resistance genes (%)											
(No positive)	*mecA*	*AacA-D*	*ermA*	*ermC*	*tetK*	*tetM*	*vatA*	*vatB*	*vatC*	*msrA*	*msrB*	*linA*
Cystitis (16)	11 (68.7)	12 (75)	6 (37.5)	3 (18.7)	7 (43.7)	7 (43.7)	1 (6.2)	-	-	5 (31.2)	3 (18.7)	3 (18.7)
Urethritis (25)	17 (68)	15 (60)	5 (20)	3 (12)	10 (40)	5 (20)	1 (4)	-	-	4 (16)	2 (8)	2 (8)
Pyelonephritis (25)	22 (88)	23 (88)	14 (56)	9 (36)	16 (64)	13 (52)	3 (12)	2 (8)	-	11 (44)	8 (32)	10 (40)
Total (66)	50 (75.7)	50 (75.7)	25 (37.8)	15 (22.7)	33 (50)	25 (37.8)	5 (7.5)	2 (3)	-	20 (30.3)	13 (19.6)	15 (22.7)

Table 6. Distribution of antibiotic resistance genes in the methicillin resistant strains of *Staphylococcus aureus* isolated from studied samples

Type of samples	Distribution of antibiotic resistance genes (%)											
(No. positive)	*mecA*	*AacA-D*	*ermA*	*ermC*	*tetK*	*tetM*	*vatA*	*vatB*	*vatC*	*msrA*	*msrB*	*linA*
Methicillin resistant strains (55)	50 (90.9)	45 (81.8)	20 (36.3)	10 (18.1)	30 (45.5)	15 (27.2)	3 (5.4)	1 (1.8)	-	15 (27.2)	9 (16.3)	10 (18.1)

Table 7. Distribution of PVL gene in the methicillin resistant *Staphylococcus aureus* isolates of studied samples

Type of samples (No. positive)	PVL gene (%)	Methicillin resistant (%)
Cystitis (16)	10 (62.5)	8 (80)
Urethritis (25)	10 (40)	7 (70)
Pyelonephritis (25)	17 (68)	16 (94.1)
Total (66)	36 (54.5)	31 (86.1)

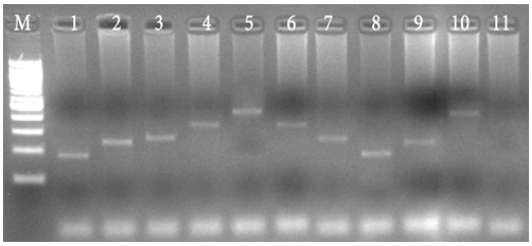

Fig. 1. Results of the gel electrophoresis for identification of antibiotic resistance genes in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1-5: Positive samples for *aacA-D* (227 bp), *ermA* (190 bp), *ermC* (299 bp), *tetK* (360 bp) and *vatC* (467 bp), Lines 6-10: Positive controls and Line 11: Negative control

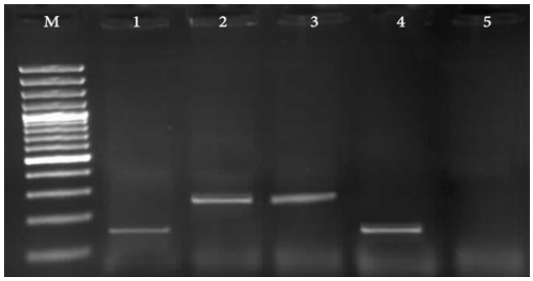

Fig. 2. Results of the gel electrophoresis for identification of antibiotic resistance genes in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1 and 2: Positive samples for *tetM* (158 bp) and *vatA* (268 bp), Lines 3 and 4: Positive controls and Line 5: Negative control

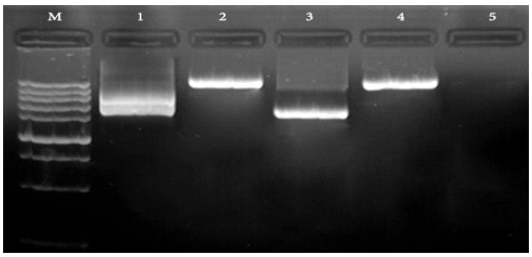

Fig. 3. Results of the gel electrophoresis for identification of antibiotic resistance genes in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1 and 2: Positive samples for *msrA* (940 bp) and *msrB* (595 bp), Lines 3 and 4: Positive controls and Line 5: Negative control.

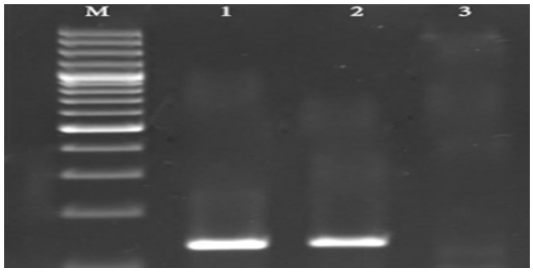

Fig. 4. Results of the gel electrophoresis for identification of antibiotic resistance genes in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Line 1: Positive samples for *vatB* (136 bp), Lines 2: Positive controls and Line 3: Negative control.

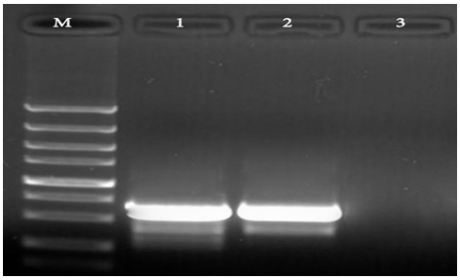

Fig. 5. Results of the gel electrophoresis for identification of antibiotic resistance genes in *S. aureus* strains. M: 100 bp DNA ladder (Fermentas, Germany), Line 1: Positive samples for *linA* (323 bp), Lines 2: Positive controls and Line 3: Negative control

prevalence of PVL gene was seen in the cases on pyelonephritis.

DISCUSSION

The results of the present study showed the high prevalence of MRSA in the urine samples of pediatrics suffered from UTIs. Totally, 42.5% of urine samples of our investigation were infected with *S. aureus* and 83.3% of isolates were resistant to methicillin. The *S. aureus* strains of our investigation were resistant to more than one antibiotics. One possible explanation for the high prevalence of resistant strains 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. Onanuga *et al*. (2012)[29] showed that the total prevalence of *S. aureus in the urine samples of patients suffered from UTIs in Nigeria was* 33.6% which was lower than our results. They showed that all isolates were resistant to methicillin and the total prevalence of resistance against tetracycline, chloramphenicol, cotrimoxazole, gentamycin, vancomycin, cefuroxime, nitrofurantoin, ofloxacin and ciprofloxacin were 97.8%, 80.4%, 73.9%, 69.6%, 54.3%, 39.1%, 34.8% and 32.6%, respectively. The prevalence of resistance against methicillin in Iran (this study), Jamaica, Australia, Spain, France and USA were 83.3%, 23%, 21.6%, 30.3%, 33.6% and 75%, respectively[12,30,31]. Deng *et al.* (2013)[32] reported the highest levels of resistance in the *S. aureus* against oxacillin and vancomycin. Another Iranian study which was conducted by Momtaz and Hafezi (2014) showed that *S. aureus* strains had the highest levels of resistance against penicillin (100%), cephalothin (100%), cefazoline (100%), ceftireaxon (100%), azitromycin (62.12%), tetracycline (57.57%) and erythromycin (54.54%)

Another important finding of our investigation is that 15.1% of S. aureus strains were resistant to imipenem. Imipenem is one of the most effective drugs in the cases of UTIs in Iran. Boyce *et al*. (1991)[33] showed that 57% of MRSA strains of human clinical samples were resistant to imipenem which was entirely higher than our results. Similar results have been reported previously by Momtaz and Hafezi (2014)[17] and Yamazaki *et al*. (2008)[10]. Our results revealed that 59% of *S. aureus* isolates were resistant to vancomycin. This part of our investigation is closely similar to the results of previous studies[11,34].

Idea of medical practitioners, using from the disk diffusion method, availability of antibiotic drugs and accuracy in prescription of antibiotics are the main factors cause differences in the prevalence of resistance in various countries. Current recommendations showed that antibiotics may trigger release of PVL and progression to bad clinical complications. Furthermore, due to the inappropriate prescription, it was not surprising that our study found that resistance to penicillin, cephalotin, cefazoline, ceftireaxon, azithromycin and tetracycline were so high.

Totally, 54.5% of the *S. aureus* isolates of our study carried the PVL gene. This gene had the highest incidence in the pyelonephritis cases of our study (68%). The PVL gene is a bicomponent cytotoxin that is preferentially linked to epithelial surfaces. Totally, 86.1% of MRSA strains of our study carried the PVL gene. A lot of studies revealed the high presence of PVL genes in the *S. aureus* strains of human clinical samples including Nigeria[35] (18%), Iran[9] (7.23%) and United Kingdom[36] (11.3%). High prevalence of PVL gene in the MRSA strains of human hospital infection sources have been reported in various clinical investigation[37,38]. The PVL toxin's ability to cause the death of polymorphonuclear cells including neutrophils, basophils and eosinophils has been known since its earliest descriptions.

Another part of our study focused on the prevalence of antibiotic resistance genes. Our results showed that the total prevalence of *mecA*, *aacA-D*, *ermA*, *tetK*, *vatA*, *msrA* and *linA* resistance genes in the bacterial strains were 75.5%, 75.5%, 37.8%, 50%, 7.5%, 30.3% and 22.7%, respectively. In fact the results of the disk diffusion method have been confirmed using the PCR amplification of antibiotic resistance genes for certain antibiotics. Momtaz and Hafezi (2014)[17] showed that *S. aureus* strains of hospital infections harbored *mecA* (80.30%), *tetK* (34.84%), *ermA* (30.30%), *vatB* (1.51%) and *vatC* (3.03%) antibiotic resistance genes which was similar to our results. A Nigerian study reported that *mecA*, *aacA-ophD* and *tetM* were the most commonly antibiotic resistance genes in the cases of UTIs[39]. They showed that the *ermA* gene was

identified in all erythromycin-resistant MRSA isolates, while two erythromycin-resistant MSSA isolates possessed the *msrA* gene. Besides, all the gentamicin-resistant isolates carried the *aacA-aphD* gene. Moreover, the *tetM* gene was detected in 11 isolates (7 MRSA and 4 MSSA) and the *tetK* gene was present in 4 MRSA and 23 MSSA isolates[39].

Our results showed that the prevalence of antibiotic resistance genes, PVL gene and antibiotic resistance pattern in the *S. aureus* strains of pyelonephritis were entirely higher than other sources of infections. It seems that, occurrence of pyelonephritis requires the presence of more virulent and resistant strains of *S. aureus*, while those of urethritis can occur by common strains of bacterium.

In conclusion, the accurate diagnosis of MRSA strains in hospitals, patients and health care units is an important need. Also the dissemination of MRSA strains with high resistance to different antibiotics in Iranian hospitals is a warning for pediatric public health. Accurate and continuous surveillance of antibiotic resistance patterns among *S. aureus* strains should be considered in pediatrics.

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