Virulence Genes Profile of Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Iranian Children with UTIs

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Abstract- Virulent and resistant strains *Pseudomonas aeruginosa (P. aeruginosa)* is one of the most important cause of UTIs in pediatrics. The present study was carried to investigate the frequency of virulence factors in the multi-drug resistant strains of *P. aeruginosa* isolated from pediatrics hospitalized due to the UTIs. One - hundred and forty three urine samples were collected from pediatric patients suffered from UTIs. Samples were cultured and those that were *P. aeruginosa* positive were analyzed for the presence of putative virulence genes. Seventy one out of 143 samples (49.65%) were positive for P. aeruginosa. Monthly, sex and age-dependent prevalence were seen for P. aeruginosa. Bacterial strains had the highest levels of resistance against ampicillin (95.77%), gentamicin (92.95%) and ciprofloxacin (81.69%). Of 71 *P. aeruginosa* isolates, 12 strains were resistant to more than 9 antibiotics (16.90%). The most commonly detected virulence factors in the cases of urethral infections were exoU and plcH while those of pyelonephritis and cystitis were were exoS and lasB. Our findings should raise awareness about antibiotic resistance in hospitalized pediatrics with UTIs in Iran. Clinicians should exercise caution in prescribing antibiotics, especially in cases of UTIs. Such information can help in identifying these virulence genes as useful diagnostic markers for clinical *P. aeruginosa* strains isolated from UTIs.

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Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections diseases in human (1-3). UTIs account for more than 8 million referrals to hospitals, 1.5 million hospitalization, and 300,000 severe clinical syndromes in the United States annually (1,4). UTIs is an important cause of mortality and morbidity in pediatrics (5,6). It has been estimated that the pooled prevalence rates of UTIs in children aged 0-19 years was 2-8% (5,6).

Pseudomonas aeruginosa (*P. aeruginosa*) is the third most common pathogen associated with hospital-acquired UTIs (7). It is a non-fermentative, aerobic, Gram-negative rod shaped bacterium (7). *P. aeruginosa* is responsible for 9% of the cases of UTIs all-around the world (6). Its high ability to cause UTIs is related to certain virulence factors. Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell associated factors like alginate (algD), flagellum, lipopolysaccharide (LPS), pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like elastase B (lasB), protease, pyocyanin, phospholipase (plcH and plcN), exotoxin A, exoenzyme S (exoS), exoenzyme U (exoU), fimbrial biogenesis protein PilB (pilB), hemolysins (rhamnolipids), neuraminidase (nan1) and siderophores (8,9).

Virulent strains of *P. aeruginosa* cause more severe clinical diseases which are mainly difficult to treatment with routine antibiotics (10). Treatment of UTIs caused by this bacterium is often started empirically, and therapy is based on information determined from the antimicrobial resistance pattern (10). However, a large proportion of uncontrolled antibiotic usage has subsidized to the development of resistance in *P. aeruginosa* strains (10). *P. aeruginosa* exhibits the

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highest rates of resistance to the fluoroquinolones, with resistance to ciprofloxacin and levofloxacin ranging from 20 to 35% (10). Higher levels of antibiotic resistance in the *P. aeruginosa* isolates of UTIs have been reported previously (11,12)

Due to the uncertain epidemiology and prevalence of *P. aeruginosa* in Iranian pediatric patients, the present study was carried out to investigate the prevalence virulent genes profile of multidrug resistant *P. aeruginosa* isolated from Iranian children suffered from UTIs.

Materials and Methods

Samples and *Pseudomonas aeruginosa* isolation from April 2013 to April 2014, a period covering seasonal variation, a total of 143 urine samples were collected from boys (n=69), and girls (n=74) patients suffered from UTIs. Samples were collected from hospitalized children under 1 year to 4 years old. The presence of UTIs was confirmed using the ultrasound technique (13). Urine samples were collected from the midstream using the suprapubic aspiration (SPA) (14).

The urine samples were transferred to the Microbiology and Infectious Diseases Research Center of Private Hospital of Tehran in a cooler with ice packs. Urine samples were inoculated on to blood, MacConkey (Merck, Germany) and Nutrient agar (Merck, Germany) and incubated at 37°C for 18 - 24 h; colonies that produce pyocyanin, pyoverdin and pyorubin pigments were transferred to nutrient agar and subcultured more than one time to obtain pure cultures. The isolates were identified using conventional biochemical tests such as oxidase test, motility test, citrate utilization test, catalase test, urease production test, gelatinase liquefaction, nitrate reduction test, triple sugar iron agar test, alkaline protease production, indole test, oxidative-fermentative test, hemolysin production and lecithinase production. The results of the bacteriological and biochemical tests were confirmed by the PCR assay (15).

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 *P. aeruginosa* strains against 22 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (16). Susceptibility of *P. aeruginosa* strains were tested against ampicillin (10 u/disk), gentamicin (10 µg/disk), amikacin (30 u/disk), imipenem (30 u/disk), mezlocillin (30 u/disk), piperacillin (30 µg/disk), cefotaxime (30 µg/disk), ciprofloxacin (5 µg/disk), norfloxacin (30 µg/disk), cotrimoxazole (30 µg/disk), meropenem (10 µg/disk), ceftazidime (30 µg/disk), tobramycin (10 µg/disk), cefepime (30 µg/disk), tazobactam (10 µg/disk), levofloxacin (5 µg/disk), cefoperazone (30 µg/disk), ceftazidime (30 µg/disk), ofloxacin (5 µg/disk), vancomycin (5 µg/disk), polymyxin B (300 U/disk) and aztreonam (30 µg/disk) antibiotic agents (Oxoid, UK). All of the inoculated plates were aerobically incubated at 37 °C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012) (16). In all reactions, the *P. aeruginosa* (ATCC 27853) was used as quality control organisms.

DNA extraction from the *Pseudomonas aeruginosa* isolates

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5ml of brain heart infusion broth and incubated overnight at 37ºC. Then 1.5 ml of a saturated culture was harvested by 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 in 100µl H2O. The stock was kept at -20ºC until use. The DNA concentration has been determined by measuring the absorbance of the sample at 260 nm using spectrophotometer (17).

Detection of putative virulence genes of *Pseudomonas aeruginosa* the PCR method was used in order to study the distribution of exoS, exoU, algD, pilB, nan1, lasB and plcH virulence factors (18, 19). Oligonucleotide primers and size of products is shown in (Table 1).

Table 1. The oligonucleotide primers and the PCR programs used for amplification of putative virulence factors in the *Pseudomonas aeruginosa* isolates of pediatrics suffered from UTIs (18, 19)

Target gene	Primer sequence (5'-3')	PCR product (bp)
algD	F: ATGCGAATCAGCATCTTTGGT R: CTACCAGCAGATGCCCTCGGC	1310
pilB	F: ATGAACGACAGCATCCAACT R: GGGTGTTGACGCGAAAGTCGAT	826
nan1	F: ATGAATACTTATTTTGATAT R: CTAAATCCATGCTCTGACCC	1317
lasB	F: GGAATGAACGAGGCGTTCTC R: GGTCCAGTAGTAGCGGTTGG	300
plcH	F: GAAGCCATGGGCTACTTCAA R: AGAGTGACGAGGAGCGGTAG	307
exoS	F: CTTGAAGGGACTCGACAAGG R: TTCAGGTCCGCGTAGTGAAT	504
exoU	F: GGGAATACTTTCCGGGAAGTT R: CGATCTCGCTGCTAATGTGTT	428

The PCR mixture contained 200 µM of each dNTP (Fermentas, Germany), PCR buffer (10 mM Tris/HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), DMSO at a final concentration of 4 %, 12.5 pmol of each primer, 1 U Taq DNA polymerase (Fermentas, Germany) and 25 ng DNA template. The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device using the following protocol: 94 ºC for 3 min, 25-30 cycles of 94 ºC for 35-45 s, 53-62 ºC for 45-60 s, 72 ºC for 45-95 s, and 72 ºC for 7 min.

Fifteen microliters of PCR products were resolved on a 1.5% 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 a significant relationship between incidences virulence genes of *P. aeruginosa* isolated from the urine samples of pediatric patients samples. 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

The urine samples of hospitalized boy and girl pediatrics were analyzed for the presence of *P. aeruginosa*. From 143 urine samples, 71 (49.65%) were positive for *P. aeruginosa* (Table 2).

Table 2. Total distribution of *Pseudomonas aeruginosa* in the urine samples of boy and girl pediatrics

Samples		No. samples collected	No. positive results (%)
Type of urine samples	**Age**		
Boy	<1	18	9 (50)
	1-2	17	9 (52.94)
	2-3	14	6 (42.85)
	3-4	20	5 (25)
	Total	69	29 (42.02)
Girl	<1	21	15 (71.42)
	1-2	18	11 (61.11)
	2-3	18	9 (50)
	3-4	17	7 (41.17)
	Total	74	42 (56.75)
Total	<1	39	24 (61.53)
	1-2	35	20 (57.14)
	2-3	32	15 (46.87)
	3-4	37	12 (32.43)
	Total	143	71 (49.65)

In addition, 29 out of 69 boy urine samples (42.02%) and 42 out of 74 girl urine samples (56.75%) were positive for *P. aeruginosa* (*P*=0.039). The age distribution of the pediatric patients with regard to infection with *P. aeruginosa* is shown in (Table 2.) We found that the less than one-year-old pediatrics had the highest incidence of *P. aeruginosa* (61.53%), while the 3-4 year-old children had the lowest incidence (32.43%) (*P* =0.024).

Total distribution of *P. aeruginosa* in the urine samples of pediatrics based on the types of infections is shown in (Table 3).

Table 3. Types of urinary tract infections in the pediatric patients of Iran

Samples		No. sample collected	Positive results (%)
Sex	**Type of disorder**		
Boy	Pyelonephritis	19	11 (57.89)
	Cystitis	20	7 (35)
	Urethral infections	30	15 (50)
Girl	Pyelonephritis	33	22 (66.66)
	Cystitis	27	13 (48.14)
	Urethral infections	14	3 (21.42)
Total	Pyelonephritis	52	33 (63.46)
	Cystitis	47	20 (42.55)
	Urethral infections	44	18 (40.90)

Total prevalence of *P. aeruginosa* in the cases of pyelonephritis, cystitis, and urethral infections were 63.64%, 42.55% and 40.90%, respectively. There were significant differences (*P*=0.042) for the prevalence of *P. aeruginosa* between pyelonephritis and urethral infections. Total prevalence of *P. aeruginosa* in the cases of urethral infections was entirely higher in boys than girls (*P*=0.035), while the prevalence of pyelonephritis and cystitis in girls were entirely higher than boys.

(Figure 1) shows the monthly prevalence of *P. aeruginosa* in boy and girl patients suffered from UTIs. We found that samples that were collected in July, August and September months had the highest prevalence of *P. aeruginosa*, while those collected in January, February, March and December months had the lowest prevalence. There were significant differences (*P*=0.040) in the prevalence of *P. aeruginosa* between the hot and cold seasons of the year.

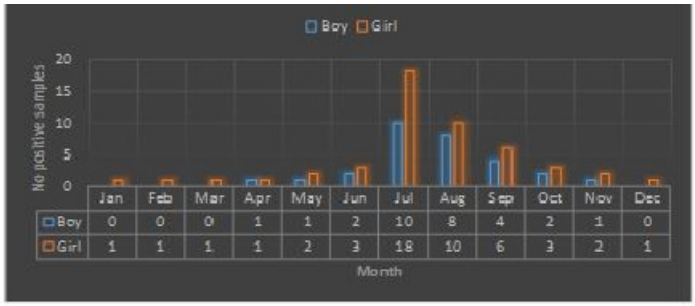

Figure 1. Monthly distribution of *Pseudomonas aeruginosa* in the urine samples of boy and girl pediatrics

Antimicrobial resistance in the *P. aeruginosa* isolated from the urine samples of boy and girl patients suffered from UTIs is shown in (Table 4). *P. aeruginosa* strains exhibited the highest level of resistance to ampicillin (95.77%), followed by gentamicin (92.95%), ciprofloxacin (81.69%) and amikacin (77.46%). Bacterial strains of girl patients had the highest levels of antibiotic resistance (*P* =0.047). There were significant differences between resistance to ampicillin and imipenem (*P*=0.015), gentamicin and piperacillin (*P*=0.022), ciprofloxacin and mezlocillin (*P*=0.024), vancomycin and cefoperazone (*P* =0.031), levofloxacin and tobramycin (*P*=0.033), amikacin and imipenem (*P* =0.028), norfloxacin and cotrimoxazole (*P*=0.035) and ampicillin and cefotaxime (*P* =0.025).

Table 4. Distribution of antibiotic resistance pattern in the *Pseudomonas aeruginosa* isolates of boy and girl pediatrics

Antimicrobial agents	Prevalence of resistance (%)		
	Boys*	Girls**	Total***
Ampicillin	27 (93.10)	41 (97.61)	68 (95.77)
Gentamicin	26 (89.65)	40 (95.23)	66 (92.95)
Amikacin	21 (30.43)	34 (80.95)	55 (77.46)
Imipenem	1 (3.44)	4 (9.52)	5 (7.04)
Mezlocillin	4 (13.79)	8 (19.04)	13 (18.30)
Piperacillin	4 (13.79)	7 (16.66)	11 (15.49)
Cefotaxime	6 (20.68)	8 (19.04)	14 (19.71)
Ciprofloxacin	22 (75.86)	36 (85.71)	58 (81.69)
Norfloxacin	19 (65.51)	26 (61.90)	45 (63.38)
Cotrimoxazole	4 (13.79)	9 (21.42)	15 (21.12)
Meropenem	4 (13.79)	6 (14.28)	10 (14.08)
Ceftazidime	6 (20.68)	11 (26.19)	17 (23.94)
Tobramycin	3 (10.34)	6 (14.28)	9 (12.67)
Cefipime	4 (13.79)	6 (14.28)	10 (14.08)
Tazobactum	5 (17.24)	6 (14.28)	11 (15.49)
Levofloxacillin	18 (62.06)	28 (66.66)	46 (64.78)
Cefoperazone	5 (17.24)	6 (14.28)	11 (15.49)
Ceftazidime	11 (37.93)	15 (35.71)	26 (36.61)
Ofloxacin	17 (58.62)	22 (52.38)	39 (54.92)
Vancomycine	17 (58.62)	23 (54.76)	40 (56.33)
Polymyxin B	6 (20.68)	9 (21.42)	15 (21.12)
Aztreonam	5 (17.24)	7 (16.66)	12 (16.90)

*Based on the total of 29 isolates

**Based on the total of 42 isolates

***Based on the total of 71 isolates

(Figure 2) shows the prevalence of resistance against more than one antibiotic agent in the *P. aeruginosa* isolates from pediatric patients. Results showed that all of the bacterial isolates were resistant to more than one antibiotic. Of 71 *P. aeruginosa* isolates, 12 strains were resistant to more than 9 antibiotics (16.90%), 17 strains were resistant to 9 antibiotics (23.94%), and 27 strains were resistant to 8 antibiotics (38.02%). Bacterial isolates of girl patients had the highest levels of resistance to more than one antibiotics (*P*=0.046).

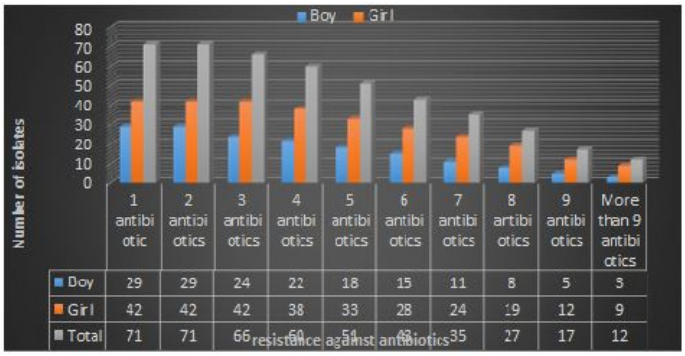

Figure 2. Prevalence of multi-drug resistant strains of *Pseudomonas aeruginosa* isolated from boy and girl pediatrics

(Figures 3,4) show the results of gel electrophoresis for amplification of virulence factors. Distribution of putative virulence factors in resistant strains of *P. aeruginosa* is shown in (Table 5). We found that exoS (92.95%), lasB (91.54%) and plcH (70.42%) were the most commonly detected virulence genes in urine samples from both groups of children. The prevalence of all virulence factors in boy patients was entirely higher than girls (*P*=0.027). Totally, *P. aeruginosa* isolates of pyelonephritis and cystitis cases harbored difference

profiles of virulence factors than those of urethral infections (Table 6). The most commonly detected virulence factors in the cases of urethral infections were exoU (83.33%) and plcH (83.33%), while those of pyelonephritis were exoS (100%) and lasB (93.93%) and those of cystitis were lasB (100%) and exoS (95%). Significant differences were seen for the prevalence of exoS and nan1 genes (P=0.037). We also found significant differences in the prevalence of exoU between the *P. aeruginosa* isolates of urethral infections and cystitis (P=0.029) and plcH between the *P. aeruginosa* isolates of urethral infections with cystitis (P=0.031) and pyelonephritis (P=0.035).

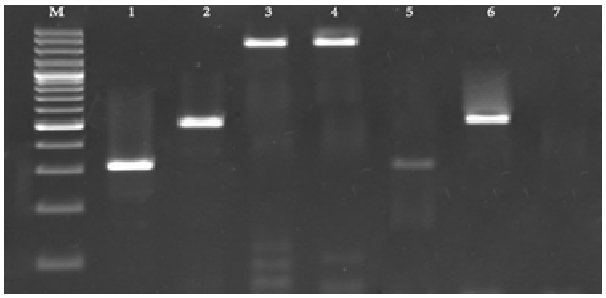

Figure 3. Results of the gel electrophoresis for identification of virulence factors in *Pseudomonas aeruginosa*. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1-3: Positive samples for *plcH* (307 bp), *exoS* (504 bp) and *nan1* (1317 bp), Lines 4-6: Positive controls and Line 7: Negative control

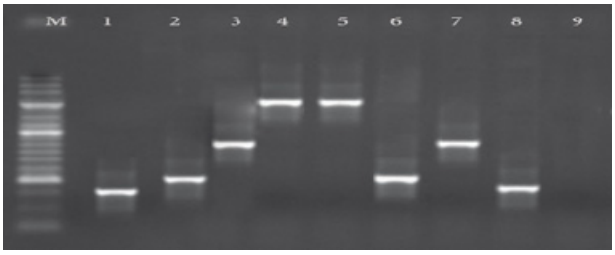

Figure 4. Results of the gel electrophoresis for identification of virulence factors in *Pseudomonas aeruginosa*. M: 100 bp DNA ladder (Fermentas, Germany), Lines 1-4: Positive samples for *lasB* (300 bp), *exoU* (428 bp), *pilB* (826 bp) and *algD* (1310 bp), Lines 5-8: Positive controls and Line 9: Negative control

Table 5. Distribution of putative virulence factors in resistant strains of *Pseudomonas aeruginosa* isolated from boy and girl pediatrics

Samples (No. positive strains)	Distribution of virulence factors (%)						
	exoS	***exoU***	***algD***	***pilB***	***nan1***	***lasB***	***plcH***
Boy (29)	28 (96.55)	23 (79.31)	25 (86.20)	26 (89.65)	15 (51.74)	29 (100)	27 (93.10)
Girl (42)	38 (90.47)	17 (40.47)	20 (47.61)	26 (61.90)	11 (26.19)	38 (90.47)	23 (54.76)
Total (71)	66 (92.95)	40 (56.33)	45 (63.38)	52 (73.23)	26 (36.61)	65 (91.54)	50 (70.42)

Table 6. Total distribution of putative virulence factors in the various types of urinary disorders

Types of samples (No. positive samples)	Distribution of virulence factors (%)						
	exoS	***exoU***	***algD***	***pilB***	***nan1***	***lasB***	***plcH***
Pyelonephritis (33)	33 (100)	16 (48.48)	26 (78.78)	27 (81.81)	11 (33.33)	31 (93.93)	27 (81.81)
Cystitis (20)	19 (95)	9 (45)	12 (60)	15 (75)	8 (40)	20 (100)	14 (70)
Urethral infections (18)	14 (77.77)	15 (83.33)	7 (38.88)	10 (55.55)	7 (38.88)	14 (77.77)	15 (83.33)
Total (71)	66 (92.95)	40 (56.33)	45 (63.38)	52 (73.23)	26 (36.61)	65 (91.54)	50 (70.42)

Discussion

Our work has identified the high prevalence of resistant and virulent strains of *P. aeruginosa* in the urine samples of pediatric patients suffered from UTIs. Totally, 49.65% of pediatrics was infected with P. aeruginosa. As far as we know, this is the highest

prevalence report of *P. aeruginosa* in the urine samples of pediatrics suffered from UTIs. Total prevalence of UTIs caused by *P. aeruginosa* in Kolkata (20), Iran (21), India (22), Iran (23) and Nigeria (24) were 13.26%, 8.7%, 4.53%, 3.6% and 15.5%, respectively. Possible explanations for the high prevalence of *P. aeruginosa* in this study is 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 P. aeruginosa. High prevalence of *P. aeruginosa* has been reported previously due to the inadequate disinfection procedures in a urology unit (25).

Our work has also identified the role of the month in the incidence of *P. aeruginosa* in pediatric patients. One possible explanation for the high prevalence of *P. aeruginosa* in warmer months like July, August, and September in Iran is that climatic variables such as heat, thunderstorms and rain, together with variable barometric pressure may have affected the patients' autonomic nervous systems. These variables could affect immunity, thus making people more susceptible to infections. Ramos *et al*., (2013) (26) reported that seasonal humidity and temperature have high effects on the prevalence of P. aeruginosa. They showed a significant correlation between urinary tract infection and temperature.

Our results showed that the total prevalence of *P. aeruginosa* in boy and girl patients were 42.2% and 56.75%, respectively. One possible explanation for the high prevalence of *P. aeruginosa* 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 (27) that can cause urinary tract infection. Our results also revealed that *P. aeruginosa* strains of boy patients were more virulent than those of girls. This part of our investigation is in agree with the results of Bitsori *et al*., (2012) (28) and Zorc *et al*., (2005) (29). Narrow and long urethra and also the higher resistance of boys to UTIs caused the lower prevalence of cystitis and pyelonephritis in boys than girls. Our results showed that the prevalence of urethral infections in boys was 50%. Similar results have been reported by Nickavar and Sotoudeh, (2011) (30) and Zorc *et al*., (2005) (29).

Another important finding of our investigation relates to the distributions of antibiotic resistance pattern in *P. aeruginosa* strains. Totally, bacterial strains of our study had the lowest resistance against imipenem (7.04%), tobramycin (12.67%), cefepime (14/08%), piperacillin (15.49%), tazobactam (15.49%) and cefoperazone (15.49%), while resistance to ampicillin (95.77%), gentamicin (92.95%) and ciprofloxacin (81.69%) were high. Of the studies that have been conducted in this field (12, 21, 23, 31-33), all have shown a high distribution of antibiotic resistance against ampicillin, gentamicin, ciprofloxacin, and amikacin. High efficacy of imipenem, tobramycin, cefepime, piperacillin, tazobactam and cefoperazone for the treatment of the cases of UTIs caused by *P. aeruginosa* strains has been reported previously from Iran (21, 23), Turkey (34), India (35) and Indonesia (36).

Onguru *et al*., (2008) (37) reported that the *P. aeruginosa* strains of various clinical sources were resistant to imipenem (44.1%) which was entirely high. They showed that imipenem resistant strains were also resistant to amikacin (70%), gentamicin (85%), tobramycin (87%), cefepime (81%), piperacillin (61%) and ciprofloxacin (77%). The results of our study showed that considerable numbers of isolates were resistant to more than one antibiotic agent. Similar investigations have been reported previously (38-40).

Higher prevalence of virulence factors in the boy patients is another interesting finding of our study. Total prevalence of exoS, exoU, algD, nan1, lasB, plcH and pilB virulence factors in the pediatric patients were 92.95%, 56.33%, 63.38%, 36.61%, 91.54%, 70.42% and 73.23%, respectively. Higher levels of the exoS gene have been reported previously by Hamood *et al*., (1996) (41) and Fazeli and Momtaz, (2014) (42). Total prevalence of nan1 and exoS genes in another Iranian investigation (43) was 47.7% and 46.6%, respectively. All of the exoU, exoS, and lasB gene are predominant in various types of infections in Australia (44). Previous study which was conducted in Bulgaria (45) showed that a total prevalence of algD, pilB, nan1, lasB, plcH, exoS and exoU factors in the clinical isolates of *P. aeruginosa* were 91.1%, 23.8%, 21.3%, 100%, 91.6%, 62.4%, and 30.2%, respectively which was entirely similar to our results.

As it showed in our results, these genes had difference prevalence in various types of infections. This may be related to the high differences in the roles of these genes. The ability of exoS to inactivate eukaryotic cell function, inducing cytoskeleton disruption, actin depolymerization, being also involved in bacterial resistance to macrophages and degradation of

immunoglobulin A and G caused the highest levels of virulence for the bacterium (46). Studies showed that the presence of the exoU gene in the *P. aeruginosa* isolates from clinical samples may be important in the development of the acute invasive infections (47). This gene has phospholipase activities and disrupts eukaryotic cell membranes (47).

We identified a large number of virulence factors and antibiotic resistance in the *P. aeruginosa* strains isolated from Iranian patients. Our data indicate that resistance against ampicillin and gentamicin and exoS virulence factors were the most commonly detected characteristics of the *P. aeruginosa* strains isolated from Iranian pediatrics with UTIs. Hence, judicious use of antibiotics is required by clinicians. It is compulsory to evaluate the prevalence of virulence factors and pattern of antibiotic resistance among clinical isolates of *P. aeruginosa* strains. Also, because of the variation of resistance pattern in each hospital, it is important for each region and even hospital to formulate their antibiotic policy according to their local resistance pattern. We recommended the initial manage of children affected with community-acquired UTIs with imipenem prescription. It seems that *P. aeruginosa* strains of various types of UTIs harbored different virulence factors, but further studies must be done to prove this finding.

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