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Community Acquired *Pseudomonas aeroginosa* Urinary Tract Infections in Children Hospitalized in a Baqiatallah Hospital, Tehran, Iran: Virulence Profile and Antibiotic Resistance Properties

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Pseudomonas aeroginosa **is the third most common pathogen associated with hospital-acquired UTIs. From a clinical perspective, it is important to know which virulence profile and antibiotic resistance properties are present in *P. aeroginosa* strains in pediatric patients suffering from urinary tract infections. The purpose of this clinical study was, virulence profile and antibiotic resistance properties Pseudomonas aeroginosa urinary tract infections in children hospitalized. Two hundred and twenty urine samples were collected from the hospitalized pediatrics of Baqiat-alah Hospital, Tehran, Iran. Samples were cultured and those that were *P. aeroginosa*-positive were analyzed for the presence of antibiotic resistance genes and bacterial virulence factors using PCR. Antimicrobial susceptibility was performed using disk diffusion method. Eight out of 90 (8.88%) male and 15 out of 130 (11.53%) female urine samples were positive for *P. aeroginosa*. The highest levels of antibiotic resistance of the *P. aeroginosa* isolates of our investigation was observed against ampicillin (52.17%), gentamycin (47.82%), ciprofloxacin (39.13%) and netilmycin (34.78%), while resistance against piperacillin and imipenem were 4.34% and 17.39%, respectively. The incidence of genes encoding resistance against â-lactamse antibiotic i.e., *blaSHV*, *blaTEM*, *blaDHA* and *blaOXA* were 69.56%, 47.82%, 34.78% and 30.43%, respectively. The most commonly detected virulence factors were *exoS* (73.91%), *toxA* (69.56%), *exoT* (65.21%) and *phzM* (43.47%).** Conclusions : **Our findings should raise awareness about antibiotic resistance in pediatrics with UTIs. Regular prescription of piperacillin, imipenem, polymyxin B and colistin has been suggested fro clinicians.**

Key words: *Pseudomonas aeroginosa*, Virulence profile, Antibiotic resistance, Urinary tract infections, Pediatrics, Tehran.

Urinary tract infections (UTIs) are the second most common infection of any organ system and the most common urological disease in many sites of the world, with a total annual cost of more than $3.5 billion[1]. UTIs account for more than 8 million visits to physician's offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually[2,3].

The organisms most commonly responsible for UTIs are *Escherichia coli* (*E. coli*), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis*[4]. However, there is paucity of literature in relation to pathogenesis of UTIs caused by *P. aeruginosa*. Despite advances in antimicrobial therapy,

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the mortality and morbidity associated with *P. aeruginosa* induced UTIs remain significantly high. *P. aeruginosa* is responsible for 9% of UTIs in children[5]. The bacterium is also responsible for 77.8 and 22.2 percent of severe UTIs in male and female children under 12 years old[6].

Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A (*exoA*), exoenzyme S, U and T (*exoS*, *exoU* and *exoT*), hemolysins (rhamnolipids), zinc metalloprotease (*lasB*), phenazine operons (*phzI* and *phzII*), the genes encode precursor proteins involved in the formation of three phenazine compounds (*phzH, phzM* and *phzS*), phospholipases C encoded by *plcH* and *plcN* (PLC-H and PLC-N, respectively) and siderophores[7-10].

One of the most critical issues about *Pseudomonas* infections is the occurrence of bacterial resistance against commonly used antibiotics. Previous study showed that the Iranian isolates of *P. aeruginosa* were resistant to trimethoprim (100%), amoxicillin (100%), ampicillin (100%), tetracycline (100%), ticarcillin (100%), carbenicillin (90%), ceftazidime (80%), clavunic acid (69.2 %), imipenem (60%), cefepime (52%), gentamycin (50%) and ciprofloxacin (40 %) (11). High resistance of *P. aeruginosa* against commonly used antibiotics and especially â-lactams including broad-spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines are mainly encode by several resistance genes including *blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M*, *blaDHA* and *blaVEB* (12). As a result, strain diversity and the evolution of new *P. aeruginosa* equipped with varying degrees of antibiotic resistance exist,

There were no widely studied investigation about the molecular characterization and antimicrobial resistance properties of *P. aeruginosa* isolated from pediatrics suffered from UTIs in Iran. Therefore, the present study was carried out in order to investigate the distribution of virulence factors and antibiotic resistance properties of *P. aeruginosa* isolated from children with severe UTIs in Iran.

MATERIALS AND METHODS

Samples collection and bacterial isolation

From January to June 2013, a total of 220 urine samples were collected from boys (n=90) and girls (n=130) patients who suffered from UTIs. All pediatrics were under 12 years old. Presence of UTIs in pediatrics was confirmed using the ultrasound technique[13]. All samples were collected from the hospitalized pediatrics of Baqiat-alah Hospital in Tehran, Iran. Most of pediatrics had been handling urine catheter for a week before they got UTIs. Strong urge to urine frequently even immediately after the bladder is emptied, painful burning sensation when urinating, cloudy and bloody urine with bad smell and in some cases fever, chills and nausea are the most commonly detected symptoms in pediatric patients. In order to decrease potential bacterial, cellular and artifactual contamination all urine samples were collected from midstream. Urine samples were collected using the Suprapubic Aspiration (SPA) method based on the standard technique of NICE (2007)[14].

For the isolation of *P. aeruginosa*, each sample was plated on the selective medium *Pseudomonas* cetrimide agar (PCA) (LABOBASI, Mendrisio, Switzerland) using a spreading technique. Plates were incubated for 18-24 hours and observed for suspected colonies of *P. aeruginosa*. Identification of *P. aeruginosa* was done by colony pigmentation, grape-like odor, motility and biochemical tests including carbohydrate fermentation (-), citrate assimilation (+), lysine decarboxylase (-), indol (-), oxidase (+), beta-hemolysis on blood-agar (+) and DNAse (-). Inocula from pure colonies on PCA were cultured on nutrient agar slants and kept at 4ºC.

Antibiotic susceptibility test

P. aeruginosa isolates to be inoculated on the surface of Mueller– Hinton agar (HiMedia Laboratories, Mumbai, India), plates were incubated into nutrient broth overnight until the turbidity is equivalent to 0.5 Mcfarland standards, allowed for few minutes at room temperature. Antimicrobial susceptibility was performed on Mueller-Hinton agar by the standard disk diffusion method recommended by the Clinical and Laboratory Standards Institute[15]. This was done by dipping a sterile swab stick in to overnight nutrient broth and carefully swabbing the entire surface of

Mueller–Hinton agar plates. The antibiotics used against the test bacteria were: imipenem (30 µg/disk), piperacillin (100 µg/disk), cefipime (30 µg/disk), gentamycin (10 µg/disk), ceftazidime (30 µg/disk), ciprofloxacin (5 µg/disk), polymyxin B (300 U/disk), ampicillin (10 u/disk), colistin (10 u/disk), aztreonam (30 µg/disk), tobramycin (10 u/disk) and netilmycin (10 u/disk).

The antibiotic multi disk (Oxoid, UK) was then placed on the surface of the inoculated plates and gently pressed. The plates were incubated at 37 ^{0}C for 18–24 h. The diameter of zone of inhibition was measured in millimeters and isolates were scored as sensitive or resistant by comparing with values recommended on standard charts. *P. aeruginosa* ATCC 10145 was used as quality control organism in antimicrobial susceptibility determination.

DNA extraction and PCR confirmation

Chromosomal DNA was extracted from each *P. aeruginosa* isolate by DNA extraction kit (Fermentase, Germany) according to manufacturer's instruction.

The bacteria were confirmed using the PCR method for *nanI* gene of the *P. aeruginosa*[16].

PCR was carried out with 2 µL template DNA, 0.25 µM of each primer (F: 5'-ATGAATACTTATTTTGATAT and R: CTAAATCCATGCTCTGACCC-3'), 0.2 mM deoxyribonucleoside triphosphates, 1x reaction buffer, 2 mM $MgCl_2$ and 1.5 U Taq DNA polymerase (Fermentas) in a total volume of 25 µL. The DNA was amplified using the following protocol: initial denaturation (94 ºC for 5 min), followed by 25cycles of denaturation (94 ºC for 35 s), annealing (53 ºC for 45 s) and extension (72 ºC for 1 min), with a single final extension of 7 min at 72 ºC.

PCR amplification of virulence genes

Three different multiplex PCR assays were used in order to amplification of various virulence genes. The programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device was used in all PCR reactions. The first multiplex PCR reaction was used in order to detection of *algD* and *algU* virulence genes of *P. aeruginosa* isolated from clinical samples. The PCR reaction was performed in a total volume of 50 µL containing 2.5 µL of DNA template, 1.5 mM $MgCl_2$, 200 µM dNTP (Fermentas) , 0.5 µM of each primers (algDF: 5'-AAGGCGGAAAT GCCATCTCC-3' and algDR: 5'-AGGGAAGTTCC GGGCGTTTG-3' [17] and also, algUF: 5'-CGCGAACCGCACCATCGCTC-3' and algUR: 5'-GCCGCACGTCACGAGC-3') (18), 1.25 U Taq DNA polymerase (Fermentas) and 5 µL PCR buffer 10X . Reactions were initiated at 1 cycle 95 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min and a final elongation step at 72 °C for 7 min.

The second multiplex PCR reaction was used in order to detection of *lasB*, *toxA*, *plcH*, *plcN* and *exoS* virulence genes of *P. aeruginosa* isolated from clinical samples. List of primers is shown in Table 1 [19]. The PCR reaction was performed in a total volume of 50 µL containing 25 ng of DNA template, 10 mM Tris/HCl, 50 Nm KCl, 1.5 mM $MgCl_2$, 200 µM dNTP (Fermentas) , 12.5 pmol of each primers, 1 U Taq DNA polymerase (Fermentas) and 5 µL PCR buffer 10X . Reactions were initiated at 1 cycle 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 55 for 1 min, 72 °C for 1.5 min and a final elongation step at 72 °C for 5 min.

The third multiplex PCR reaction based on the method of Finnan et al. (2004)[20] was used in order to detection of *exoT*, *exoY*, *exoU*, *phzH*, *phzM*, *phzI*, *phzII*, *phzS*, *lasA*, *pilA* and *pilB* virulence genes. List of primers is shown in Table 1.

PCR amplification of antibiotic resistance genes

List of primers used for detection of various â-lactamase genes is shown in Table 2 (21). The PCR reaction was performed in a total volume of 25 µL containing 2 µL of DNA template, 1.4 mM $MgCl_2$, 150 µM of each dNTP (Fermentas), 0.3 µM of each primers and 1 U Taq DNA polymerase (Fermentas). The cycling parameters used were as previously described[21].

Gel electrophoresis

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 significant relationship between incidences of bacteria in males and females, virulence gene and pattern of resistance of *P. aeruginosa* isolated from clinical samples. A χ^2 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 Baqiat-alah Hospital, Tehran, Iran. Written informed consent was obtained from all of the study patients or their parents.

RESULTS

Of 220 samples studied, 23 (10.45%) samples were positive for *P. aeruginosa* including 8.88% of males and 11.53% of female (Table 3). Antibiotic resistance pattern *P. aeruginosa* isolates is shown in Table 4. Bacterial strains exhibited the highest level of resistance to ampicillin (52.17%), gentamycin (47.82%), ciprofloxacin (39.13%) and netilmycin (34.78%). The most effective tested antibiotics against *P. aeruginosa* were piperacillin and imipenem.

The distribution of antimicrobial resistance genes within the *P. aeruginosa* isolated from pediatrics with UTIs is shown in Table 5. Several genes that encode resistance to β-lactams antibiotics i.e., *blaSHV* (69.56%), *blaTEM* (47.82%), *blaDHA* (34.78%) and *blaOXA* (30.43%) were the most commonly detected antibiotic resistance genes in *P. aeruginosa* isolates . The distribution of virulence factors in the *P. aeruginosa* isolates is shown in Table 6. We found that *exoS* (73.91%), *toxA* (69.56%), *exoT* (65.21%) and *phzM* (43.47%)

Table 1. Oligonucleotide primers used for virulence genes amplification.

Gene	Sequence (5'–3')	Size of product (bp)
lasB	lasB F: GGAATGAACGAAGCGTTCTCCGAC lasB R: TGGCGTCGACGAACACCTCG	284
toxA	toxA F: CTGCGCGGGTCTATGTGCC toxA R: GATGCTGGACGGGTCGAG	270
plcH	plcH F: GCACGTGGTCATCCTGATGC plcH R: TCCGTAGGCGTCGACGTAC	608
plcN	plcN F: TCCGTTATCGCAACCAGCCCTACG plcN R: TCGCTGTCGAGCAGGTCGAAC	481
exoS	exoS F: CGTCGTGTTCAAGCAGATGGTGCTG exoS R: CCGAACCGCTTCACCAGGC	444
exoT	exoT F: CAATCATCTCAGCAGAACCC exoT R: TGTCGTAGAGGATCTCCTG	1159
exoY	exoY F: TATCGACGGTCATCGTCAGGT exoY R: TTGATGCACTCGACCAGCAAG	1035
exoU	exoU F: GATTCCATCACAGGCTCG exoU R: CTAGCAATGGCACTAATCG	3308
phzII	phzII F: GCCAAGGTTTGTTGTCGG phzII R: CGCATTGACGATATGGAAC	1036
phzM	phzM F: ATGGAGAGCGGGATCGACAG phzM R: ATGCGGGTTTCCATCGGCAG	875
phzS	phzS F: TCGCCATGACCGATACGCTC phzS R: ACAACCTGAGCCAGCCTTCC	1752
phzI	phzI F: CATCAGCTTAGCAATCCC phzI R: CGGAGAAACTTTTCCCTC	392
phzH	phzII F: GGGTTGGGTGGATTACAC phzII R: CTCACCTGGGTGTTGAAG	1752
pilA	pilA F: ACAGCATCCAACTGAGCG pilA R: TTGACTTCCTCCAGGCTG	1675
pilB	pilB F: TCGAACTGATGATCGTGG pilB R: CTTTCGGAGTGAACATCG	408

Table 2. Oligonucleotide primers used for β-lactamase genes amplification.

Antimicrobial agent	Resistance gene	Sequence (5'–3')	Size of product (bp)
β-lactamase	*blaTEM*	F: ATGAGTATTCAACATTTCCG R: GGACTCTGCAACAAATACGC R: CTGACAGTTACCAATGCTTA	867
β-lactamase	*blaSHV*	F: GGTTATGCGTTATATTCGCC R: TTAGCGTTGCCAGTGCTC	867
β-lactamase	*blaOXA*	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	814
β-lactamase	*blaCTX-M*	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	593
β-lactamase	*blaDHA*	F: CACACGGAAGGTTAATTCTGA R: CGGTTARACGGCTGAACCTG	970
β-lactamase	*blaVEB*	F: CGACTTCCATTTCCCGATGC	642

Table 3. Prevalence of *Pseudomonas aeruginosa* in pediatrics suffered from UTIs

Source of isolation	Sex of patients	N. patients	N. positive sample (%)
Urinary tract infection	Male	90	8 (8.88)
	Female	130	15 (11.53)
Total		220	23 (10.45)

were the most commonly detected virulence factors in urine samples of hospitalized children. There were no positive results for *algD* and *plcH* factors. Interestingly, we found that females had the highest frequency of *P. aeruginosa*, antibiotic resistance, antibiotic resistance genes and putative virulence factors.

DISCUSSION

P. aeruginosa is the third most common pathogen associated with hospital-acquired catheter associated UTIs[22]. Our results indicated that 8.88% of male and 11.53% of female pediatrics with UTIs were infected with *P. aeruginosa*. Girls were more prone to get UTIs than boys. It is because of the relatively short, straight anatomy of the urethra in women. In addition, retrograde ascent of bacteria from the perineum is the most common cause of UTIs in women. Genetic factors, including expression of Lewis blood group Le (a+b-) and Le (a-b-) and HLA-A3 may also put women at higher risk for recurrent UTIs. Similar results have been reported by Bradbury *et al*. (2010)[23] and Goldman *et al*. (2007) [24].

Pathogenesis of this bacterium is based on the presence of various virulence factors. These factors have been shown to play an important role in pathogenesis of *P. aeruginosa* induced infections like respiratory tract infections, burn wound infections and keratitis[25, 26]. However, limited reports are available regarding role of these virulence traits in urinary tract infections[24, 27]. High presence of virulence factors in our investigation has been confirmed the results of previous studies[28, 23]. The results of our study showed that *exoS* (73.91%), *toxA* (69.56%), *exoT* (65.21%) and *phzM* (43.47%) were the most commonly detected virulence factors in pediatrics suffered from UTIs. Wolska and Szweda (2009)[27] reported that the frequency of *algD*, *lasB*, *toxA*, *plcN*, *plcH* and *exoS* virulence factors in clinical isolates of *P. aeroginosa* were 84.6%, 76.9%, 76.95, 76.9%, 76.9% and 46.15%, respectively. Endimiani et al. (2006)[29] indicated that 100% of *P. aeroginosa* isolates from clinical infections were positive for *exoT*, *exoU*, *lasB*, *plcH*, *toxA* and *plcN*, while *exoS* and *exoY* were detected in 78.9% and 73.7% of isolates. Over 60% of studied isolates from urine samples of pediatrics of our investigation

Table 4. Antimicrobial resistance properties in *Pseudomonas aeruginosa* isolated from pediatrics suffered from UTIs

Source of isolation	Sex of patients	*P. aeruginosa* positive	IMP 30	PIP	CFP	GM10	CFTZ	CIP5	POL B	AM10	Col	AZT	TOB	NTL
Urinarytract	Male	8	1	-	2	4	2	3	1	4	1	2	3	2
infection	Female	15	3	1	4	7	4	6	4	8	4	4	4	6
Total		23	4	1	6	11	6	9	5	12	5	6	7	8
			(17.39)	(4.34)	(26.08)	(47.82)	(26.08)	(39.13)	(21.73)	(52.17)	(21.73)	(26.08)	(30.43)	(34.78)

*In this table: IMP30= imipenem (30 µg/disk); PIP= piperacillin (100 µg/disk); CFP= cefipime (30 µg/disk); GM10= gentamycin (10 µg/disk); CF30= ceftazidime (30 µg/disk); CIP5= ciprofloxacin (5 µg/disk); POL B= polymyxin B (300 U/disk); AM10= ampicillin (10 u/disk); Col= colistin (10 u/disk); AZT= aztreonam (30 µg/disk); TOB= tobramycin (10 u/disk); NTL= netilmycin (10 u/disk).

Table 5. Distribution of antimicrobial resistance genes in *Pseudomonas aeruginosa* isolated from pediatrics suffered from UTIs

Source of isolation	Sex of patients	*P. aeruginosa* isolates	bla_{TEM}	bla_{SHV}	bla_{OXA}	$bla_{CTX\text{-}M}$	bla_{DHA}	bla_{VEB}
Urinary	Male	8	3	5	2	1	2	2
tract infection	Female	15	8	11	5	5	6	4
Total		23	11	16	7	6	8	6
			(47.82)	(69.56)	(30.43)	(26.08)	(34.78)	(26.08)

Table 6. Distribution of putative virulence factors in *Pseudomonas aeroginosa* isolated from pediatrics suffered from UTIs.

Source of isolation	Sex of patients	*P. aeruginosa* isolates	*exoT*	*exoY*	*exoS*	*exoU*	*phzH*	*phzII*	*phzM*	*phzI*	*phzS*	*lasA*	*lasB*	*pilA*	*pilB*	*toxA*	*algD*	*algU*	*plcH*	*plcN*
Urinary tract infection	Male	8	4	2	5	1	1	2	3	2	3	1	-	1	-	5	-	2	-	1
	Female	15	11	4	12	3	2	4	7	5	8	1	1	-	1	11	-	6	-	4
Total		23	15 (65.21)	6 (26.08)	17 (73.91)	4 (17.39)	3 (13.04)	6 (26.08)	10 (43.47)	7 (30.43)	11 (25.58)	2 (8.69)	1 (4.34)	1 (4.34)	1 (4.34)	16 (69.56)	-	8 (34.78)	-	5 (21.73)

contained *toxA* gene. In all probability, this toxin can play a significant role as a virulence factor of *P. aeroginosa* within catheter-associated UTIs[30]. Prevalence of *algD*, *pilB*, *lasB*, *plcH* and *exoU* genes among clinical isolates of *P. aeroginosa* in Bulgaria were 91.1%, 23.8%, 100%, 91.6%, 62.4% and 30.2%, respectively[31].

The *toxA* factor which was detected in 69.56% of *Pseudomonas* strains of our study is a chromosomally encoded secreted toxin capable of inhibiting protein synthesis of infected patients through the ADP-ribosylation of cellular elongation factor[30]. Ultimately, the action of this toxin results in localized tissue damage and bacterial invasion[30]

The *exoS* gene which was detected in 73.91% of *Pseudomonas* strains of our study is directly translocated into eukaryotic cells by the contact-dependent type III secretory process and, as such, it provides the bacterium with a mechanism for manipulating the eukaryotic cells it encounters. In support of *exoS* contributing to *P. aeruginosa* pathogenicity, bacterial translocation of *exoS* into epithelial cells results in a general inactivation of cellular function, as recognized by the inhibition of DNA synthesis, loss of focal adhesion, cell rounding, and microvillus effacement[32, 33].

Our results harbored the high presence of antibiotic resistance in *P. aeruginosa strains isolated from pediatrics with UTIs. The highest bacterial resistances were observed against* ampicillin (52.17%), gentamycin (47.82%), ciprofloxacin (39.13%) and netilmycin (34.78%). Majority of the *P. aeruginosa* strains of the Japoni *et al*. (2006)[34] investigation were resistant to more than 5 antibiotics which was similar to our results. Shiny *et al*. (2013)[35] screened 500 pus and 500 urine samples. They showed that 12.8% of pus and 4% of urine samples were positive for *P. aeruginosa* and the prevalence of resistance against cefotaxime was 93.75%, while all isolates were sensitive to imipenem. In a study of Viedma et al. (2012) (36) which was conducted on 2007 to 2010 years, the prevalence of *P. aeruginosa* was increased over this period from 2.8% to 15.3% and all of the isolates were susceptible only to colistin (100%) and amikacin (75%). Fazeli *et al*. (2012)[37] showed that 29% and 32.2% of the *P. aeruginosa* strain were resistant to ciprofloxacin and gentamycin which was similar to our results. Resistance of *P. aeruginosa* isolates of our investigation was 39.13%

against ciprofloxacin compared to 26.8% in Latin America[38] and 10–32% in Europe[39, 40]. Akingbade *et al.* (2012)[41] reported that the resistance rate of the clinical isolates of *P. aeruginosa* against amoxicillin, ampicillin, cloxacillin, cotrimoxazole, erythromycin, tetracycline and ofloxacin were 92.7%, 90%, 88.2%, 77.3%, 72.7%, 70.9% and 690%, respectively.

Several genes that encode resistance to β-lactams antibiotics i.e., bla_{TEM} (47.82%), bla_{DHA} (34.78%), bla_{OXA} (30.43%), bla_{VEB} (17.64%), bla_{SHV} (69.56%) and $bla_{CTX\text{-}M}$ (26.08%) were detected in bacterial strains of our study. A recent study performed within our goals in Egypt reported that the majority (97%) of *P. aeruginosa* isolates were beta-lactamase producers[42]. Du *et al*. (2010)[43] showed the high incidence of antibiotic resistance genes including *bla*TEM-1 (100%), *bla*PSE-1 (100%), *bla*OXA-2 (96.2%), *bla*SHV-18 (91.3%), *bla*OXA-17 (78.3%), *bla*VIM-3 (26.1%), *bla*OXA-10 (21.7%) and *bla*SHV-1 (8.7%) which was similar to our results.

We found that bla_{SHV} (69.56%) were the most commonly detected genes in *P. aeruginosa* isolates. Similar investigation in Taiwan[44] reported the high presence of *bla*SHV-5 and *bla*SHV-12 in the *P. aeruginosa* isolates of clinical specimens.

As far as we know, this investigation is the most widely report of virulence profile and antibiotic resistance properties of *P. aeruginosa* isolated from children suffered from UTIs in Iran. Our results revealed that all of the *exoS*, *toxA*, *exoT* and *phzM* virulence factors and *blaSHV*, *blaTEM*, *blaDHA* and *blaOXA* antibiotic resistance genes were the most commonly detected characteristics of bacterial isolates. Due to the irregular high prescription of ampicillin, gentamycin and ciprofloxacin antibiotics, such amounts of resistance were observed against these antibiotics. Hence, judicious use of antibiotics is required by clinicians. It is compulsory to evaluate the prevalence of virulence factors, antibiotic resistance genes and pattern of antibiotic resistance among clinical isolates of *P. aeruginosa*. 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 initially manage of children affected with a community acquired UTIs with piperacillin, imipenem, polymyxin B and colistin prescription.

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