Int. J. Adv. Res. Biol. Sci. 2(11): (2015): 272–279

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-11-34

# Virulence genes and antimicrobial resistance properties of *Acinetobacter baumannii* isolated from pediatrics suffered from UTIs

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## Abstract

**Background and objectives:** One of the main problems in the treatment of cases of *A. baumannii* especially in the cases of UTIs in pediatrics is the occurrence of high antibiotic resistance. The present investigation was carried out in order to study the distribution of virulence genes and antimicrobial resistance properties of *A. baumannii* isolated from pediatrics suffered from UTIs. **Methods:** Two hundred and eighty urine samples were collected from pediatrics suffered from UTIs. Samples were cultured immediately and those that were *A. baumannii*-positive were analyzed by the disk diffusion method and PCR-based amplification of virulence factors. **Results:** Twenty out of 280 samples (7.1%) were positive for *A.baumannii*. The prevalence of *A.baumannii* in boy and girl urine samples were 4.6% and 9.3%, respectively ($P$ =0.028). The highest levels of infection was seen in less than one year old pediatrics (12.7%). *A. baumannii* strains harbored the highest levels of resistance against ampicillin (85%), gentamycin (80%), ciprofloxacin (70%) and trimethoprim/sulfamethoxazole (65%). *Afa/draBC* (80%), *csgA* (40%) and *cnf1* (30%) were the most commonly detected virulence factors. **Conclusions:** It is logical to primary identification of best antibiotic choice for treatment of cases of UTIs using the disk diffusion method. In the current situation, prescription of imipenem, tobramycin and ceftriaxone can be effective for treatment of cases of *A.baumannii*-based UTIs in pediatrics

**Keywords:** *Acinetobacter baumannii*, Virulence genes, Antimicrobial resistance pattern, Pediatrics, Urinary tract infections.

## Introduction

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).

One of the most important pathogenic bacteria which is responsible for majority of the cases of human clinical infections is *Acinetobacter baumannii* (*A. baumannii*) (5).*A. baumannii* is a Gram-negative, non-motile, catalase positive, oxidase-negative, non-spore forming and strictly aerobic cocobacilli, which is a causative agent of wound, skin and soft tissue infections, endocarditis, septicemia, meningitis, pneumonia and respiratory tract infections and finally UTIs (6, 7).

Pathogenesis of diseases caused by *A. baumannii* is depend on the presence of latent virulence factors (5). Some of the most important virulence of the *A. baumannii* strains of clinical infections are curli fibers (*csg*), drantigen family (*afa*/*dra*), cytotoxic necrotizing factor (*cnf*), siderophores like aerobactin (*iutA*), colicin V production (*cvaC*) (5, 8, 9).

Another important key factor to study the epidemiological features of *A. baumannii* is its antibiotic resistance pattern. In the past, virulent cases of *A. baumannii* are mainly treated with traditional antibiotic therapy (10) but now a days, it exerts resistance to nearly all major classes of antibiotics, including cephalosporins, broad-spectrum penicillins, fluoroquinolones, carbapenems, aminoglycosides, and tetracyclines (10-12).

Due to the rapid emergence of resistance, increased incidence and the worldwide spread of drug resistant *A. baumannii*, the present investigation was carried out to study the prevalence and distribution of virulence factors and antibiotic resistance properties of *A. baumannii* isolated from the Iranian pediatric patients suffered from UTIs.

## Materials and Methods

### Ethical considerations

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

### Samples and *Acinetobacter baumannii* isolation

From February 2015 to August 2015, a total of 280 urine samples were collected from boys (n=130) and girls (n=150) patients suffered from UTIs. Samples were collected from hospitalized children less than 10 years old. 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 laboratory in a cooler with ice-packs. Urine samples were inoculated on to blood agar (Merck, Germany) and MacConkey agar (Merck, Germany) and incubated aerobically at 37°C for 24 hours. Non-hemolytic, opaque and creamy colonies on blood agar and nonlactose fermenting colonies on MacConkey agar were further sub-cultured on MacConkey agar and incubated for another 24 hours at 37ºC to obtained pure colonies. The isolated organisms were identified based on colonial and microscopic characteristics and various biochemical tests according to standard laboratory methods (15). Further identification of isolates was done using Gram stain, oxidase test and API 20NE identification strip (Biomérieux, Marcy l'Etoile, France). Finally, the results of the bacteriological and biochemical tests were confirmed by the PCR assay amplification of *recA* gene of the *A.baumannii* (F: 5′-CCTGAATCTTCTG GTAAAAC-3′ and R: 5′-GTTTCTGGGCTGCCAAA CATTAC-3′) (336bp fragment) (16).

### Antimicrobial susceptibility test of *Acinetobacter baumannii* strains

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 *A.baumannii* strains against 13 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (17). Susceptibility of *A.baumannii* strains were tested against ampicillin (10 u/disk), gentamycin (10 µg/disk), cephalexin (10 µg/disk), imipenem (30 u/disk), cefotaxime (30 µg/disk), ciprofloxacin (5 µg/disk), ceftazidime (30 µg/disk), tobramycin (10 µg/disk), levofloxacillin (5 µg/disk), cephalothin (30 µg/disk), trimethoprim/sulfamethoxazole (25 µg/disk), tetracycline (30 µg/disk), and ceftriaxone (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) (17). In all reactions, the *A.baumannii* (ATCC 17978) was used as quality control organisms.

### DNA extraction from the *Acinetobacter baumannii* isolates

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5ml of nutrient 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 mMTris-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 5 minutes. 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 (18).

### Virulence genes amplification of *Acinetobacter baumannii* isolates

The multiplex PCR assay was done to detection of putative virulence factors in the *A.baumannii* strains isolated from the urine samples of pediatrics suffered from UTIs. Table 1 shows the list of primer, PCR program and volume of PCR ingredients used for detection of virulence factors (19). The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany).

**Table 1.** Primer sequence, size of products, PCR program and volume of ingredients used for detection of virulence factors in *Acinetobacter baumannii* isolates of pediatrics suffered from UTIs (19).

| Target gene | Primer sequence (5'-3')* | PCR product (bp) | PCR programs | PCR Volume (50µL) |
|---|---|---|---|---|
| *afa/draBC* | F: GCTGGGCAGCAAACTGATAACTCTC R: CATCAAGCTGTTTGTTCGTCCGCCG | 750 | 1 cycle: 95 $^{0C}$ ---------- 4 min. 30 cycle: 95 $^{0C}$ ---------- 50 s 58 $^{0C}$ ---------- 60 s 72 $^{0C}$ ---------- 45 s 1 cycle: 72 $^{0C}$ ---------- 8 min | 5 µL PCR buffer 10X 1.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template |
| *cnf1* | F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT | 498 | | |
| *csgA* | F: ACTCTGACTTGACTATTACC R: AGATGCAGTCTGGTCAAC | 200 | | |
| *cvaC* | F: CACACACAAACGGGAGCTGTT R: CTTCCCGCAGCATAGTTCCAT | 680 | | |
| *iutA* | F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG | 300 | | |

### Agarose gel electrophoresis

Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/ml of SYBR Green in Tris–borate–EDTA buffer at 90 V for 40 min, also using suitable molecular weight markers. The products were examined under ultraviolet illumination. *A.baumannii* ATCC 17978 and *A.baumannii* ATCC 19606 and rough strains purchased from the Pasteur Institute (Tehran, Iran) were used as positive controls and distilled water (D.W, Merck, Germany) was used as a negative control.

### 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 of *A.baumannii* 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

The results of our study revealed the high prevalence of *A.baumannii* in the urine samples of pediatrics suffered from UTIs. Table 2 shows the total distribution of *A.baumannii* in the urine samples of pediatric patients. Of 280 samples collected, 20 samples (7.1%) were positive for *A.baumannii*. The prevalence of *A.baumannii* in boy and girl urine samples was 4.6% and 9.3%, respectively. Significant statistical difference was seen for the prevalence of *A.baumannii* between boys and girls ($P$ =0.028).

Less than one year old pediatric patients had the lowest prevalence of *A.baumannii* (12.7%). Significant statistical difference was seen for the prevalence of *A.baumannii* between less than one year old and 7-10 years old pediatrics ($P$ =0.033) and also between 1-3 years old and 7-10 years old pediatrics ($P$ =0.041).

**Table 2.**Total distribution of *Acinetobacter baumannii* in the urine samples of pediatric patients suffered from UTIs.

| Different criteria | | No. samples collected | No. *A. baumannii* (%) |
|---|---|---|---|
| Urine samples | Boy | 130 | 6 (4.6) |
| | Girl | 150 | 14 (9.3) |
| | Total | 280 | 20 (7.1) |
| | <1 Year | 55 | 7 (12.7) |
| | 1-3 Years | 56 | 6 (10.7) |
| | 3-5 Years | 56 | 4 (7.1) |
| | 5-7 Years | 55 | 2 (3.6) |
| | 7-10 Years | 58 | 1 (1.7) |
| | Total | 280 | 20 (7.1) |
| | Total | 280 | 20 (7.1) |

**Table 3.**Antibiotic resistance pattern of *Acinetobacter baumannii* isolated from the urine samples of pediatric patients suffered from UTIs.

| Samples (No. positive strains) | | Pattern of antibiotic resistance (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Amp* | Gen | Cephl | Imp | Cefo | Cip | Ceft | Tob | Lev | Ceph | TmSx | Tet | Cefr |
| Urine | Boy (6) | 5 (83.3) | 5 (83.3) | 4 (66.6) | 1 (16.6) | 4 (66.6) | 5 (83.3) | 4 (66.6) | 3 (50) | 5 (83.3) | 4 (66.6) | 5 (83.3) | 4 (66.6) | 3 (50) |
| | Girl (14) | 12 (85.7) | 11 (78.5) | 6 (42.8) | 1 (7.1) | 8 (57.1) | 9 (64.2) | 7 (50) | 6 (42.8) | 7 (50) | 6 (42.8) | 8 (57.1) | 9 (64.2) | 6 (42.8) |
| | <1 Year (7) | 5 (71.4) | 4 (57.1) | 2 (28.5) | - | 3 (42.8) | 3 (42.8) | 1 (14.2) | - | 2 (28.5) | 1 (14.2) | 3 (42.8) | 2 (28.5) | 1 (14.2) |
| | 1-3 Years (6) | 5 (83.3) | 5 (83.3) | 2 (33.3) | - | 3 (50) | 4 (66.6) | 4 (66.6) | 2 (33.3) | 3 (50) | 3 (50) | 3 (50) | 4 (66.6) | 2 (33.3) |
| | 3-5 Years (4) | 4 (100) | 4 (100) | 3 (75) | - | 3 (75) | 4 (100) | 3 (75) | 4 (100) | 4 (100) | 3 (75) | 4 (100) | 4 (100) | 3 (75) |
| | 5-7 Years (2) | 2 (100) | 2 (100) | 2 (100) | 1 (50) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) |
| | 7-10 Years (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| | **Total** (20) | 17 (85) | 16 (80) | 10 (50) | 2 (10) | 12 (60) | 14 (70) | 11 (55) | 9 (45) | 12 (60) | 10 (50) | 13 (65) | 13 (65) | 9 (45) |

*Amp=ampicillin (10 u/disk), Gen= gentamycin (10 µg/disk), Cephl= cephalexin (10 µg/disk), Imp= imipenem (30 u/disk), Cefo= cefotaxime (30 µg/disk), Cip= ciprofloxacin (5 µg/disk), Ceft= ceftazidime (30 µg/disk), Tob= tobramycin (10 µg/disk), Lev= levofloxacillin (5 µg/disk), Ceph= cephalothin (30 µg/disk), TmSx= trimethoprim/sulfamethoxazole (25 µg/disk), Tet= tetracycline (30 µg/disk), and Cefr= ceftriaxone (30 µg/disk).

Table 3 shows the antibiotic resistance pattern of the *A. baumannii* strains of the cases of pediatrics UTIs. *A. baumannii* strains of our study revealed the highest levels of resistance against ampicillin (85%), gentamycin (80%), ciprofloxacin (70%), trimethoprim/sulfamethoxazole (65%), and cefotaxime (60%). *A. baumannii* strains of boys had the higher levels of resistance than those of girls ($P$ =0.036). Statistically significant differences were seen for the prevalence of bacterial resistance between ampicillin and imipenem ($P$ =0.026), gentamycin and imipenem ($P$ =0.029), ampicillin and tobramycin ($P$ =0.037) and ciprofloxacin and ceftriaxone ($P$ =0.040). *A. baumannii* strains of 7-10 years old pediatrics had the highest levels of resistance ($P$<0.05).

**Table 4.**Distribution of virulence factors of *Acinetobacter baumannii* isolated from the urine samples of pediatric patients suffered from UTIs.

| Samples (No. positive strains) | | Virulence factors (%) | | | | |
|---|---|---|---|---|---|---|
| | | ***afa/draBC*** | ***cnf1*** | ***csgA*** | ***cvaC*** | ***iutA*** |
| **Urine** | Boy (6) | 5 (83.3) | 4 (66.6) | 4 (66.6) | 1 (16.6) | 4 (66.6) |
| | Girl (14) | 11 (78.5) | 4 (28.5) | 5 (35.5) | 1 (7.14) | 2 (14.2) |
| | <1 Year (7) | 4 (57.1) | 1 (14.2) | 2 (28.5) | - | - |
| | 1-3 Years (6) | 5 (83.3) | 1 (16.6) | 3 (50) | - | 1 (16.6) |
| | 3-5 Years (4) | 4 (100) | 3 (75) | 3 (75) | 1 (25) | 2 (50) |
| | 5-7 Years (2) | 2 (100) | 2 (100) | 2 (100) | 1 (50) | 2 (100) |
| | 7-10 Years (1) | 1 (100) | 1 (100) | 1 (100) | - | 1 (100) |
| | **Total**(20) | 16 (80) | 8 (40) | 11 (55) | 2 (10) | 6 (30) |

Table 4 represents the distribution of virulence factors in the *A. baumannii* strains isolated from the urine samples of pediatric patients suffered from UTIs. The most commonly detected virulence factors in the *A. baumannii* strains of pediatric patients were *afa/draBC* (80%), followed by *csgA* (40%) and *cnf1* (30%). *A. baumannii* strains of boy patients had the higher prevalence of virulence factors ($P$<0.05). Seven to ten years old pediatric patients also had the higher prevalence of virulence factors ($P$<0.05). Significant difference was seen between the prevalence of *afa/draBC* and *cvaC* ($P$ =0.043) and *afa/draBC* and *iutA* ($P$ =0.047).

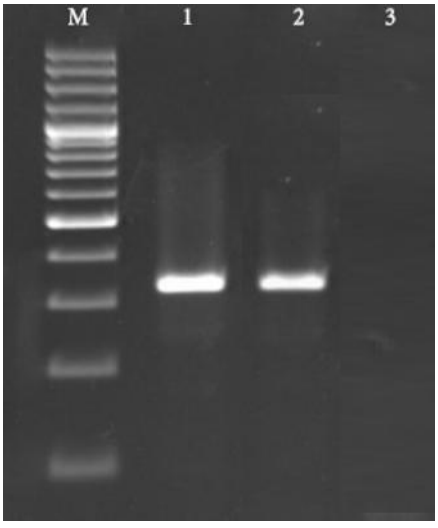


**Figure 1.**Results of the gel electrophoresis for identification of *Acinetobacter baumannii* in the urine samples of pediatric patients suffered from UTIs. M: 100 bp DNA ladder (Fermentas, Germany), Line 1: Positive sample (336bp), Line2: Positive controls and Line 3: Negative control.

## Discussion

The results of the present investigation showed the high prevalence of resistant and virulent strains of *A. baumannii* in the urine samples collected from girls and boys suffered from UTIs. Our results revealed the higher prevalence of *A. baumannii* in females than males and higher prevalence of virulence factors and antibiotic resistance in males than females. Besides, older pediatrics had the highest levels of virulence genes and antibiotic resistance than those who were younger. Conceivable clarifications for the high distribution of *A. baumannii* in our study is due to the low levels of health care and lack of sanitary conditions in hospitals, unnecessary application of urine catheter, surge the age of circumcision in boys, indecorous prescription of operative drugs and existence of antibiotic resistance in bacterial strains. A possible reason for the lower prevalence of antibiotic resistance in younger patients is that these patients were not usually used from antibiotics. It is because of the number of diseases in these patients is much less than those of elderly. Therefore, the frequency of using antibiotics is less. Higher prevalence of virulent strains of *A. baumannii* in older pediatrics than younger is the fact that the younger pediatrics has the weaker immune system and can easily get UTIs with non-virulent strains of bacteria. A possible reason for the higher prevalence of *A. baumannii* in females is that they have relatively short and wide urethra. Therefore, bacterial penetration is easier in females. Also, host factors such as changes in normal vaginal flora may put females at higher risk for UTIs. Therefore, females are more prone to get UTIs. Narrow and long urethra and also higher resistance of males to get UTIs caused the lower prevalence of UTIs in them. One of the reasons for the high prevalence of antibiotic resistance and virulence factors in boys than girls is the fact that they have relatively narrow and long urethra. In addition, boys are more resistance than women to get UTIs. Therefore, the virulent and resistant strains of *A. baumannii* have the higher capacity to cause UTIs in boys.

We found that 7.1% of studied samples were infected with *A. baumannii*. In a study which was conducted in Iran by Momtaz et al. (2014) (19), *A. baumannii* was detected in 121 out of 500 human clinical samples (24.2%). The rate of *A. baumannii* in the urine samples of mentioned study was 23.91% which was higher than our results. In a study which was conducted in India, Jaggi et al. (2012) (20) reported that the prevalence of *A. baumannii* in human clinical infections were 9.4% which was higher than our results. In a Korean investigation which was conducted by Siau et al. (1996) (21) the prevalence rate of *A. baumannii* in the cases of infections in the hospitals of Hong Kong was 11% which was higher than our results, too. High differences in the type of samples, method of sampling, number of samples collected, method of experiment, sex and age of patients and geographical area which the samples were collected are the main factors for differences in the prevalence of *A. baumannii* in various investigations.

Our results showed that majority of *A. baumannii* were resistant to more than one antibiotic. Excessive and indiscriminate prescription of antibiotics caused to the *A. baumannii* strains of our research become resistant to majority of antibiotics including ampicillin (85%), gentamycin (80%), ciprofloxacin (70%), trimethoprim/sulfamethoxazole (65%), and cefotaxime (60%). In an extensive investigation which was conducted by Moradi et al. (2015) (10) reported that there was an increase in antimicrobial resistance of *A. baumannii* in Iran. They showed that during the initial time point of their studies (2001-2007) there was a high rate of resistance to all antibiotics, with the exception of carbapenems, lipopeptides, and aminoglycosides (10). Also, the resistance rate was increased in one group of these three antimicrobial groups from 2010 to 2013 (10). In particular, there was an increase in resistance to carbapenems (imipenem and meropenem) from 2010-2011 and 2012-2013, whereas no significant change in the resistance rate of the other two antimicrobial groups (lipopeptides and aminoglycosides) during the study time was observed, although they did observe certain trends in amikacin (aminoglycoside group antibiotic) between 2011-2012 and 2012-2013 (10). Jaggi et al. (2012) (20) reported that the prevalence of antibiotic resistance in the *A. baumannii* strains of clinical samples against amikacin, gentamicin, tobramycin, aztreonem, cefipime, ceftazidime, ciprofloxacin, Levofloxacin and imepenem were 90.3%, 85.8%, 80%, 94.2%, 90.3%, 92.1%, 67.4% and 67.1%, respectively which was higher than our results. In a study which was conducted in Tehran, Iran (22), *A. baumannii* strains of clinical samples had the high levels of resistance against piperacillin-Tazobactam, ciprofloxacin, cefotaxime, cefepime, ceftriaxone and ceftazidime which was similar to or results. Similar results have been reported previously by Mirnejad and Vafaei (2013) (Iran) (23), Văduva et al. (2008) (Denmark) (24), Reguero et al. (2013) (Colombia) (25) and Zhao et al.(2015) (China) (26). In fact, differences in the idea of medical practitioners in

antibiotic prescription causes variations in the levels of antibiotic resistance against different antibiotics.

The final part of our investigation showed that the *afa/draBC* (80%), *csgA* (40%) and *cnf1* (30%) genes had the highest prevalence among all studied virulence factors. These genes are mainly associated with adherence, biofilm formation, colonization and invasion of *A. baumannii* in the host's epithelium (27, 28). In a study of Momtaz et al. (2015) (19) the prevalence of *afa/draBC*, *csgA* and *cnf1* genes in the *A. baumannii* strains of human clinical infections were 42.97%, 12.39% and 35.53%, respectively. In a study which was conducted in the West part of Iran by Mohajeri et al. (2014) (27) the prevalence of *csgA* and *fimH* genes of *A. baumannii* were 54% and 60%, respectively. Adhesive virulence factors are considered an important factor in adhesion, biofilm formation and survival of most bacteria and their virulence in human (28). Similar results have been reported previously by Eraç et al. (2014) (8), McConnell et al. (2013) (29) and Eijkelkamp et al. (2014) (30).

## Conclusion

In conclusion, we identified a large number of resistant and virulent strains of *A. baumannii* isolated from the urine samples of pediatric patients suffered from severe UTIs in Iran with respect to the high prevalence of bacterium in less than one year old girls. Prevalence of *afa/draBC*, *csgA* and *cnf1* genes and resistance against ampicillin, gentamycin, ciprofloxacin, trimethoprim/sulfamethoxazole and cefotaxime were high. Antibiotic prescription should more accurately administered in 7-10 years old boy patients because they had the higher prevalence of resistance. Less than 1 year old girls are at a higher risk of infection. Therefore, the highest levels of health care should be performed for them. We recommended the initially identification of *A. baumannii* and then application of disk diffusion method to choose the best type of antimicrobial agents in the cases of UTIs.

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