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# Changes in Antimicrobial Resistance Patterns of Pediatric Uropathogens in the South of Iran

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***Abstract***

***Background:*** The increasing prevalence of antimicrobial resistance has emerged as a critical global public health issue. This study aimed to identify the predominant bacterial pathogens causing community-acquired urinary tract infections (UTIs) in children and to evaluate the evolving patterns of antimicrobial resistance among pediatric uropathogens in Shiraz, southern Iran.

***Methods:*** This research involved two prospective cross-sectional studies conducted among pediatric patients diagnosed with UTIs. The first study was carried out from 2005 to 2006 and included 435 children, while the second study, conducted from April 2010 to March 2011, enrolled175 children. Participants were children aged 1 month to 10 years suspected of having UTIs and referred to various outpatient pediatric clinics. Informed consent was obtained, and demographic data and laboratory test results were recorded using a standardized checklist. Urine samples were collected via urine bags for children under 2 years old and midstream clean-catch for older children. Patients were included if they presented with pyuria (white blood cell count >10 cells/µL) and UTI symptoms, such as dysuria, frequency, fever, etc. Bacterial culture was performed on blood agar and eosin methylene blue (EMB) agar, followed by antimicrobial susceptibility testing on isolated pathogens. Data were analyzed using SPSS version 19. Statistical analysis involved Pearson correlation and Chi-square tests, with a p-value of less than 0.05 considered significant.

***Results:*** In the initial study (2005-2006), Escherichia coli was the most common pathogen (69.2%), followed by Klebsiella (13.33%) and Enterobacter (12.18%). The male-to-female ratio was 0.417, with the highest infection rate in infants under 12 months ($p = 0.002$). Resistance was highest for Amoxicillin (93.83%), Ampicillin (84.39%), Co-trimoxazole (60%), and Cephalotin (58.71%), and lowest for Nitrofurantoin (12.63%) and Ciprofloxacin (7.77%). In the follow-up study (2010-2011), E. coli remained dominant (67.43%), followed by Proteus (11.43%) and Klebsiella (6.85%). The male-to-female ratio was 0.2 and infections were highest in children under 12 months ($p = 0.016$). Resistance increased for Co-trimoxazole (64.85%), Cefixime (53.42%), Nalidixic acid (52.98%), and Nitrofurantoin (44.77%), while moderate resistance was noted for Gentamicin (39.19%) and Ciprofloxacin (22.55). A significant association existed between bacterial type and age groups in the second study ($p = 0.002$).

***Conclusion:*** An upward trend in resistance was observed for commonly used oral antibiotics such as Co-trimoxazole, Cefixime, Nalidixic acid, and Nitrofurantoin. However, resistance remained lower for parenteral agents including Gentamicin, Cefotaxime, Ceftizoxime, and Ciprofloxacin. This highlights the ongoing challenge in selecting effective empirical antibiotic therapies for pediatric UTIs.

***Key Words:*** Antibiotic, Pediatric, Resistance, Urinary tract infection.

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## 1- INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting children globally (1). It is crucial to promptly diagnose and treat UTIs, as delayed therapy, especially in cases of pyelonephritis, can result in severe complications such as renal scarring and permanent kidney damage (2). Therefore, starting antibiotic therapy as soon as UTI symptoms are recognized is essential to prevent these adverse outcomes (3).

However, the increasing prevalence of antimicrobial resistance among uropathogenic bacteria presents a significant challenge in choosing effective empirical antibiotics (4). Identification of the causative bacteria through urine culture and assessment of their antibiotic susceptibility patterns are necessary steps to guide appropriate treatment and reduce the risk of therapeutic failure (5). While empirical treatment should be begin immediately based on clinical suspicion, adjustments should be made according to laboratory results to ensure optimal care (6).

Given the global increase in antibiotic resistance and its impact on clinical management, continuous local surveillance is crucial (7). This study aimed to identify the bacterial species causing community-acquired UTIs and evaluate their antibiotic resistance profiles in children in Shiraz, southern Iran, during two distinct time periods (2005–2006 and 2010–2011). The findings of this research are essential for helping clinicians choose the most effective empirical antibiotic treatments, reducing inappropriate antibiotic use, preventing treatment failures, and ultimately limiting the spread of resistant bacteria in the community.

## 2- MATERIALS AND METHODS

This study utilized a prospective cross-sectional design, covering two distinct periods, to investigate urinary tract infections in pediatric patients. The first study took place between 2005 and 2006, and the second between April 2010 and March 2011. The participants were children aged 1 month to 10 years suspected of having UTIs and referred to various outpatient pediatric clinics in Shiraz, Iran.

Upon referral, informed consent was obtained from the parents or guardians of potential participants. The study objectives were thoroughly explained, and demographic data (including age and gender) and laboratory test results were meticulously recorded by the researcher using a standardized checklist. Participants' contact information was collected for potential follow-up purposes. Children whose parents or guardians declined to continue participation at any stage were excluded from the study. Ethical approval for this research was granted by the Ethics Committee of Azad University of Kazerun (IR.IAU.KAU.REC.1403.264).

Urine samples were collected using appropriate methods for the respective age groups: urine bags were utilized for children under 2 years old while midstream clean-catch specimens were obtained from older children. Only patients presenting with pyuria (white blood cell count >10 cells/µL) and clinical symptoms of a urinary tract infection such as dysuria , frequency, FTT, fever, etc., were included in the study. Bacterial culture was performed on all collected urine samples. A calibrated 1 µL loop was used to inoculate the samples onto both blood agar and eosin methylene blue (EMB) agar, alongside routine urine analysis. Following isolation, antimicrobial susceptibility testing was subsequently performed on all identified pathogens to determine their resistance profiles.

Data were categorized and analyzed separately across three specific age categories: 1–12 months, 13–60 months, and over 60 months. Statistical analysis was conducted using SPSS software version 19. Pearson correlation and Chi-square tests were employed for statistical comparisons, and a p-value of less than 0.05 was considered statistically significant.

## 3- RESULTS

In the first study, conducted from 2005 to 2006, a total of 435 urine samples yielded positive bacterial cultures. The predominant bacterial isolates included Escherichia coli (69.20%), Klebsiella spp. (13.33%), Enterobacter spp. (12.18%), and Proteus spp. (2.07%). Of these positive cultures, 70.57% were from girls and 29.43% from boys, resulting in a male-to-female ratio of 0.417. The highest incidence of infection was observed in children under 12 months, with 71.09% of boys and 57.98% of girls in this age group affected. However, no significant association was found between the type of bacterial isolate and age groups in this study (p = 0.616).

The second study, conducted from April 2010 to March 2011, included 175 culture-positive samples. The most frequent isolates were E. coli (67.43%), Proteus spp. (11.43%), and Klebsiella spp. (6.85%). In this period, 82.86% of the positive cultures were from girls and 17.14% from boys, yielding a male-to-female ratio of 0.2. Similar to the first study, the majority of infections occurred in children younger than 12 months (45.14%), and this age distribution was statistically significant (p = 0.016). Specifically, 60% of boys and 42% of girls were under 12 months (Table 1).

Antimicrobial susceptibility results are summarized in Tables 2 and 3 for the first and second studies, respectively. In the first study (2005-2006), antibiotics demonstrating the highest efficacy included cefixime (88.11% sensitive), ciprofloxacin (86.89% sensitive), ceftizoxime (84.61% sensitive) , amikacin(79.23% sensitive), and gentamicin (78.93% sensitive). Conversely, amoxicillin (93.83% resistance), ampicillin (84.39% resistance), cephalothin (58.71% resistance), and co-trimoxazole (60% resistance) exhibited lower effectiveness.

**Table-1**. Demographic and pathogen distribution data findings in two studies.

| First study | Sample | Pathogen Distribution | Second study | Sample | Pathogen Distribution |
|---|---|---|---|---|---|
| Samples | 435 cases | | Samples | 175 cases | |
| Male/female | 128/307(0/417) | | Male/female | 30/145(0/2) | |
| Mean age | 31/29±24/17 | | Mean age | 32/32±42/44 | |
| <12months | 269cases | 61/84% | <12months | 79 cases | 45/14% |
| 13-60months | 101cases | 23/22% | 13-60months | 65 cases | 37/14% |
| >60months | 65cases | 14/94% | >60months | 31cases | 17/71% |
| Ecoli | | 69/20% | Ecoli | | 67/43% |
| Klebsiella | | 13/33% | Proteus | | 11/43% |
| Entrobacter | | 12/18% | Klebsiella | | 6/85% |
| Proteus | | 2/07% | Streptococcus | | 4/57% |
| Citrrobacter | | 0/92% | Entrobacter | | 4% |
| Pseudomonas | | 0/69% | Citrrobacter | | 2/85% |
| Staphylococcus | | 1/61% | Pseudomonas | | 2/85% |

**Table-2**. Distribution of antimicrobial susceptibility test, regardless of the cause of urinary tract infections in patients (2005-2006) the first study.

| Antibiotics | Sensitive% | Intermediate% | Resistance% |
|---|---|---|---|
| **Cefixime** | 88/11 | 6/76 | 5/13 |
| **Gentamycin** | 78/93 | 11/57 | 9/50 |
| **Ceftizoxime** | 84/61 | 6/95 | 8/44 |
| **Cotrimoxazole** | 27/2 | 12/8 | 60 |
| **Nalidixic acid** | 75 | 13/74 | 11/26 |
| **Chloramphenicol** | 50/86 | 19/40 | 29/74 |
| **Nitrofurantoin** | 72/70 | 14/67 | 12/63 |
| **Amikacin** | 79/23 | 11/63 | 9/14 |
| **Cephalothin** | 14/84 | 26/45 | 58/71 |
| **Ampicillin** | 6/94 | 8/67 | 84/39 |
| **Amoxicillin** | 0 | 6/17 | 93/83 |
| **Ciprofloxa** | 86/89 | 5/34 | 7/77 |

Highly effective antibiotics were Cefixime, Ciprofloxacin, Ceftizoxime, Amikacin, Gentamycin; Less effective antibiotics were Amoxicillin, Ampicillin, cephalotin, co trimoxazole.

In the second study (2010-2011), ciprofloxacin (72.84% sensitive), norfloxacin (68.82% sensitive), and Ceftizoxime (62.43% sensitive) remained highly effective. However, co-trimoxazole (64.85% resistance), cefixime (53.42% resistance), nalidixic acid (52.98% resistance), and Nitrofurantoin (44.77% resistance) showed reduced susceptibility. Moderate resistance was noted for gentamicin (39.19% resistance).

A significant difference was observed between bacterial isolates and age groups in the second study (p = 0.002, Table 4). E. coli remained the most common pathogen across all age groups. Notably, Proteus spp. were more frequently isolated in children aged 13–60 months, whereas Klebsiella spp. were predominant in children under 12 months (Table 4).

Comparing antibiotic resistance profiles between E. coli and non-E. coli isolates revealed that E. coli had a significantly lower resistance rate to nitrofurantoin compared to other pathogens (p < 0.001, Table 5).

**Table-3.** Distribution of antimicrobial susceptibility test, regardless of the cause of urinary tract infections in patients (2011-2012) second study.

| Antibiotic | Sensitive% | Intermediate% | Resistan%t |
|---|---|---|---|
| **ciprofloxacin** | 72/84 | 4/63 | 22/55 |
| **norfloxacine** | 68/82 | 6/46 | 24/74 |
| **ceftizoxime** | 62/43 | 7/88 | 29/7 |
| **cefotaxime** | 53/97 | 7/15 | 38/89 |
| **Nalidixic acid** | 36/31 | 10/72 | 52/98 |
| **Nitrofurantoin** | 29/66 | 25/59 | 44/77 |
| **Cefixime** | 29/2 | 17/4 | 53/42 |
| **Co.trimoxazol** | 26/06 | 9/1 | 64/85 |
| **Gentamycine** | 8/78 | 52/04 | 39/19 |

Highly effective antibiotics, Ciprofloxacin, Norfloxacilin, Ceftizoxime; Less effective antibiotics, co trimoxazole, cefixime.nalidixic acid, Nitrofurantoin.

**Table-4.**Comparing the microbial agents distribution between different age groups in 2ndstudy (2011-2012).

| Microbial agents | Age group 12≥% | Age group 13-60% | Age group 60<% | Total | p.value |
|---|---|---|---|---|---|
| **Ecoli** | 56/96 | 72/31 | 83/87 | 118 | 0/002 |
| **proteus** | 8/86 | 20 | 0 | 20 | |
| **klebsiella** | 12/65 | 1/54 | 3/22 | 12 | |
| **streptococcus** | 3/8 | 3/07 | 9/68 | 8 | |
| **Enterobacter** | 6/32 | 1/54 | 3/22 | 7 | |
| **Citrobacter** | 5/06 | 1/54 | 0 | 5 | |
| **pseudomonas** | 6/32 | 0 | 0 | 5 | |
| **Total** | 100 | 100 | 100 | 175 | |

**Table-5.** Comparing antibiotic resistance between Ecoli and Non Ecoli in 2ndstudy.

| Antibiotics | Antibiotic resistant % for Ecoli | Antibiotic resistant % for NonEcoli | P-value |
|---|---|---|---|
| **Cefixime** | 52 | 55.7 | 0.786 |
| **Gentamycine** | 37 | 43.85 | 0.484 |
| **Nitroforantoien** | 31 | 73.21 | <0.001 |
| **Ciprofloxacine** | 25 | 25 | 0.832 |
| **Nalidixic acid** | 53 | 52.94 | 0.872 |
| **Cotrimoxazol** | 70 | 54.54 | 0.073 |
| **Cefotoxime** | 37.5 | 42.10 | 0.774 |
| **Ceftizoxime** | 28 | 33.33 | 0.603 |
| **Norfloxacin** | 24.5 | 25 | 0.841 |

When evaluating trends in antibiotic resistance between the two study periods regardless of bacterial species, a significant increase in resistance rates for most commonly prescribed antibiotics was observed, with the exception of co-trimoxazole, for which resistance remained stable (p = 0.362) (Table 6).

**Table-6.**Comparing antibiotic resistance between two studies regardless of etiologic agents.

| Antibiotics | Antibiotic resistant % in first study(435 samples) | Antibiotic resistant % in second study(175 samples) | p-value |
|---|---|---|---|
| **Cefixime** | 5.13 | 53.42 | <0.001 |
| **Gentamycine** | 9.50 | 39.19 | <0.001 |
| **Ceftizoxime** | 8.44 | 29.7 | <0.001 |
| **Cotrimoxazol** | 60 | 64.85 | 0.362 |
| **Nalidixic acid** | 11.26 | 52.98 | <0.001 |
| **Chloramphenicol** | 29.74 | - | |
| **Nitroforantoien** | 12.63 | 44.77 | <0.001 |
| **Amikacine** | 9.14 | - | |
| **Cephalotin** | 58.71 | - | |
| **Ampicillin** | 84.39 | - | |
| **Amoxicillin** | 93.83 | - | |
| **Ciprofloxacin** | 7.77 | 22.55 | <0.001 |
| **Cefotoxime** | - | 38.89 | |
| **Norfloxacin** | - | 24.74 | |

## 4- DISCUSSION

The present study investigated the antibiotic susceptibility profiles of uropathogens isolated from pediatric patients with UTIs in southern Iran. Escherichia coli consistently emerged as the predominant pathogen across both study periods. The antimicrobial resistance data serve as a valuable benchmark for regional and international comparisons.

A significant rise in antibiotic resistance was observed between the two study periods, highlighting the need for careful empirical treatment choices in pediatric UTIs. Notably, resistance to nitrofurantoin was significantly lower in E. coli isolates compared to other pathogens in the second phase ($p < 0.001$). Resistance to most antibiotics increased over time, except for co-trimoxazole, which remained relatively stable (60% in the first period vs. 64.85% in the second). These findings challenge traditional guidelines recommending TMP-SMX and ampicillin as first-line agents, as current resistance rates render them ineffective for community-acquired UTIs in this region.

Comparable findings have been reported in other Iranian studies. In Mashhad, Ismaili et al. noted a female predominance (86.1%) in pediatric UTIs and identified E. coli (74.1%) as the leading pathogen, followed by Klebsiella (7.2%) and Proteus (6%). High sensitivity (>90%) was observed for ciprofloxacin, amikacin, cephalotin, cefixime, cefotaxime, nitrofurantoin, and gentamicin, while only 3.4% of isolates were resistant to co-trimoxazole (8). Similarly, Mashouf et al. found E. coli and Klebsiella to be the most prevalent, with Klebsiella strains showing greater resistance to ampicillin, co-trimoxazole, and nalidixic acid (9).

A Brazilian study by Biondi et al. revealed E. coli as the primary pathogen (77%) during 1986–1989 and 2004–2005, with resistance rates of 55% for ampicillin and 51% for TMP-SMX. In contrast, resistance to nitrofurantoin, nalidixic acid, and cephalosporins remained low. TMP-SMX resistance increased significantly over time ($p = 0.03$), and nitrofurantoin resistance was notably higher among non-E. coli isolates, aligning with our findings (10).

A Turkish study reported E. coli (87%) and Klebsiella (10%) as the most common pathogens. Due to high resistance rates, ampicillin, co-trimoxazole, and amoxicillin were discouraged, while amikacin and nitrofurantoin were recommended, particularly for older children (11). Another prospective study in Istanbul confirmed E. coli (81.7%) as the leading uropathogen, with significant resistance to ampicillin (85%) and TMP-SMX (42.9%). Although resistance to nitrofurantoin and cefixime was relatively low, the authors cautioned against their empirical use (12).

In the UAE, a four-year surveillance reported rising resistance, particularly to nitrofurantoin (from 5.4% to 35.6%), while nalidixic acid resistance declined from 27.8% to 21% (13). A retrospective analysis from Taiwan (1991–2005) found an increase in ampicillin resistance, while resistance to gentamicin and cephalosporins remained stable. The study endorsed parenteral first-generation cephalosporins, gentamicin, and oral nitrofurantoin as suitable treatments (14).

The 2009 U.S. Surveillance Network study (n = 25,418) confirmed E. coli as the most common uropathogen, with relatively high TMP-SMX resistance (24%) and very low nitrofurantoin resistance (<1%). Over time, TMP-SMX resistance rose in both males (23% to 31%) and females (20% to 23%), prompting a shift towards cephalosporins and nitrofurantoin for empirical therapy (15).

A recent review also identified third-generation cephalosporins (e.g., ceftriaxone, cefotaxime) as first-line options for febrile UTIs, often used with aminoglycosides like gentamicin for broader coverage. Due to increasing resistance, oral agents like amoxicillin-clavulanate and TMP-SMX are no longer recommended for empirical use in many regions (4).

Alkhawaldeh et al., Wenzler et al., and Ahmed et al. highlighted the most commonly prescribed antibiotics for empiric treatment of pediatric UTIs, including third-generation cephalosporins, amoxicillin-clavulanate in low-resistance areas, and TMP-SMX only when susceptibility is confirmed (5–7). Alsaywid et al. emphasized selecting antibiotics based on patient age, symptom severity, clinical condition, infection site, and local resistance patterns, recommending cefixime, cefpodoxime, and cefdinir for mild-to-moderate infections. Due to widespread resistance, amoxicillin-clavulanate, TMP-SMX, and amoxicillin are no longer suitable for first-line treatment (3).

Maringhini et al. noted a rise in ESBL-producing pathogens in developing countries, particularly in children with prior antibiotic use. They recommended broader-spectrum agents, such as carbapenems, in complicated or resistant cases, while cefixime and ceftriaxone remained viable for uncomplicated infections (16).

## 5- LIMITATIONS AND FUTURE DIRECTIONS

Several limitations merit consideration. First, the study excluded information on urinary tract anomalies, which can influence infection recurrence and pathogen profiles, limiting generalizability to children with complicated UTIs. Second, infection frequency and radiological data were not collected, which would have provided a more comprehensive clinical picture. Third, urine sample collection in infants was performed using urine bags, which are prone to contamination; more accurate methods such as catheterization or suprapubic aspiration are often declined by parents during outpatient visits. Fourth, although the study compared two time periods, the most recent data are from 2010–2011. Given the evolving nature of antimicrobial resistance, more recent studies are essential to accurately capture current patterns.

Future studies should incorporate detailed clinical and radiological parameters, employ more reliable sampling techniques for infants, and conduct ongoing antimicrobial surveillance with up-to-date methodologies to inform regional empirical treatment guidelines.

## 6- CONCLUSION

The rise in antimicrobial resistance among pediatric uropathogens, particularly to co-trimoxazole, cefixime, nalidixic acid, and nitrofurantoin, complicates empirical therapy in southern Iran. However, there is low resistance to parenteral agents such as gentamicin, third-generation cephalosporins (e.g., cefotaxime, ceftizoxime), and ciprofloxacin, which supports their use in severe or complicated infections. Routine culture and sensitivity testing is essential for guiding treatment and curbing antibiotic resistance. Continuous local surveillance is necessary to adapt empirical therapy protocols to evolving resistance patterns.

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