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Etiology and Antimicrobial Susceptibility Pattern of Pathogenic Bacteria in Children Subjected to UTI

A Referral Hospital-Based Study in Northwest of Iran

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Abstract: Urinary tract infection (UTI) is 1 of the most common bacterial diseases in children with a considerable resistance to antimicrobials.

This 5 years prospective study was carried out to determine the frequency of isolation and antimicrobial resistance patterns of uropathogens among children subjected to urine culture at Tabriz Children Educational-Health Care Center, in the northwest of Iran. Organisms were isolated using standard culture techniques.

Frequency of UTI among children examined by urine culture was 3.6%. The isolated bacteria were *Escherichia coli* (71.4%), followed by *Klebsiella* spp. (9.6%), *Enterococcus* spp. (6.4%), *Pseudomonas aeruginosa* (4.2%), *Serratia* spp. (4.2%), and *Enterobacter* spp. (4.2%). *E coli* resistance levels were 11% for nitrofurantoin, 15% for ciprofloxacin, 25% for nalidixic acid, and 30% to 75% for amikacin, gentamicin, ceftriaxone, ceftizoxime, cefotaxime, and co-trimoxazole. Among the tested antibiotics, ciprofloxacin, showed the highest activity (100%) against *Klebsiella* and *P aeruginosa* isolates followed by amikacin, nalidixic acid, and gentamicin.

Overall, the highly active antibiotic against Gram-negative and Gram-positive organisms was amikacin and then ciprofloxacin. On the other hand, the empirical initial therapy with co-trimoxazole and third-generation cephalosporins would be inadequate for more cases of UTI in the study area. Moreover, susceptibility testing should be carried out on all clinical isolates, and the empirical antibiotic treatment changed accordingly.

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Abbreviations: AN = amikacin, CLSI = Clinical and Laboratory Standards Institute, CP = ciprofloxacin, CRO = ceftriaxone, CT = ceftizoxime, CTX = cefotaxime, FD = nitrofurantoin, GM = gentamicin, hpfh = igh power field, MDRm = ultiple drug resistance, NA = nalidixic acid, SXT = co-trimoxazole, UTIu = rinary tract infection.

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INTRODUCTION

Urinary tract infection (UTI) is the most common serious bacterial infection in infants and children.[1,2] UTI is an infection of the lower urinary tract, the upper urinary tract, or both.[3] It is acquired by an estimated 3% to 5% of girls and 1% of boys in childhood.[4] Boys are more susceptible during the first year of life; thereafter the incidence is substantially higher in girls.[5,6]

Rapid diagnosis and prompt antimicrobial treatment are required to minimize renal scarring and progressive kidney damage.[7] Reporting of antimicrobial susceptibility testing of the urinary tract is usually achieved 48 hr following sampling, and therefore, in the majority of UTI cases, the treatment decision is empirical, being influenced by available data reflecting antibiotic resistance. Since the initiation of antimicrobial therapy in UTI is empirical, knowledge of the antimicrobial resistance patterns of common uropathogens in each region is essential to provide clinically appropriate therapy. Hence, there exists a great need for antimicrobial resistance surveillance at the local, national, and international levels.[8] Unfortunately, data regarding the isolation frequency and antimicrobial susceptibility patterns of endemic uropathogens are scarce in Iran.

The aim of the present study was to determine the frequency of isolation and antimicrobial resistance patterns of uropathogens among children subjected to urine culture at the Tabriz Children hospital, a teaching and referral hospital in the northwest of Iran.

MATERIALS AND METHODS

Study Design

The prospective study was carried out with uropathogenic bacteria isolated from outpatients and inpatients children with UTI submitted to Tabriz Children Educational-Health Care Center—the only Children's referral hospital in northwest of Iran—from April 2010 to March 2014. During the study period, a total of 25,811 urine samples were tested.

UTI was considered by the presence of a pure bacterial growth of $>10^5$ colony forming units/mL by properly collected urine specimen (suprapubic aspiration, transurethral catheterization or clean-catch midstream voiding) in children with urinary symptoms such as fever $\geq 38^{\circ}$C, chills, frequency, urgency, dysuria, suprapubic, and/or flank tenderness, pyuria (defined as ≥ 5 leukocytes/hpf), and in neonates clinical evidences of sepsis.[5,7] Data about age and sex of patients were also collected. To use the data from this study, parents have been allowed.

Isolation and Identification of Bacteria

One calibrated loopful (0.01 mL) of well-mixed urine samples were cultured on 5% blood agar plates (Merck KGaA,

Tabriz, Germany) and incubated aerobically at 37°C for 24 hr. The isolates obtained were further processed as per the standard procedures to identify the pathogens.[9]

Antimicrobial Susceptibility Testing

The antibiotic susceptibility of urine isolates bacteria was determined by the disc diffusion method (Kirby–Bauer) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations.[10]

The sensitivity of isolates to 9 antibiotics, commonly used in the UTIs, was determined. The antibiotics used were amikacin (AN), nalidixic acid (NA), ciprofloxacin (CP), gentamicin (GM), co-trimoxazole (SXT), cefotaxime (CTX), ceftriaxone (CRO), ceftizoxime (CT), and nitrofurantoin (FD). For statistical analysis, bacteria with intermediate susceptibility were considered as resistant.

Multiple drug resistance (MDR) was defined as resistance in an isolate to 3 or more unrelated drugs tested.[11] *Escherichia coli* ATCC 25922 was used as a control strain. Muller–Hinton agar medium for antimicrobial testing and antibiotic discs were obtained from Hi-media Laboratories, Mumbai, India.

RESULTS

Of the 25,811 children were included, 940 had UTI. Sixty-five (69%) of them were girls and 29 (31%) were boys, with mean age of 4.5 years and 4.2 months for them, respectively. Frequency of UTI among the children examined by urine culture was 3.6% (n = 940). Only 79% of our cases showed a high white blood cell count (pyuria) in urinalysis. Furthermore, nitrites and leukocyte esterase and were positive in only 85% and 67% of our patients, respectively. Approximately two-thirds of patients (69%) had all 3 positive findings present on urinalysis.

The most frequently isolated bacteria included *E coli* (71.4%), *Klebsiella* spp. (9.6%), and *Enterococcus* spp. (6.4%) other than *Serratia* spp., *Enterobacter* spp., and *Pseudomonas aeruginosa* (4.2% each 1). Of the total of 940 confirmed UTIs, 550 (58.5%) was in outpatients and 390 (41.5%) from inpatients.

E coli isolates were resistant to co-trimoxazole in 75.3% of cases, followed by cefotaxime (48.1%), ceftizoxime (43.2%), ceftriaxone (42%), gentamicin (32%), amikacin (30%), nalidixic acid (24.6%), ciprofloxacin (14.8%), and nitrofurantoin (11.1%). Among the antibiotics used for susceptibility testing of the isolates, ciprofloxacin, nitrofurantoin, and nalidixic acid showed the highest activity (100%) against *Klebsiella* spp. isolates and gentamicin, amikacin as well as ciprofloxacin (100%) against *P aeruginosa*.

The antibiotic susceptibility pattern of other isolates is shown in Table 1 and Figure 1.

As indicated in Table 1, alarming antimicrobial resistance was seen in *Serratia* and *Enterobacter* isolates against the most antibiotics tested.

MDR of the uropathogenic isolates was analyzed and 48.2% of *E coli* isolates, 50% of *Klebsiella* spp. isolates, and 100% of *P aeruginosa*, *Serratia* spp., *Enterobacter* spp. as well as *Enterococcus* spp. isolates.

As is shown in Table 2, 89%, 87%, and 81% of MDR isolates were resistant to cefotaxime (CTX), ceftizoxime (CT), and ceftriaxone (CRO) as well as co-trimoxazole (SXT), respectively.

The antibiotic resistance pattern of the isolated uropathogens was also evaluated by patient sex. Isolated bacteria in male patients tended to be more resistant to tested antibiotics (data not showed); however, this difference did not reach statistical significance.

DISCUSSION

To the best of our knowledge, there was no comprehensive study before this study in Northwest of Iran to estimate the most common uropathogens and their resistance pattern in pediatrics.

This study showed the prevalence of isolation and antibiotic resistance pattern of uropathogenic bacteria in a referral pediatric hospital in Tabriz in the northwest of Iran, during 5 years period. According to our findings, frequency of UTI among children was 3.6% and the girls were affected by UTI more often than boys, but boys were significantly younger than girls; these results are similar to the most of previous reports.[7,12] However, in a study from southern Iran, about 7.9% of children were positive for UTI and UTI was reported more frequent in male patients possibly because the most of their participants were neonates and male infants are usually infected more than girls during the first 3 months of their life.[13]

As indicated in the previous studies,[13–16] *E coli* and *Klebsiella* spp. have also been isolated as the most common pathogens responsible for UTI among children. However, *E coli* was the most frequent organism isolated (71.4%). This is similar to results of investigations in other countries.[17,18]

Uropathogenic *E coli* isolates, showed the most resistance to co-trimoxazole (75.3%) and then to third-generation cephalosporins (42% to 48%), used to testing.

As indicated, the most strains of isolated *E coli* were resistant to co-trimoxazole. Unfortunately, this is a significant

TABLE 1. Antimicrobial Susceptibility (%) of Isolated Uropathogenic Bacteria

Antibiotics	Disc Content, μg/mL	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Serratia* spp.	*Enterobacter* spp.	*Enterococcus* spp.
Co-trimoxazole	25	24.7	50	0.0	75	0.0	0.0
Gentamicin	10	67.9	50	100	50	50	0.0
Amikacin	30	70.3	75	100	75	100	75
Nitrofurantoin	300	88.9	100	0.0	0.0	0.0	100
Nalidixic acid	30	75.3	100	50	50	0.0	0.0
Ceftriaxone	30	58	50	25	0.0	50	0.0
Cefotaxime	30	51.9	37.5	0.0	0.0	0.0	0.0
Ceftizoxime	30	56.8	50	25	0.0	25	0.0
Ciprofloxacin	5	85.1	100	100	100	100	25

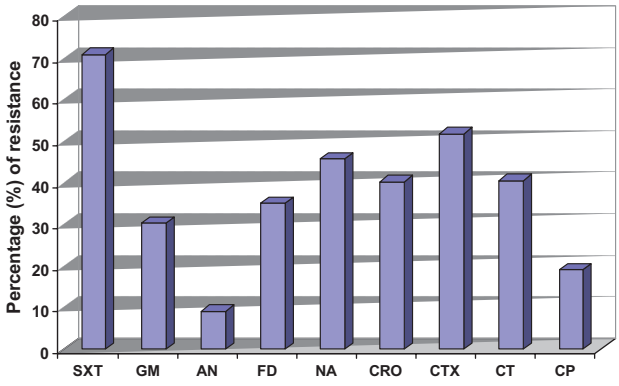

FIGURE 1. Overall resistance of urinary pathogens to tested antibiotics.

problem in the northwest of Iran and has also been reported by other laboratories in Iran.[19,20] On the other hand, greater concern is that increasing resistance is eroding the usefulness of third-generation cephalosporins, aminoglycosides and nalidixic acid for the management of UTI in our region. However, it has also been shown in previous studies of Iran, for example, Mirsoleymani et al[13] have reported a high rate resistance to third-generation cephalosporins including ceftriaxone and cefixime from southern Iran.

Our observation supports previous studies indicating that nitrofurantoin still exhibited low resistance rates in the most countries investigated.[8,21–23] In this study, a considerable portion of the uropathogens, especially *E coli* isolates, were found to be sensitive to nitrofurantoin (Tables 1 and 2). This may have been due to the fact that this antibiotic has not been widely used in treating UTI cases. Therefore, nitrofurantoin may be a good option for the empirical treatment of UTI in the northwest of Iran. However, it should not be used to treat UTI in febrile infants because it is excreted in the urine and do not achieve therapeutic concentrations in the bloodstream.[7]

Overall, our findings showed that the highly active antibiotic against Gram-negative and Gram-positive uropathogens was amikacin and then ciprofloxacin with susceptibility rates of 90.9% and 80.9%, respectively (Figure 1). Ciprofloxacin was also the most effective antimicrobial against Gram-negative uropathogens with susceptibility rate of 97.0% (results not showed).

Among the total isolates that were tested against all 9 antibiotics, the half (50%) of them were resistant to 3 or more antibiotics and considered MDR. As indicated in Table 2, 89%, 87%, and 81% of MDR isolates were resistant to cefotaxime, ceftizoxime, and ceftriaxone as well as co-trimoxazole, respectively. Overall, the evaluation of antimicrobial resistance among MDR isolates, indicated a high resistance against all antibiotics tested, except nitrofurantoin and ciprofloxacin. Our finding about nitrofurantoin is similar to previous report from Turkey.[8] However, the multidrug-resistant isolates ratio in that study was lower than in our observation (24.5% vs 50%).

TABLE 2. Resistance of MDR Isolates to Antimicrobials

Antibiotics	SXT	GM	AN	FD	NA	CRO	CTX	CT	CP
Resistance*, %	81	62	55	19	38	81	89	87	25.5

AN = amikacin; CP = ciprofloxacin; CRO = ceftriaxone; CT = ceftizoxime; CTX = cefotaxime; FD = nitrofurantoin; GM = gentamicin; MDR = multiple drug resistance; NA = nalidixic acid; SXT = co-trimoxazole.

* The percentage is calculated among resistant isolates in all species.

In this study, higher resistance rates to all antibiotics tested with the exception of amikacin, ciprofloxacin, and nitrofurantoin may be explained by high and uncontrolled usage of these antimicrobial agents, especially third-generation cephalosporins, during the past few years in our country. Unfortunately, these antibiotics were widely prescribed not only for UTI but also for other infections in Iran.

In conclusion, in the northwest of Iran including Tabriz, children, who are receiving prophylactic antibiotics and are admitted to the hospital for a UTI, are often infected with an organism that is resistant to third-generation cephalosporins and co-trimoxazole. Therefore, a larger number of these children may be empirically treated with ciprofloxacin, nitrofurantoin, or amikacin.

However, it is necessary to regular monitoring of the usage of antibiotics in order to make reliable information available for optimal empirical therapy for children with UTIs. Moreover, regular reporting of the antimicrobial susceptibility patterns of uropathogens can be a useful guide for appropriate initiating empiric therapy of UTIs in children.

Although our study was the first investigation about epidemiology of children UTI in northwestern part of Iran, nonetheless, we could investigate the uropathogens from other parts of Iran to find more accurate and more comprehensive results about etiology and their antimicrobial resistance pattern and it can be our limitation of this research.

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