

ANTIMICROBIAL RESISTANCE PATTERNS OF UROPATHOGENS AMONG CHILDREN IN ISTANBUL, TURKEY

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Abstract. Urinary tract infections are a common cause of end-stage renal disease in Turkey. This prospective study investigated the antibiotic resistance patterns of uropathogens in order to recommend appropriate therapeutic protocols for children with urinary tract infections in Istanbul, Turkey. Between October 2007 and October 2008, children presenting with a first episode of urinary tract infection to a pediatric outpatient clinic were enrolled in the study. Urine samples were cultured, and antimicrobial susceptibility testing was performed. Children with proven urinary tract infections underwent imaging studies where available. A total of 126 children with a first episode of community-acquired urinary tract infection were enrolled in the study. The median age was 60.6 months; 84.1% of the children were female. Of the 126 urine samples, *Escherichia coli* was the leading uropathogen (81.7%), followed by *Proteus* spp (7.1%), *Klebsiella* spp (4.0%), *Enterococcus* spp (3.2%), *Enterobacter* spp (2.4%), and *Pseudomonas* spp (1.6%). Among the isolated uropathogens, resistance to ampicillin (85.0%), amoxicillin-clavulanate (73.8%), cefazolin (37.3%) and trimethoprim-sulfamethoxazole (42.9%) was remarkable. A large number of *Enterococcus* species were resistant to all antimicrobial agents except vancomycin. A country-based evaluation of antibiotic susceptibility is needed to modify antibiotic treatment. Resistance to antimicrobial agents commonly used to treat urinary tract infections (nitrofurantoin, cefixime) is less a problem than resistance to other antimicrobials (aminopenicillins, cephalosporins, trimethoprim-sulfamethoxazole) frequently prescribed for other indications.

Keywords: antibiotic resistance, uropathogens, children, Turkey

INTRODUCTION

Urinary tract infections (UTI) are common bacterial disorders in childhood. Approximately 3-5% of girls and 1-2% of

boys develop UTI (Elder, 2000). Vesicoureteral reflux (VUR)-associated UTIs are reported as an important cause of end-stage renal disease in Turkey (Bakkaloglu *et al*, 2005). Since initial antibiotic treatment is empirical, knowledge regarding the causative organisms and their sensitivity patterns is mandatory for effective treatment. Geographic variations and different antibiotic prescribing practices

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result in varying patterns of antimicrobial resistance among urinary tract pathogens. A country-based evaluation of antibiotic susceptibility was needed to modify antimicrobial treatment.

This prospective study investigated the types and antibiotic resistance patterns of uropathogens among children with UTI in Istanbul, Turkey.

MATERIALS AND METHODS

Children younger than 14 years old with a culture-proven UTI evaluated at pediatric outpatient clinics from October 2007 to October 2008, were prospectively included in the study. Patients were excluded if they used a prophylactic antibiotic for a known urinary tract malformation and/or had a previous history of UTI and/or used catheters and/or received outpatient antibiotics prior to admission.

Children with a suspected UTI had urinalysis analyzed within 30 minutes of providing the specimen, and if the dipstick or microscopy was abnormal, the urine was cultured. The presence of 5 or more leukocytes per high power field of urine using a x40 objective was considered as pyuria. Children with pyuria were started on antibiotics before the culture results were obtained following the guidelines of the WHO Pocketbook of Hospital Care for Children (WHO, 2005). Children below 3 months of age and those with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$), vomiting or flank pain were examined with a complete blood count, C-reactive protein (CRP), and erythrocyte sedimentation rate. Ultrasonographic (USG) examination was performed in all patients. Further imaging studies, a voiding cystourethrogram (VCUG), and technetium-99m-labeled

dimercaptosuccinic acid (DMSA) were performed on children with pathological ultrasonographic results and/or diagnosed as having pyelonephritis and/or were under 12 months old. Children with clinical deterioration, leukocytosis, age less than 3 months, or were suspected to have pyelonephritis or urosepsis were hospitalized, while the rest were followed up in the outpatient clinic.

Urine samples were obtained by transurethral catheterization in non-toilet trained children and midstream clean-catch urine in toilet trained children. All samples were cultured on blood and eosin-methylene blue agar plates with a standard loop. The plates were incubated at 37°C for 24 hours, and bacteria were identified using standard methods. A UTI was defined as $\geq 10^5$ colony forming units/ml of midstream urine or $\geq 10^4$ colony forming units/ml of urine obtained by transurethral catheterization.

Antimicrobial susceptibility testing was performed by the disc diffusion technique according to the guidelines of the National Committee of Clinical Laboratory Standards. All bacteria were tested against ampicillin (AMP), amoxicillin-clavulanate (AMC), cefazolin, cefuroxime, ceftriaxone, cefixime, cefepime, aztreonam, imipenem, gentamicin, ciprofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole (TMP-SMX). *Enterococcus* species were also tested against vancomycin.

Statistical analysis was performed using SPSS software, version 10.0. Data were recorded as mean (standard deviation) with a p -value < 0.05 indicating significance. Statistical analysis was performed with the Pearson's χ^2 test. Comparisons were analyzed using 95% confidence intervals.

Table 1
Antibiotic resistance patterns of isolated uropathogens.

	Isolated uropathogens <i>n</i> (%)						Total resistance (%)
	<i>E.coli</i>	<i>Proteus</i> spp	<i>Klebsiella</i> spp	<i>Pseudomonas</i> spp	<i>Enterobacter</i> spp	<i>Enterococcus</i> spp	
	103 (81.7)	9 (7.1)	5 (4.0)	2 (1.6)	3 (2.4)	4 (3.2)	
Antimicrobial resistance <i>n</i> (%)							
Ampicillin	87 (84.5)	8 (88.9)	5 (100.0)	2 (100.0)	2 (66.7)	3 (75.0)	0.783
AMC	75 (72.8)	8 (88.9)	4 (80.0)	2 (100.0)	1 (33.3)	3 (75.0)	0.485
Cefazolin	36 (35.0)	3 (33.3)	3 (60.0)	2 (100.0)	0 (0.0)	3 (75.0)	0.110
Cefuroxime	20 (19.4)	0 (0.0)	2 (40.0)	2 (100.0)	0 (0.0)	3 (75.0)	0.002
Ceftriaxone	11 (10.7)	0 (0.0)	1 (10.0)	1 (50.0)	0 (0.0)	3 (75.0)	0.002
Cefixime	8 (7.8)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	3 (75.0)	0.001
Cefepime	8 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)	0.001
Aztreonam	10 (9.7)	0 (0.0)	2 (40.0)	2 (100.0)	0 (0.0)	3 (75.0)	0.001
Imipenem	14 (13.6)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)	0.017
Gentamicin	13 (12.6)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	0.261
Ciprofloxacin	5 (4.9)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	2 (50.0)	0.008
Nitrofurantoin	5 (4.9)	2 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0.205
TMP-SMX	41 (39.8)	5 (55.6)	1 (20.0)	1 (50.0)	3 (100.0)	3 (75.0)	0.169
Vancomycin	ND	ND	ND	ND	ND	0 (0.0)	ND

TMP-SMX, trimethoprim-sulfamethoxazole; ND, not done

Table 2
Age-related distribution of uropathogens.

Uropathogens	Age of patient in month		
	0-12	13-60 <i>n</i> (%)	>60
<i>E. coli</i>	17 (16.5)	34 (33)	52 (50.5)
<i>Proteus</i>	4 (44.4)	3 (33.3)	2 (22.2)
<i>Klebsiella</i>	2 (40)	2 (40)	1 (20)
<i>Pseudomonas</i>	1 (50)	0 (0.0)	1 (50)
<i>Enterobacter</i>	1 (33.3)	1 (33.3)	1 (33.3)
<i>Enterococcus</i>	1 (25)	2 (50)	1 (25)

RESULTS

A total of 126 children with a first episode of community-acquired UTI were enrolled in the study. The ages of the children ranged from 1 month to 14 years (168 months). The median age was 60.6 (44.1) months, and 84.1% of the children were female. The age-related distribution of the children was as follows: 20.6% were younger than 1 year, 33.4% were 13-60 months old, and 46% were older than 5 years. The signs and symptoms reported at the time of admission were fever (62.7%), dysuria (49.2%), abdominal pain (48.4%), vomiting (46.0%), nocturnal enuresis (23.8%), constipation (16.0%), and costovertebral angle tenderness (16.0%). Nocturnal enuresis was significantly higher in children older than 5 years ($p < 0.01$); the rest of the symptoms were not significantly different by age.

Of the 126 urine samples, *Escherichia coli* was the leading uropathogen (81.7%), followed by *Proteus* spp (7.1%), *Klebsiella* spp (4.0%), *Enterococcus* spp (3.2%), *Enterobacter* spp (2.4%), and *Pseudomonas* spp (1.6%). The antibiotic resistance patterns of the isolated uropathogens and age-related distribution of the uropathogens

are reported in Tables 1 and 2. Among the isolated uropathogens, resistance to AMP (85.0%), AMC (73.8%), cefazolin (37.3%), and TMP-SMX (42.9%) was remarkable. A high proportion of *Enterococcus* spp was resistant to all antimicrobial agents except vancomycin.

Comparing antimicrobial resistance, there was no statistical difference among the resistance of causative agents to AMP, AMC, cefazolin, gentamicin, nitrofurantoin, and TMP-SMX ($p > 0.05$). However, cefuroxime and aztreonam resistance were statistically more common among isolated *Klebsiella*, *Pseudomonas*, and *Enterococcus* species ($p < 0.01$). Cefixime, cefepime and imipenem resistance were significantly more common among *Enterococcus* species ($p < 0.01$). In addition to the *Enterococcus* species, ceftriaxone resistance was significantly more common among *Pseudomonas* species and ciprofloxacin resistance was significantly more common among *Klebsiella* species ($p < 0.01$).

Forty-two patients underwent VCUG and DMSA scans, and VUR was detected in 11 (26.2%). Renal cortical defects were detected in 16 patients by the DMSA scan (38%). There was no difference in resistance of isolated uropathogens between

Table 3
Urinary tract infection related epidemiological and antibiotic resistant studies carried out in various countries among pediatric age groups (7).

Pathogen (%)	Younis 09 Jordan (N = 336) Apr 04-Dec 06	Rabasa 09 Nigeria (N = 529) Jan 06-Apr 07	Tseng 08 Taiwan (N = 368) Jan 91-Dec 05	Guidoni 08 Brazil (N = 100) Aug 04-Dec 05	Rai 08 Nepal (N = 538) Apr 07-Nov 07	Anatoliotaki 07 Greece (N = 262) Jan 00-Dec 04	Yuksel 06 Turkey (N = 165) Jan 03-Jan 04	Lutter 05 USA (N = 361) 1997-2001	Marcus 05 Israel (N = 175) Jan 01-Dec 02	Pape 04 Germany (N = 100) 2000-2002	Ghiro 02 Italy (N = 1,333) Jan 94-Dec 98
<i>E. coli</i>	58	65	81	77	93.3	79.4	87	87	60	47	89.9
<i>Klebsiella</i>	15.2	15	6.5	3	1.5	8.4	10	3	12.6	4	2.1
<i>Proteus</i>	9.8	10	3.5	15	2.3	4.2	ND	ND	4.5	8	3.6
<i>Pseudomonas</i>	15	ND	ND	ND	0.7	1.5	ND	2	9.7	5	1.4
<i>Enterococcus</i>	4.8	ND	6	1	ND	1.9	ND	2	8	23	1.3
<i>S. aureus</i>	0.3	10	ND	ND	0.7	ND	ND	ND	ND	ND	ND

Antibiotics	Antibiotic resistance (%)	
	<i>E. coli</i>	<i>E. coli</i>
Ampicillin	73.3	86.8
Amoxicillin-clavulanate	ND	69.3
Cephalexin	41.1	81.3
Cefuroxime	ND	ND
Ceftriaxone	11.8	ND
Cefotaxime	ND	ND
Gentamicin	43.2	12.6
Ciprofloxacin	3.8	5
Nalidixic acid	29.3	86.7
Nitrofurantoin	13.3	ND
TMP-SMX	59.5	74.5

Antibiotics	Antibiotic resistance (%)	
	<i>E. coli</i>	<i>E. coli</i>
Cephalexin	28.9	96.9
Cefuroxime	ND	ND
Ceftriaxone	ND	61.6
Cefotaxime	ND	61.3
Gentamicin	19.8	52.2
Ciprofloxacin	ND	59.7
Nalidixic acid	ND	78.2
Nitrofurantoin	9	34.2
TMP-SMX	46.6	76.9

Antibiotics	Antibiotic resistance (%)	
	<i>E. coli</i>	<i>E. coli</i>
Cephalexin	26	1 st generation
Cefuroxime	6	48
Ceftriaxone	16	33
Cefotaxime	15	ND
Gentamicin	12	ND
Ciprofloxacin	4	ND
Nalidixic acid	14	ND
Nitrofurantoin	7	ND
TMP-SMX	38	40

TMP-SMX, trimethoprim-sulfamethoxazole; ND, no data

patients with radiologically detected lesions and those with no lesions.

DISCUSSION

Urine cultures and antibiotic susceptibilities provide guidance for empirical antibiotic treatment of UTIs. Treatment must start before urine cultures provide results in 24-48 hours. Knowledge of the predominant uropathogens in the age group of the patient and local bacterial susceptibility patterns affect the choice of antibiotics.

Urinary tract infection is a leading cause of chronic renal failure in Turkey (Bakkaloglu *et al*, 2005). The frequency of *E. coli* in childhood UTIs ranges from 54% to 89% in different regions of Turkey (Gökçe *et al*, 2006; Yüksel *et al*, 2006; Catal *et al*, 2009).

Epidemiological and antimicrobial resistance studies carried out in various countries are shown in Table 3 (Ipek and Bozaykut, 2010). As expected, the most common causative agent was *E. coli*, which has been isolated at rates varying between 47% and 93.3%, followed by *Klebsiella* spp (1.5-15.2%), *Pseudomonas* spp (0.7-15%), *Proteus* spp (2.3-15%), and *Enterococcus* spp (1-23%). A high rate of resistance against AMP and TMP-SMX was found in all the studies. Rabasa *et al* (2009) and Rai *et al* (2008) attributed their findings of high-level resistance to AMP and TMP-SMX to the habit of self-medication and sales of non-prescribed medicine in their countries, similar to our country. Tseng *et al* (2008) studied antibiotic resistance in 1991-2000 and 2001-2005 in Taiwan, and found increased resistance to all antibiotics over time, but only AMP resistance increased significantly ($p < 0.05$). In contrast, Guidoni *et al* (2008) found no change in antibiotic resistance between

1986-1989 and 2004-2005, other than TMP-SMX was more resistant during the second period. Resistance to TMP-SMX, often used in pediatric UTIs, is common in almost all studies. Resistance to TMP-SMX in infections among toddlers and preteens is more common than among adults, and the resistance decreases as age increases (Gaspari *et al*, 2003; Abelson Storby *et al*, 2004).

Our findings of 84.9% AMP-resistance, 73.8% AMC-resistance, and 42.8% TMP-SMX-resistance among uropathogens underscores the fact that AMP, AMC, and TMP-SMX should no longer be used for empiric treatment. Uropathogens were also highly resistant to cefuroxime and cefazolin similar to the findings of Gökçe *et al* (2006). High resistance rates against AMP, AMC, 1st generation cephalosporins and TMP-SMX have not only been reported in Turkey but in other international studies (Pape *et al*, 2004; Anatoliotaki *et al*, 2007). Gentamicin sensitivity, which has varied among studies, was reported to be as low as 2.4% by Ghiri *et al* (2002) and as high as 24.9% by Wu *et al* (2004).

Since *E. coli* is the leading cause of UTI, empiric treatment should be based on *E. coli* susceptibility patterns. For our region, nitrofurantoin, cefixime, and gentamicin seem the best choices for initial therapy. In the published literature, *E. coli* has generally been reported to have a low resistance rate (0-6%) to nitrofurantoin, except for the findings of Gökçe *et al* (2006) (15%) and Al-Mardeni *et al* (2009) (20.7%). *E. coli* resistance to other antibiotics, such as AMP, TMP-SMX, gentamicin, and ceftriaxone, was also remarkably high in the Al-Mardeni *et al* (2009) study from Jordan. This study also recommended nitrofurantoin for empiric treatment of uncomplicated UTIs, due to the low resistance rate. Abelson Storby *et al* (2004) from

Sweden, also suggested nitrofurantoin as a good initial empiric treatment for uncomplicated UTIs because of its low level of resistance (<2%).

Aminoglycosides still seem effective for most isolated uropathogens. Studies from Germany, Greece, Jordan, and Tunisia have also reported low *E. coli* resistance rates to aminoglycosides (Bouallégué *et al*, 2004; Pape *et al*, 2004; Gökçe *et al*, 2006; Anatoliotaki *et al*, 2007; Al-Mardeni *et al*, 2009).

Increasing antibiotic resistance among urinary tract isolates is a worldwide problem. As the habit of uncontrolled antibiotic use plays an important role in the emergence of resistant isolates, current interventions aimed at reducing unnecessary antibiotic prescribing, especially in underdeveloped and developing countries, must be supported. It is crucial to establish an international surveillance system to assess uropathogen frequencies and resistance patterns among pediatric patients.

In conclusion, prevention of further morbidity in pediatric UTI is related to initiating treatment with a correct first-line antimicrobial agent. Countries should establish systems for evaluating local resistance rates of uropathogens, and existing empiric treatment protocols should be reviewed periodically to determine changing patterns of antibiotic sensitivity.

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Antimicrobial resistance patterns of uropathogens among children in Istanbul, Turkey. Southeast Asian J Trop Med Public Health. 2011;42:355-62. 20. Yolbas I, Tekin R, Kelekci S, Tekin A, Okur MH, Ece A, et al. Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. Eur Rev Med Pharmacol Sci. 2013;17. Antibiotic resistance patterns of *Escherichia coli* strains isolated from urine cultures in Turkey: a meta-analysis. Mikrobiyol Bul. 2013; 47:603-18. 24. Schlager TA. Urinary tract infections in infants and children. Infect Dis Clin N Am. The prevalence of uropathogens among children attending hospitals in all the study areas according to urine samples culture, showed that four Gram-negative organisms were isolated and they are *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas* spp. and *Proteus mirabilis* (Figure 1(a)). *Klebsiella pneumoniae*, was the most predominant organism (88.8%) and it was more isolated from male children, followed by *E. coli*. The resistant patterns of the isolates to some of the antibiotics show that the first line of antibiotics for treatment of UTI in children in these areas should be Ofloxacin and Gentamicin. The observed antibiotic resistant patterns may be due to long term use of some of these drugs over the years. Monitoring regional antibiotic resistance patterns of uropathogens are important for deciding suitable empirical antibiotics for urinary tract infections (UTIs) in children. This study aimed to investigate regional differences in antimicrobial susceptibility patterns of *E. coli* and *Klebsiella* spp. in children below 24 months old, diagnosed with their first episode of UTI, and to find factors associated with an increased risk for UTI caused by extended-spectrum β -lactamase (ESBL)-producing uropathogens. Impact of Antibiotic Prescribing Patterns on Susceptibilities of Uropathogens in Children below 24 Months Old. by. Ji Young Park. Antimicrobial resistance patterns of uropathogens among children in Istanbul, Turkey. Article. Full-text available. *Escherichia coli* was the most common causative agent both in total and among different age groups. Ampicillin had the highest resistance rate from all the pathogens isolated (63.8%), followed by piperacillin (51.8%) and trimethoprim-sulfamethoxazole (TMP-SMX; 48.6%). Cephalotin also had a high resistance rate (32.7%).