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Original Article

In vitro Antimicrobial Susceptibility of Urinary Tract Infection Pathogens in Children

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ABSTRACT

Aim: Urinary tract infection (UTI) is one of the most common bacterial infections in children. Empirical treatment is commenced according to the patient's characteristics and the antimicrobial susceptibility patterns in the region. Therefore, a determination of antimicrobial resistance patterns has a great importance in effective treatment. The aim of this study was to determine the pathogens which cause UTIs in patients admitted to a university hospital in Izmir and to determine their antimicrobial susceptibility pattern.

Materials and Methods: The files of patients aged between 0-18 years, followed up with a diagnosis of UTI, vesicoureteral reflux and neurogenic bladder in Ege University Faculty of Medicine Paediatric Nephrology Unit between February, 2013 and November, 2018 were retrospectively reviewed.

Results: A total of 1,126 positive urine cultures from 729 patients (65% female) were included in this study. Gram-negative pathogens constituted 88.2% of the cultures. *Escherichia coli* (*E. coli*) was the most commonly isolated bacteria with a prevalence of 59.1%, followed by *Klebsiella pneumonia* with 17.9%, and *Enterococcus faecalis* with 8.3% (n=93). Ampicillin, cefuroxime and trimethoprim-sulfamethoxazole with susceptibility rates of 18.6%, 39.6%, 49.0% respectively, constituted the highest resistant antimicrobials to *Enterobacteriaceae*. *Enterococcus* spp. showed the highest resistance to gentamycin with 50% resistance in tested cases. *Pseudomonas* spp. with 64.3% susceptibility showed the highest resistance to piperacillin-tazobactam.

Conclusion: This study revealed that bacterial resistance to commonly used antimicrobials in UTI is an important and challenging problem which requires planning.

Keywords: Antibiotic resistance, antimicrobial resistance, antimicrobial susceptibility, *E. coli*, paediatric, urinary tract infections, urine culture

Introduction

Urinary tract infection (UTI) is the most common bacterial infection in children under 2 years of age and also one of the most common bacterial infections in children (1). Although the numbers vary in studies conducted in different populations, UTI is common in males under one year of age, whereas it is common in girls above one year of age during their childhood (2,3). Empirical treatment is commenced according to the patient's age, antimicrobial susceptibility patterns in the region, the clinical condition of the patient

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and the presence of any underlying diseases (2,4). Therefore, the determination of antimicrobial resistance patterns by regional centre studies is of great importance in terms of effective treatment and the prevention of complications. The aim of this study was to determine the pathogens which cause UTIs in patients admitted to a university hospital in Izmir and to determine their antimicrobial susceptibility patterns.

Materials and Methods

Patients and Urine Samples

The outpatient clinic files of 729 patients aged between 0-18 years, followed up with a diagnosis of UTI, vesicoureteral reflux and neurogenic bladder in Ege University Faculty of Medicine Paediatric Nephrology Unit between February, 2013 and November, 2018 were retrospectively reviewed. The urine culture results of the patients treated with a diagnosis of UTI were evaluated. The urine cultures of those patients who were not considered to be clinically diagnosed with UTI and were not treated, were excluded from this study. The diagnosis of UTI was made with positive urine cultures accompanied by the presence of leukocytes and/or nitrite and/or leukocyte esterase in the urinalysis. The significance of the positive urine culture was also evaluated with the clinical findings in the patient's file. In those patients with no urinary control or those who were not toilet trained, urine samples were collected according to a standard hygiene protocol via urine bags. Catheterization or a clean catch method were used in cases of urine cultures which were thought to be contaminated. The urine samples were collected mid-stream in those who were toilet trained. Positive urine culture was accepted as $\geq 10^3$ CFU/mL samples taken with the catheterization method, $>10^4$ CFU/mL with the mid-stream or clean catch in patients with symptoms, or $>10^5$ in those without symptoms (1). All the information regarding the culture results and antimicrobial susceptibility for the isolated pathogens was recorded. Those with intermediate susceptibility to antimicrobials were included in the resistant group (5). Second urine cultures from the same patient within one month were not included in this study. The urine culture results which were studied in laboratories other than Ege University Faculty of Medicine Bacteriology Laboratory were not included in this study.

Ethical Consideration

Ethical approval for this study was obtained from the Medical Research Ethics Committee of Ege University Faculty of Medicine (approval no: 22-12.1T/16, date: 16.12.2022).

Antimicrobial Susceptibility Tests

The cultures of the urine samples were studied in Ege University Faculty of Medicine Bacteriology Laboratory of the Medical Microbiology Department. MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry) was used to identify the bacteria. The antimicrobial susceptibilities of these isolates were determined by VITEK 2 (bioMerieux, France) and EUCAST clinical breakpoints were used.

Statistical Analysis

For the evaluation of the study data, the SPSS (Statistical Package for the Social Sciences, version 22.0 for Windows, SPSS® Inc., Chicago, IL, USA) statistical analysis program was used. Frequencies, and means ± standard deviations of the data are provided.

Results

A total of 1,126 positive urine cultures were included in this study. Two hundred and seventy-four (24.3%) of the urine samples were taken via urine bag, 762 (67.7%) of them were obtained via mid-stream with the clean catch method and 90 (8%) with a catheter. Among the 729 cases, 64.7% of the samples were obtained from female and 35.3% from male patients. The mean infection time was 56±53 months. Most of the cultures were gram negative with a percentage of 88.2% (n=993). *Escherichia coli* (*E. coli*) was the most commonly isolated bacteria with a prevalence of 59.1% (n=666) followed by *Klebsiella pneumonia* with 17.9% (n=202), *Enterococcus faecalis* with 8.3% (n=93), *Proteus mirabilis* with 3.2% (n=37), *Enterococcus faecium* with 2.9% (n=33) and *Pseudomonas aeruginosa* with 2.5 % (n=28). The isolated bacteria and their frequencies are shown in Table I.

Enterobacteriaceae such as *Escherichia* spp., *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Morganella* spp., *Citrobacter* spp., and *Serratia* spp., were the causative pathogens in 962 cultures (85.4%) and they constitute most of the bacteria in this study, followed by *Enterococcus* spp. in 129 (11.5%), *Pseudomonas* spp. in 28 (2.5%), *Staphylococcus* spp. in 4 (0.4%) and *Acinetobacter* spp. in 3 cultures (0.3%).

There were 333 cases (29.5%) younger than one year old, among whom male gender was more prominent with a ratio of 61.6%. However, the male ratio declined to 24.2% in cases older than one year old. *Enterobacteriaceae* are the most common bacteria, followed by *Enterococcus* spp. and *Pseudomonas* spp. in both sexes.

Ampicillin, cefuroxime and trimethoprim-sulfamethoxazole with susceptibility rates of 18.6%, 39.6%, and 49.0% respectively, saw the highest resistance with

Table I. The frequency of isolated bacteria in culture positive patients

Microorganism	No of subtypes	%		n=1126	%
E. coli	666	59.1	*Escherichia* spp.	666	59.1
Klebsiella pneumoniae	202	17.9	*Klebsiella* spp.	209	18.6
Klebsiella oxytoca	7	0.6			
Enterococcus faecalis	93	8.3	*Enterococcus* spp.	129	11.5
Enterococcus faecium	33	2.9			
Enterococcus raffinosus	2	0.2			
Enterococcus avium	1	0.1			
Proteus mirabilis	37	3.2	*Proteus* spp.	44	3.9
Proteus vulgaris	7	0.6			
Pseudomonas aeruginosa	28	2.5	*Pseudomonas* spp.	28	2.5
Enterobacter cloacae	13	1.2	*Enterobacter* spp.	18	1.6
Enterobacter aerogenes	5	0.4			
Morganella morganii	16	1.4	*Morganella* spp.	16	1.4
Citrobacter freundii	6	0.5	*Citrobacter* spp	8	0.7
Citrobacter amalonaticus	2	0.2			
Staphylococcus aureus	2	0.2	*Staphylococcus* spp.	4	0.4
Coagulase negative staphylococcus	2	0.2			
Acinetobacter baumanii complex	3	0.3	*Acinetobacter* spp.	3	0.3
Serratia marcescens	1	0.1	*Serratia* spp.	1	0.1

Enterobacteriaceae. The antimicrobials with the highest susceptibility in this group were meropenem, imipenem and ertapenem with rates of 99.2%, 97.1% and 96.1% respectively. The antimicrobial susceptibility rates of microorganisms according to extended-spectrum beta-lactamase positivity are shown in Table II.

Enterococcus spp. showed the highest resistance to gentamycin with 50% resistance in tested cases. None of the tested cases showed resistance to vancomycin or linezolid. *Pseudomonas* spp. with 64.3% susceptibility, showed the highest resistance to piperacillin-tazobactam. With a rate of 96.4%, *Pseudomonas* spp. showed the highest

Table II. *In vitro* antimicrobial resistance patterns of *Enterobacteriaceae* to common antimicrobial agents according to ESBL positivity

			% Antimicrobial susceptibility of isolated microorganisms							
			***Enterobacteriaceae* n=962**		***Escherichia* spp. n=666**		***Klebsiella* spp. n=209**		***Proteus* spp. n=44**	
Antimicrobial agent			n=340	n=622	n=246	n=420	n=85	n=124	n=2	n=42
Nitrofurantoin	Total		90.8		96.8		83.6		54.5	
	ESBL+	ESBL-	92.8	89.7	96.1	97.2	87.0	80.4	50.0	54.8
Ampicillin	Total		18.6		25.3		0.0		20.5	
	ESBL+	ESBL-	1.5	28.1	1.6	39.1	0.0	0.0	50.0	19.0
Amoxicillin-Clavulanate	Total		61.2		61.4		64.1		79.5	
	ESBL+	ESBL-	37.4	74.5	34.8	77.1	44.7	77.6	100.0	78.6
Piperacillin-Tazobactam	Total		61.4		63.7		46.9		97.7	
	ESBL+	ESBL-	40.9	72.9	44.3	75.2	29.4	59.2	100.0	97.6
Cefuroxime	Total		39.6		44.2		22.5		79.5	
	ESBL+	ESBL-	3.2	59.8	3.7	68.8	1.2	36.8	50.0	81.0

Table II. Continued										
			% Antimicrobial susceptibility of isolated microorganisms							
			***Enterobacteriaceae* n=962**		***Escherichia* spp. n=666**		***Klebsiella* spp. n=209**		***Proteus* spp. n=44**	
Ceftriaxone	Total		43.2		45.1		26.3		69	
	ESBL+	ESBL-	2.9	65.4	2	70.4	4.7	41.6	50.0	90.5
Cefixime	Total		90.9		91.5		88.4		100.0	
	ESBL+	ESBL-	82.5	95.5	84.5	95.5	77.9	96.2	100.0	100.0
Amikacin	Total		85.9		86.3		82.3		93.2	
	ESBL+	ESBL-	76.2	91.1	77.5	91.4	71.8	89.7	100.0	92.9
Gentamycin	Total		68.0		67.7		65.6		70.5	
	ESBL+	ESBL-	47.6	79.0	46.3	80.0	50.6	76.9	50.0	71.4
Trimethoprim-Sulfamethoxazole	Total		49.0		50.0		42.1		36.4	
	ESBL+	ESBL-	29.7	59.5	29.9	61.7	22.3	56.2	50.0	35.7
Imipenem	Total		97.1		100.0		95.7		70.5	
	ESBL+	ESBL-	98.5	96.3	100.0	100.0	100.0	92.8	0.0	73.8
Meropenem	Total		99.2		100.0		96.6		100.0	
	ESBL+	ESBL-	99.7	98.9	100.0	100.0	100.0	94.4	100.0	100.0
Ertapenem	Total		96.1		98.5		88.9		100.0	
	ESBL+	ESBL-	95.6	96.6	97.5	99.3	91.8	87.1	100.0	100.0

ESBL: Extended-spectrum beta-lactamase

susceptibility to imipenem and meropenem. The *in vitro* antimicrobial susceptibility rates of *Pseudomonas* spp. to common antimicrobials are shown in Table III.

Table III. Antimicrobial susceptibility rates of *Pseudomonas* spp.

Antimicrobials	**% Antimicrobial susceptibility** ***Pseudomonas* spp. n=28**
Piperacillin-Tazobactam	64.3
Amikacin	89.3
Gentamycin	89.3
Imipenem	96.4
Meropenem	96.4
Ciprofloxacin	89.3
Ceftazidime	89.3
Cefepime	89.3

Discussion

Bacterial resistance is an important challenging problem in UTI. Antimicrobial-resistant infections have been predicted to reach 10 million lives per year by 2050 (6). Especially in the paediatric population, antimicrobial prescriptions are higher, mostly in cases of over-diagnosed viral upper respiratory infections. If paediatricians have a symptomatic child with a positive urinalysis, it is inevitable to start the initial treatment of acute UTI without obtaining culture results (6-8). Certainly, early diagnosis and proper treatment is important, not only to prevent complications such as renal abscess or septicaemia, but also it is crucial to avoid renal scarring, and even renal failure (9). Additionally, the loss of schooltime as well as the loss of parental worktime are the indirect costs which cannot be underestimated (10,11).

The increment in the resistance to common antimicrobials, especially in paediatric uropathogens, has led to challenges due to the more difficult treatment of UTI over time (9). Antimicrobial resistance has variabilities such as geographic differences in bacterial patterns and local antimicrobial prescription practices (7,12-14).

The frequency of *E. coli* has been reported as having a wide range in the literature. Rai et al. (15) reported *E. coli* at a very high level of 93.3%. We also found that *E. coli* was the most common organism isolated with 59.1%, which was quite low compared to their paper. Other studies such as Akram et al. (16) reported a lower prevalence (21.4%) of

E. coli. *E. coli* is reported as the most common pathogen in those studies conducted in the adult population. While the prevalence of *E. coli* in UTI was reported as 50% in a multicentre study conducted in China, in a meta-analysis conducted in Iran, the most common pathogen causing UTI was reported to be *E. coli* with a frequency of 62% (17,18). Catal et al. (12) demonstrated an increasing rate of *Klebsiella* spp. in urine cultures between 2000 and 2006, with rates of 7.2% and 18%, respectively. Gunduz and Uludağ Altun (19) reported the *Klebsiella* spp. rate to be 14.9%. In this study, we have a higher rate (18.6%) of *Klebsiella* spp. Unfortunately, *non-E. coli* pathogens are more resistant to most antimicrobial agents. Therefore, those patients with *Klebsiella* spp. needed hospitalization and parenteral antibiotic therapy also increases the treatment costs (20).

The current American Academy of Pediatrics guideline suggests giving oral or parenteral antimicrobials for 7-14 days for the management of UTIs in febrile infants and young children. Ceftriaxone, cefotaxime, ceftazidime, gentamicin, tobramycin, and piperacillin are the drugs of choice for parenteral therapy; amoxicillin-clavulanate, sulfonamide (trimethoprim-sulfamethoxazole or sulfisoxazole), or cephalosporin (cefixime, cefpodoxime, cefprozil, cefuroxime axetil, or cephalexin) are recommended as oral agents for treating UTI (7,8). For an antimicrobial to be considered as a first-line empirical treatment for UTI, resistance should not exceed 20% (13). In a meta-analysis conducted by Bryce et al. (14), this threshold was reached for many first-line antimicrobials used for paediatric *E. coli* UTIs. In most countries, half of all isolates were resistant to ampicillin, 1/3 to co-trimoxazole, and 1/4 to trimethoprim. This supports the increased risk of *E. coli* resistance to that particular antimicrobial due to previous antimicrobial usage (14). In our study, we also had similar resistance results with the exception of increased rates to cefuroxime. Our results showed the first resistance rates to ampicillin, cefuroxime, and trimethoprim-sulfamethoxazole, which have the highest resistance for *Enterobacteriaceae*, with resistance rates of 81.4%, 58.8% and 51.0% respectively.

Gunduz and Uludağ Altun (19) reported lower resistance rates of *E. coli* to ampicillin, co-trimoxazole, ceftriaxone, amikacin, amoxicillin-clavulanate and the same resistance rates for gentamicin, nitrofurantoin, and ciprofloxacin in comparison to Bryce's meta-analysis. Al Benwan and Jamal (21) also showed that approximately 62% of *E. coli* and 77% of *non-Coliform* bacteria were resistant to ampicillin, similar to our study. Dejonckheere et al. (22) also reported that resistance to amoxicillin-clavulanic acid for *E. coli* strains had increased from 16% to 36% (2015-2019) over the last 20 years. They pointed out that the problem of increasing antimicrobial resistance to antimicrobials commonly used in UTIs is a growing health problem (22). Although it is known that *in vivo* antibiotic responses are important in the treatment of UTI, it is thought that the development of *in vitro* antimicrobial resistance may pose a risk for *in vivo* responses in the coming years.

Study Limitations

The retrospective nature of this study is its limitation.

Conclusion

This study, in line with the literature, shows that resistance to commonly used antimicrobials is still very important. This highlights the necessity of planning in this regard.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Medical Research Ethics Committee of Ege University Faculty of Medicine (approval no: 22-12.1T/16, date: 16.12.2022).

Informed Consent: Written informed consent was obtained from all the patients or their parents/guardians.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.K.B., A.K., A.T., C.K., Concept: İ.K.B., C.K., A.K., Design: S.T., Data Collection or Processing: S.T., E.A., Analysis or Interpretation: S.T., İ.K.B., S.Ö., Literature Search: S.T., S.Ş.A., Writing: S.T.

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References

1. Okarska-Napierała M, Wasilewska A, Kuchar E. Urinary tract infection in children: Diagnosis, treatment, imaging - Comparison of current guidelines. J Pediatr Urol 2017;13:567-73.
2. Schmidt B, Copp HL. Work-up of Pediatric Urinary Tract Infection. Urol Clin North Am 2015;42:519-26.
3. Stein R, Dogan HS, Hoebeke P, et al. Urinary tract infections in children: EAU/ESPU guidelines. Eur Urol 2015;67:546-58.
4. Hodson EM, Willis NS, Craig JC. Antibiotics for acute pyelonephritis in children. Cochrane Database Syst Rev 2007;(4):CD003772.
5. Abejew AA, Denboba AA, Mekonnen AG. Prevalence and antibiotic resistance pattern of urinary tract bacterial infections in Dessie area, North-East Ethiopia. BMC Res Notes 2014;7:687.

6. Elder JS. Urologic disorders in infants and children. In: Behrman RE, Kliegman RM, Jenson HB (eds). Nelson textbook of pediatrics 18th ed. Philadelphia, WB Saunders, 2010;1829-34.
7. Alberici I, Bayazit AK, Drozdz D, et al. Pathogens causing urinary tract infections in infants: a European overview by the ESCAPE study group. Eur J Pediatr 2015;174:783-90.
8. Yolbaş I, Tekin R, Kelekci S, et al. Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. Eur Rev Med Pharmacol Sci 2013;17:971-6.
9. Ipek IO, Bozaykut A, Arman DC, Sezer RG. Antimicrobial resistance patterns of uropathogens among children in Istanbul, Turkey. Southeast Asian J Trop Med Public Health 2011;42:355-62.
10. Çoban B, Ülkü N, Kaplan H, Topal B, Erdoğan H, Baskın E. Five-year assessment of causative agents and antibiotic resistances in urinary tract infections. Turk Pediatri Ars 2014;49:124-9.
11. Senel S, Karacan C, Erkek N, Göl N. A single-center experience of antimicrobial resistance patterns in pediatric urinary tract infection. Med Princ Pract 2010;19:359-63.
12. Catal F, Bavbek N, Bayrak O, et al. Antimicrobial resistance patterns of urinary tract pathogens and rationale for empirical therapy in Turkish children for the years 2000-2006. Int Urol Nephrol 2009;41:953-7.
13. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:103-20.
14. Bryce A, Hay AD, Lane IF, Thornton HV, Wootton M, Costelloe C. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. BMJ 2016;352:939.
15. Rai S, Pant ND, Bhandari R, et al. AmpC and extended spectrum beta-lactamases production among urinary isolates from a tertiary care hospital in Lalitpur, Nepal. BMC Res Notes 2017;10:467.
16. Akram M, Shakil S, Khan AU. Prevalence of integrons, blaCTX-M and blaTEM resistance markers among ESBL-producing uropathogenic Escherichia coli isolates: first report of genomic blaCTX-M from India. J Chemother 2011;23:131-4.
17. Qiao LD, Chen S, Yang Y, et al. Characteristics of urinary tract infection pathogens and their in vitro susceptibility to antimicrobial agents in China: data from a multicenter study. BMJ Open 2013;3:e004152.
18. Mortazavi-Tabatabaei SAR, Ghaderkhani J, Nazari A, Sayehmiri K, Sayehmiri F, Pakzad I. Pattern of Antibacterial Resistance in Urinary Tract Infections: A Systematic Review and Meta-analysis. Int J Prev Med 2019;10:169.
19. Gunduz S, Uludağ Altun H. Antibiotic resistance patterns of urinary tract pathogens in Turkish children. Glob Health Res Policy 2018;3:10.
20. Marcus N, Ashkenazi S, Yaari A, Samra Z, Livni G. Non-Escherichia coli versus Escherichia coli community-acquired urinary tract infections in children hospitalized in a tertiary center: relative frequency, risk factors, antimicrobial resistance and outcome. Pediatr Infect Dis J 2005;24:581-5.
21. Al Benwan K, Jamal W. Etiology and Antibiotic Susceptibility Patterns of Urinary Tract Infections in Children in a General Hospital in Kuwait: A 5-Year Retrospective Study. Med Princ Pract 2022;31:562-9.
22. Dejonckheere Y, Desmet S, Knops N. A study of the 20-year evolution of antimicrobial resistance patterns of pediatric urinary tract infections in a single center. Eur J Pediatr 2022;181:3271-81.