Iranian Journal of Microbiology
Volume 6 Number 6 (December 2014) 387-391

ORIGINAL ARTICLE

Choosing the correct empirical antibiotic for urinary tract infection in pediatric: Surveillance of antimicrobial susceptibility pattern of *Escherichia coli* by E-Test method

Iraj Sedighi[1], Abbas Solgi[1], Ali Amanati[2], Mohammad Yousef Alikhani[3, 4*]

[1]*Department of Pediatrics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran.*
[2]*Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.*
[3]*Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.*
[4]*Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran.*

Received: September 2014, **Accepted:** November 2014

ABSTRACT

Background and Objectives: Urinary Tract Infections (UTIs) are of the most common bacterial diseases worldwide. We investigate the antibiotic susceptibility patterns of *Escherichia coli (E. coli)* strains isolated from pediatric patients with community acquired urinary tract infection (UTI) to find a clinical guidance for choosing a right empirical antibiotic in these patients.

Materials and Methods: In this cross sectional study, 100 urine specimens which were positive for *E. coli* had been investigated for antibiotics susceptibility pattern. The susceptibility to Co-trimoxazol (25µg), Amikacin (30µg), Ceftriaxone (30µg), Nalidixic Acid (30µg), Cefixime (5µg), and Nitrofurantoin (300µg) tested with Disk diffusion agar and MIC determined with the E-test.

Results: Mean age of patients was 38 Months. Girls had greater proportion than boys (74 versus 26%). In Disk diffusion method, 26% of the isolates were susceptible to cotrimoxazole. Susceptibility to amikacin, ceftriaxone, nitrofurantoin, nalidixic acid and cefixime was 94%, 66%, 97%, 62% and 52%, respectively. By E-Test method and according to CLSI criteria susceptibility for co-trimoxazol, amikacin, ceftriaxone and nalidixic acid was 37%, 97%, 67% and 50%, respectively. The highest percentage of agreement between Disk diffusion and E-Test method was found for amikacin (96%) and the lowest percentage for co-trimoxazole (89%).

Conclusions: Treatment failure, prolonged or repeated hospitalization, increased costs of care, and increased mortality are some consequence of bacterial resistance in UTIs. Misuse of antibiotics in each geographic location directly affects antibiotic resistance pattern. In the treatment of UTI, proper selection of antimicrobial agents should be relevant to the bacterial susceptibility testing surveillance. According to our results, amikacin as an injectable drug and nitrofurantoin as an oral agent could be used as a drug of choice in our region for children with UTIs.

Keywords: Antibiotic susceptibility, Escherichia coli, Urinary Tract Infections, Disk diffusion agar, E-Test

*Corresponding author: Mohammad Yousef Alikhani
Address: Brucellosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
Tel: +98811 8276295
Fax: +98811 8276299
E-mail: alikhani43@yahoo.com & alikhani@umsha.ac.ir

INTRODUCTION

Urinary Tract Infections (UTIs) is one of the most common infections during childhood and *E. coli* is one of the more predominant pathogen recovered in UTIs (1). Given that the vast majority of UTIs are treated empirically, the choice of an antimicrobial

Table 1. Antimicrobial resistance of *E. coli* isolates by E. test (n = 100)

Antibiotic	Break point(µg/ml)	Resistant	Intermediate	Susceptible	MIC-50	MIC-90	MIC Range
Co-trimoxazole	S ≤ 8 R ≥ 32	63%	0%	37%	0.094	0.61	0.016-2
Nalidixic Acid	S ≤ 8 R ≥ 32	42%	8%	50%	2	3	1-192
ceftriaxone	S ≤ 8 R ≥ 64	21%	12%	67%	0.032	0.2	0.016-128
Amikacin	S ≤ 16 R ≥ 32	3%	0%	97%	0.75	1.6	0.125-32

agent should be adjusted by local susceptibility pattern. The rising prevalence of multi-drug resistant infections makes more complexity in the empirical treatment of these infections (2). The antimicrobial resistance patterns of community-acquired UTIs have been studied by some researcher around the world (1, 2). Awareness of local antimicrobial agent's resistance trends among urinary isolates is important to notice clinicians for appropriate use of antibiotics and also for evidence based recommendations in empirical antibiotic treatment of UTI. (3-6)

Proper application of sensitive and specific methods in determination of susceptibility of antimicrobial agents is necessary for exact diagnosis and early effective treatment. Determination of antibiotic susceptibility patterns of *E. coli* strains is one of the most concerns in empirical management of community acquired UTIs in pediatric practice.

This study was conducted to determine the minimal inhibitory concentration (MIC) of urinary *E. coli* isolates by E-test.

MATERIALS AND METHODS

Bacterial Strains. This cross-sectional descriptive study was conducted between June 2009 and September 2010. A total of 100 *E. coli* (one specimen per patient) isolated from children admitted with community-acquired UTIs at the Besat teaching Hospital. Choosing the right method of specimen collecting was according to physician decision, clinical status and need for treatment in patients. Supra-pubic, catheter and midstream sampling have been utilized in this study. After appropriate sampling, specimen were sent to the microbiology laboratory in a standard manner and processed by trained personnel. Isolation performed according to standard laboratory protocols and Uropathogenic *Escherichia coli* (UPEC) isolated from true positive urine cultures.

Diagnostic criteria. Community acquired urinary tract infections were defined as recovery of 100 000 CFU/mL or more bacteria in midstream clean-catch method, 50 000 CFU/mL or more by catheter and any count by supra-pubic rout (7).

Antimicrobial Susceptibility Testing. Antimicrobial sensitivity testing to the following four antimicrobial agents was performed with both Disk diffusion agar (Mast Co, UK) and E-Test (AB biomerienx solna Sweden), according to the Clinical and Laboratory Standards Institute (CLSI) criteria (8): Co-trimoxazol (25µg), Amikacin (30µg), Ceftriaxone (30µg) and Nalidixic acid (30µg). For Cefixime (5µg) and Nitrofurantoin (300µg) only Disk diffusion agar were utilized. This study was approved by the Research Committee of Hamadan University of Medical Sciences.

Statistical Analysis. All data were processed using Statistical Package for Social Sciences (SPSS), version 17.0 software. The significance of the results was established using MC- Nemar Test. the level of significance was considered at $P < 0.05$.

RESULTS

During 14 months surveillance period 100 *Esherichia coli* isolated from urine specimens of patients with clinically and laboratory diagnosis of UTI were investigated for antibiotic susceptibility testing. Mean age of patients was 38 Months. Girls had greater proportion than boys (74 versus 26%). Among patients who have positive culture for *E. coli*, E-test susceptibility testing was performed and the following results obtained: 37% susceptible

Table 2. Antimicrobial resistance of *E. coli* isolates by Disk Diffusion Agar (n = 100)

Antibiotic	Susceptible (٪)n	Intermediat (٪)n	Resistance (٪)n
Co-trimoxazole	26 (26)	4 (4)	70 (70)
Cefixime	62 (62)	3 (3)	35 (35)
Nalidixic Acid	52 (52)	1 (1)	47 (47)
Nitrofurantoin	97 (97)	3 (3)	(0) 0
Ceftriaxone	66 (66)	4 (4)	30 (30)
Amikacin	94 (94)	2 (2)	4 (4)

to Co-trimoxazole, 50% susceptible to Nalidixic Acid, 67% susceptible to ceftriaxone and 97% to Amikacin (Table1).

In Disk diffusion agar test, Drug susceptibility of the isolates was 66%, 94%, 62%, and 97% to ceftriaxone, Amikacin, cefixime, and Nitrofurantoin, respectively (Table2).

Co-trimoxazol obtained the lowest and Amikacin and Nitrofurantoin had the highest sensitivity with both methods which were used in our study.

According to the MICs of co-trimoxazol (MIC≥32), 63% of *E. coli* strains were classified as resistance. The MICs of other antimicrobial agents showed that *E. coli* strains were highly susceptible to Amikacin. Their MIC_{90} was 1.6 µg/ml.

The highest and the lowest overall agreement were found between E-Test and Disk diffusion agar test for amikacin (96%) and co-trimoxazol (89%), respectively (Table 3).

In comparison with results of E-Test susceptibility testing, no statistically significant differences were found in Disk diffusion agar test, with the exception of co-trimoxazol (*P.value*<0.05).

DISCUSSION

Understanding the impacts of drug susceptibility pattern is crucial as the changing rate of antibiotic susceptibility has a large impact on the treatment of UTI. Treatment failure, prolonged or repeated hospitalization, increased costs of care, and increased mortality are some consequence of bacterial resistance in UTIs. Although, several factors (such as bacterial virulence, bacterial biofilms formation in urinary tract, previous inappropriate use of antimicrobial agents and structural anomalies) may play a role in antimicrobial resistance, misuse of antibiotics in each geographic location directly affects antibiotic resistance pattern.

The purpose of this study was to investigate the antimicrobial susceptibility pattern of uropathogenic *E. coli* in pediatric patients in our province for better usage of empiric antimicrobial agents. Sex distribution in our study was compatible to other study and reference textbooks (1, 9). The lowest sensitivity result, or the most resistance, belongs to Co-trimoxazole in both Disk diffusion agar

Table 3. Comparison of Antimicrobial susceptibility pattern of *E. coli* isolates by Disk Diffusion Agar and E. test (n = 100)

	Susceptibility testing		
Antibiotic	**Disk Diffusion Agar (٪)n**	**E-Test (٪)n**	***P-Value***
Co-trimoxazole	26(26%)	37(37%)	0.001
Nalidixic Acid	52(52%)	50(50%)	0.500
ceftriaxone	66(66%)	67(67%)	1.000
Amikacin	94(94%)	97(97%)	0.250

and E-Test (susceptibility rates were 26 and 37%; respectively). Same results reported by other study in our country such as Khorvash *et al.* 2008 (10), Prais *et al.* 2002 (11) and Farajnia *et al.* 2009 (9).

Nitrofurantoin, after Amikacin, has attained subsequent rank of susceptibility (97% by Disk diffusion agar test). These results were same to other study in our country (12-14), and is comparable to the 94.9% susceptible *E. coli* isolated from 240 recurrent UTIs other studies (15-18).

Interestingly, one of the most relevant antibiotics, ceftriaxone had changed susceptibility pattern in the base of previous findings demonstrate that reported sensitivity were more than 90% (12, 16). In our study, sensitivity of ceftriaxone as determined as 66% and 67% in Disk diffusion agar and E-Tests, respectively. The reduced sensitivity may be consequence of unbridle use of this agent in outpatient as well as inpatient management of pediatric infectious disease (19, 20). On the other hand, emergence of community-acquired *E. coli* producing extended spectrum beta-lactamases (ESBL) should be keep in mind and could be scope of further investigation for researchers (21, 22). These *E. coli* producing beta-lactamases also could exhibit co-resistance to co-trimoxazole (15). Moyo *et al.* (23) showed more resistance to co-trimoxazole and nalidixic acid than previous reports (more than 90% and 61%, respectively). Noemia *et al.* (24) proposed initial empirical oral treatment with nitrofurantoin or nalidixic acid in children with febrile UTI, but according to finding of our study nalidixic acid could not be the drug of choice in our region. Sensitivity of nalidixic acid in other studies was greater than our survey (18).

We conclude that former agents such as co-trimoxazole and nalidixic acid which have been used widely had more resistant rate. On the other hand, those (nitrofurantoin, amikacin) with lesser usage have the little resistant rate. Whereas *E. coli* is the most common uropathogen in pediatric patients (18, 25), it is advisable that co-trimoxazole and nalidixic acid do not used routinely for empiric practice. In afebrile UTIs (lower UTI or cystitis) and also in vesicourethral reflux prophylaxis, nitrofurantoin could be considered. If patient is febrile (upper UTI) and no concern exists about renal function, amikacin will be the drug of choice because of its high efficacy to *E. coli*. Another advantage of amikacin in primary treatment of febrile UTI in contrast with ceftriaxone is that ceftriaxone can induce beta-lactamase resistance instead of *in vitro* sensitivity which may cause treatment failure. Authors recommended that pediatrician should review antibiotic resistance pattern in their region and investigate susceptibility of oral and parenteral agents, as this study revealed that empirical initial treatment with co-trimoxazole is inadequate in approximately one third of UTI cases. Notable in this context is that in comparison between results of E-Test and Disk diffusion agar, no statistically significant differences were found, with the exception of co-trimoxazol ($P.value < 0.05$) which implies that antibiotic resistance for co-trimoxazol over estimated by Disk diffusion method but this method is reliable for other antibiotics (nitrofurantoin, nalidixic acid and amikacin).

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deputy of research of Hamadan University of medical sciences, Hamadan, Iran.

REFERENCES

1. Kliegman RM, Behrman RE, Jenson HB, Stanton BF. Nelson text book of pediatrics. 18 Editions. Philadelphia: Saunders; 2007.
2. Steinke DT, Seaton RA, Phillips G, MacDonald TM, Davey PG. Prior trimethoprim use and trimethoprim-resistant urinary tract infection: a nested case-control study with multivariate analysis for other risk factors. *J Antimicrob Chemother* 2001;47:781-787.
3. Dapeng Y. The drug resistance of pathogenic bacteria collected from urinary tract infection patients subjected to the Allied Hospital of Beihua University. *China J Misdiagn* 2007; 7:2492-2493.
4. Zhao SB, Song L, Hu H. Distribution of bacteria in urinary tract infection and analysis of drug resistance of these bacteria. *Ningxia Med J*2007; 29:750-752.
5. Wang J, Liu H, Xuefeng Z. Drug resistance supervision to pathogens from urinary tract in mountain area of western Hubei province. *China J Nosocomial* 2007; 17:732-733.
6. Wan X, Wenfang W, Yu C, Changchun C, Yifeng L, Yongxiang x. The pathogenetic characteristics of community-acquired urinary tract infection in female in Nanjing. *Jiangsu Med J* 2008; 34:571-573.
7. Wald ER. Cystitis and Pyelonephritis, in Feigin & Cherry's Textbook of Pediatric Infectious Diseases, WB saunders; 2014: p. 542.
8. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth informational supplement. Clinical and Laboratory Standards Institute 2014; M100-S24.

9. Khorvash F, Zarefar S, Mobasherizadeh S, Mostafavizadeh K. Antibiotic Susceptibility Pattern of Organisms Causing Urinary Tract Infection in Spinal Cord Injured Patients with E test. *Tabibe Shargh J* 2008; 9:305-311.
10. Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. *Arch Dis Child* 2003;88:215-218.
11. Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. *Int J Infect Dis* 2009;13: 140-144.
12. Vaezzadeh F, sharifi-yazdi MK. Laboratory evaluation of urine culture and drug resistance in children clinically suspected of urinary tract infection (UTI). *Iran J Public Health* 2001; 30: 123-124.
13. Erfani Y, Choobineh H, Safdari H, Rasti A, Alizadeh S. comparison of E. test and disk diffusion agar in antibiotic susceptibility of *E.coli* isolated from patients with urinary tract infection in Shariati hospital (Iran). *Journal of Biological Sciences* 2008; 3: 24-27.
14. Haghi-Ashteiani M, Sadeghifard N. Etiology and antibacterial resistance of bacterial Urinary tract infection in children's medical center ,Tehran. Iran. *Acta Medica Iranica* 2007;45:153-157.
15. Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L, Marchese A. The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 2009; 34:407-413.
16. Lixiang Z, Xiang C, Xiaoping Z, Weixia Y, Lanmei D, Xiaojing X et al. prevalance of virulence factors and antimicrobial of uropathogenic *E.coli* in jiangsu province (China). *J Urology* 2009; 74:702-707.
17. Matute AJ, Hak E, Schurink C. Resistancy of uropathogens in symptomatic urinary tract infections in Leon, Nicaragua. *Int J Antimicrob agents* 2004; 23: 506-509.
18. Dimitrov TS, Udo EE, Emara M, Awni F, Passadilla R. Etiology and antibiotic susceptibility patterns of community-acquired urinary tract infections in a Kuwait Hospital. *Med Princ Pract* 2004; 13:334 –339.
19. Hsueh PR, Chen WH, Luh KT. Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991–2003 at a university hospital in Taiwan. *Int J Antimicrobial Agents* 2005;26:463–472.
20. Bergman M, Nyberg ST, Huovinen P, Paakkari P, Hakanen AJ,. Association between antimicrobial consumption and resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 2009;53: 912–917.
21. Pitout JDD, Nordmann P, Laupland KB and Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum b-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy* 2005;56: 52–59.
22. Colodner R, Rock W, Chazan B, Keller N, Guy N, Sakran W, et al. Risk factors for the development of extended-spectrum b-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* 2004; 23: 163–167.
23. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, Maselle SY. Antimicrobial resistance among producers and non-producers of extended spectrum betalactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC Research Notes* 2010;3:348-352.
24. GoldraichNP and Manfroi A. Febrile urinary tract infection: *Escherichia coli* susceptibility to oral antimicrobials. *Pediatr Nephrol* 2002;17:173–176.
25. Wagenlehner FME, Naber KG. Treatment of Bacterial Urinary Tract Infections: Presence and Future. *European Urology* 2006;49:235–244.