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**Original Article** 

**URINARY INFECTION** 

# Antimicrobial susceptibilities of *Escherichia coli* isolates as agents of community-acquired urinary tract infection (2008–2014)

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#### ABSTRACT

**Objective:** Urinary tract infections (UTIs) are among the most frequently seen community-acquired infections worldwide. *E. coli* causes 90% of urinary system infections. To guide the empirical therapy, the resistance pattern of *E. coli* responsible for community-acquired UTI was evaluated throughout a seven-year period in this study.

**Material and methods:** The urine cultures of patients with urinary tract infections admitted to outpatient clinics between 1<sup>st</sup> January 2008 and 31<sup>st</sup> December 2014 were analyzed. Presence of  $\geq 10^5$  colony-forming units/mL in urine culture media was considered as significant for UTI. Isolated bacteria were identified by standard laboratory techniques or automated system VITEK2 (BioMerieux, France) and BD PhoenixTM 100 (BD, USA), as required. Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method using Clinical Laboratory Standard Institute (CLSI) criteria.

**Results:** A total of 13281 uropathogens were isolated. Overall *E. coli* accounted for 8975 (67%) of all isolates. Resistance rates of *E. coli* to antimicrobial agents was demonstrated to be as follows: ampicillin 66.9%, cefazolin 30.9%, cefuroxime 30.9%, ceftazidime 14.9%, cefotaxime 28%, cefepime 12%, amoxicillin-clavulanic acid 36.9%, trimethoprim-sulfamethoxazole (TMP-SXT) 20%, ciprofloxacin 49.9%, amikacin 0.3%, gentamycin 24%, nitrofurantoin 0.9%, and fosfomycin 4.3%. There was no resistance to imipenem nor meropenem. The frequency of ESBL-producing *E. coli* strains was 24%.

**Conclusion:** It is concluded that fosfomycin and nitrofurantoin are appropriate empirical therapy for community-acquired UTI empirical therapy, but the fluoroquinolones and the TMP-SXT shall not be used in the emprical treatment of UTI at this stage. In conclusion, as resistance rates show regional differences, it is necessary to regularly examine regional resistance rates to determine the appropriate empiric antibiotic treatment and national antibiotic usage policies must be reorganized according to data obtained from these studies.

Keywords: Antimicrobial susceptibility; community-acquired urinary tract infections; Escherichia coli

#### Introduction

Urinary tract infection (UTIs) is one of the frequently seen infections both in the world and in our country as well. Canbaz et al.[1] reported that every year approximately five million patients experience episodes of cystitis in our country, and 17.8% of the patients who consulted to primary healthcare centers are diagnosed as urinary system infection.<sup>[2]</sup> Most of the causative bacteria consist of members of the Enterobacteriaceae family. Most frequently Escherichia coli is isolated in complicated or uncomplicated nosocomial -or community acquired urinary system infections.<sup>[2-6]</sup> Therefore for the empirical treatment of urinary infections most frequently oral forms of E. coli susceptible trimethoprim-sulfametoxale (TMP-SXT), and fluoroquinolones have been

preferred. However it has been recommended that guiding data adjusted to epidemiological data should be used in the selection of routine treatment.<sup>[2,7]</sup> Therefore region-specific microorganisms, and their antimicrobial susceptibilitis should be determined. To that end *E. coli* isolates identified in urine cultures of patients who consulted to outpatient clinics of our hospital were investigated.

## Material and methods

Urine samples sent from outpatient clinics to the Medical Microbiology Laboratory of Izmir Tepecik Training and Research Hospital between January 1<sup>st</sup>, 2008, and December 31<sup>st</sup>, 2014 were retropectively analyzed. Culture plates were incubated in incubators at a mean ambient temperature of 35±2°C, and under

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Table 1. Resistance rates of 8975 E. coli isolates (%)																
Antibiotics	Ampicillin	Cefazolin	Cefaclor	Ceftazidime	Ceftriaxone	Cefepime	Amoxicillin-clavulanate	Ciprofloxacin	Co-trimaxazole	Amikacin	Gentamycine	Nitrofurantoin	Fosfomycine	Imipenem	Meropenem	ESBL
R (%)	67	43	31	15	28	12	37	50	20	0.3	24	0.9	4.3	0	0	24
ESBL: exte	ESBL: extended-spectrum beta-lactamase															

normal atmospheric conditions for 18-24 hours, culture plates with bacterial growth of  $\geq 10^5$  CFU/mL of a single species on their surfaces were examined. The isolates obtained were identified at a species level using conventional methods, and fully automated bacterial identification system (VITEK 2 Compact; bioMérieux, Paris, France, and USA Phoenix<sup>TM</sup> 100; Becton Dickinson, MD, USA). Antimicrobial susceptibilities of isolated *E. coli* strains were determined using Kirby-Bauer disc diffusion system, and fully automated systems in compliance with the recommendations of Clinical and Laboratory Standards Institute (CLSI). The presence of extended- spectrum beta- lactamase (ESBL) was determined using ceftazidime, ceftazidimeclavulanic acid, and cefotaxime- cefotaxime clavulanic acid discs.<sup>[8]</sup> As a control ATCC 25923 strain of *E. coli* was used.

#### Statistical analysis

In the comparison of antibacterial susceptibilities, *chi*-square, and Fisher's exact test were used.

### Results

In urine cultures accepted by the laboratory between January 1st 2014, and 31st December 2014 a total of 13281 uropathogens were isolated, and E. coli accounted for 8975 (67%) of all isolates. Resistance rates of E. coli to antimicrobial agents was demonstrated to be as follows: ampicillin 66.9% (n=6013), cefazolin 30.9% (n=3859), cefuroxime 30.9% (n=2782), ceftazidime 14.9% (n=1981), ceftriaxone 28% (n=2513) cefepime 12% (n=1077), amoxicillin-clavulanic acid 36.9% (n=3320), trimethoprim-sulfamethoxazole (TMP-SXT) 20% (n=1795), ciprofloxacin 49.9% (n=4487), amikacin 0.3% (n=270), gentamicin 24% (n=54), nitrofurantoin 0.9% (n=81), fosfomycin 4.3% (n=386). There was no resistance to imipenem nor meropenem. Production of ESBL was detected in 24% (n=2154) of the samples. Antimicrobial resistance rates of 8975 E.coli strains isolated from urine culture samples between the years 2008, and 2014 are shown in Table 1.

## Discussion

*E. coli* are among the most frequently identified bacterial agents in community-, and hospital-acquired UTIs. Its rate has been reported to range between 55, and 95 percent in community-acquired infections.<sup>[2-6,9,10]</sup>

In this study *E. coli* was identified in 8975 (67%) out of 13281 samples sent to microbiology laboratory of our hospital which was in compliance with the literature.

Determination of empirical treatment to be initiated during the time elapsed from the detection of urinary tract infection up to the results of the urine culture were obtained in a patient who consulted to a health center for the first time carries utmost importance. In order to start empirical treatment, resistance rate against this antibacterial drug in this region of application should not exceed 10-20 percent. Based on this recommendation, regional data should be constructed, or even antibacterial resistance rates of each hospital should be known in order to plan appropriate treatment accordingly. According to a guideline published by Infectious Diseases Society of America (IDSA) in the year 2011, if regional resistance rates against antibiotics in the empirical treatment of acute uncomplicated cystitis in women does not exceed 20% then initiation of therapy with nitrofurantoin, fosfomycin, trimethoprim-sulfamethoxazole, and pivmecillinam, and in cases of suspect acute pyelonephritis fluoroquinolones or beta-lactame antibiotics are recommended.<sup>[7]</sup>

Resistance rates related to TMP-SXT, and ciprofloxacin which were most frequently used in our country were detected to be 20%, and 49.9%, respectively. Studies investigating antimicrobial susceptibility rates of *E. coli* isolates as agents of community- acquired urinary system infections in Turkey have reported resistance rates to TMP-SXT ranging between 29, and 49 percent.<sup>[11,12]</sup> However resistance rates to TMP-SXT in other countries have demonstrated significant differences. According to ECO.SENS II data median resistance rate to TMP-STX in cases with uncomplicated UTIs was found to be 14. 4 percent.<sup>[13]</sup> In a study performed by Kashef et al.<sup>[6]</sup> in Iran resistance rate to TMP-SXT was detected as 61.8 percent. In our country, resistance rates of *E. coli* strains isolated from the patients with community-acquired urinary system infection to quinolones varied between 17, and 45 percent.<sup>[3,14]</sup> In a study by Gupta et al.<sup>[5]</sup> in India, resistance to ciprofloxacin was observed at higher levels (80.7%), while in the ECO.SENS study which encompassed European countries, resistance rates ranging between 0.5, and 7.6% were detected.<sup>[9]</sup> Resistance rates are known to change among countries, regions or hospitals.<sup>[7]</sup>

When compared with the data of our country, resistance rates of microorganisms against TMP-SXT detected in this study were lower, while higher resistance rates against ciprofloxacin were remarkable. In a study where susceptibility of *E. coli* isolates grown on urine cultures of the patients who consulted to the outpatient clinics of five hospitals in Izmir in the year 2007 was investigated, rates of resistance to TMP-SXT, and ciprofloxacin were 45.8, and 41.1%, respectively.<sup>[10]</sup> When compared with the results of our former study, with time, decrease in the resistance to TMP-SXT, while increase in the resistance to ciprofloxacin have been seen (p<0.0001). In a meta-analysis performed by Işıkgöz Taşbakan et al.<sup>[15]</sup>, the authors detected decrease in the resistance to co-trimoxazole, and increase in the resistance to ciprofloxacin were detected. Decrease in the resistance to TMP-SXT might be due to its decreased use. With introduction of quinolones into clinical use, utilization of TMP-SXT has gradually declined. In a study conducted by Arman et al.<sup>[4]</sup> it has been observed that quinolones has been prescribed as the first-line empirical treatment of the patients who consulted to the primary healthcare centers with complaints of UTI, while, nitrofurantoin, and TMP-SXT have been the least prescribed antibiotics. Decrease in the rates of resistance to TMP-SXT within years might be related to their lower levels of preference. Therefore, it has been concluded that in cases where alterations in regional resistance rates against TMP-SXT are lower than 20%, TMP-STX can be preferred for the empirical treatment of urinary system infections.

Nowadays, frequent use of quinolones as a primary-line treatment of UTI, quinolone-resistant *E. coli* has become a serious problem. Primary mechanisms involving in the development of resistance against quinolones include decrease in the intracellular deposition of the antibiotics because of chromosomal mutations occurring in the genes encoding DNA girase, and topoisomerase IV enzymes, decrease in efflux pumps or permeability of the outer membrane of the bacteria. However in recent years, plasmid-mediated quinolone resistance has emerged in addition to these mechanisms of resistance. Though these genes of resistance termed as *Qnr* do not induce quinolone resistance per se they cause decrease in the susceptibility to quinolone, and increase in minimum inhibitor concentration values of the drug. Plasmid-mediated quinolone resistance in the Enterobacteriaceae family has been increasingly reported from different parts of the world.<sup>[16]</sup> These plasmids can convey resistance genes like beta-lactamases which induce resistance against other antibiotics. In recent years increase in the ESBL content of E. coli which is a microbial agent of communityacquired urinary system infections, and transport of qnr genes on these plasmids might be the reasons for increased quinolone resistance.<sup>[13]</sup> Besides, in our country nonprescription widespread use of ciprofloxacin which has an increased bioavailability, higher urinary concentration, and broader antibacterial spectrum is another reason for increased resistance. In conclusion, rapidly increasing numbers of quinolone-resistant E. coli isolates suggest that quinolones are not an appropriate choice in the empirical treatment of UTI which obviates the need to use quinolones before antibiotic susceptibility test results are obtained. Therefore rational antibiotic use is a must so as to possibly decrease antibiotic resistance.

Increase in the bacterial resistance against TMP-SXT, and quinolones brings treatment alternatives into question. Espeially in the ambulatory oral treatment of infections caused by ESBL producing E. coli isolates novel oral treatment alternatives are needed. One of these alternatives is fosfomycin. Though fosfomycin has been used in the treatment of mainly UTI since the year 1988, it is one of the antibiotics used worldwide against which resistance rates of E. coli still remain at extremely low levels. Since it can reach higher concentrations in urine with its lower toxicity, and cross-resistance with other antibiotics, it is used in the treatment of uncomplicated UTIs.<sup>[4]</sup> Only 4.3% of uropathogen E. coli isolates have demonstrated resistance to fosfomycin. In a study performed by Pullukçu et al.<sup>[17]</sup>, 95.5% of the ESBL producing E. coli isolates were found to be susceptible to fosfomycin, and the authors emphasized that fosfomycin is an effective alternative in the treatment of uncomplicated urinary system infections. In this study 52 patients with complicated UTI infections caused by ESBL producing E. coli spp. were given daily doses of 3 g fosfomycin for 3 days, and 94.3% clinical, and 78.5% microbiological success rates were achieved. Fosfomycin susceptibilities of uropathogen E. coli isolates were investigated in various studies. Among them Saltoğlu et al.<sup>[18]</sup> could not detect any E. coli strains resistant to fosfomycin, while Arman et al.<sup>[4]</sup> reported very low resistance rates as 3.6 percent. In European countries similar rates have been reported.<sup>[13]</sup> Senol et al.<sup>[19]</sup> compared fosfomycin, and carbapenem in the treatment of complicated lower UTIs caused by E. coli, and despite comparable clinical, and microbiological success rates, the authors reported favourable cost-effectiveness with fosfomycin treatment. Fosfomycin appears to be an effective alternative in the current treatment of community-acquired

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urinary system infections thanks to ease of single dose applications, better patient compliance, lower complication rates, and its safe use in children, and pregnants.

Nitrofurantoin is an antibacterial agent specific to urinary tract infections. It has bacteriostatic effects in lower, and bactericidal effects in higher concentrations.<sup>[20]</sup> In our study a 0.9% bacterial resistance against nitrofurantoin was detected. Among other relevant studies performed in our country, various resistance rates were reported related to nitrofurantoin use as 0.9% by Arman et al.<sup>[4]</sup>, and 19.1% by Işıkgöz Taşbakan et al.<sup>[15]</sup> (meta-analysis). Nitrofurantoin with relatively lower cost has generally yielded improved outcomes with 5 days of therapy. In their study encompasing 75 patients with lower UTIs caused by ESBL producing E. coli strains, Işıkgöz Taşbakan et al.<sup>[20]</sup> detected clinical, and microbiological success rates of 69, and 68% with nitrofurantoin therapy, and observed reinfection in 2, and relapse in one patient. In studies performed in other countries, nitrofurantoin resistance rates also remained at lower levels. In an ECO-SENS study its incidence was reported as lower than 2 precent.<sup>[9]</sup> Nitrofurantoin seems to be a good alternative for uncomplicated lower urinary tract infections when given in appropriate dosages and at suitable time intervals.

Use of antibiotics containing beta-lactam ring, and broad spectrum cephalosporins have increased during the last two decades in the whole world which facilitated emergence of ESBL producing microorganisms. ESBL contained in uropathogen E. coli isolates induces resistance against oral penicillines, and cephalosporins, and also higher resistance against other groups of antibiotics including primarily aminoglycosides, and quinolones which creates problems in clinical use.<sup>[21]</sup> In our study, resistance rates of uropathogen E. coli isolates against various antibiotics were as follows: ampi-cillin (66.9%), cephazolin (42%), cefuroxime (36.9%), ceftazidime (14.9%), ceftriaxone (28%), cefepime (12%), amoxicillin-clavulanic acid (36.9%), While resistance againat imipenem, and meropenem was not detected. ESBL was detected in 24% of E. coli isolates. In our country, rates of resistance against ampicillin were detected as 37% Yuluğkural et al.<sup>[11]</sup>, and 70% Yousefi Rad.<sup>[22]</sup> Resistance rates change among regions. In developing countries rates of resistance against ampicillin rise up to 98%, while as indicated in ECO-SENS study, in European countries it varies between 21, and 34 percent.<sup>[5,9]</sup> In a meta-analysis performed by Aykan et al.<sup>[12]</sup>, and Işıkgöz Taşbakan et al.<sup>[15]</sup> rates of resistance were found to vary between 18.5, and 22.%. Rates of resistance against amoxicillin-clavulanic acid (AMC) were found to be 21, and 42.5% in studies performed by Yuluğkural et al.[11] and Yılmaz et al.<sup>[10]</sup>, respectively. In our study, resistance rates of beta-lactam antibiotics were observedly consistent with relevant data of our country. These higher rates of resistance

especially against ampicillin, and AMC were interpreted as consequences of frequent preference of these antibiotics in empirical treatment, and inappropriate antibiotic policy. In studies performed in our country ESBL production was detected in 1-54.4% of *E. coli* isolates.<sup>[21]</sup> In other countries lower percentages of ESBL producing uropathogen *E. coli* isolates were remarkable.<sup>[23]</sup> In this study higher rates of resistance against oral ampicillin, and 1., and 2. generation cephalosporins suggest that they are not suitable candidates for empirical treatment.

Aminoglycosides have an important place in the treatment of infections caused especially by gram-negative bacteria. Since agents of urinary tract infections are gram-ngative bacteria, aminoglycosides are preferred in the empirical treatment of UTI infections. Although an incorrect application, in the ambulatory treatment of community-acquired urinary system infections single dose of aminoglycosides have been frequently used.In our study resistance to gentamycin, and amikacin was detected in 24, and 0.3% of the patients. These rates have demonstrated variations in studies performed in our country. Resistance rates to gentamycin were found to be 8, and 28% in studies performed by Arslan et al.<sup>[14]</sup>, and Yılmaz et al.<sup>[10]</sup>, respectively. Resistance rates to amikacin were detected as 0.7, and 9.5% in studies conducted by Doğru et al.<sup>[3]</sup>, and Yılmaz et al.<sup>[10]</sup>, respectively. In some studies performed abroad resistance rates to gentamycin ranged between 1.3% (ECO.SENS<sup>[13]</sup>) and 63.4% (Gupta et al.<sup>[5]</sup> India). Huang et al.<sup>[23]</sup> could not detect antimicrobial resistance against amikacin, contrary to the findings of Sharma et al.<sup>[24]</sup> (33.3%). In our study higher rates of resistance to gentamycin can be related to widespread use of this antibiotic in the treatment of infections of other systems.

Based on above-mentioned recommendations in order to initiate empirical treatment in community-acquired urinary system infections, regional resistance to the antibacterial drug to be used should not exceed 10-20%, and based on our study outcomes we can use fosfomycin, and nitrofurantoin in empirical treatment. Increasing rates of resistance to quinolones can be an alarming sign if we consider unnecessary, widwespread, use of antibiotics, and random prescription of antibiotherapy. When we have taken both importance of monitorization of regional resistance into account, and position of our hospital as one of the final and the most biggest tertiary health center in the referral chain of our region, we think that study data will also reflect the relevant data of a large population.

In conclusion, as is the case with the whole world increasing rates of antibacterial resistance in our region which is thought to be related to erroneous strategies of antibiotherapy should be closely monitored both at the regional, and national levels. Based on the results obtained antibiotherapy strategies should be developed in no time at all. Ethics Committee Approval: This manuscript is retrospective data analysis so ethics committee approval were not included.

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