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# Susceptibility of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Urine Isolates to Fosfomycin, Ciprofloxacin, Amikacin and Trimethoprim-Sulfamethoxazole

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**Aim:** Urinary tract infections (UTIs) caused in particular by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* strains are related with high morbidity and mortality, and treatment is quite difficult. These infections generally are treated by carbapenems, and their costs are high. We aimed in this study to investigate the susceptibilities of ESBL-producing *E. coli* strains isolated from urine cultures to fosfomycin, ciprofloxacin, amikacin and trimethoprim-sulfamethoxazole and to determine the general resistance profile in our region of these strains isolated from UTIs.

**Materials and Methods:** Between January 2005-December 2005, ESBL-producing *E. coli* strains isolated from urine samples sent from various outpatient and inpatient clinics to the Bacteriology Laboratory of the Department of Microbiology and Clinical Microbiology were included prospectively in the study. ESBL production was detected using the double disk synergy test. Antibiotic susceptibility testing was performed for ESBL-producing isolates by disk diffusion test according to Clinical and Laboratory Standards Institute (CLSI) criteria. *Escherichia coli* ATCC 35218 and ATCC 25922 were used as control strains. The diagnosis of nosocomial UTIs was established according to the Centers for Disease Control and Prevention criteria. The data were assessed using the SPSS 11.0 packet program.

**Results:** A total of 344 ESBL-producing *E. coli* isolates (241 nosocomial isolates; 103 outpatient isolates) were included in the study. The rates of resistance were 3.5% for fosfomycin, 76.5% for ciprofloxacin, 11% for amikacin, and 74.4% for trimethoprim-sulfamethoxazole. While resistance rates of nosocomial strains were 4.1%, 81.3%, 11.2%, and 71%, respectively, resistance rates of the strains isolated from outpatients were 1.9%, 65%, 10.7%, and 82.5%, respectively. There were statistically significant differences between the two groups for ciprofloxacin and trimethoprim-sulfamethoxazole.

**Conclusions:** Because of the high antibiotic resistance rates in our country, we think that fosfomycin and amikacin may have priority in the treatment of non-complicated UTIs caused by ESBL-producing *E. coli* strains due to ease of use and high concentration in the urine.

Key Words: Urinary tract infections, ESBL, *Escherichia coli*, fosfomycin

## İdrar Kültürlerinden Soyutlanan Genişlemiş Spektrumlu Beta-Laktamaz Üreten *Escherichia coli* Kökenlerinin Fosfomisin, Siprofloksasin, Amikasin ve Trimetoprim-Sulfametoksazol'e Duyarlılıkları

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**Amaç:** Özellikle genişlemiş spektrumlu beta-laktamaz (GSBL) üreten *Escherichia coli* kökenlerinin neden olduğu üriner sistem enfeksiyonlarının tedavisi oldukça güç, mortalite ve morbiditesi de yüksektir. Bu enfeksiyonlar genellikle karbapenemlerle tedavi edilmekte ve yüksek tedavi maliyeti görülmektedir. Çalışmamızda idrar kültürlerinden soyutlanan GSBL üreten *E. coli* kökenlerinin fosfomisin, siprofloksasin, amikasin ve trimethoprim-sülfametoksazole duyarlılığının araştırılması ve bölgemizdeki üriner sistem enfeksiyonlarından soyutlanan bu kökenlerin genel direnç durumunun belirlenmesi amaçlanmıştır.

**Yöntem ve Gereç:** Ocak 2005-Aralık 2005 tarihleri arasında bakteriyoloji laboratuvarımıza gönderilmiş olan üriner sistem enfeksiyonlarından soyutlanan GSBL üreten kökenler prospektif olarak çalışmaya alınmıştır. GSBL üretimi çift disk sinerji testi ile belirlenmiştir. GSBL üreten suşların antibiyotik duyarlılık testleri "Clinical and Laboratory Standards Institute (CLSI)" kriterlerine göre disk diffüzyon yöntemiyle yapılmıştır. Kontrol kökeni olarak *E. coli* ATCC 35218 ve ATCC 25922 kullanılmıştır. Nozokomiyal üriner sistem enfeksiyonu tanısı Centers of Disease Control and Prevention kriterlerine gore değerlendirilmiştir. Veriler SPSS11,0 paket programı kullanılarak değerlendirilmiştir.

**Bulgular:** 2005 yılında laboratuvarımızda soyutlanan 344 GSBL üreten *E. coli* kökeni (241 nozokomiyal köken, 103 poliklinik hastalarından soyutlanan köken) çalışmaya dahil edilmiştir. Direnç oranları fosfomisin için % 3,5, siprofloksasin için % 76,5, amikasin için % 11 ve trimetoprimsülfametoksazol için % 74.4'tür. Hastane kaynaklı kökenlerde direnç oranları sırasıyla % 4,1, % 81,3, % 11,2, % 71 iken poliklinik hastalarından soyutlanan kökenlerde % 1,9, % 65, % 10,7, % 82,5'tur. Siprofloksasin ve trimethoprim-sülfametoksazol için iki grup arasında istatistiksel olarak fark vardır.

**Sonuç:** Ülkemizdeki antibiyotik direnç oranlarının yüksek olması nedeniyle, fosfomisin ve amikasinin GSBL üreten *E. coli*'nin neden olduğu komplike olmayan üriner sistem enfeksiyonlarının tedavisinde ilk seçenekler arasında olduğunu düşünmekteyiz.

**Anahtar Sözcükler:** Üriner sistem enfeksiyonu, GSBL, *Escherichia coli*, fosfomisin.

## Introduction

Community- and hospital-acquired urinary tract infections (UTI) are among the frequently encountered infectious diseases (1). The antimicrobials most often used in the treatment of UTI are amoxicillin, trimethoprim-sulfamethoxazole, aminoglycosides, cephalosporins and fluoroquinolones. In recent years, fosfomycin-tromethamine (FT) has also become available.

It is acknowledged that today there is an increase in the antimicrobial-resistance rates of UTI pathogens. The treatment of nosocomial (hospital-acquired) UTIs caused in particular by extended- spectrum beta-lactamase (ESBL)-producing *Escherichia coli* strains is quite difficult. Those infections are associated with increased morbidity and mortality (2). Associated with plasmid-mediated ESBL, resistance to β-lactam antibiotics in *Enterobacteriaceae* has become a worldwide problem (3,4). ESBL confers clinically significant resistance to broad-spectrum penicillins, monobactams and cephalosporins (except cephamycins), and are often associated with resistance to other non-related antibiotics in multiresistant pathogens (5).

In our study, we aimed to investigate the susceptibilities of ESBL-producing *E. coli* strains isolated from urine cultures to FT, ciprofloxacin, amikacin and trimethoprim-sulfamethoxazole, and thus to determine the general resistance profile in our region of these strains isolated from UTIs.

## Materials and Methods

A total of 27122 urine samples were sent from various outpatient and inpatient clinics to the Bacteriology Laboratory of the Department of Microbiology and Clinical Microbiology of Ege University Medical Faculty between January 2005 and December 2005. Of these 27122, 2983 samples yielded *E. coli.* Three hundred forty-four of these 2983 *E. coli* samples were included in this study.

Mid-stream urine samples collected in a sterile container were inoculated onto 5% sheep blood agar and eosin methylene blue agar by using quantitative method. The bacteria were defined by conventional and automated methods (API / VITEK 2, Bio Mérieux Marcy L'etoile, France) in samples in which bacterial growth was detected. Antibiotic susceptibility to gentamicin, ampicillin, amoxicillin/clavulanate, cephazolin, cefuroxime, ceftriaxone, ceftazidime, cefepime, imipenem, and meropenem was determined via disk diffusion tests with antibiotic-containing disks (Oxoid Ltd., Besingstoke, Hampshire, UK) on Mueller-Hinton agar plate. The results were expressed as susceptible or resistant according to criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) (6). ESBL production was detected using the double disk synergy test (DDST)(7). DDST with amoxicillin-clavulanate, cefotaxime, ceftazidime, cefpodoxime (ceftriaxone) and aztreonam (by CLSI recommendation) were performed to detect the ESBL producers. DDST was done on Mueller-Hinton agar plates (Oxoid Ltd., Besingstoke, Hampshire, UK) streaked with a 0.5 McFarland bacterial suspension of isolates by placing a disk containing 20 µg amoxicillin plus 10 µg clavulanic acid (AMC) in the center of the plate. This central disk was then surrounded by a disk of ceftazidime, cefotaxime, cefpodoxime (or ceftriaxone) and aztreonam (OXOID), each at the distance of 30 mm from the central disk (center to center). The DDST was considered positive when decreased susceptibility to the broad-spectrum β-lactam agent on the outer disk was associated with synergy towards the central disk. Synergy was defined when the inhibition zone of the disk of cephalosporin plus clavulanic acid was ≥5 mm greater than that of the disk of cephalosporin alone.

Strains that did not produce ESBL were not included in the study. Only one isolate from each patient was included in the study; recurrent isolates were excluded.

The following antimicrobial agents were tested by disk diffusion method: FT (200 µg), ciprofloxacin (5 µg), amikacin (30 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg). *E. coli* ATCC 35218 and *E. coli* ATCC 25922 were used as control strains.

The data were evaluated with the SPSS 11.0 packet program. Resistance patterns were compared by chi-square test.

## Results

A total of 344 ESBL-producing *E. coli* isolates (241 nosocomial isolates, 103 outpatient isolates) were included in the study. All strains were resistant to beta-lactam antibiotics, and susceptible to carbapenems.

The resistance rates detected were 3.5% (12/344) for FT, 76.5% (263/344) for ciprofloxacin, 11% (38/344) for amikacin, and 74.4% (256/344) for trimethoprim-sulfamethoxazole. While the resistance rates of strains isolated from inpatients were 4.1%, 81.3%, 11.2%, and 71%, respectively, the resistance rates of strains isolated from outpatients were 1.9%, 65%, 10.7%, and 82.5%, respectively. The resistance rates of nosocomial and outpatient isolates are summarized in Table. There was a statistically significant difference in resistance rates of *E. coli* isolates to ciprofloxacin and trimethoprim-sulfamethoxazole according to those causing nosocomial or outpatient UTIs ($P < 0.05$).

## Discussion

The treatment of UTIs caused in particular by ESBL-producing *E. coli,* is difficult and more expensive due to the multiple-drug resistance. In this study, the resistance rates of *E. coli* strains to non-beta-lactam antibiotics were determined and other possible treatment options were reviewed. In the many studies conducted in Turkey on urinary tract pathogens, the resistance rates for fluoroquinolones, trimethoprim-sulfamethoxazole and amikacin have been demonstrated. However, since fosfomycin has only very recently been introduced, the number of studies with this agent is inadequate.

It is noted that the effectiveness of ciprofloxacin on ESBL-producing strains has diminished. The increased resistance rates in recent years of Gram-negative bacteria to fluoroquinolones can account for this condition (8-10). In studies carried out in Turkey, fluoroquinolone resistance has been found to range between 21.8% and 47% in community-acquired UTIs (11-15). In our study, ciprofloxacin resistance was found as 81.3% in strains isolated from inpatients and as 65% from outpatients. These rates are higher than those found in other studies conducted in our region, but are consistent with our previous data. In our hospital, ciprofloxacin resistance in *E. coli* strains isolated from urine cultures in 2004 was detected as 47% (n = 1116) in hospitalized patients and 30.2% (n = 1448) in outpatients (11). Similarly, ciprofloxacin resistance in 357 *E. coli* strains isolated from blood cultures in our hospital in 2004-2005 was reported to be 52.6% (16). In studies reported from

Table. Resistance rates of *E. coli* strains against antimicrobial agents.

| | ESBL-producing *E. coli* strains isolated from hospitalized patients n = 241 (%) | ESBL-producing *E. coli* strains isolated from outpatients n = 103 (%) | P |
|---|---|---|---|
| Amikacin | 27 (11.2) | 11 (10.7) | NS |
| Ciprofloxacin | 196 (81.3) | 67 (65) | P < 0.01 |
| Cotrimoxazole | 171 (71) | 85 (82.5) | P < 0.025 |
| Fosfomycin | 10 (4.1) | 2 ( 1.9) | NS |

NS: Not significant.

various countries, there are variable resistance rates. Marchese et al. (17) reported a 12% ciprofloxacin resistance in *E. coli* strains isolated as UTI pathogens. In another study reported from Italy, the same resistance rate was reported in 387 *E. coli* strains that were the causative agents of non-complicated UTIs (18). Puerto et al. (19) reported high resistance rates in a region experiencing resistance problems in Spain, similar to our rates. Widespread fluoroquinolone use and occurrence of cross resistance among fluoroquinolones may account for the high resistance rates in our country.

Trimethoprim-sulfamethoxazole is an agent that has been used in the treatment of UTIs for a very long period, before quinolones entered the market. However, because of widespread resistance rates, fluoroquinolones are now more frequently preferred. In our study, a resistance rate of 74% was detected in ESBL-producing strains. In another study, a trimethoprim-sulfamethoxazole resistance of 19.4% (577/2980) was reported in strains not producing ESBL and a rate of 7.1% (12/169) in ESBL- producing strains (20). While the resistance rate to trimethoprim-sulfamethoxazole is 24% in studies reported from Italy, in a study by Kahlmeter (21), the resistance rates in strains obtained from various countries ranged between 17.9% and 54.3%. Ungheri et al. (22) reported trimethoprim-sulfamethoxazole resistance in strains resistant to quinolones as 48.1%. In the study by Puerto et al. (19), similar to our own rates, there was a trimethoprim-sulfamethoxazole resistance rate of 49.3% in strains not producing ESBL and a resistance rate of 53.9% in ESBL-producing strains.

Amikacin has been found to be effective against *E. coli* strains (Table). Similarly, the resistance rate in our settings of *E. coli* strains that caused nosocomial bacteremia in 2004-2005 was 8.4% (16). While Puerto et al. (19) and Alhambra et al. (23) did not report amikacin resistant strains from Spain, Hernandez et al. (24) reported a resistance rate of 6.5% in ESBL-producing strains. On the other hand, in the study conducted by Tonkic et al. (20), amikacin resistance of 83.9% (146/174) was reported in ESBL-producing *E. coli* strains. Our results are similar to those of Mediterranean countries. In our study, although there was a difference between the two groups, it was not statistically significant (probably due to low resistance rates in both groups.).

Fosfomycin is a phosphonic acid derivative effective against urinary tract pathogens that has been in use for a long time in European countries. Upon oral administration, it is rapidly metabolized and excreted in the urine unchanged. It has the advantage of single dose administration. Since it has been introduced in recent years in our country, it is being regarded as a new treatment alternative. In a previous study, fosfomycin resistance was not detected in 72 community-acquired *E. coli* strains not producing ESBL (25). Arca and Karabiber (26) reported 0.8% resistance in 120 *E. coli* strains and Deveci et al. (27) reported no fosfomycin resistance among 100 *E. coli* strains, 13 of them being ESBL-producers. In the ECO.SENS project covering 16 European countries and Canada, fosfomycin resistance in *E. coli* strains isolated from non-complicated UTIs was detected as 0.7% in 2000 (21). In studies reported from Spain, Daza et al. (2000) (28) detected a fosfomycin resistance rate of 1% and Alhambra et al. (2004) (23) detected a resistance rate of 2.2%. In our study, a resistance rate of 3.5% was detected in ESBL-producing strains. Similarly, in studies reported from Italy, while Ungheri et al. (22) did not report any resistance, in another two studies, a resistance rate of 1% was detected (17,18). In a recent study, Pullukcu et al. (29) reported 94.3% clinical and 78.5% microbiological success in 52 patients with ESBL-producing *E. coli*-related lower UTIs using FT (3g x 1 every other night, three times).

In conclusion, UTIs, especially those caused by ESBL-producing *E. coli* strains, are frequently encountered infections necessitating long treatment periods and high costs. When the high resistance rates in our country are taken into consideration, fosfomycin and amikacin will probably be favored due to ease of use and high concentrations in the urine.

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