ORIGINAL ARTICLE

In vitro activity of fosfomycin against *Escherichia coli* strains isolated from recurrent urinary tract infections

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ABSTRACT

Aim To investigate the prevalence of susceptibility of *E.coli* isolates causing recurrent urinary tract infections (UTIs) to fosfomycin.

Methods A total of 679 urine samples obtained from 524 patients with UTI prediagnosis, which revealed *E.coli* in the microbiological culture in the period between August 2011 and January 2013 was included in the study. Antimicrobial susceptibility was determined by disk diffusion method according to Clinical and Laboratory Standard Institute (CLSI). Recurrent UTI was defined as UTI which occurred in the same patients at least twice at two different time periods that were more than two months after the previous infection in the 18-month-period of the study, all of which were caused by *E.coli*.

Results Among 524 patients, ten isolates (1.9%) were found resistant to fosfomycin. With respect to fosfomycin resistance, no significant differences were found between extended-spectrum beta-lactamase (ESBL)-producing and ESBL-negative isolates (p=0.23). Resistance to fosfomycin was significantly higher in the recurrent UTI group (3/31; 9.7%) compared to the non-recurrent UTI group, (7/493; 1.4%) (p=0.03).

Conclusion Fosfomycin is still a good alternative in *E.coli*-caused UTIs. However, in recurrent UTI cases, resistance can develop to fosfomycin, so susceptibility to this agent should be determined.

Keywords: UTI, ESBL, recurrent, Turkey.

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INTRODUCTION

Urinary tract infections (UTI) are among the most common bacterial infections in humans (1,2). *Escherichia coli* is the most frequent causative microorganism in UTI (1,3). Extended-spectrum beta-lactamase (ESBL) produced by *E. coli* limits the treatment options in infections caused by this species (1,2). ESBL-producing *E. coli* is accepted to be resistant to penicillins, cephalosporins and monobactams (1,2). In addition, cross-resistance can occur to floroquinolones, cotrimoxazoles and aminoglycozides all of which are frequently preferred in UTI treatment (1,4). Because of the increasing rate of resistance, there are studies focused on effective, easy-to-use, and low-resistance-forming antimicrobials for UTI antibiotherapy (1,3). Fosfomycin, a broad-spectrum phosphoenolpyruvate analog antimicrobial, which prevents the first step of cell wall synthesis of the bacteria with inhibiting UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) enzyme is preferred as an alternative agent for uncomplicated UTI due to the advantage of single-dose use, rare side effects, and low resistance rates in Enterobacteriaceae (5,6).

Usage of fosfomycin as an alternative drug in treatment of uncomplicated UTI has started. This agent has been used as a single-dose in UTI treatment in various European countries since 1988 (4,6). Fosfomycin is well-tolerated and leads to little nephrotoxicity (6,9). The oral form is fosfomycin-tromethamine (9).

There are no studies conducted on the relationship between fosfomycin resistance and recurrent UTI caused by *E. coli*. This study investigated the susceptibility rate of *E.coli* strains isolated in recurrent and non-recurrent UTI to fosfomycin. The aim of this study was to demonstrate whether *E. coli* strains gain resistance to fosfomycin in cases of recurrent UTIs.

MATERIALS AND METHODS

Urine samples from patients with UTI prediagnosis were collected from various clinics of Abant Izzet Baysal University Hospital between August 2011 to January 2013. The specimens were inoculated onto 5% sheep blood agar and eosin methylene blue agar media (Oxoid, Basingstoke, United Kingdom) with 0.001 mL-loops and were incubated on 37°C for 24h. After the incubation, microorganism growth of > 10^4 CFU/mL was considered to be a marker of infection. The enteric Gram-negative bacteria were identified to the species level according to standard biochemical test results. Conventional methods such as oxidase, citrate, urease, indole, methyl red, and Voges-Proskauer tests and triple-sugar iron agar (for lactose and glucose fermentation) were used for identification (10). A total number of 679 urine samples showing *E. coli* in culture was obtained from 524 patients.

Antimicrobial susceptibility was determined using disks of 14 antimicrobials (Oxoid, England) on Mueller-Hinton agar media by Kirby-Bauer method (7). Fosfomycin trometamol disk (200 µg fosfomycin/50 µg glucose-6-phosphate) (Oxoid, Basingstoke, United Kingdom) was used to determine the susceptibility to fosfomycin and growth-inhibition zone was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) criteria (7). ESBL productivity was investigated with double-disk synergy method (7). *E. coli* ATCC 25922 standard strain was used for quality control.

Urinary tract infection was classified into two groups, recurrent and non-recurrent. Recurrent UTI for this study was defined as UTI which occurred in the same patients at least three times at three different time periods with intervals of more than two months in the 12-month-period of the study, all of which were caused by *E. coli* (11). Non-recurrent UTIs were accepted when only one UTI occurred in the period investigated. Non-recurrent UTIs were limited to the patients admitted to our hospital only, admittances of patients to other medical centers were ignored because of the difficulties of obtaining data from the records.

The resistance rates were calculated after excluding recurrent culture results of the same patients.

This study was approved by the Ethical Committee of Abant Izzet Baysal University Clinical Researches, Turkey. All the data of the

patients and isolates were obtained from the hospital and laboratory records retrospectively.

Descriptive statistics was expressed as numbers and percentages. Differences between the groups and correlations between the variables according to categorical variables were analyzed with $\chi 2$ test and Fisher's Exact test. The results were evaluated within 95% confidence interval and a p value of <0.05 was accepted as significant.

RESULTS

Among 524 patients with UTI, a total of 31 (5.9%) patients admitted to the hospital three times or more with a result of *E. coli*-revealed urine culture were accepted as recurrent UTI group, and 493 patients were accepted as non-recurrent UTI group.

A total of ten (1.9%) isolates were found fosfomycin-resistant. With respect to susceptibility rates, fosfomycin was the most effective agent secondly after imipenem, which showed no resistance. Four of the fosfomycin-resistant isolates were ESBL-producing isolates and the other six were ESBL-negative. No significant difference was found between fosfomycin susceptibility rates according to ESBL positivity (p=0.2318). Three (9.7%; 3/31) of the fosfomycin-resistant isolates were in the recurrent UTI group, and the other seven (1.4%; 7/493) were in the non-recurrent UTI group. (p= 0.017).

No significant relatedness was found between resistance to fosfomycin and other antimicrobials, e.g. levofloxacin, trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, imipenem, gentamicin, ceftriaxone, and cefuroxime (p>0.05 for each) (Table 1).

Table 1. Resistance rates of 524 *E. coli* strains to the antimicrobials

Antibiotics	Resistance rate (%)
Imipenem (10 μg)	0
Fosfomycin (200 μg)	1.9
Nitrofurantoin (300 μg)	7.1
Amoxicillin/clavulanic acid (20/10 μg)	9.4
Amikacin (30 μg)	15.2
Cefoxitin (30 μg)	22.6
Ceftriaxone (30 μg)	23.4
Levofloxacin (5 μg)	33.3
Ciprofloxacin (5 μg)	36.1
Gentamicin (10 μg)	36.1
Norfloxacin (10 μg)	37.8
Trimethoprime/Sulfamethoxazole (23.75/1.25 μg)	42.2
Tetracycline (30 μg)	48.7
Ampicillin (10 μg)	63.1

DISCUSSION

Members of Enterobacteriaceae are most commonly isolated as the causative agents in UTI. The antimicrobial susceptibility patterns of these microorganisms have changed due to the uncontrolled antibiotic use (1,3). Recent studies have reported a decrease in susceptibility rates to frequently used antimicrobials (1,3,8). Urinary tract infection cases are frequently treated with co-trimoxazoles and quinolones, however, high resistance rates suggest that new antimicrobial should be used in some cases (3,8).

The CLSI criteria for fosfomycin are accepted just for *E. coli* strains isolated from UTI (7). However, the British Society for Antimicrobial Chemotherapy (BSAC) recommends this agent for other Enterobacteriaceae too, but some reports state that fosfomycin is a promising therapeutic option for *E. coli* strains including ESBL-producing ones rather than *Klebsiella* spp. (2,8,9).

Studies from outside of Turkey have revealed resistance rates between 1.2-4.5% (1,3,9,19). Among the studies conducted in Turkey, the resistance rates were reported between 0-8% (12-18). In our study the resistance rate to fosfomycin of 1.9% was detected, which is within the range of previous data from our country (12-18). All those reports demonstrate the low resistance rate to fosfomycin though this agent has been used for a long time. Resistance to fosfomycin develops rarely and most of these are chromosomal or plasmid-mediated. The chromosomal resistance is caused with mutations in structural genes which code bacterial proteins helping to transport the agent into the cell (4). These mutations corrupt L-alpha glycerolophosphate and hexose phosphate systems both of which are basic transport mechanisms of the bacteria. Such modification reduces the passage of fosfomycin into the cell thus decreasing its effect on the target region (2,4). Several mechanisms have been hypothesized to expla-

in the low rates of resistance to fosfomycin. Reduction of bacterial adhesion by fosfomycin can prevent development of resistance (2,21). The absence of use of fosfomycin in (surrounding) veterinary clinics may also keep this rate low (4).

In our study, resistance rate to fosfomycin in recurrent UTI was statistically significantly higher than the rate of non-recurrent cases (9.7% versus 1.4%, respectively). We found that only one isolate among the *E. coli* strains of recurrent UTI of the same patients showed resistance to fosfomycin and that it was the last one isolated to the date. Despite the bare significance and the low number of resistant isolates and the need for larger studies, this observation and analysis may demonstrate that *E. coli* strains, which cause recurrent UTI, might be more prone to gain antibiotic resistance to fosfomycin. To our knowledge, such observations have not been reported before. Besides this, our study was limited to the records of our hospital, which is the only tertiary care center of Bolu Province. We could not find out whether the patients had been admitted to other medical centers in or outside our province; so the "recurrence" term accepted in our study might not be accurate if the patients of non-recurrent UTI group had been admitted to other centers. Our significant result about the recurrence and resistance to fosfomycin might lose the significance if our patients' urine cultures revealed fosfomycin-susceptible *E. coli* in other medical centers. In addition, we checked out the admissions of each patient backwards within one year before their first culture was included in our study in order to rule out the repetitions and to distinguish the groups more accurately.

In this study, no resistant isolates were found to imipenem according to CLSI 2009 criteria, which were changed after 2007. In addition, no relationship was found between resistance rate to fosfomycin and resistance to other drugs included. This finding supports the report of Ko et al. (22), who demonstrated that fosfomycin did not have cross-resistance with other antimicrobials.

In our study, we used a cut-off value of 10^4 CFU/mL for determination of UTI. Although, the most appropriate cut-off value of UTI is still controversial, guidelines using 10^4 CFU/ml as cut-off value are generally accepted for UTI caused by *E. coli* (23). For example, de Backer et al. (1) used the cut-off value of 10^5 in their study, because of better comparison of the results with previous surveillance.

In the present study, we did not classify UTI cases into complicated or uncomplicated infections. We aimed to get the resistance rate among all *E. coli* isolates as we consider that virulence factors or antibiotic resistance profiles of *E. coli* strains do not vary according to potential complications of the infection (1,3,6). Indeed, the term of "complicated UTI" does not include the type of the causative pathogen (1,3,6,15). Besides, further molecular trials should be done to demonstrate this consideration accurately.

One of the limitations in the present study was performing only disk diffusion method for determining the antimicrobial susceptibility profiles. We did not keep all isolates for further testing to determine minimal inhibitory concentration values. However, de Cueto et al. (24) demonstrated that there was no discrepancy between different methods to determine the susceptibilities of *E. coli* strains to fosfomycin. Therefore, our findings are expected to be reliable.

In conclusion, the data obtained from our study suggest that fosfomycin, which has advantages as ease of use and low resistance rates, is still a good alternative in *E.coli*-caused UTI. However, in recurrent UTI cases, resistance can develop to fosfomycin, so susceptibility to this agent should be determined.

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