KÜ Tıp Fak Derg 2008;10(1)
ISSN 1302-3314
Orijinal Makale

The Susceptibility to Fosfomycin Tromethamine of Ciprofloxacin Sensitive and/or Resistant *Escherichia Coli* Strains Isolated from Urine Cultures, and Comparison of Disk Diffusion and Agar Microdilution Tests in Detection of Fosfomycin Tromethamine Susceptibility

Selda CANVER*, Jülide Sedef GÖÇMEN*

*Departments of Microbiology and Clinical Microbiology, Faculty of Medicine, Kirikkale University, Kirikkale, TURKEY

Abstract

Aim: To detect the susceptibility to fosfomycin of Escherichia coli strains, the most common infective agent of the urinary system, which develops resistance to ciprofloxacin with an increasing trend and to compare the effectiveness of two different detection in-vivo methods of the susceptibility to fosfomycin.

Material: Three hundred and seven E.coli strains those isolated from urinary samples of patients with urinary infection, attempted to polyclinic and clinics at microbiology laboratory between January 2007 and June 2007. The susceptibility to fosfomycin and ciprofloxacin by E.coli strains was studied by Kirby Bauer disk diffusion test. In addition, the susceptibility to fosfomycin of randomly selected 50 ciprofloxacin resistant and 50 sensitive E.coli strains was evaluated by microdilution agar method. The results of two detection methods were compared subsequently.

Results: Of these studied 307 E. coli strains, 303 (98.7%) strains were found to be sensitive to fosfomycin tromethamine, whereas 196 (63.8%) strains showed sensitivity to ciprofloxacin. The susceptibility to fosfomycin of randomly selected 50 ciprofloxacin resistant E.coli strains was 100% by disk diffusion method, and 98% by agar microdilution method. The susceptibility to fosfomycin of randomly selected 50 ciprofloxacin sensitive E.coli strains was 94 % by both methods.

Conclusion: The susceptibility to fosfomycin of E.coli strains was found to be independent of ciprofloxacin susceptibility and/or resistance, in addition, there were no significant differences between the detection rates of disk diffusion and agar microdilution tests in detecting the susceptibility to fosfomycin of E.coli strains.

Key words: fosfomycin tromethamine, urinary infection, Escherichia coli, ciprofloxacin resistance

İdrar Kültürlerinden İzole Edilen Siprofloksasin Dirençli ve Duyarlı *Escherichia Coli* Suşlarında Fosfomisin Trometamol Duyarlılığı Ve Bu Duyarlılığın Disk Difüzyon ve Agar Mikrodilüsyon Yöntemleriyle Karşılaştırmalı olarak İn Vitro Araştırılması

Özet

Amaç: Çalışmamızda siprofloksasine karşı direnç oranı giderek artan üriner sistem enfeksiyonu etkeni olan E.coli suşlarında fosfomisin trometamol duyarlılığını belirlemeyi ve bu duyarlılığı belirlemede kullanılan iki farklı yöntemin etkinliğini karşılaştırmayı amaçladık.

Materyal Metod: Bu çalışmada; Ocak 2007–Haziran2007 tarihleri arasında Mikrobiyoloji laboratuarına poliklinik ve servislerden gelen, üriner sistem enfeksiyonu ön tanısı almış hastaların idrar örneklerinden izole edilen 307 E.coli suşunda Kirby Bauer disk difüzyon yöntemi ile sirofloksasin ve fosfomisin duyarlılığı belirlendi. Metot karşılaştırması amacıyla rastgele seçilen Siprofloksasin duyarlı ve dirençli 50'şer E.coli suşunda fosfomisin trometamol duyarlılığı ayrıca agar mikrodilüsyon yöntemi ile de çalışıldı.

Bulgular: Çalışılan 307 suşun 303 tanesi (%98.7) Fosfomisin trometamol'e, 196 tanesi (%63.8) siprofloksasin'e duyarlı bulundu. Rastgele seçilen Siprofloksasine dirençli 50 E.coli suşunda disk difüzyon yöntemiyle Fosfomisin trometamol duyarlılığı %100 olarak saptanırken agar mikrodilüsyon yöntemiyle bu oran %98 olarak tespit edilmiştir. Siprofloksasine duyarlı 50 E.coli suşunda ise duyarlılık her iki yöntemle de %94 olarak bulunmuştur.

Sonuç: Fosfomisin trometamol duyarlılığı suşların siprofloksasin'e duyarlı olup olmamalarından etkilenmediği gibi bu duyarlılığı belirlemede disk difüzyon ve agar mikrodilüsyon metotları arasında anlamlı bir farklılık görülmemiştir.

Anahtar Sözcükler : Fosfomisin trometamol, İdrar yolu enfeksiyonu, Escherichia coli, Siprofloksasin direnci

Introduction

Infections of the urinary tract are an important cause of mortality worldwide. In the development of urinary tract infections, host related factors such as gender, pregnancy, use of a urinary drain, and presence of diabetes mellitus as well as the virulence of uropathogen bacteria play an important role [1].

In recent years, resistance of uropathogen E coli strains to the antibiotics used for the treatment of urinary tract infections has increased. Thus, treatment with antibiotics fail because of the resistance of these strains, which ten results in increased cost of prescriptions, prolonged hospitalization, social costs, and increased morbidity and mortality rates [2].

This study aimed to detect the susceptibility to fosfomycin tromethamine (FOS) of Escherichia coli Strains, the most common infective agent of the urinary system, which develops resistance to ciprofloxacin (CIP) with an increasing trend and to compare the effectiveness of two different in-vivo detection methods of the susceptibility to fosfomycin tromethamine.

KÜ Tıp Fak Derg 2008;10(1)
ISSN 1302-3314
Orijinal Makale

Material and Method

In this study, 307 E.coli strains isolated from the urinary samples of 230 outpatients with prediagnosis of urinary tract infection and 77 inpatients with prediagnosis of infection (95 male, 212 female) were used. Obtained in the form of pure culture, the biochemical characteristics of the strains were studied and their species were identified. E.coli ATCC 25922 was used as the control strain.

Antibiotic susceptibility was studied by Kirby-Bauer disk diffusion method. The diameters of the inhibition zones that formed around the antibiotic discs were interpreted according to recommendations by Clinical and Laboratory Standards Institute (CLSI) [3]. Simultaneous wide-spectrum beta lactamas production of the strains was studied by double disk synergy method.

The CIP and FOS Minimal inhibition concentration (MIC) values of 50 randomly selected bacteria resistant and sensitive to CIP were also studied. For CIP, broth microdilution defined by CLSI and for FOS, agar microdilution methods were used. The range of sensitive-resistant MIC was ≤1 - ≥4 microgram/ml for ciprofloxacin and ≤64 - ≥256 microgram/ml for fosfomycin. The ciprofloxacin used in the study was obtained from Fluka company as a potency clear active agent and fosfomycin tromethamine, from Sigma Company as a potency clear active agent. CIP was studied at a concentration range of 0.0125-32 µg/ml, and FOS, at a concentration range of 16-2048 µg/ml.

The data were recorded using SPSS 15.0 package program. For the statistical analyses of the data, X^2 (Chi-square) test was used. $P<0.05$ was considered statistically significant.

Results

The study was performed on 307 E. coli strains isolated from the urinary samples of the patients with prediagnosis of urinary tract infection. Of the patients, 230 were outpatients, and 77 were inpatients. There were 95 male and 212 female patients. Twenty-seven strains (8.8%) formed extended spectrum beta-lactamases (ESBL). Thirteen strains (5.7%) isolated from the outpatients and 14 strains (18.2%) isolated from the inpatients were ESBL positive. The rate of ESBL formation in the inpatients was statistically significantly higher ($p=0.001$).

The distribution of antibiotic susceptibility and resistance rates of 307 E. coli strains according to Kirby-Bauer disk diffusion method is presented in Table 1

Comparisons of the antibacterial susceptibility rates of the outpatients and inpatients showed that there were no statistically significant differences between the susceptibility rates of the groups for nitrofurantoin, imipenem, amikacin and fosfomycin ($P>0.05$), while there were statistically significant differences between the susceptibility rates of the two groups for the other antibiotics tested ($p<0.05$) (Table 2).

In 50 E.coli strains determined to be resistant to ciprofloxacin by disk diffusion method, MIC was studied by broth microdilution method, and ciprofloxacin MIC value was determined to be over 32µg/ml.

In 50 E.coli strains determined to be sensitive to ciprofloxacin by disk diffusion method, MIC was studied by method and all of these strains were found to be sensitive to ciprofloxacin by MIC studies.

Comparisons of the antibacterial susceptibility rates of the E.coli strains resistant and sensitive to ciprofloxacin showed that there were no statistically significant differences between the susceptibility rates of the groups for imipenem, nitrofurantoin, cephoxitin, and fosfomycin trometamol ($P>0.05$). There were statistically significant differences between the susceptibility rates of the two groups for the other antibiotics tested ($p<0.05$) (Table 3).

At 64-128-256 µg/ml concentrations of fosfomycin trometamol, the susceptibility rates of 50 ciprofloxacin resistant E.coli strains were 82%, 98%, and 98% respectively. At the same concentrations of fosfomycin trometamol, the susceptibility rates of 50 ciprofloxacin-sensitive E.coli strains were 82%, 88%, and 94% (Table 4).

Discussion

In our times, the slow but constant increase in the resistance of uropathogenic bacteria to various antibiotics is noteworthy. Particularly the susceptibility of uropathogenic E.coli to ampicillin, amoxicillin, sulphanamids, trimetoprim sulphamethoxazole and in recent years, to fluoroquinolones have shown a tendency to gradually decline [2, 4,5].

Fosfomycin presents its effect by inhibiting the synthesis of bacterial cell wall. It is a derivative of fosfonic acid, which rapidly metabolizes upon oral intake and is excreted in urine without undergoing change. With its advantages of being used as a single dose and having a low resistance rate, it is one of the primary choices in the treatment of urinary tractinfections [6].

In this study, the susceptibility of E.coli strains, isolated from urine cultures and sensitive and resistant to ciprofloxacin, to fosfomycin tromethamine was evaluated by agar microdilution and disk diffusion methods.

The susceptibility to fosfomycin tromethamine of ciprofloxacin resistant E.coli strains was found to be 100% by disk diffusion method, while it was 98% by agar microdilution method. The susceptibility to fosfomycin tromethamine of ciprofloxacin sensitive E.coli strains was 94 % by both methods.

Similarly, Ko et al investigated the fosfomycin susceptibility of ciprofloxacin resistant E.coli strains isolated from urine cultures by agar microdilution method and determined resistance to fosfomycin in only one strain out of 97 strains [7]. In our study, only one of the 50 ciprofloxacin resistant strains was resistant to fosfomycin, and 3 of the 50 ciprofloxacin sensitive strains were resistant to fosfomycin.

Table 1: The Antibiotic Resistance Rates of E. coli Strains by Kirby-Bauer Disc Diffusion Method

Antibiotics	Sensitive		Resistant	
	Number	%	Number	%
Nitrofurantoin	307	100	0	0
İmipenem	307	100	0	0
Fosfomycin-Trometamol	303	98.7	4	1.3
Cephoxitin	290	94.5	17	5.5
Amikacin	286	93.2	21	6.8
Gentamicin	244	79.5	63	20.5
Cefepim	240	78.2	67	21.8
Aztreonam	228	74.3	79	25.7
Cephotaxim	217	70.7	90	29.3
Ciprofloxacin	216	70.4	91	29.6
Cephuroxime	196	63.8	111	36.2
Cephazolin	186	60.6	121	39.4
Amoxicillin-clavulanic acid	181	59.0	126	41.0
Trimetoprim-sulphamethoxazole	167	54.4	140	45.6
Amoxacillin	118	38.4	189	61.6

ISSN 1302-3314

Table 2: The Differences between the Antibiotic Susceptibility Rates of the Outpatients and Inpatients

	Outpatients		Inpatients		p value
	Sensitive		Sensitive		
	Number	%	Number	%	
Amoxicillin	106	46.1	12	15.6	p=0.000
Amoxicillin-clavulanic acid	147	63.9	34	44.2	p=0.002
Amikacin	218	94.8	68	88.3	p=0.052
Gentamicin	198	86.1	46	59.7	p=0.000
Trimetoprim-sulphamethoxazole	136	59.1	31	40.3	p=0.004
Ciprofloxacin	178	4-77.4	38	49.4	p=0.000
Cephuroxime	168	73.0	28	36.4	p=0.000
Cephazolin	160	69.6	26	33.8	p=0.000
Cephoxitin	224	97.4	66	85.7	p=0.000
Cephotaxim	184	80.0	33	42.9	p=0.000
Cefepim	201	87.4	39	50.6	p=0.000
Nitrofurantoin	230	100	77	100	p=1.000
Fosfomycin-Trometamol	228	99.1	75	97.4	p=0.248
Aztreonam	193	83.9	35	45.5	p=0.000
Imipenem	230	100	77	100	p=1.000

KÜ Tıp Fak Derg 2008;10(1)
ISSN 1302-3314
Orijinal Makale

Table 3: The Differences in the Antibiotic Resistance Rates of Ciprofloxacin Resistant and Sensitive E. coli Strains

Susceptibility	Cip Sensitive		Cip Resistant		p value
	Number	%	Number	%	
Nitrofurantoin	50	100	50	100	p=1.000
İmipenem	50	100	50	100	p=1.000
Amikacin	48	96.0	37	74.0	p=0.002
Fosfomycin Trometamol	47	94.0	50	100	p=0.080
Gentamicin	46	92.0	30	60.0	p=0.000
Cephoxitin	46	92.0	46	92.0	p=1.000
Aztreonam	43	86.0	23	46.0	p=0.000
Cefepim	42	84.0	26	52.0	p=0.001
Cephotaxim	41	82.0	19	38.0	p=0.000
Cephuroxime	37	74.0	9	18.0	p=0.000
Trimetoprim-sulphamethoxazole	36	72.0	13	26.0	p=0.000
Cephazolin	35	70.0	12	24.0	p=0.000
Amoxicillin-clavulanic acid	32	64.0	21	42.0	p=0.028
Amoxacillin	24	48.0	3	6.0	p=0.000

Table 4: The susceptibility of Ciprofloxacin Sensitive and Resistant E. coli Strains to Fosfomycin Concentrations

Fosfomycin	Cip resistant (n:50)		Cip sensitive (n:50)	
	Sensitive n (%)	Resistant n (%)	Sensitive n (%)	Resistant n (%)
64 µg/ml	41 (82)	9 (18)	41 (82)	9 (18)
128 µg/ml	49 (98)	1 (2)	44 (88)	6 (12)
256 µg/ml	49 (98)	1 (2)	47 (94)	3 (6)

Ungheri et al studied 79 kinolon resistant isolates from urine samples and found no fosfomycin resistance in any of the strains by agar microdilution method. In their study, the resistance to amoxicillin was 63.3%, and 48.1% to trimetoprim sulphamethoxazole [8]. In our study, the resistance of ciprofloxacin resistant E.coli strains to amoxicillin was 94%, and to trimetoprim sulphamethoxazole, 74%.

In the study by Fuchs et al, fosfomycin susceptibility of E.coli strains isolated from urine samples were studied by disk diffusion, agar microdilution, and E test methods, and by all three methods, a susceptibility rate of 100% was determined. In the same study, susceptibility of the strains to ciprofloxacin was 95.3%. High susceptibility to fosfomycin was accounted for by widespread use of fosfomycin in the USA [9].

Kahlmeter, in ECO SENS project involving 16 European countries and Canada, determined a rate of 0.7% for fosfomycin resistance and 0.3% for ciprofloxacin resistance by E.coli strains isolated from uncomplicated urinary tractinfections. The highest resistance to kinolons was determined in Spain and Portugal, which was accounted for by the increased rate of antibiotics use in these countries [10].

In the study by Lobel, the rate of fosfomycin susceptibility was 98.6% [11].

Marchese et al found 99% fosfomycin susceptibility and 88% ciprofloxacin susceptibility by uropathogen E.coli strains [12]. In our study, the rate of fosfomycin susceptibility was 98.7%, and ciprofloxacin susceptibility, 70.4%.

In our study, 27 (8.8%) trains formed ESBL. Thirteen (5.7%) strains in the outpatients and 14 (18.2%) in the inpatients were ESBL positive. The rate of ESBL production was statistically significantly higher among the inpatients (p=0.001). This may be accounted for by higher inhibition rates of strains in the hospitals due to high rate of antibiotics use. In our study, the resistance rates of E.coli strains isolated from the urinary tractinfections to common antimicrobials were high. The resistance rate to ciprofloxacin was 29.6%. In the comparisons of the resistance to some antimicrobials and ESBL production of E.coli strains isolated from the outpatients and in patients showed significant differences. In the outpatient group, the rate of ciprofloxacin susceptibility was higher than in the inpatient group (p=0.000).

As shown by the findings of our study, the rate of ciprofloxacin resistance by uropathogen E.coli starins has been increasing. Resistant origins lead to infections that are more serious and difficult to treat. To prevent rapid development of resistance associated with widespread and improper antibiotics use, the antibiotic susceptibility of agent to be used in the treatment should be investigated, and random use of antibiotics should be avoided.

Our results have shown that fosfomycin is a better alternative to other antibiotics in the treatment of urinary tract infections due to uropathogen E.coli strains. fosfomycin may be a good alternative at places where the rate of resistance to ciprofloxacin is high.

In our study, fosfomycin tromethamine, independent from ciprofloxacin resistance, was highly effective. Moreover, both disk diffusion and agar microdilution methods were found to be equally valuable in determining susceptibility.

References

1. Hooton TM: (2000) Pathogenesis of urinary tract infections: an update. *J Antimicrob Chemother*. ;46 Suppl 1:1-7; discussion 63-65.
2. Goettsch W, van Pelt W, Nagelkerke N, Hendrix MG, Buiting AG, Petit PL,et al: (2000) Increasing resistance to fluoroquinolones in Escherichia coli from urinary tract infections in the Netherlands. *J Antimicrob Chemother*. ;46 (2):223-228.
3. Clinical and Laboratory Standards İnstitute: Performance Standards for Antimicrobial Susceptibility Testing; Fiffeenth Informational Suplement M100-S15, CLSI, Wayne, PA (2005).
4. Chomarat M: (2000) Resistance of bacteria in urinary tract infections. *Int J Antimicrob Agents*.;16 (4):483-487
5. Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, et al: (2000) Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). *J Antimicrob Chemother*. ;45 (3):295-303.
6. Marchese A, Bozzolasco M, Gualco L, Debbia EA, Schito GC, Schito AM: (2003) Effect of fosfomycin alone and in combination with N-acetylcysteine on E. coli biofilms. *Int J Antimicrob Agents*. ;22 Suppl 2:95-100.
7. Ko KS, Suh JY, Peck KR, Lee MY, Oh WS, Kwon KT,et al: (2007) In vitro activity of fosfomycin against ciprofloxacin-resistant or extended-spectrum beta-lactamase-producing Escherichia coli isolated from urine and blood. *Diagn Microbiol Infect Dis*.; 58 (1):111-5.

ISSN 1302-3314

8. Ungheri D, Albini E, Belluco G: (2002) In-vitro susceptibility of quinolone-resistant clinical isolates of Escherichia coli to fosfomycin trometamol.*J Chemother*;14(3):237-40.
9. Fuchs CP, Barry LA, Brown SD: (1999) Fosfomycin tromethamine susceptibility of outpatient urine isolates of Escherichia coli and Enterococcus faecalis from ten North American medical centres by three methods. *J. Antimicrob. Chemother*; 43: 137 140.
10. Kahlmeter G: (2003) ECO.SENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project.*J Antimicrob Chemother*. ;51(1):69-76.
11. Lobel B: (2003) Short term therapy for uncomplicated urinary tract infection today. Clinical outcome upholds the theories. *Int J Antimicrob Agents*. ;22 Suppl 2:85-7.
12. Marchese A, Gualco L, Debbia EA, Schito GC, Schito AM. (2003) In vitro activity of fosfomycin against gram-negative urinary pathogens and the biological cost of fosfomycin resistance. *Int J Antimicrob Agents.* ;22 Suppl 2:53-9.

Corresponding Author:
J. Sedef Gocmen
Kırıkkale Üniversitesi Tıp Fakültesi
Mikrobiyoloji ve Klinik Mikrobiyoloji A.D.
71100 Kirikkale, TURKIYE
Phone: +905359433616
Fax: +903182252819
E-mail: jsedef@yahoo.com