

Original article

In Vitro Efficacy of Nitrofurantoin and Some Antibiotics in *Escherichia coli* Strains Isolated from Urine Cultures

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ÖZET

İdrar kültürlerinden izole edilen *Escherichia coli* suşlarında nitrofurantoin ve bazı antibiyotiklerin in vitro etkinliği

Amaç: Bu retrospektif çalışmada, *Escherichia coli*'nin neden olduğu üriner sistem enfeksiyonlarının (ÜSE) tedavisinde yaygın olarak kullanılan bazı antibiyotikler ile nitrofurantoinin in vitro etkinliğini saptamayı amaçladık.

Materyal ve metot: Toplam 3279 *E.coli* izolatu [2157'si (%65,8) ayakta tedavi edilen ve 1122'si (%34,2) yatan hasta olmak üzere] çalışma kapsamına alındı. *E.coli* izolatlarının identifikasyonları geleneksel yöntemler ve BD Phoenix™ 100 (Becton Dickinson, MD, USA) tam otomatik mikrobiyoloji sistemi ile yapıldı. Antimikrobik duyarlılık testleri Kirby-Bauer disk difüzyon yöntemi ile çalışıldı. Genişlemiş-spektrumlu beta-laktamaz (GSBL) aktivitesi ise çift-disk sinerji yöntemi kullanılarak belirlendi.

Bulgular: Amosisilin-klavulanat, trimetoprim-sulfametoksazol ve siprofloksasin için duyarlılık oranları; sırasıyla %22,2, %39,8 ve %41,1 bulundu. Nitrofurantoin duyarlılık oranı ayakta tedavi edilen hastalar için %93,1 (n=2009) ve yatan hastalar için %89,2 (n=1001) olarak belirlendi. Ayakta tedavi edilen ve yatan hastaların nitrofurantoin duyarlılık oranları arasındaki fark istatistiksel olarak anlamlıydı (p<0,001). Ancak, nitrofurantoin duyarlılığı ile duyarlılık oranlarının yıllara göre dağılımı arasında anlamlı bir ilişki yoktu (p=0,755). GSBL üreten *E.coli* izolatlarının oranı %38 (n=1246) olarak saptandı. GSBL üreten ve üretmeyen izolatlar için nitrofurantoin duyarlılık oranları arasında da istatistiksel olarak anlamlı fark vardı (p<0,001).

Sonuç: Bu çalışmanın ışığı altında, en azından hastane-mizde ÜSE'nin tedavisi için nitrofurantoinin güvenli bir şekilde kullanılabileceğini düşünmekteyiz.

Anahtar Kelimeler: Üriner sistem enfeksiyonu; *Escherichia coli*; nitrofurantoin; antibiyotikler; mikrobiyal duyarlılık testi

ABSTRACT

Objective: In this retrospective study, we aimed to detect the in vitro efficacy of nitrofurantoin and commonly used antibiotics in the treatment of urinary tract infections (UTIs) that are caused by *Escherichia coli*.

Material and method: A total of 3,279 urine isolates of *E.coli* [2,157 (65.8%) outpatients, 1,122 (34.2%) inpatients] were included in this study. Identifications of *E.coli* isolates were performed by conventional methods and the BD Phoenix™ 100 (Becton Dickinson, MD, USA) fully automated microbiology system. Antimicrobial susceptibility tests were examined with Kirby-Bauer's disc diffusion method. The double-disk synergy method was used for the detection of extended-spectrum beta-lactamase (ESBL) activity.

Results: The susceptibility rates for amoxicillin-clavulanate, trimetoprim-sulfametoksazol, and ciprofloxacin were detected as 22.2%, 39.8%, and 41.1%, respectively. Nitrofurantoin susceptibility rate was 93.1% (n=2,009) in outpatients and 89.2% (n=1,001) in inpatients. The difference among nitrofurantoin susceptibility rates of outpatients and inpatients was statistically significant (p<0.001). But, there was no statistically significant relationship between the nitrofurantoin susceptibility and distribution according to the years of susceptibility rate (p=0.755). The rate of ESBL-producing *E.coli* isolates was detected as 38% (n=1,246). There was also statistically significant difference between ESBL-producing and ESBL-non-producing isolates for nitrofurantoin susceptibility rates (p<0.001).

Conclusion: In the light of present study, we consider that nitrofurantoin may be used safely for the therapy of UTIs at least in our hospital.

Key Words: Urinary tract infections; *Escherichia coli*; nitrofurantoin; antibiotics; microbial sensitivity test

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections among the community and hospital-acquired infections.

UTIs that have high morbidity rate are fairly common especially among women in our country as all over the world. *Escherichia coli* is the most frequently encountered microorganism in urine cultures. Trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, and ciprofloxacin are commonly used antibiotics in empiric treatment of UTIs. Broad-spectrum beta-lactams, fluoroquinolones, beta-lactam and beta-lactamase inhibitor combinations, and carbapenems are the parenteral antibiotics

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that are most frequently used for treatment of UTIs in hospitalized patients. Today, inappropriate use of antibiotics led to increasing resistance rates of Gram-negative bacteria against standard antibiotics. With the increasing the rates of extended-spectrum beta-lactamase (ESBL) in *E. coli*, the treatment of UTIs has become more complex and more difficult. Therefore it is essential to reassess the treatment of UTIs^{1,2}.

We aimed to analyze the resistance status of our urine isolates of *E.coli* against nitrofurantoin and commonly used antibiotics such as amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin and also ESBL-producing rates in our hospital.

MATERIAL AND METHOD

The present study is a retrospective analysis of the medical records of patients that were diagnosed with UTIs and isolated *E.coli* from urine cultures between the dates of January 2006 and September 2011 in Dicle University Hospital. The urine cultures were performed from mid-stream specimens of urine that were sent to clinical microbiology laboratory. The mid-stream specimens of urine were obtained from patients with a preliminary diagnosis of UTIs. The urine specimens were inoculated quantitatively onto 5% sheep blood agar and Eosin-Methylen Blue (EMB) agar (Merck KGaA, Darmstadt, Germany). Then, media plates were incubated aerobically at 35±2°C for 18-24 hours. A total of 3,279 *E.coli* isolates [2,157 outpatients (65.8%) and 1,122 inpatients (34.2%)] with urine cultures yielding growth of ≥10⁵ colony forming units/mL (CFU/mL) included in this study. Identifications of *E.coli* isolates were performed by conventional methods and the BD Phoenix™ 100 (Becton Dickinson, MD, USA) fully automated microbiology system.

Antimicrobial susceptibility tests were performed with Kirby-Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoint. Pure culture of *E.coli* isolates which had been grown onto 5% sheep blood agar and EMB agar was suspended in normal sterile saline to form a suspension equivalent to 0.5 McFarland turbidity standard. The bacterial suspension was inoculated onto Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany) and antibiotic impregnated discs (Oxoid, Basingstoke, UK) were applied onto the media plates. After incubation aerobically at 35±2°C for 16-18 hours, inhibition zone diameters were measured and susceptibility results were determined according to CLSI breakpoint criteria. The double-disc synergy method was used for the

detection of ESBL activity. For the quality controls of fully automated microbiology system, antimicrobial susceptibility test and double-disc synergy method, *E.coli* ATCC 25922 and ESBLs positive *E. coli* ATCC 35218 were used.

Data of this study were analyzed by Epi Info™ 7-Community Edition (Centers for Diseases Control and Prevention, Atlanta, GA, USA) statistical package program. Statistical evaluation of difference between outpatients and inpatients for nitrofurantoin susceptibility was performed with the Chi-square test. In addition, statistical evaluation of difference between ESBL-producing and non-producing isolates for nitrofurantoin susceptibility rates was performed with the Chi-square test, too. Additionally, statistical evaluation of distribution according to the years of nitrofurantoin susceptibility rates was performed with the Chi-square trend analysis. The p value of <0.05 was selected for statistical significance.

RESULTS

A total of 3,279 *E.coli* strains isolated from urine cultures were included in this study. Of total 3,279 strains, 2,157 (65.8%) strains were isolated from the outpatients and 1,122 (34.2%) strains were isolated from hospitalized patients.

According to the antimicrobial susceptibility testing, nitrofurantoin-intermediate susceptible isolates were considered as resistant to nitrofurantoin. The rates of nitrofurantoin-sensitive and nitrofurantoin-resistant isolates were determined as 91.8% (n=3,010) and 8.2% (n=269), respectively.

In this present study, the nitrofurantoin susceptibility rates were 93.1% (n=2,009) in outpatients and 89.2% (n=1,001) in inpatients. The difference among nitrofurantoin susceptibility rates of outpatients and inpatients were found statistically significant (p<0.001) (Table 1). The rate of ESBL-producing *E.coli* isolates was detected as 38% (n=1,246). There was statistically significant difference between ESBL-producing and ESBL-non-producing *E.coli* strains for nitrofurantoin susceptibility rates (p<0.001) (Table 2).

Table 1. Distribution of Nitrofurantoin Susceptibility and Resistance Rates of *E.coli* Isolates for Outpatients and Inpatients

Property of patient	S n (%)	R n (%)	p value	Total n (%)
Outpatient	2,009 (93.1)	148 (6.9)	<0.001	2,157 (65.8)
Inpatient	1,001 (89.2)	121 (10.8)		1,122 (34.2)
Total	3,010 (91.8)	269 (8.2)		3,279 (100)

S: Sensitive; R: Resistant

Table 2. Distribution of Nitrofurantoin Susceptibility and Resistance Rates of *E. coli* Isolates According to ESBL of Producing/Not-Producing

Property	S n (%)	R n (%)	p value	Total n (%)
ESBL (+)	1,012 (81.2)	234 (18.8)	<0.001	1,246 (38)
ESBL (-)	1,998 (98.3)	35 (1.7)		2,033 (62)
Total	3,010 (91.8)	269 (8.2)		3,279 (100)

S: Sensitive; R: Resistant

Nitrofurantoin susceptibility rate does not show significant changes over the years. The increasing and/or decreasing of nitrofurantoin susceptibility rates over the years were evaluated by the Chi-square trend analysis. There was no statistically significant relationship between nitrofurantoin susceptibility and distribution according to the years of susceptibility rates ($p=0.755$) (Table 3).

Table 3. Distribution According To the Years of Nitrofurantoin Susceptibility Rates

Year	Nitrofurantoin n (%)	p value	Total
2006	618 (92.4)	0.755	669
2007	619 (92.3)		671
2008	588 (90.5)		650
2009	370 (91.8)		403
2010	395 (92.3)		428
2011*	420 (91.7)		458
Total	3,010 (91.8)		3,279

*Data for the first nine months of 2011

In present study according to antimicrobial susceptibility testing results between the years 2005 and 2011, the average rates of susceptibility for amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin were detected as 22.2%, 39.8%, and 41.1%, respectively (Table 4).

Table 4. Distribution of Nitrofurantoin and Commonly Used Antibiotics Susceptibility Rates and ESBL-Producing Rates Over the Years

Year	Amoxicillin-clavulanate n (%)	Trimethoprim-sulfamethoxazole n (%)	Ciprofloxacin n (%)	Nitrofurantoin n (%)	Total
2006	154 (23)	281 (42)	308 (46)	618 (92.4)	669
2007	134 (20)	282 (42)	315 (47)	619 (92.3)	671
2008	162 (25)	240 (36.9)	234 (36)	588 (90.5)	650
2009	80 (19.9)	153 (38)	145 (36)	370 (91.8)	403
2010	94 (22)	167 (39)	150 (35)	395 (92.3)	428
2011*	105 (23)	183 (40)	197 (43)	420 (91.7)	458
Total	729 (22.2)	1,306 (39.8)	1,349 (41.1)	3,010 (91.8)	3,279

*Data for the first nine months of 2011

DISCUSSION

UTIs are the most common bacterial infections among the community and hospital-acquired infections. In recent years, resistance rates to commonly used antibiotics increase rapidly and the treatment of UTIs has become more difficult. In our study, resistance rate for amoxicillin-clavulanate was detected as $\geq 70\%$, for trimethoprim-sulfamethoxazole and ciprofloxacin as $\geq 60\%$. The rate of ESBL-producing *E. coli* strains in our hospital was 38%. It is known that ESBL-producing *E. coli* isolates are resistant to penicillins, cephalosporins, and monobactams and these isolates can also develop co-resistance to other classes of antibiotics which are frequently used for UTIs⁴.

Because of increasing rates of resistance it has become essential to determine new alternative treatment options in UTIs. In our study, 91.8% of our *E. coli* isolates was also determined as susceptible to nitrofurantoin and resistance rate to nitrofurantoin in ESBL-producing isolates (18.8%) is higher than ESBL-non-producing (1.7%) isolates.

Antibiotic susceptibility rates vary in regional,

in community-acquired or hospital-acquired infections. In 2000, by ECO.SENS Project, the prevalence and susceptibility of *E. coli* strains causing community-acquired, uncomplicated UTIs in women was investigated. The bacteria were identified and their susceptibility to 12 antibiotics commonly used in the treatment of UTIs was determined in this project. The rates of resistance among *E. coli* strains were: ampicillin and sulfamethoxazole 30%; trimethoprim alone or with sulfamethoxazole 15%; nalidixic acid 6%; ciprofloxacin 3%; amoxicillin-clavulanate, mecillinam, cefadroxil, nitrofurantoin and fosfomycin, less than or equal to 2%⁵.

In 2005, resistance rates for the *E. coli* isolates were reported as ampicillin 37.7%, trimethoprim-sulfamethoxazole 21.3%, ciprofloxacin 5.5%, levofloxacin 5.1%, and nitrofurantoin 1.1% by North American Urinary Tract Infection Collaborative Alliance (NAUTICA)⁶.

The reported data in recent years showed that antibiotic resistance has increased rapidly. In a study from USA in 2008, antibiotic susceptibility rates of urine isolates of *E. coli* to ciprofloxacin,

levofloxacin and trimethoprim-sulfamethoxazole reported as 75%, 76%, and 80% respectively. According to this study, nitrofurantoin susceptibility rate (96%) was found remarkably higher than the other antibiotics⁷.

The next few years data from Kuwait, *E.coli* resistance rates against antibiotics (community-acquired/hospital-acquired) were amoxicillin (66/75%), trimethoprim-sulfamethoxazole (47/52%), ciprofloxacin (28/46%), cephalothin (47/60%), amoxicillin-clavulanate (27/41%), cefotaxime (12/26%), amikacin (1/3%), piperacillin-tazobactam (1/4%), and nitrofurantoin was observed (7/8%) have excellent activities against *E. coli*⁸. According to this study, the prevalence of ESBL-producing *E. coli* in community-acquired-UTIs was 12% and 26% in hospital-acquired-UTIs⁸. European Committee on Antimicrobial Susceptibility Testing (EUCAST) investigated antibiotic susceptibility rates in *E. coli* strains isolated from community-acquired-UTIs of women in Austria, Greece, Portugal, Sweden and UK. In this study resistance rates to mecillinam, cefadroxil, nitrofurantoin, fosfomycin trometamol, gentamicin and the third-generation cephalosporins cefotaxime and ceftazidime was <2%, with the following exceptions; gentamicin in Portugal (2.8%), fosfomycin in Greece (2.9%); and cephalosporins in Austria (2.7-4.1%). In addition, *E. coli* resistance levels were higher for amoxicillin-clavulanate (2.0-8.9%) and ciprofloxacin (0.5-7.6%) and much higher to ampicillin (21.2-34.0%), sulfamethoxazole (21.2-31.3%), trimethoprim (14.9-19.1%) and trimethoprim-sulfamethoxazole (14.4-18.2%) in this study⁹.

Many of studies showed that nitrofurantoin has good efficacy against the bacteria isolated from

UTIs, therefore this antibiotic can be recommended for the treatment of UTIs, as a good alternative³. However, nitrofurantoin is a nephrotoxic agent and this feature has limited use of nitrofurantoin⁵.

It is possible that the development of antibiotic resistance in near future. Some data provides the first signs of this opinion. In a study from Taiwan the antibiotic susceptibility rates to ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, amikacin, fosfomycin were reported as 29%, 45%, 24%, 97%, and 95% in ESBL-producing isolates, respectively⁴.

According to this study, in 20% of *E. coli* isolates resistance to nitrofurantoin was observed. Some investigators recommended fosfomycin combined therapy with other antibiotics in UTIs. Therefore to be informed about fosfomycin susceptibility will contribute to the treatment approach of UTIs¹⁰.

In conclusion, due to vary of susceptibility patterns to antibiotics over the time and in different geographical regions, antibiotic treatment of UTIs should be based on local experience of susceptibility and resistance patterns.

Nitrofurantoin is an antibiotic that shows good efficacy against *E. coli* and the other bacteria isolated from urine cultures. Because of low-resistance to nitrofurantoin, it is recommended for the treatment of UTIs, as a good alternative drug³.

In the light of present study, we considered that nitrofurantoin may be used as an appropriate oral antibiotics for the therapy of UTIs at least in our region. In addition, the importance of antimicrobial susceptibility testing was emphasized once again, with the present study.

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