

An Investigation on Uropathogen *Escherichia coli* Strains with Regard to Antimicrobial Susceptibility and Extended Spectrum Beta- Lactamase

Banu KAŞKATEPE*^o, Sulhiye YILDIZ*

An Investigation on Uropathogen Escherichia coli Strains with regard to Antimicrobial Susceptibility and Extended Spectrum Beta-Lactamase

Summary

Urinary tract infections (UTI) are among the most commonly encountered infections and are thus associated with widespread usage of antibiotics. *Escherichia coli* is the commonest bacteria isolated in UTI and treatment alternatives are limited in extended spectrum beta-lactamase (ESBL) producers.

The aim of this study was to determine antibiotic susceptibility, ESBL production of the *Escherichia coli* strains and comparison of (ESBL) (+) and ESBL (-) strains, those were isolated in the Clinical Microbiology Laboratory, from urine samples of patients who presented to Ankara Dışkapı Yıldırım Beyazıt Training Hospital with urinary tract infections, in a period between January 2007 and June 2007. Conventional bacteriologic methods were used in identification and a total of 130 *E.coli* species were isolated. Sensitivity tests were performed according to the guidelines and criteria of National Committee for Clinical Laboratory Standards (NCCLS) using Kirby-Bauer disk diffusion method. The highest susceptibility rates were detected in amikacin (95 %) and cefoperazone- sulbactam (91 %). ESBL production was investigated by double disk synergy method and 27 (21 %) of the isolated *E.coli* strains were found to be ESBL producers. Gentamicin and piperacillin-tazobactam susceptibility in ESBL (-) strains were found higher than ESBL (+) strains. An increase in the rate of resistance to ciprofloxacin (96 %) in ESBL (+) strains was noted as well.

Key Words: Antimicrobial susceptibility, *E.coli*, ESBL

Received: 25.10.2010

Revised: 05.02.2011

Accepted: 18.02.2011

Üropatojen Escherichia coli Suşlarının Antibiyotik Duyarlılıklarının Belirlenmesi ve Genişlemiş Spektrumlu Beta Laktamazlar Yönünden İncelenmesi

Özet

Üriner sistem infeksiyonları en sık görülen infeksiyonlardandır ve yaygın antibiyotik kullanımına neden olmaktadır. *Escherichia coli* üriner sistem infeksiyonlarından en sık izole edilen bakteridir ve Genişlemiş spektrumlu betalaktamaz (GSBL) üreten kökenlerde tedavi seçenekleri sınırlıdır.

Bu çalışmada Ankara Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Klinik Mikrobiyoloji Laboratuvarı'nda Ocak 2007- Haziran 2007 tarihleri arasında üriner sistem infeksiyonu etkeni olarak izole edilen *Escherichia coli* suşlarının antibiyotik duyarlılıklarının belirlenmesi, GSBL üretimi ve GSBL pozitif ve GSBL negatif suşların karşılaştırılması amaçlanmıştır.

Üriner sistem infeksiyonu etkeni olarak izole edilen 130 adet *E.coli* suşunun identifikasyonu klasik bakteriyolojik yöntemlerle yapılmıştır. Antibiyotik duyarlılığı "Kirby Bauer" disk difüzyon yöntemi kullanılarak, NCCLS kriterlerine göre yürütülmüştür (1). En yüksek duyarlılık oranı amikasin (%95) ve sefoperazon-sulbaktam (%91) için saptanmıştır. 130 *E.coli* suşunun 27(%21)' sinde GSBL saptanmıştır. GSBL pozitif ve negatif *E.coli*' lerin antibiyotik duyarlılıkları karşılaştırıldığında, ampirik tedavide ilk seçenek olarak kullanılan antibiyotiklerden olan siprofloksasine karşı GSBL pozitif suşlardaki direnç oranı (%96) dikkat çekicidir. Ayrıca GSBL pozitif suşlarda piperasilin-tazobaktam (%44) ve gentamisin (%41) duyarlılıkları GSBL negatif suşlara göre daha düşüktür.

Anahtar Kelimeler: Antimikrobiyal duyarlılık, *E.coli*, GSBL

* Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06100 Ankara/ TURKEY.

^o Corresponding Author E-mail: banukaskatepe@gmail.com

INTRODUCTION

Urinary tract infections (UTI) are one of the most common bacterial infectious diseases and *Escherichia coli* is the most frequently isolated bacteria among Gram negative agents in UTI (2-5). Growing resistance to commonly used antibiotics has been a big problem in empirical treatment (6). Especially treatment is limited for strains which produce extended spectrum beta-lactamase (ESBL). ESBLs are plasmid-mediated enzymes that hydrolyze broad-spectrum β -lactams. The first ESBL producing organisms were isolated in Germany in 1983 (7,8). ESBL production provides the most common mechanism of resistance to beta-lactam antimicrobial agents among gram-negative bacteria (9). The treatment of serious infections related with these organisms is a big problem and the mortality rate in patients that infected with ESBL producing organisms is significantly higher than those with non-ESBL isolates (8).

The aim of this study was to determine the susceptibility pattern to commonly used antibiotics and ESBL production of *E.coli* isolated from urinary tract infections and to compare of ESBL (+) and ESBL (-) *E coli* strains.

MATERIALS AND METHODS

In this study, 130 *Escherichia coli* strains isolated in the Clinical Microbiology Laboratory, from urine samples of patients who presented themselves to Ankara Dışkapı Yıldırım Beyazıt Training Hospital with urinary tract infections, in a period between January 2007 and June 2007. Conventional bacteriological methods were used for identification. Antimicrobial susceptibility test of the isolates was performed by the disk diffusion method

according to the recommendations of the National Committee for Clinical Laboratory Standards. The positivity of ESBL was determined by double disc synergy test. For this purpose, an amoxicillin-clavulanate disc (AMC) was placed in the centre and cefotaxime (CTX), ceftriaxone (CRO) and aztreonam (ATM) discs were placed at a distance of 20 mm from the AMC disc. After incubation for 16-18 hours at 35 °C, ESBL production was interpreted as positive by a clear extension of the edge of the inhibition zone produced by CTX, CRO or ATM towards the AMC disc. Positive and negative strains were analyzed statistically by "Pearson Chi-Square" and "Fisher's Exact Test".

RESULTS

130 uropathogen *E.coli* strains' antibiotic susceptibilities were determined and it was found that amikacin (95%) and cefoperazone-sulbactam (91%) are the most effective antimicrobials. Antibiotic susceptibilities are reported at Table 1. 27 (21%) *E.coli* isolates were positive for ESBL. For ESBL positive isolates the most effective antimicrobials were amikacin and cefoperazone-sulbactam. Ciprofloxacin resistance was higher (96%) in ESBL (+) strains when compared to ESBL (-). ESBL (+) and ESBL (-) strains' antibiotic susceptibilities are listed in Table 2.

DISCUSSION

The occurrence of ESBL among clinical isolates varies greatly worldwide and is rapidly changing over time. In our study we found 21% ESBL producing of uropathogen *E.coli* isolates. Similar rates have been found in other studies in our country; Yetkin et al. (10) found 30.3%, Pullukçu at al. (11) in their study, the comparing ESBL producing strains, between

Table1. Antibiotic susceptibility of *E.coli*

Antibiyotikler	S	%	I	%	R	%
Amikacin	124	95	6	5	-	-
Gentamicin	97	75	-	-	33	25
Piperacillin- tazobactam	99	76	19	15	12	9
Cefoperazone-sulbactam	118	91	9	7	3	2
Cefotaxime	99	76	4	3	27	21
Ceftriaxone	100	77	2	1	28	22
Ciprofloxacin	68	52	-	-	62	48
Trimethoprim-sulfamethoxazole	63	48	1	1	66	51

Table 2: Comparison of ESBL (+) and ESBL (-) isolates. (%)

Antimikrobiyaller	GSBL +						GSBL -						<i>p value</i>
	S	%	I	%	R	%	S	%	I	%	R	%	<i>p value</i>
Amikacin	22	81	5	19	-	-	102	99	1	1	-	-	<i>p</i> = 0.001
Gentamicin	11	41	-	-	16	59	86	83	-	-	17	17	<i>p</i> <0.001
Piperacillin- tazobactam	12	44	10	37	5	19	87	84	9	9	7	7	<i>p</i> <0.001
Cefoperazone-sulbactam	22	81	3	11	2	8	96	93	6	6	1	1	<i>p</i> = 0.126
Cefotaxime	-	-	1	4	26	96	99	96	3	3	1	1	-
Ceftriaxone	-	-	1	4	26	96	100	97	1	1	2	2	-
Ciprofloxacin	1	4	-	-	26	96	67	65	-	-	36	35	<i>p</i> <0.001
Trimethoprim-sulfamethoxazole	11	41	-	-	16	59	52	50	1	1	40	49	<i>p</i> = 0.367

S: Susceptible I: Intermediate R: Resistant

inpatient and outpatient group were detected 24.8 and 17.7% respectively. On the other hand, lower ESBL production rates were found in different studies; In Kocoğlu et al.'s study (3.4%), in Özden et al.'s study (8.3%) (12,13).

Zhao et al. (14) in their study made in China, they reported 35.7% ESBL positive rates in *E.coli*. However, Schito et al.'s study (15) in which sixty two centers from nine countries were participated, they found ESBL positive rate 1.7% in *E.coli* in a period between 2003- 2006.

In recent years, development of resistance to commonly used antibiotics for UTI is a big problem in the treatment. Increasing resistance to ampicillin, trimethoprim-sulfamethoxazole and fluoroquinolones has been reported (10,16-19) which is supported by our results as well. The resistance rate of *E.coli* to TMP-SXT is found 51%. In this study it was also found that there was no statistically significant difference between the ESBL (+) and ESBL (-) isolates and there were high resistance rates in two groups. According to these findings, TMP- SXT use should be taken into consideration.

Various rates of quinolones have been reported in a number of studies. These rates vary according to centers and usage profiles. Although very high rates of susceptibility have been reported as 91.8%(20), resistance has been increasing in time (10,21,22). In this study we have found a 48% ciprofloxacin resistance. Especially the rate of resistance in ESBL (+) strains is very high (96%) when compared to

ESBL (-) (35%). In Auer et al.'s study (23) it is reported 78% ciprofloxacin resistance rate in ESBL (+) 100 *E.coli* strains, which are isolated from urinary tract infections.

The susceptibility of uropathogen *E.coli* to amikacin and gentamicin is found 95% and 75%, respectively. These results show that susceptibility to aminoglycosides is still high. Again in our study regarding ESBL (+) strains as compared to ESBL (-) strains, there was a statistically significant higher gentamicin resistance in ESBL (+) strains (59%) than ESBL (-) strains (17%). Similar rates have been found in Uyanık et al.'s study (24) conducted with 139 *E.coli* strains isolated from urinary tract infections. They found 53% and 10% gentamicin resistance in ESBL (+) and ESBL (-) strains, respectively.

In different studies it is noted that piperacillin-tazobactam (TZP) and cefoperazone-sulbactam (SCF) are the most effective inhibitory combinations (25,26). In Yetkin et al.'s study (10), susceptibility rate to TZP is 50% in ESBL producing isolates however, SCF susceptibility is high in both ESBL (+) (81%) and ESBL (-) strains (93%). We found TZP and SCF susceptibility 76% and 91%, respectively. In our study TZP susceptibility in ESBL (+) strains (44%) is significantly lower than ESBL (-) strains (84%).

In conclusion, taking into account the development of resistance, we recommend regular monitoring of uropathogens for susceptibility and use that as a guideline for the empiric treatment protocols.

REFERENCES

1. National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial susceptibility testing. 11th Informational Supplement. NCCLS Document M100-S11. National Committee for Clinical Laboratory Standards. Wayne, Pa, 2001.
2. Barrett SP, Savage MA, Rebec MP, Guyot A, Andrews N, Shrimpton SB. Antibiotic sensitivity of bacteria associated with community-acquired urinary tract infection in Britain. *J Antimicrob Chemother* 44: 359-365, 1999.
3. Chomarat M: Resistance of bacteria in urinary tract infections. *Int J Antimicrob Agents* 16: 483-487, 2000.
4. Daza D, Gutierrez J, Piedrola G. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. *Int J Antimicrob Agents* 18: 211-215, 2001.
5. Hryniewicz K, Szczypa K, Sulikowski K, Betlejewska K. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother* 47: 773-780, 2001.
6. Gales AC, Sader HS, Jones RN. Urinary tract infection trends in Latin American hospitals: Report from the SENTRY antimicrobial surveillance program (1997-2000). *Diagn Microbiol Infect Dis* Nov, 44: 289-299, 2002.
7. Mulvey MR, Bryce E, Boyd D, Ofner-Agostini M, Christianson S, Simor AE, Paton S. Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp.* in Canadian hospitals. *Antimicrob Agents Chemother* 48: 1204-1214, 2004.
8. Marra AR, Wey SB, Castelo A, Gales AC, Cal RGR, Filho JRC, Edmond MB, Pereira CA. Nosocomial bloodstream infections caused by *Klebsiella pneumoniae*: impact of extended-spectrum β -lactamase (ESBL) production on clinical outcome in a hospital with high ESBL prevalence. *BMC Infectious Diseases* 6: 24, 2006.
9. Cormican MG, Marshall SA, Jones RN. Detection of extended-spectrum β -lactamase (ESBL)-producing strains by the Etest ESBL screen. *J Clin Microbiol* 34: 1880-4, 1996.
10. Yetkin G, Kuzucu Ç, Çalışkan A. İdrarda üreyen *Escherichia coli*' lerin geniş spektrumlu beta laktamazlar önünden irdelenmesi. *İnönü Üniversitesi Tıp Fakültesi Dergisi* 13: 249-252, 2006.
11. Pullukçu H, Taşbakan M, Sipahi OR, Turhan A, Özinel MA, Ulusoy S. İdrar kültürlerinden soyutlanan bakteriler ve çeşitli antibiyotiklere in vitro duyarlılıklarının değerlendirilmesi. *ANKEM Derg* 20: 26-30, 2006.
12. Koçoğlu E, Karabay O, Koç İnce N, Özkardeş F, Yıldırım R. Toplum kaynaklı üriner sistem infeksiyonlarından izole edilen *Escherichia coli* suşlarında genişlemiş spektrumlu beta-laktamaz ve bazı antibiyotiklere direnç sıklığının araştırılması. *ANKEM Derg* 21: 5-9, 2007.
13. Ozden M, Kalkan A, Demirdag K, Kilic SS, Ozdarendeli A. Ciprofloxacin and cotrimoxazole resistance and extended spectrum beta-lactamase production in *Escherichia coli* strains isolated from urinary tract infections. *Int J Antimicrob Agents* 21: 492-493, 2003.
14. Zhao DN, Xiao YH, Zhang SL, Wang XX. Bacterial composition and resistance from urinary tract infections in females. *Zhonghua Fu Chan Ke Za Zhi* 44: 32-37, 2009.
15. Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L, Marchese A. The ARESC Study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *International Journal of Antimicrobial Agents* 34: 407-413, 2009.
16. Yılmaz E, Özakın C, Sınırtaş M, Gedikoğlu S. Uludağ Üniversitesi Tıp Fakültesi Bakteriyoloji Laboratuvarı'nda 1999-2002 yılları arasında idrar örneklerinden izole edilen Mikroorganizmalar ve antibiyotik duyarlılıkları. *İnfeksiyon Dergisi* 19: 91-96, 2005.
17. Kalem F, Gündem NS, Arslan U, Tuncer İ. İdrar örneklerinden izole edilen *Escherichia coli* suşlarında antimikrobiyal duyarlılığı. *ANKEM Derg* 22: 193-197, 2008.
18. Kaya O, Akçam FZ, Uyar C, Demir C, Yaylı G. 2002-2004 yılları arasında izole edilen üropatojen *Escherichia coli* suşlarında artan antibiyotik direnci. *S D Ü Tıp Fak Derg* 13: 22-26, 2006.

19. Erođlu M, Koçođlu E, Karabay O, Semerciöz A. Toplum kaynaklı erişkin üriner sistem infeksiyonlarında izole edilen *Enterobacteriaceae* türlerinin bazı antibiyotiklere duyarlılıkları: Geriye dönük çalışma. *Türk Üroloji Dergisi* 33: 100-103, 2007.
20. Akay H, Duranay M, Akay A. Üriner sistem enfeksiyonlarından izole edilen mikroorganizmaların dağılımı ve *Escherichia coli* suşlarında antibiyotik duyarlılığı. *İst Tıp Fak Derg* 69: 1-4, 2006.
21. Goettsch W, Van Pelt W, Nagelkerke N, Hendrix MGR, Buiting AGM, Petit PL, Sabbe LJM, Van Griethuysen AJA, Neeling AJ. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. *J Antimicrob Chemother* 46: 223-228, 2000.
22. Yılmaz FF, Ermertcan Ş. İdrar yolu infeksiyonlarından izole edilen *Escherichia coli* kökenlerinde florokinolon direncinin araştırılması. *İnfeksiyon Dergisi* 19: 429-433, 2005.
23. Auer S, Wojna A, Hell M. ESBL producing *Escherichia coli* strains in ambulatory urinary tract infections – Oral treatment options?. *Antimicrob. Agents Chemother.* Doi:10.1128/AAC.01760-09, 2010
24. Uyanık MH, Hancı H, Yazgı H. Üriner sistem infeksiyonlarından soyutlanan toplum kökenli *Escherichia coli* suşlarına fosfomisin trometamolün ve bazı antibiyotiklerin in-vitro etkinliği. *ANKEM Derg* 23: 172-179, 2009
25. Gür D. Toplumdan kazanılmış infeksiyonlarda beta-laktamazların rolü. *ANKEM Derg* 20 (Ek 2): 274-277. 2006.
26. Küçükbayrak A, Behçet M, Güler S, Özdemir D. Üriner semptomu olan poliklinik hastaların idrarında üreyen *E.coli* suşlarının antibiyotik duyarlılığı. *TAD* 4: 18-21, 2006.

