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The Urinary System Infections caused by Extended-Spectrum-B-Lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* Strains

Kerem Taken[1,*], Mehmet Parlak[2], Mustafa Günes[1], Muslum Ergün[3], Recep Eryılmaz[4], Murat Demir[1], Ilhan Gecit[1]

[1]*Department of Urology, Yüzüncü Yıl University Faculty of Medicine, Van, Turkey*
[2]*Department of Medical Microbiology, Yüzüncü Yıl University Faculty of Medicine, Van, Turkey*
[3]*Mus State Hospital, Department of Urology, Muş, Turkey*
[4]*Bitlis State Hospital, Department of Urology, Bitlis, Turkey*

ABSTRACT

Production of β-Lactamase enzymes is an important mechanism of resistance in Gram-negative bacteria. The aim of this study was to investigate the relationship between the Extended-Spectrum β-Lactamase (ESBL)-positive *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* strains isolated from the urine cultures and the underlying diseases and also to assess the antibiotic resistance.

The bacteria were identified according to conventional methods including colony morphology, gram stains, oxidase test and biochemical tests (indole production, Triple Sugar Iron (TSI) agar, Simmon²s Citrate agar, Christensen's Urea agar, and motility test medium). BD Phoenix automated system (Becton Dickinson, USA) was used for further identification of the bacteria that were identified as *E. coli* and *K. pneumoniae* and for assessing the resistance rates and ESBL production.

A total of 401 *E. coli* and 58 *K. pneumoniae* strains were isolated from the urinary specimens. Of these, 159 (39.6%) of *E. coli* and 26 (44.8%) of *K. pneumoniae* strains were ESBL-positive. The most active antibiotics against ESBL-positive *E. coli* and *K. pneumoniae* strains were carbapenems. Underlying diseases were detected in a total of 148 ESBL-positive strains (80%), including 127 (79.8%) of *E. coli* and 21 (80.8%) of *K. pneumoniae* strains. Common underlying diseases were benign prostate hyperplasia (BPH), urolithiasis, neurogenic bladder, urethral stricture, vesicoureteral reflux (VUR), hypospadias, ureteropelvic junction (UPJ) obstruction, vesicointestinal fistula, and cystocele ($p<0.05$).

In the treatment of urinary system infections, ESBL-positive *E. coli* and *K. pneumoniae* strains should be kept in mind and the underlying diseases should be studied and treated.

Key Words: Extended-Spectrum-β-Lactamase, *Escherichia coli*, *K. pneumonia,* Urinary System Infections

Introduction

Urinary system infections (USI) are mostly caused by *Escherichia coli* (*E.coli*) and *Klebsiella pneumoniae (K. pneumoniae),* which belonging to the family *Enterobacteriaceae* (1). In the treatment of USI, antibiotic resistance is a challenging problem. In many USI, the treatment fails due to increased bacterial resistance against antibiotics. Moreover, this resistance may also be increased by the antibiotics used in the treatment (2,3).

Production of β-Lactamase enzymes is an important mechanism of resistance in Gram-negative bacteria. Many bacterial species such as *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis,* and *Salmonella* carry genes that encode ESBL enzymes (4). β-Lactamase enzymes deactivate the β-Lactam antibiotics by destroying the amide of the β-Lactam ring of the antibiotics. Moreover, these enzymes are synthesized by bacteria via chromosomes, plasmids, or mobile genetic agents known as transposons. Therefore, these enzymes are called extended-spectrum β-Lactamase (ESBL) enzymes (5). ESBL-mediated resistance exhibits resistance to the antibiotics used in the treatment and may lead to serious treatment failure results and high rates of resistance to ESBL-producing bacteria, especially fluoroquinolones, have been reported. However, carbapenems are highly active in the treatment of these infections (6). Identification of ESBL-producing *E. coli* and *K. pneumoniae* strains and the knowledge of their rates are of prime importance for the selection of an appropriate antibiotics to the treatment of infectious diseases.

***Corresponding Author:** Dr. Kerem Taken, Yuzuncu Yil University Faculty of Medicine, Department of Urology, 65090 Tuşba, Van, Turkey, Hospital phone: +90 (432) 215 04 74, Mobil Phone: +90 (533) 127 77 09, Fax: 0 (432) 216 75 19, E-mail: takenyyu@yahoo.com
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In this study, we aimed to investigate the relationship between the ESBL-positive *E. coli* and *K. pneumoniae* strains isolated from the urine cultures obtained from the outpatients and the inpatients treated in our clinic and the underlying diseases and also to assess the antibiotic resistance of these isolates.

Material and method

The study included the *E. coli* and *K. pneumoniae* strains isolated from the urine cultures obtained from the outpatients and the inpatients treated at Yuzuncu Yil University Dursun Odabas Medical School Urology Clinic between March 2012 and April 2014. The study complies with the Helsinki Declaration. Antibiotic resistance rates, ESBL production, and underlying diseases were retrospectively analyzed.

The urinary specimens were plated on blood agar with 5% sheep blood and Eosin-Methylene Blue Agar by quantitative plating method and then incubated at 37 °C for 18-24 h. Subsequently, the bacteria were identified according to conventional methods including colony morphology, gram stains, oxidase test and biochemical tests (indole production, Triple Sugar Iron (TSI) agar, Simmon's Citrate agar, Christensen's Urea agar, and motility test medium). BD Phoenix automated system (Becton Dickinson, USA) was used for further identification of the bacteria that were identified as *E. coli* and *K. pneumoniae* and also for assessing their resistance rates and ESBL production. Selection of antibiotics and the resistance rates were evaluated based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

The results were expressed as mean±SEM. The statistical analysis of the data was carried out with statistical package of social sciences, version 16.0, SPSS Inc, Chicago, Illinois, USA and Graph pad Prism version-5. Statistical comparisons of the rates were performed using the chi-square test and Z test. A 95% confidence interval was used. P values less than 0.05 were considered as statistically significant.

Results

A total of 401 *E. coli* and 58 *K. pneumoniae* strains were isolated from the urinary specimens. Of these, 159 (39.6%) of *E. coli* and 26 (44.8%) of *K. pneumoniae* strains were ESBL-positive. The most active antibiotics against ESBL-positive *E. coli* were carbapenems (imipenem, meropenem, and ertapenem), followed by nitrofurantoin, amikacin, and piperacillin/tasobactam, whereas the most active agents against ESBL-positive *K. pneumoniae* were carbapenems, amikacin, and cefoperason/sulbactam. Table 1 presents the comparison of resistance rates of *E. coli* and *K. pneumoniae* strains.

Table 1. Comparison of antibiotic resistance rates between ESBL-positive *E. coli* and *K. pneumoniae*

Antibiotics	*E. coli* (n=159)	*K. pneumonia* (n=26)	Total (n=185)
	R (%)	R (%)	R (%)
AMX/CLA	159 (100)	25 (96.1)	184 (99.4)
AMP/SLB	159 (100)	25 (96.1)	184 (99.4)
Aztreonam	159 (100)	25 (96.1)	184 (99.4)
Ceftriaxone	158 (99.4)	26 (100)	184 (99.4)
Ceftazidime	158 (99.4)	25 (96.1)	183 (98.9)
Ciprofloxacin	112 (70.4)	15 (57.6)	127 (68.6)
TMP-SXT	108 (67.9)	19 (73)	123 (66.4)
CEF-SUL	70 (44)	12 (9.5)	82 (44.3)
Gentamicin	61 (38.3)	10 (38.4)	71 (38.3)
PIP-TAZ	25 (15.7)	9 (34.6)	34 (18.3)
Amikacin	21 (13.2)	1 (3.8)	22 (11.9)
Nitrofurantoin	11 (6.9)	16 (61.5)	27 (14.5)
Ertapenem	3 (1.9)	1 (3.8)	4 (2.2)
Imipenem	0 (0)	0 (0)	0 (0)
Meropenem	0 (0)	0 (0)	0 (0)

R: Number of resistant strains, AMX/CLA: Amoxicillin/Clavulanic acid, AMP/SLB: Ampicillin/Sulbactam, PIP/TAZ: Piperacillin-Tazobactam, CEF-SUL: Cefoperazone-Sulbactam, TMP-SXT: Trimethoprim-Sulfamethoxazole.

Underlying diseases were detected in a total of 148 ESBL-positive strains (80%), including 127 (79.8%) of *E. coli* and 21 (80.8%) of *K. pneumoniae* strains. The underlying diseases included BPH, urolithiasis, neurogenic bladder, urethral stricture and others; VUR, hypospadias, UPJ obstruction, vesicointestinal fistula, and cystocele ($p<0.05$) (Table 2). In the patients with underlying diseases, the resistance rates against amikacin in the *E. coli* strains and the resistance rates against ciprofloxacin and trimethoprim/sulfamethoxazole in the *K. pneumoniae* strains were found statistically significant ($p<0.05$) (Table 3).

Discussion

Recent studies have revealed that there is an increase in the antibiotic resistance in the treatment for urinary system infections in the world (7). This increase is associated with the increase in ESBL-positive bacteria and also with a number of risk factors including length of stay in the intensive care unit, surgical procedures, catheterization period, longer hospital stay, use of cephalosporin and aminoglycoside, and infections caused by ESBL-producing bacteria (8,9).

ESBL is most commonly found in *E. coli* and *K. pneumoniae* strains, and the antibiotics that can be used in the treatment of infections caused by these microorganisms are limited. ESBL-producing strains have been reported by numerous studies in different parts of the world. In multicenter studies, the rates of ESBL-producing bacteria vary between 1-74% depending on the countries and regions (3,10,11). In Turkey, however, the rates of ESBL-positive *E. coli* and *K. pneumoniae* strains have been reported between 21-29% and 35-49%, respectively (5,12-14). In our study, we found the rates for *E. coli* and *K. pneumoniae* as 39.6% and 44.8%, respectively, which revealed that the rate for *E. coli* strains was higher than the ones reported for Turkey. This situation could be best explained by the fact that our study only included the urinary samples and the patients who were treated in the urology clinic. Moreover, the high prevalence of underlying diseases (80%) in our

Table 2. Comparison of underlying diseases between ESBL-positive *E. coli* and *K. pneumoniae* strains

Etiologic Factor	*E. coli* (n=159)	*K. pneumonia* (n=26)	Total (n=185)
Neurogenic Bladder	29 (18.2%)	8 (30.7%)	37 (20%)
Urolithiasis	27 (17%)	2 (7.7%)	29 (15.6%)
BPH	23 (14.5%)	2 (7.7%)	25 (13.5%)
Urethral Stricture	11 (6.9%)	2 (7.7%)	13 (7%)
VUR	10 (6.3%)	3 (11.5%)	13 (7%)
Tumor	7 (4.4%)	2 (7.7%)	9 (4.9%)
Others	20 (12.6%)	2 (7.7%)	22 (11.9%)
Total	127 (79.8%)	21 (80.8%)	148 (80%)

USI: Urinary System Infection, BPH: Benign Prostate Hyperplasia, VUR: Vesicoureteral Reflux, Others: hypospadias, UPJ obstruction, vesicointestinal fistula, and cystocele.

Table 3. Comparison of antibiotic resistance profiles in association with the presence of underlying diseases

Antibiotics	*E. coli* (n=159)			*K. pneumonia* (n=26)		
	Presence of Underlying Diseases		*p*	Presence of Underlying Diseases		*p*
	NO (n=32)	YES (n=127)		NO (n=5)	YES (n=21)	
Ciprofloxacin	21 (65.6%)	91 (71.6%)	NS	1 (20%)	14 (66.6 %)	**<0.05**
Ceftriaxone	31 (96.8%)	127 (100%)	NS	5 (100%)	21 (100%)	NS
TMP/SXT	22 (68.7%)	86 (67.7%)	NS	5 (100%)	14 (66.6%)	**<0.05**
AMX/CLV	32 (100%)	127 (100%)	NS	5 (100%)	20 (95.2%)	NS
Nitrofurantoin	1(%0,6)	10 (7.8%)	NS	4 (80%)	12 (57.1%)	NS
Amikacin	0 (%0)	21 (16.5%)	**<0.05**	0 (0%)	1 (4.7%)	NS
Imipenem	0 (%0)	0 (0%)	NS	0 (0%)	0 (0)	NS
Meropenem	0 (%0)	0 (0%)	NS	0 (0%)	0 (0)	NS
Ertapenem	0 (%0)	3 (2.3%)	NS	0(0%)	1 (4.7%)	NS

AMX/CLA: Amoxicillin+Clavulanic acid, TMP-SXT: Trimethoprim-Sulfamethoxazole, NS: Not statistically significant.

patients may be an indication for the increased ESBL rate which was induced by irregular use of antibiotics due to recurrent infections.

In the studies conducted in Turkey, the resistance rates against Trimethoprim-Sulfamethoxazole, Ciprofloxacin, and Amoxicillin+Clavulanic acid in ESBL-positive *E. coli* strains isolated from urinary samples have been reported between 50-80%, 55.6-80.3%, and 89-94.4%, respectively (5,15-17). In our study, we found similar rates for resistance to Trimethoprim-Sulfamethoxazole and Ciprofloxacin, whereas the rate for Amoxicillin+Clavulanic acid was found as 100%. On the other hand, in *K. pneumoniae* strains, the resistance rates of Amoxicillin+Clavulanic acid, Ciprofloxacin, and Trimethoprim-Sulfametocsazole have been reported up to 90.5%, 47%, and 72%, respectively (5,15). However, the rates we found were slightly higher than the ones reported in the studies. We consider that this situation is associated with the irregular use of antibiotics in the treatment or prophylaxis of urinary system infections.

Resistance rates of Piperacillin-Tazobactam in ESBL-producing *E. coli* and *K. pneumoniae* strains have been reported between 20-70% (14,16). In our study, we found 15.7% for *E. Coli* and 34.6% for *K. pneumoniae* strains, both of which were consistent with the rates reported in the literature.

Carbapenems are potent antibiotics which possess the broadest spectrum of activity. Carbapenems, such as imipenem and meropenem, are commonly used for the treatment of ESBL-positive and Gram-negative bacterial infections (1,3,18). Resistance rates of carbapenems have been reported between 0-11.1% (1,18). In our study, although no resistance was observed against imipenem and meropenem in *E. coli* and *K. pneumoniae* strains, the resistance rate of ertapenem was assessed as 1.9% and 3.8%, respectively.

Amikacin is the second most active antibiotic following Carbapenems (12,18). In our study, the resistance rates of Amikacin in ESBL-positive *E. coli* and *K. pneumoniae* strains were 13.2% and 3.8%, respectively. Although the rate in *E. coli* strains (13.2%) was remarkably high, Amikacin is a useful agent for the treatment of infections caused by ESBL-positive pathogens.

We suggest to be carefulness in urological practises because of freguency of urinary system infections. In our study; the most common complicated factors were the presence of recurrence USI for more than 3 times in the preceding year and urological pathology in the urinary system. In this clinical study, neurogenic bladder, BPH, urolithiasis, ureteral obstruction were detected as the most common underlying diseases.

ESBL-positive *E. coli* and *K. pneumoniae* strains should be kept in mind for the treatment of urinary system infections. Amikacin is one of the most active antibiotics following Carbapenems. Investigation and treatment of underlying diseases is of prime importance. Regional antibiotic resistance rates should be determined and these rates should be kept in mind for the treatment of urinary system infections.

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