JMID/
Journal of Microbiology and Infectious Diseases

2019; 9 (3):112-115
doi: 10.5799/jmid.614195

In Vitro Activities of Ceftazidime-Avibactam and Comparator Antimicrobial Agents Tested against ESBL Producing Urinary *E. coli* Isolates

Estelle Caine[1], Ozlem Koyuncu Ozyurt[2], Gozde Ongut[2], Kubra Kasaroglu[3], Emre Yıldız[2], Dilara Ogunc[2], Filiz Gunseren[4], Dilek Colak[2], Ozge Turhan[4], Betil Ozhak[2]

[1]*Nottingham Trent University, England*
[2]*Akdeniz University, Faculty of Medicine, Department of Medical Microbiology, Antalya, Turkey*
[3]*Akdeniz University Central Laboratory*
[4]*Akdeniz University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Antalya, Turkey*

ABSTRACT

Objectives: Urinary tract infections (UTIs) remain one of the most common infections for antibiotic prescription. Increased resistance to antibiotics currently used to treat these infections may lead to significant problems. Extended spectrum beta-lactamase (ESBL) production in organisms causing UTIs are often responsible for the increase in resistance. The aim of this study was to investigate the in vitro antimicrobial susceptibility of ESBL producing community acquired urinary *E. coli* isolates to Ceftazidime/Avibactam (CAZ/AVI) and other antimicrobial agents using disk diffusion method.

Methods: Between 2016 and 2017, a total of 100 ESBL producing *E. coli* urine isolates were collected from outpatients who had no history of hospitalization in the last three months in Akdeniz University Hospital. All isolates were tested for in vitro susceptibility to the CAZ/AVI and other antimicrobial agents using disk diffusion method. **Results:** No CAZ/AVI and carbapenem resistance were observed. Resistance rates to other antimicrobial agents were as follows: ampicillin 100%, amoxicillin/clavulanate 85%, cefotaxime 98%, ceftazidime 89%, cefepime 82%, gentamicin 41%, ciprofloxacin 65%, levofloxacin 39%, fosfomycin 3%, nitrofurantoin 2%, trimethoprim/sulfamethoxazole 72% and Piperacillin/Tazobactam 41%.

Conclusions: Our results showed that ceftazidime / avibactam, carbapenems, nitrofurantoin and fosfomycin may be among the first line treatment options in the empirical therapy. In selected patients, CAZ/AVI could be an option for the treatment of UTI caused by ESBL producing *E.coli* which may reduce the use of carbapenems. *J Microbiol Infect Dis 2019; 9(2):112-115.*

Keywords: *Ceftazidime-avibactam, ESBL, Urinary tract infections, in vitro susceptibility test*

INTRODUCTION

Each year, 150 million people are affected by urinary tract infections (UTIs) worldwide. It is the most common infection for prescribing antibiotics. Although, the microbial spectrum of UTIs consists mainly of *Escherichia coli* (75%–95%), other species of *Enterobacteriaceae*, such as *Proteus mirabilis* and *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus* occasionally cause UTIs [1,2]. Increased resistance to antibiotics currently used to treat these infections may lead to significant problems. Extended spectrum beta-lactamase (ESBL) production in organisms causing UTIs, particularly *E. coli* and *Klebsiella spp.* are often responsible for the increase in resistance [3].

ESBLs, generally encoded by plasmid-borne genes, are capable of hydrolyzing the beta-lactam antibiotics such as penicillins, broad-spectrum cephalosporins and monobactams. ESBL producing organisms often show multidrug-resistance phenotypes such as resistance to fluoroquinolones, aminoglycosides and co-trimoxazole. Most ESBLs belong to the Ambler class A of β-lactamases and are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam and avibactam) [4,5].

Correspondence: Dr. Dilara Ogunc, Akdeniz University Faculty of Medicine, Department of Medical Microbiology, Antalya,
E-mail: dogunc@akdeniz.edu.tr
Received: 15 February 2019 Accepted: 09 June 2019

ESBL producing gram-negative bacteria has been associated with increased mortality, length of hospitalization, and hospital costs compared to non ESBL producing bacterial infections.

ESBL-producing strains have increased the use of carbapenems which causes an increase in carbapenem resistance.

New beta-lactam/beta-lactamase inhibitor combinations may be useful for the prevention of carbapenem overuse [6]. CAZ/AVI has not yet been licensed for use in our country.

The aim of this study was to investigate the in vitro antimicrobial susceptibility of ESBL producing community acquired urinary *E. coli* isolates to CAZ/AVI and other antimicrobial agents using disk diffusion method.

METHODS

Between 2016 and 2017, a total of 100 ESBL producing *E. coli* urine isolates from outpatients with no history of hospitalization in the last three months in Akdeniz University Hospital were included in the study. *E. coli* strains were isolated from clean-voided midstream urine. Identification of the strains were performed using MALDI-TOF MS (Bruker Daltonics, Germany) according to the manufacturer's instructions. Detection of ESBL was performed using phenotyping confirmatory disk diffusion test according to EUCAST recommendation's [7]. Both cefotaxime and ceftazidime, alone and in combination with clavulanate were used for ESBL confirmatory test. A ≥5mm increase in a zone diameter of cefotaxime-clavulanic acid and/or ceftazidime/clavulanic acid, compared with zone diameters when the cefotaxime and ceftazidime were tested alone, was considered to be phenotypic confirmation of ESBL production. Antimicrobial susceptibility testing and interpretation of results were performed in accordance with EUCAST recommendation's [8]. All isolates were tested for in vitro susceptibility to CAZ/AVI, ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, ertapenem, imipenem, meropenem, gentamicin, ciprofloxacin, levofloxacin, fosfomycin, nitrofurantoin and trimethoprim-sulfamethoxazole by disk diffusion method. Quality control was performed as recommended in EUCAST documents using *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603.

This study was approved by the Akdeniz University School of Medicine Ethical Committee of Clinical Research (Desicion number: 70904504/65/91).

RESULTS

No CAZ/AVI and carbapenem resistance were observed in any of the isolates.

The least active agents were ampicillin, cefotaxime, ceftazidime and cefepime.

All isolates were resistant to ampicillin, whereas resistance to cefotaxime (98%), ceftazidime (89%) and cefepime (82%) all exceeded 80%.

Fluoroquinolone resistance to ciprofloxacin and levofloxacin was determined as 65% and 39%, respectively.

Amoxicillin/clavulanate has very poor activity with a resistance of 85%, while piperacillin/tazobactam was moderately active with 41% resistance to ESBL producing *E. coli* isolates.

Resistance rates to other antimicrobial agents were found to be as follows: gentamicin 41%, fosfomycin 3%, nitrofurantoin 2%, and trimethoprim-sulfamethoxazole 72% (Table 1).

Table 1. Antibiotic resistance in ESBL producing *E. coli* isolates

Antibiotics	Resistance n (%)
Ampicillin	100 (100)
Amoxicillin/clavulanic acid	85 (85)
Piperacillin/tazobactam	41 (41)
Cefotaxime	98 (98)
Ceftazidime	89 (89)
Cefepime	82 (82)
Ceftazidime/avibactam	0 (0)
Ertapenem	0 (0)
Imipenem	0 (0)
Meropenem	0 (0)
Gentamicin	41 (41)
Ciprofloxacin,	65 (65)
Levofloxacin,	39 (39)
Fosfomycin,	3 (3)
Nitrofurantoin	2 (2)
Trimethoprim/sulfamethoxazole	72 (72)

DISCUSSION

Antibiotic resistance has become an increasing problem worldwide, use and abuse of antibiotics in medicine and agriculture has amplified the resistance especially in bacteria known to cause community and hospital acquired infections. The CDC considers Gram-negative bacilli that produce ESBLs as serious threats [9,10].

In our study, CAZ/AVI and carbapenems possess excellent activity against ESBL producing *E. coli* isolates. CAZ/AVI has been approved by the Food and Drug Administration and European Medicines Agency for cUTI [11]. According the results from the INFORM global surveillance study conducted in 2012 to 2014, CAZ/AVI inhibited 99.9% of molecularly confirmed ESBL-producing isolates of *E. coli*, *K. pneumoniae, K. oxytoca*, and *P. mirabilis* [12]. The INFORM Global Surveillance Program results in Asia-Pacific Countries, susceptibility to CAZ/AVI for *Enterobacteriaceae* isolates from each of the Asia-Pacific countries surveyed ranged from 99.1–100%, except China, Thailand, and Philippines. CAZ/AVI inhibited 99.6%of ESBL-positive isolates [13]. In a study conducted to determine the in vitro activity of avibactam in combination with beta-lactams against Gram-negative bacteria, all *E. coli* and K. pneumoniae isolates with CTX-M-15 -lactamase were susceptible to CAZ/AVI [14]. In one study from China, the susceptibility of *E. coli* to CAZ/AVI was found as 96.8%, but for carbapenem -resistant *E. coli* susceptibility to CAZ/AVI was reduced to 28.6%. The authors attributed this to the fact that 67.9% of carbapenem-resistant *E. coli* was bla_{NDM}-positive [15]. In addition to bla_{NDM} gene, mechanisms of CAZ/AVI resistance in carbapenem-resistant *Enterobacteriaceae* include, but are not limited to, mutations in the bla_{KPC} gene, differences in susceptibilities of KPC subtypes and other resistance determinants - e.g. outer membrane proteins (OMPs) such as *omp*K36 and ESBLs [16]. In our study, we think that the reason for not having resistance to CAZ/AVI was the lack of carbapenem resistance in our isolates.

IDSA recommends Nitrofurantoin, Trimethoprim-sulfamethoxazole (if resistance prevalence is known to exceed 20 or if used for UTI in previous 3 months) or fosfomycin for empiric treatment of uncomplicated cystitis [17].

Although the frequency of urinary multidrug resistant (MDR) *E. coli* among US outpatients increased between 2001 and 2010, nitrofurantoin demonstrated consistent antimicrobial activity against MDR *E. coli* [18].

In our study, ESBL producing *E. coli* isolates demonstrated high susceptibility for nitrofurantoin and fosfomycin. Resistance trimethoprim-sulfamethoxazole exceeded 20%, suggesting that these agent may not be reliable for use as empiric therapy.

In 2015, Detection and Monitoring of Antimicrobial Drug Susceptibility (ADSI) Working Group was performed laboratory-based resistance surveillance to determine the antimicrobial susceptibility to various antimicrobial agents for urinary *E. coli* isolates obtained from various geographic regions in Turkey. In this study, 37% of the isolates were found to be ESBL positive and antimicrobial resistance rates of ESBL producing strains were found as: 62.1% to norfloxacin, 68.3 % to trimethoprim-sulfamethoxazole, 12.1% to nitrofurantoin and 4 % to fosfomycin [19].

In our study, CAZ/AVI and carbapenems showed excellent in vitro activity against ESBL producing *E. coli* strains. High rates of resistance to trimethoprim/sulphamethoxazole and ciprofloxacin indicate that these antibiotics cannot be recommended for empirical treatment. Therapy of urinary tract infections with ESBL producing strains is problematic. An understanding of local antimicrobial resistance patterns of *E. coli* and other uropathogens is essential for the management of empirical antimicrobial treatment. Our result showed that CAZ/AVI, carbapenems, nitrofurantoin and fosfomycin may be among the first line treatment options in empirical therapy. In selected patients, CAZ/AVI could represent an option for the treatment UTI caused by ESBL producing *E. coli* which may reduce the use of carbapenems.

ACKNOWLEDGMENTS

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

Funding source: Not declared.

REFERENCES

1. Picozzi SC, Casellato S, Rossini M, et al. Extended-spectrum beta-lactamase positive *Escherichia coli* causing complicated upper urinary tract infection: Urologist should act in time. Urol Ann 2014; 6(2):107-12.

2. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103-20.

3. Keepers TR, Gomez M, Celeri C, Krause KM, Biek D, Critchley I. Fosfomycin and Comparator Activity Against Select Enterobacteriaceae, Pseudomonas, and Enterococcus Urinary Tract Infection Isolates from the United States in 2012. Infect Dis Ther. 2017; 6(2):233-243.

4. Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. Clinical and economic I mpact of bacteremia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother. 2006; 50(4):1257-1262.

5. Popejoy MW, Paterson DL, Cloutier D, et al. Efficacy of ceftolozane/tazobactam against urinary tract and intra-abdominal infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: a pooled analysis of Phase 3 clinical trials. J Antimicrob Chemother 2017; 72(1):268-272.

6. D'Angelo RG, Johnson JK, Bork JT, Heil EL. Treatment options for extended-spectrum beta-lactamase (ESBL) and AmpC-producing bacteria. Expert Opin Pharmacother. 2016; 17(7):953-967.

7. The European Committee on Antimicrobial Susceptibility Testing. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0, 2017. http://www.eucast.org.

8. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. http://www.eucast.org.

9. Forster CS, Courter J, Jackson EC, Mortensen JE, Haslam DB. Frequency of Multidrug-Resistant Organisms Cultured From Urine of Children Undergoing Clean Intermittent Catheterization. J Pediatric Infect Dis Soc 2017; 6(4):332-338.

10. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci. 2015; 22(1):90-101.

11. Mendes RE, Castanheira M, Woosley LN, Stone GG, Bradford PA, Flamm RK. Molecular β-lactamase characterization of gram-negative pathogens recovered from patients enrolled in the ceftazidime-avibactam phase 3 trials (RECAPTURE 1 and 2) for complicated urinary tract infections: Efficacies analysed against susceptible and resistant subsets. Int J Antimicrob Agent 2018; S0924-8579(18)30103-1.

12. Karlowsky JA, Biedenbach DJ, Kazmierczak KM, Stone GG, Sahm DF. Activity of Ceftazidime-Avibactam against Extended-Spectrum- and AmpC β-Lactamase-Producing Enterobacteriaceae Collected in the INFORM Global Surveillance Study from 2012 to 2014. Antimicrob Agents Chemother 2016; 60(5):2849-2857.

13. Karlowsky JA, Kazmierczak KM, Bouchillon SK, de Jonge BLM, Stone GG, Sahm DF. In Vitro Activity of Ceftazidime-Avibactam against Clinical Isolates of Enterobacteriaceae and Pseudomonas aeruginosa Collected in Asia-Pacific Countries: Results from the INFORM Global Surveillance Program, 2012 to 2015. Antimicrob Agents Chemother. 2018; 62(7). pii: e02569- e02517.

14. Aktaş Z, Kayacan C, Oncul O. In vitro activity of avibactam (NXL104) in combination with β-lactams against Gram-negative bacteria, including OXA-48 β-lactamase-producing *Klebsiella pneumoniae*. Int J Antimicrob Agents. 2012; 39(1):86-89.

15. Yin D, Wu S, Yang Y, et al. China Antimicrobial Surveillance Network (CHINET) Study Group. Results from the China Antimicrobial Surveillance Network (CHINET) in 2017 of the In VitroActivities of Ceftazidime-Avibactam and Ceftolozane-Tazobactam against Clinical Isolates of Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother 2019; 63(4):e02431-e02418.

16. European Centre for Disease Prevention and Control. Emergence of resistance to ceftazidime-avibactam in carbapenem-resistant Enterobacteriaceae – 12 June 2018. Stockholm, ECDC; 2018.

17. Gupta K, Hooton TM, Naber KG, et al. Infectious Diseases Society of America; European Society for Microbiology and Infectious Diseases. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011; 52(5):e103-e120.

18. Sanchez GV, Baird AM, Karlowsky JA, Master RN, Bordon JM. Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary *Escherichia coli* from US outpatients. J Antimicrob Chemother. 2014; 69(12):3259-3262.

19. .http://www.tmc-online.org/pdf/Pinar_Ciragil.pdf