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Evaluation of ceftazidime-avibactam efficacy in gram negative bacteria

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Abstract

In recent years, the emergence of multidrug-resistant *Enterobacterales* and *Pseudomonas aeruginosa* strains has become a serious problem due to high morbidity/mortality rates and difficulty in treatment.Treatment options are very limited in infections caused by carbapenem-resistant microorganisms.Ceftazidime/avibactam (CZA) is a newly developed cephalosporin/beta-lactamase inhibitor combination for the treatment of infections caused by resistant Gram negative microorganisms.In this study, the in vitro activity of CZAagainst various carbapenem resistant Gram negative microorganisms was evaluated.122 carbapenem-resistant Gram negative bacteria species were included in the study. Identification of the strains and their antimicrobial susceptibility were performed using conventional methods as well as a BD Phoenix (BectonDickinson, MD, USA) fully automated system. Ceftazidime-avibactam susceptibility was determined using the disk diffusion method (Bioanalyse, Ankara, Turkey) and the gradient diffusion test (Liofilchem MIC strip test, Italy).In carbapenem-resistant *K. pneumoniae* strains, gentamicin/amikacin susceptibility was found in 14 strains (14.3%) and ceftolozane-tazobactam susceptibility was found in only one strain. In *P. aeruginosa* strains, gentamicin/amikacin susceptibility was seen in 8 strains (33.3%) and piperacillin tazobactam susceptibility was found in 7 strains (29.2%), and other than these all strains were found to be resistant to all the other antibiotics studied. CZA susceptibility rates in carbapenem resistant *K. pneumoniae* and *P. aeruginosa* strains were found to be 85.7% and 83.3%, respectively.ESBL production and carbapenemase activity were positive in the strains included in the study.The results obtained from this study show that CZA can be a treatment alternative in carbapenem-resistant Gram negative bacterial infections. More studies are needed in order to monitor the resistance status of these new treatment options against the increasing resistance threat in hospitals and to determine the appropriate treatment option.

Keywords: Antibiotic resistance, gram negative bacteria, ceftazidime/avibactam

Introduction

Multi-drug-resistant and widely drug-resistant Gram negative bacterial infections, which have been increasing in hospitalized patients in recent years, pose a great threat. These infections, whose treatment options are generally inadequate, can cause high mortality and morbidity. This increase in resistance, which results from the increased and sometimes inappropriate use of antibiotics, raises the interest in new antibiotic options that are in use or being developed for the treatment of infections with resistant Gram negative bacteria. While effective antibiotic alternatives are declining, the number of newly developed antibiotics remains low in the face of growing resistance [1].

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While methicillin-resistant *Staphylococcus aureus* (MRSA) was the main bacterium causing resistant infections about 20 years ago, today multi-drug resistant Gram negative bacteria have become the primary agents [1]. When the list of priority factors threatening human health of the World Health Organization is examined, it is seen that carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *P. aeruginosa* and *Enterobacterales* infections resistant to carbapenem and 3rd generation cephalosporins are at the top [2]. Due to these resistant bacterial infections, which cause the death of many people and often do not have an effective treatment option, there has been a serious increase in inappropriate and widespread use of antibiotics, and the whole world has to face the negative consequences of this situation [3].

Although the number of new antibiotics used in recent years is quite limited, data on some newly developed and under development antibiotics are increasing. Recently, a new β-lactam combination, ceftazidime/avibactam (CZA), has been approved for the treatment of infections due to Gram negative bacteria producing class A,

class C, and class D β-lactamases. CZA is a β-lactam/β-lactamase inhibitor combination with activity against antibiotic-resistant Gram negative microorganisms, including many carbapenem-resistant strains. Ceftazidime is a 3rd generation cephalosporin active against *P. aeruginosa*. Avibactam is an inhibitor of class A, class C and some class D β-lactamases. The antibacterial spectrum of CZA covers 95% of *P. aeruginosaisolates* and >99% of *Enterobacteriaceae*, including strains carrying broad-spectrum β-lactamases (ESBLs) [4]. However, its effects on *Acinetobacter spp*, anaerobes and Gram positive bacteria are quite low. Its combined use with ceftazidime was approved by the US Food and Drug Administration (FDA) in 2015 for intra-abdominal infections and complicated urinary tract infections, and in 2018 for hospital-acquired and ventilator- associated pneumonia [4]. CZA appears to be a promising treatment option today, where there are limited alternatives for infections caused by various resistant Gram negative microorganisms. The aim of this study is to evaluate the in vitro activity of CZA against various carbapenem-resistant Gram negative microorganisms.

Materials and Methods

In this study, culture samples taken from hospitalized patients in clinics at Ordu University Medical Faculty Training and Research Hospital between January 2021 and December 2021 were investigated. The isolated bacteria and their antibiotic susceptibility were evaluated and in vitro activity of CZA against carbapenem-resistant Gram negative microorganisms was investigated.

In our study, 122 carbapenem-resistant bacterial species cultured from samples sent from inpatient clinics to the microbiology laboratory of our hospital were included. Patient samples were cultured on 5% sheep blood agar (RTA, Kocaeli, Turkey), chocolate agar (RTA, Kocaeli, Turkey), eosin methylene blueagar (RTA, Kocaeli, Turkey), sabouraud dextrose agar (RTA, Kocaeli, Turkey).

Afterwards, these cultivars were incubated aerobically at 37 °C for 24-48 hours, and at the end of this period, all isolates were identified by standard microbiological procedures. After the growth characteristics of Gram-negative bacteria isolated from the samples with growth, Gram staining, oxidase test and biochemical tests (reactions in Triple sugar iron agar, Simmon's citrate agar, Christensen urea agar, movement medium and indole medium). If necessary, these Gram-negative bacteria isolates were identified with an automated system. Bacteria in cultures with pure colonies showing growth were studied and identified in accordance with the manufacturer's recommendations with the BD Phoenix (Becton Dickinson, MD, USA) fully automatic system as well as conventional methods. Antibiotic susceptibility of the isolates was determined by studying pure bacterial cultures obtained from the culture with the BD Phoenix (Becton Dickinson, MD, USA) automated system in line with company recommendations.

ESBL and carbapenemase phenotypic confirmation tests were performed according to the screening test results. For ESBL phenotypic confirmation tests; the susceptibility of bacteria resistant to either or both of the cefotaxime (<21 mm) and ceftazidime (<22 mm) discs was evaluated against the cefotaxime/clavulanic acid (30/10 µg) and ceftazidime/clavulanic acid (30/10 µg) discs. ESBL was considered positive if the zone around the combination disk was ≥5 mm wider than the zone of inhibition of the disk containing cephalosporins alone. For ESBL phenotypic confirmation of isolates resistant to either or both cefotaxime and ceftazidime discs, the double-disc synergy method (DDS) was applied. When cefepime (30 µg) and clavulanic acid (10 µg) were added together, the enlargement of the zone diameter of the cefepime disc on the side facing the clavulanic acid disc was considered ESBL positive [5]. The antimicrobial susceptibility of the strains was interpreted according to the European Antimicrobial Susceptibility Test standards (EUCAST), and carbapenemase production was determined using the EUCAST meropenem disc scanning method [5]. For carbapenemase phenotypic confirmation tests; production for; in accordance with EUCAST recommendations, 10 µl of 100 mg/l dipicolinic acid (DPA), 0.2 M EDTA, 60 mg/ml aminophenyl boronic acid (APBA), 75 mg/l cloxacillin solution were dropped onto meropenem discs in sterile petri dishes and left for 30 minutes. The inoculum prepared according to the 0.5 McFarland standard was spread on Mueller Hinton agar plates and one of each meropenem disc with or without inhibitor was placed on it. According to the carbapenemase combination disc test, it was tested whether there was synergy in the discs with inhibitör [5].

CZA susceptibility was determined on Mueller Hinton agar using the Kirby-Bauer disk diffusion method (Bioanalyse, Ankara, Turkey) and the gradient diffusion test (Liofilchem MIC strip test, Italy). In the disk diffusion method, the zone diameters were evaluated as S ≤13 and R >13 for *Enterobacterales* species and S ≤17 and R >17 for *P. aeruginosa*, while the MIC values were taken as S ≤8 and R >8 for *Enterobacterales* species and *P. aeruginosa* [6].

Results

All of the 122 strains included in the study were isolated from patients in the intensive care units including 70 (57.4%) females and 52 (42.6%) males. While 98 (80.3%) of the strains studied were found to be carbapenem-resistant *K. pneumoniae*, 24 (19.7%) strains were found to be *P. aeruginosa*. Considering the distribution of clinics where the isolates were sent, it was observed that 70.2% were sent from internal medicine critical care units and 29.8% from surgical intensive care units. When the distribution of the sample sites was examined, it was seen that they were most commonly isolated from endotracheal aspirate (ETA) with 40.9%, blood samples with 26.2% and urine samples with 22.9%.

ESBL production and carbapenemase activity were positive in the strains included in the study. In carbapenem-resistant *K. pneumoniae* strainsgentamicin/amikacin susceptibility was detected in 14 strains (14.3%), ceftolozane-tazobactam susceptibility was detected in only 1 strain, and resistance to all antibiotics was observed in all other strains [Table 1]. In *P. aeruginosa* strains, gentamicin/amikacin susceptibility was seen in 8 strains (33.3%) and piperacillin tazobactam susceptibility was found in 7 strains (29.2%), and other than these all strains were found to be resistant to all the other antibiotics (carbapenems, cephalosporins, aminoglycosides, quinolones) studied. CZA susceptibility rates in carbapenem resistant *K. pneumoniae* and *P. aeruginosastrains* were found to be 85.7% (84/98) and 83.3% (20/24), respectively

[Table 2]. In addition, when the susceptibility results for CZA were evaluated with concerning for to testing methods, full concordance was observed between the disc diffusion and MIC methods for all strains. The MIC50/MIC90 values of CZA for *K. pneumoniae* and *P. aeruginosa* were ≤0.25 to >32 µg/mL and 0.25 to >32 µg/mL, respectively [Table 3].

Table 1. Antibiotic susceptibility results of isolates *K. pneumoniae*

	Resistant		Susceptible	
	n	%	n	%
Ceftazidime-avibactam	14	14.3	84	85.7
Gentamicin/amikacin	84	85.7	14	14.3
Ceftolozane-tazobactam	97	98.97	1	1.02
Piperacillin-tazobactam	98	100	0	0
Cephalosporins	98	100	0	0
Carbapenems	98	100	0	0
Quinolones	98	100	0	0
Trimethoprim/Sulfamethoxazole	**98**	**100**	**0**	**0**

Table 2. Antibiotic susceptibility results of isolates*P. aeruginosa*

	Resistant		Susceptible	
	n	%	n	%
Ceftazidime-avibactam	4	16.7	20	83.3
Gentamicin/amikacin	16	66.6	8	33.3
Piperacillin-tazobactam	17	70.8	7	29.2
Cephalosporins	24	100	0	0
Carbapenems	24	100	0	0
Quinolones	24	100	0	0

Table 3. MIC50/MIC90 values of Ceftazidime/avibactam

Species (no. of isolates)	MIC[a](mg/liter)			Percentage of Susceptible Strains
	Range	50%	90%	
CR[b] *K. pneumoniae* (98)	≤0.25 to >32	1	>32	14.3%
CR *P. aeruginosa* (24)	0.25 to >32	1	>32	16.6%

[a] MIC, minimum inhibitory concentration, 50% and 90%, MICs at which 50% and 90% of isolates, respectively, are inhibited. [b]CR. Carbapenem resistant

Discussion

Although the resistance to multiple antimicrobial agents in Gram negative bacteria producing carbapenemase may vary according to epidemiological characteristics, it is seen at an increasing rate in the world. Infections caused by these resistant microorganisms are associated with long hospital stays, leading to increased health care costs as well as high mortality rates [7,8]. It has been reported that metallo-betalactamase (MBL) and *K. pneumoniae* carbapenemase (KPC)-mediated carbapenem resistance has started being seen in enteric Gram negative bacteria since 1996 in the United States and European countries, and it has begun to spread to other countries around the world [9]. Carbapenem resistance was detected in *K. pneumoniae* isolate for the first time in our country in 2001, and other studies showed that MBL (VIM, IMP, NDM-1), KPC and especially OXA carbapenemases started to increase rapidly [10,11]. In European countries, the problem of carbapenemase started with the presence of MBLs, but with time OXA-48 carbapenemase became endemic [12].

CZA is a good alternative therapeutic option for carbapenem-resistant strains of *K. pneumoniae* and *P. aeruginosa*, which has recently been approved by the FDA. However, its reimbursement is only valid for intensive care patients and strains not susceptible to 3rd generation cephalosporins, aminoglycosides and carbapenems. In a multicenter study on CZA susceptibility in Europe, data from 96 centers from 18 countries were collected and 24.750

Enterobactererales isolates were included in the study. CZA was found to be the most effective antibiotic in all strains studied. While it was 96.7% susceptible in MDRs, 98.5% in MBL-negative isolates in CRE strains were found to be susceptible. Naturally, as expected, it was found to be ineffective against MBL carrying isolates [13]. In another study, treatment of carbapenem-resistant *K. pneumoniae* bacteremia with CZA was associated with higher rates of clinical success (P= 0.006) and survival (P= 0.01) compared to other treatment regimens [14]. However, 3 patients developed CZA-resistant *K. pneumoniae* after 10 to 19 days of CZA treatment, with the first cases of CZA resistance developing during treatment of CRE infections. Whole genome sequencing was performed and previously undetected blaKPC-3 mutations were found. Here, strikingly, the susceptibility of mutations in *K. pneumoniae* was restored in two patients by reducing the meropenem MICs by ≥4-fold from baseline. While drawing attention to the resistance that may develop in some other studies, it has been stated that strains that can develop resistance can be seen without any exposure to CZA. Along with high efficacy in *K. pneumoniae* infections producing KPC, CZA resistance can be observed in 10% of cases due to a mutation in the blaKPC gene during the treatment process. However, it was determined as a remarkable finding that the same strains no longer show the same level of carbapenem resistance after this mutation [15,16].

In a study conducted in Turkey in which resistance was evaluated, CZA and carbapenem resistance were not observed in any of the ESBL-producing *E.coli* strains. In this study, it was emphasized that CZA could be an option in the treatment of UTI caused by ESBL-producing *E.coli* [17]. In another study investigating the in vitro sensitivity of CZA in MDR *P. aeruginosa* strains producing carbapenemase, CZA resistance was found in 21.8% of the strains [18]. Again, in a study conducted in our country, a CZA sensitivity rate of 86% was found for *P. aeruginosa* (PER-1 beta-lactamase producing) strains. Terzi et al. observed CZA resistance in 27% of carbapenemase-producing multidrug-resistant *K. pneumoniae* isolates [19]. In a recent pilot study in Turkey, 95.2% of the total 318 isolates whose CZA susceptibility was examined among carbapenem-resistant *Enterobacterales* were susceptible to CZA; It was found that 4.76% of the isolates were resistant to CZA [20]. Although a higher rate of CZA resistance was found in our study with a resistance rate of 14.8% compared to the last study, this rate is compatible with other studies. These different resistance results can be attributed to MDR of the strains included in our study and

possible differences in carbapenemase genes or different resistance mechanisms. Considering the increasing resistance rates, taking into account the resistance status while deciding on the treatment is one of the important parameters that will affect the treatment results [21].

In a study evaluating the results of disk diffusion, gradient diffusion test and reference method broth microdilution for CZA against *Enterobacterales* clinical isolates, 458 *Enterobacterales* isolates isolated from 54 medical centers were examined and it was found that the results of disk diffusion and gradient diffusion test performed well against *Enterobacterales* clinical isolates [22]. However, in another study, the sensitivity results of 302 clinical *Enterobacterales* isolates and *P. aeruginosa* isolates for CZA were compared with broth microdilution, gradient diffusion test and disk diffusion method, and it was found that the performance of the gradient diffusion test was better than the disk diffusion method [23]. We urgently need an economical and practical method for the accurate detection of CZA activity. In our study, the disc diffusion method and gradient diffusion test were used to determine the in vitro activity of CZA, and the results were found to be fully compatible. In the light of these findings, it has been determined that the disk diffusion method is a practical method that can be routinely applied to alleviate the workload of the laboratory and reduce the cost.

Conclusion

In conclusion, although our study showed that CZA is a good alternative therapeutic option for carbapenem-resistant *K. pneumoniae* and *P. aeruginosa* strains, the 14.8% rate of CZA resistance detected is remarkable and should be considered. It is known that obtaining susceptibility test results early increases clinical success by enabling early treatment. The principles of rational antibiotic use should be followed to prevent the development of resistance which can be seen even in the last option of antibiotics in treatment. Susceptibility testing for CZA should be routinely performed in laboratories and results should be followed up to date. In this area, there is a need for larger multicenter studies with a combination of laboratory and clinical data.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

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Ethical approval
Ethics committee approval is not required for this study

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