RESEARCH ARTICLE

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Comparison of in Vitro Antimicrobial Efficacy of Ceftolozane-Tazobactam and Ceftazidime-Avibactam Combination Against Carbapenem-Resistant *Enterobacteriaceae* Species Isolated from Various Clinical Specimens

ABSTRACT

Objective: The increase in resistant gram-negative bacteria is a major concern and has led to difficulties in the treatment of infections. The aim of this study was to compare the in vitro efficacy of CLZ-TAZ and CAZ-AVB combinations against carbapenem-resistant *Enterobacteriaceae* strains.

Methods: Eighty, carbapenem-resistant *Enterobacteriaceae* species isolated from various samples sent to our laboratory were included in the study. Identification and antimicrobial susceptibility of strains were performed using automated systems. The presence of carbapenemases in all isolates was tested using the CarbaNP test and the carbapenem inactivation method. The presence of carbapenemase genes was tested by multiplex PCR.

Results: The presence of carbapenemases was detected in 60 % *E. coli* isolates and in 78.5% *K. pneumoniae* isolates via phenotypic tests. OXA-48 enzyme was found in 73.7% of isolates containing carbapenemase. The second most common enzyme was NDM. The assessment of the efficacy of the β-lactam/β-lactamase inhibitor combinations against CRE isolates revealed that the activity of CAZ-AVB (77%) was higher than CLZ-TAZ (48%).

Conclusions: Our findings suggest that CAZ-AVB and CLZ-TAZ may be promising in the treatment of infections caused by CRE strains. Sensitivity rates were higher with ceftazidime-avibactam than with ceftolozane-tazobactam. The data obtained in this study will contribute to the clinical use of these agents in our country.

Keywords: Carbapenem-Resistant *Enterobacteriaceae*, Ceftolozane/Tazobactam Ceftazidime/Avibactam, *bla*OXA-48, *bla* NDM.

Çeşitli Klinik Örneklerden İzole Edilen Karbapenem Dirençli *Enterobacteriaceae* İzolatlarına Karşı Seftolozan-Tazobaktam ve Seftazidim-Avibaktam Kombinasyonlarının In Vitro Antimikrobiyal Etkinliğinin Karşılaştırılması

ÖZET

Amaç: Dirençli gram negatif bakterilerdeki artış önemli bir endişe kaynağıdır ve enfeksiyonların tedavisinde zorluklara yol açmıştır. Bu çalışmanın amacı, carbapenem-resistant *Enterobacteriaceae* suşlarına karşı CLZ-TAZ ve CAZ-AVB kombinasyonlarının in vitro etkinliğini karşılaştırmaktır.

Gereç ve Yöntem: Çalışmaya, laboratuvarımıza gönderilen çeşitli örneklerden izole edilen, karbapenemlere dirençli 80 *Enterobacteriaceae* türü dahil edildi. İzolatların tanımlanması ve antimikrobiyal duyarlılıkları otomatize sistemler kullanılarak gerçekleştirildi. Tüm izolatlarda karbapenemazların varlığı, CarbaNP testi ve karbapenem inaktivasyon yöntemi kullanılarak test edildi. Karbapenemaz genlerinin varlığı multipleks PCR ile test edildi.

Bulgular: Fenotipik testler ile karbapenemazların varlığı %60 *E. coli* izolatında ve %78.5 *K. pneumoniae* izolatında tespit edildi. Karbapenemaz içeren izolatların %73.7'sinde OXA-48 enzimi bulundu. İkinci en yaygın enzim NDM idi. β-laktam/β-laktamaz inhibitör kombinasyonlarının CRE izolatlarına karşı etkinliğinin değerlendirilmesinde ise, CAZ-AVB'nin (%77) aktivitesinin CLZ-TAZ'dan (%48) daha yüksek olduğu tespit edildi.

Sonuç: Bulgularımız, CRE suşlarının neden olduğu enfeksiyonların tedavisinde CAZ-AVB ve CLZ-TAZ'ın umut verici olabileceğini düşündürmektedir. Seftazidim-avibaktam ile duyarlılık oranları, seftolozan-tazobaktamınkinden daha yüksekti. Bu çalışmada elde edilen veriler ülkemizde de bu ajanların klinik kullanımına katkı sağlayacaktır.

Anahtar Kelimeler: Karbapenem dirençli *Enterobacteriaceae*, Seftolozan/Tazobaktam, Seftazidim/Avibaktam, *bla*OXA-48, *bla* NDM.

INTRODUCTION

Antibiotic resistance in gram-negative bacteria has increased over time and led to treatment failures by limiting clinical treatment options. Antibiotic resistance rates can vary significantly between countries and regions. Multidrug-resistant (MDR) gram-negative bacteria-related infections are very difficult to treat and they represent a serious health emergency, especially in patients having comorbid diseases (1,2,3). Because carbapenems are highly effective, they are frequently used as first-line antibiotics for the treatment of infections caused by microorganisms that produce extended-spectrum beta-lactamases (ESBL). However, the increased rates of infections caused by ESBL-producing *Enterobacteriaceae* members have caused a rise in the frequency of use of carbapenems over the years, contributing to carbapenem resistance rates (1,3). Nosocomial infections caused by *Enterobacteriaceae,* which produce different types of carbapenemases, are now common in many countries posing major limitations for antimicrobial therapy. Over the last decade, carbapenem-resistant *Enterobacteriaceae* has been found to be spreading worldwide (3-5). The high morbidity and mortality of infections caused by MDR gram-negative bacteria result from the unavailability of safe and effective antibacterial treatment options.

This necessitates further research to develop new antibiotics. For this reason, the World Health Organization has published the global priority list of antibiotic-resistant bacteria to guide the research, discovery, and development of new antibiotics. The global priority list of antibiotic-resistant bacteria includes carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* in the category1 as the critical priority list of pathogens in urgent need of new antibiotics (6,7). Among the newest agents developed to combat antimicrobial resistance, β-lactam/β-lactamase inhibitor combinations have attracted considerable interest with promising results through *in vitro* activity against MDR gram-negative bacteria (6). Ceftazidime/avibactam (CAZ-AVB) is a third generation cephalosporin and a new β-lactamase inhibitor combination. Ceftolozane/tazobactam (CLZ-TAZ) is a fourth generation cephalosporin and β-lactamase inhibitor combination. Both drugs are often used as salvage therapy when the infectious agent is resistant to all other antibiotics available (8).

The aim of this study was to investigate the *in vitro* antimicrobial efficacy of these two new combinations of β-lactam-β-lactamase inhibitors against carbapenemase gene-containing carbapenem-resistant *Enterobacteriaceae* isolate that were isolated from various clinical specimens.

MATERIAL AND METHODS

Bacterial Isolates and Antimicrobial Sensitivity Tests: Various clinical specimens (tracheal aspirates, urine and blood samples, and wound swabs) submitted to our laboratory in the period between August 2017 and October 2017 were examined for eligibility. A total of 80 isolates from the *Enterobacterales* family were included in the study. The included isolates were found to be resistant to carbapenems in antimicrobial sensitivity tests and carbapenemase positive in phenotypic tests. Only one isolate from one patient was included in the study. The BD Phoenix™ (Becton Dickinson, US) automated identification system was used to identify the isolates. The antimicrobial sensitivity of the isolates was tested using the BD Phoenix™ (Becton Dickinson, US) automated identification and antibiogram analysis system and through the gradient test method. The minimum inhibitory concentrations (MIC) of CAZ-AVB and CLZ-TAZ were tested using the gradient test method (Liofilchem, Italy). The results were interpreted in accordance with the EUCAST 2017 criteria.

Phenotypic Tests to Discover the Presence of Carbapenemases: The presence of carbapenemases in *K. pneumoniae* and *E. coli* isolates resistant to at least one of the following antibiotics imipenem, meropenem, and ertapenem was tested using the CarbaNP test and carbapenem inactivation method (CIM).

CarbaNP Test: A loopful of bacterial colonies from a blood agar plate was suspended in a 200 µl Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific, USA). After incubating at room temperature for 30 minutes, 100µl of the suspension was taken and mixed with a phenol red solution containing 6 mg/L imipenem monohydrate (Sigma, France). The bacterial suspension was examined to detect any color changes after a 2-hour incubation period at 35 °C. A color change from red to yellow at the end of the incubation period was considered a positive result. When no changes were observed in the color, the result was accepted as negative.

Carbapenem Inactivation Method (CIM): A 10 µL loopful of the bacterial colonies to be tested was suspended in 400 µL sterile distilled water. A 10 µg BD BBL™ Sensi-Disc™ meropenem disc (Becton Dickinson, ABD) is added to the suspension and incubated at 35±2°C for 2 hours. The *E. coli* ATCC 25922 suspension prepared at 0.5 McFarland turbidity was streaked onto Mueller-Hinton agar plates. The discs incubated in the suspension were placed onto the plate as they were placed in the disc diffusion test. The results of the CIM test were evaluated after the incubation. When the efficacy of the meropenem disc was preserved and an inhibition zone occurred

within the limits of susceptibility, the CIM test result was accepted negative. The test result of the CIM test was interpreted as positive when the efficacy of the meropenem disc was not preserved due to carbapenemase activity.

Examination of the Presence of Carbapenemases through Molecular Methods: Total DNA extraction from the isolates was performed by the 'sand method' (8). The presence of carbapenemase genes (blaVIM, blaIMP, blaKPC, blaOXA48, and blaNDM) was tested by multiplex PCR. The primer sequences used in the molecular determination of the presence of carbapenemase and the band sizes obtained are shown in Table 1.

Table 1. Primer sequences used in the molecular determination of carbapenemase presence and obtained band sizes

Primer	Sequence 5'-3'	Product size(bp)
***bla* KPC**	5'-TGTCACTGTATCGCGGTC-3' 5'-CTCAGTGCTCTACAGAAAAAC-3'	900
***bla* NDM**	5'-CTCAGTGCTCTACAGAAAAAC-3' 5'-GCAGCTTGTCGGCCATGCGGGC-3'	782
***bla* VIM**	5'-GATGGTGTTTGGTCGCATA-3' 5'-CGAATGCGCAGCACCAG-3'	390
***bla* IMP**	5'-GGAATAGAGTGGCTTAAYTCT-3' 5'-CCAAACYACTASGTTATCT-3'	188
***bla*OXA-48**	5'-GCGTGGTTAAGGATGAACAC-3' 5'-CATCAAGTTCAACCCAACCG-3'5'-	438

RESULTS

Of the species isolated during the study period, 1221 were *E. coli* and 378 were *K. pneumoniae*. Of these isolates, 80 were carbapenem resistant isolates. Of these isolates, 70 were *K. pneumoniae* and 10 were *E. coli*. Of the isolates; 43 (51%) were recovered from urine samples, 17 (24%) were recovered from blood samples, 12 (15%) were recovered from wound swab samples, and 8 (10%) were recovered from tracheal aspirates. Of the 80 isolates suspected to have carbapenemases, 61 (76.2%) had at least one carbapenemase gene that was identified via genotypic tests. The presence of carbapenemases was detected in 6 out of 10 (60 %) suspected *E. coli* isolates and in 55 out of 70 (78.5%) *K. pneumoniae* isolates via phenotypic tests. The presence of carbapenemases detected in phenotypic tests was confirmed by the multiplex PCR analysis. It was observed that phenotypic tests were 100% compatible with molecular methods to detect the presence of carbapenemases. Of the isolates with carbapenemases, 45 (73.7%) were found to have the OXA-48 enzyme. The second most common enzyme was NDM. The distribution of identified carbapenemases by species is presented in Table 2.

Table 2. Distribution of detected carbapenemase enzymes by species

Carbapenemase gene	*K.pneumoniae*	*E. coli*	Total
Gene undetectable	15	4	19
OXA-48	40	5	45
VIM	4	_	4
NDM	6	_	6
KPC	_	_	_
OXA-48 + VIM	3	1	4
OXA-48 + NDM	2	_	2
Total	70	10	80

MIC values of imipenem, meropenem, and ertapenem were tested in the isolates via the microdilution method. All isolates (100%) were found to be resistant to ertapenem. The assessment of the efficacy of the β-lactam/β-lactamase inhibitor combinations against CRE isolates revealed that the activity of CAZ-AVB (77%) was higher than CLZ-TAZ (48%). The sensitivity rates to CAZ-AVB were 76% (0.094-256 μg/ml) and 78% (0.094-16 μg / ml) for *K. pneumoniae* and *E. coli*, respectively. Sensitivity rates to CLZ-TAZ were 52% (1-256 μg / ml) for *K. pneumoniae* and 44% (0.38-16 μg / ml) for *E. coli*.

DISCUSSION

Enterobacteriaceae are common pathogens causing a variety of severe infections. Carbapenemase-producing *Enterobacteriaceae* strains cause infections that are treated with combined antibiotherapy regimens but the mortality is high (2). Many carbapenem hydrolyzing enzymes have been identified in gram-negative bacilli. The most common ones that are responsible for resistance are Ambler class A (KPC type), class B (VIM, NDM, and IMP types), and class D (OXA-48-like) enzymes (4).

Of a total of 80 carbapenemase-producing *Enterobacteriaceae* species included in our study, 52 (73.2%) had at least one carbapenemase gene identified through genotypic methods. The most common species was *Klebsiella* spp. having carbapenemase genes at a rate of 87.3%. The results of studies in the literature are compatible with our study results. Those studies reported carbapenemase production most commonly in *K. pneumoniae* followed by *E. coli* (4,5,10,11). Çaycı et al. reported that carbapenem-resistant Klebsiella spp. and *E. coli* were found at rates of 71.43% and

1.54% in their study, respectively (4). A multi-center study conducted in Turkey reported rates of carbapenem-resistant *Klebsiella* spp. and *E. coli* as 86.5% and 13.5%, respectively (5). Similar to other studies, the rates of carbapenem-resistant *Klebsiella* spp. and *E. coli* were 87.3% and 12.6% in our study, respectively.

When we evaluated the distribution of the clinical samples, we observed that carbapenemase-producing *Enterobacteriaceae* strains were found in urine, blood, wound swab, and tracheal aspirate cultures in the decreasing order of frequency at rates of 51%, 24%, 15%, and 10%, respectively. Similar to our study results, other studies reported that urine and blood samples were the leading sample types with the highest frequencies (4,12-14).

OXA-48 has remained to be an enzyme locally found only in our country, however, isolates with OXA-48 have started to cause outbreaks in many countries with increasing prevalence rates. The spread of bacteria with acquired carbapenemases constitutes a major public health issue. Therefore, the identification of such isolates is critical to control infections (5,12). In our study, the blaOXA-48 gene was found in 73.7% of CRE in total. The blaOXA-48 gene was found in 88.8% of *K. pneumoniae* isolates and 11.1% of *E. coli* isolates. In a study, Çelikbilek et al. found out that more than 90% of carbapenem-resistant *K. pneumoniae* isolates were blaOXA-48 positive (13). A multicenter study that included patients from various regions of Turkey reported a high prevalence rate (83%) for the presence of the blaOXA-48 gene in CRE. The blaOXA-48 gene was found in 85.1% of *K. pneumoniae* isolates and 14.8% of *E. coli* isolates (4). In the study conducted by Üsküdar et al., of the 130 samples, 121 (78%) were positive for the blaOXA-48 gene. The blaOXA-48 gene was found in 103 *K. pneumoniae* samples and in 18 *E. coli* samples in that study (14). Another study reported that, out of 181 clinical samples, 88 (%47.5) were positive for the blaOXA-48 gene with rates of 38.1% and 7.1% for *K. pneumoniae* and *E. coli*, respectively (12).

In our study, 6 isolates were positive for the blaNDM-1gene, 4 isolates were positive for the blaVIM gene, and 3 isolates were positive for the blaNDM1 and blaOXA-48 genes concomitantly. A multicenter study reported that 9 (6.3%) isolates were positive for the blaNDM-1gene, 4 (2.8%) isolates were positive for the blaVIM gene, and 3 (2.1%) isolates were positive for the blaNDM1 and blaOXA-48 genes concomitantly (4). A study by Irmak et al. reported that, of the CRE isolates, 6 (3.3%) were positive for the blaNDM-1 gene, 1 (1.45%) isolate was positive for the blaVIM gene, and 3 (1.6%) blaNDM-1 positive isolates had the blaOXA-48 gene concomitantly (12). Another study reported the presence of blaNDM-1in 6 (6.5%) isolates but the concomitant presence of the blaNDM1 and blaOXA-48 genes was not detected in any of the isolates (13). A study by Üsküdar et al. reported that 9 (7.1%) isolates were positive for the blaNDM-1gene but no isolates had the blaVIM resistance genes. No isolates had the blaNDM1 and blaOXA-48 genes concomitantly (14).

Carbapenems are considered the last resort in the treatment of infections caused by MDR-gram-negative bacteria (3,4). The emergence of carbapenem-resistant pathogens in parallel to the increasing use of carbapenems in clinical practice poses a major threat to human health. Therefore, antibiotic resistance of such bacteria is associated with critical clinical and socioeconomic effects (3,15). Because of the limited availability of therapeutic options, colistin, administered alone or in combination, has become the main antimicrobial agent for the treatment of such infections (15). However, previous studies have reported high rates of treatment failure in association with colistin therapy (16). New combinations of β-lactams, including CAZ–AVB and CLZ–TAZ, have strong activity *in vitro* against CPE and have the potential to replace colistin (15,16).

Viala et al. found the sensitivity rates of OXA-48-beta-lactamase-producing *Enterobacteriaceae* as 26/27 (96%) to CAZ–AVB and 8/27 (30%) to CLZ–TAZ. The study reported that CAZ–AVB was more effective than CLZ–TAZ in OXA-48-beta-lactamase-producing Enterobacteriaceae infections. Viala et al. demonstrated the benefits of CAZ–AVB in the empirical treatment of suspected bacterial infections (17). In another study, CAZ–AVB (MIC 50/90, 1/2 mg/L) exhibited 97.5% activity while CLZ–TAZ (MIC50/90, 0.25/2 mg/L) showed 86.9% activity against CRE according to EUCAST criteria (18).

Alatoom et al. reported 45% sensitivity to CAZ–AVB and 10% to CLZ–TAZ in their study, in which 60 CRE isolates (49 *K. pneumoniae* and 11 *E. coli*) were tested (19). Yin et al., in their study on 372 CRE isolates, found that CAZ-AVB (75%) had better activity than CLZ -TAZ (6.2%) when efficacy against all CRE isolates was evaluated. On the other hand, CAZ-AVB had much higher antibacterial activity against carbapenem-resistant *K. pneumoniae* (85%) than that against carbapenem-resistant *E. coli* (28.6%). While 28.6% of *E. coli* isolates and 85% of *K. pneumoniae* isolates were susceptible to CAZ-AVB, only 7.1% and 1.9% of them were susceptible to CLZ-TAZ, respectively (20). Shortridge et al. found that, against *Enterobacteriaceae*, CLZ-TAZ showed favorable activity with 87.5% sensitivity of non-CRE phenotype strains but lacked activity against CRE with 2.4% sensitivity (21). Zhang et al found that CAZ -AVB showed high antibacterial activity with 96.3% sensitivity in their study on a total of 872 carbapenemase-positive *Klebsiella* isolates (22). In our study, the sensitivity rate of the CAZ-AVB combination for carbapenem-resistant

Enterobacteriaceae strains isolated from various clinical samples was higher than that of CLZ-TAZ. CAZ-AVB sensitivity rates were 76% (0.094-256 μg /ml) for *K. pneumoniae* and 78% (0.094-16 μg / ml) for *E. coli*. CLZ-TAZ sensitivity rates were 52% (1-256 μg / ml) for *K. pneumoniae* and 44% (0.38-16 μg / ml) for *E. coli*.

CONCLUSION

The development of novel agents for the treatment of highly resistant gram-negative pathogens is critical for the availability of therapeutic options. The sensitivity rates with ceftazidime-avibactam were higher than those of ceftolozane-tazobactam. It has been found that ceftazidime-avibactam exhibits better activity against other carbapenem-resistant isolates, except those carrying the NDM-1 enzyme. The data obtained in this study will soon be used in our country to guide the clinical use of these agents. Our findings suggest that CAZ-AVB and CLZ-TAZ may be promising for the treatment of infections caused by CRE strains.

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