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Review of *In Vitro* Efficacy of Ceftazidime-Avibactam, Meropenem-Vaborbactam and Imipenem-Relebactam in Carbapenemase Producing *Klebsiella pneumoniae* Isolates

Karbapenemaz Üreten *Klebsiella pneumoniae* İzolatlarında Seftazidim-Avibaktam, Meropenem-Vaborbaktam ve İmipenem-Relebaktam *İn Vitro* Etkinliğinin Gözden Geçirilmesi

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Abstract

Introduction: To investigate the *in vitro* efficacy of three new antimicrobial agents [ceftazidime-avibactam (CAZ-AVI), meropenem-vaborbactam (V/R) and imipenem/cilastatin-relebactam] in isolates of carbapenemase-producing *Klebsiella pneumoniae* (CPKP).
Materials and Methods: Thirty CPKP strains were included in our study. The strains were identified at the species level using Vitek MS, and antimicrobial susceptibility tests were performed using the Vitek 2 automated system. Carbapenemase production in all strains was confirmed by the combination disc test. The blaKPC, blaNDM-1, blaOXA-48 and blaVIM genes were identified using the Gene-Xpert® System Carba R® kit (Cepheid, Sunnyvale, USA). Imipenem/relebactam (I/R), V/R, CAZ-AVI susceptibilities were investigated by using gradient strip test. Antimicrobial susceptibility results were interpreted according to the recommendations the EUCAST. Minimal inhibitory concentration (MIC) cut-off values were as follows: For IR, MIC >2 susceptible, MIC ≤2 resistant; for M/V, MIC >8 resistant, MIC ≤8 susceptible; and for CAZ-AVI, MIC >8 resistant, MIC ≤8 susceptible.
Results: All of the strains included in the study were resistant to all antibiotics except colistin. BlaOXA-48 was detected in 76.6% of the isolates; NDM-1 in 16.6%; NDM-1 and OXA-48 in 3.3%, and KPC in 3.3%. Twenty (66.6%) strains were susceptible to I/R, 18 (60%) were susceptible to M/V, and 21 (70%) were susceptible to CAZ/AVI. While 90% of the I/R susceptible isolates carried OXA-48, the other two isolates had KPC and NDM-1. Of the I/R resistant isolates 50% carried OXA-48, 40% NDM-1, and 1 NDM-1 and OXA-48. Of the M/V susceptible isolates, 88.8% carried OXA-48, other isolates NDM-1 and KPC. Of the resistant isolates, 58.3% carried OXA-48, 33.3% NDM-1, and 8.1% NDM-1/OXA-48. Of the isolates susceptible to CAZ/AVI 95.2% carried OXA-48 and 4.7% NDM-1. Of the I/R resistant isolates with a MIC >8, two carried the blaOXA-48 gene and two carried the NDM-1 gene. NDM-1 gene was present in four of the M/V resistant isolates with MIC >8. It was determined that 56.6% of the strains were susceptible to all antimicrobial agents and 23.3% were found to be resistant to all agents. While 4 (57.1%) of these strains carried NDM gene, blaOXA-48 gene was detected in 3 (42.8%) strains.
Conclusion: The most common gene detected in CPKP strains inour center was found to be OXA-48 gene. These results were similar to the data for Turkey. This situation should be considered during the clinical use of I/R and M/V, which are known to have low susceptibility in OXA-48 carrying strains in our country. Our study showed that CAZ/AVI susceptibility was higher in KPC strains.
Keywords: Ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, OXA-48

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Öz

Giriş: Karbapenemaz üreten *Klebsiella pneumoniae* (CPKP) izolatlarında üç yeni antimikrobiyal ajanın [seftazidim-avibaktam (CAZ/AVİ), meropenem-vaborbaktam (V/R) ve imipenem/silastatin-relebaktam] *in vitro* etkinliğini araştırmaktır.

Gereç ve Yöntem: Çalışmamıza 30 CPKP suşu dahil edildi. Suşların tür düzeyinde tanımlaması Vitek MS ile, antimikrobiyal duyarlılık testleri ise Vitek 2 otomatize sistemi kullanılarak yapıldı. Tüm suşlarda karbapenemaz üretimi kombinasyon disk testi ile teyit edildi. BlaKPC, blaNDM-1, blaOXA-48 ve blaVIM genleri, Gene-Xpert® System Carba R® kiti (Cepheid, Sunnyvale, ABD) kullanılarak belirlendi. İmipenem/relebaktam (I/R), V/R, CAZ/AVİ duyarlılıkları gradient strip test ile araştırıldı. Antimikrobiyal duyarlılık sonuçları, EUCAST 2022 kriterleri kullanılarak belirlendi. İmipenem-relabaktam (I/R) için minimal inhibitör konsantrasyon (MİK) değeri >2 mg/l duyarlı, ≤2 mg/l dirençli; meropenem-vaborbaktam (M/V) için MİK değeri >8 mg/l dirençli, ≤8 mg/l duyarlı; CAZ/AVİ için MİK değeri >8 mg/l dirençli, ≤8 mg/l duyarlı olarak değerlendirildi.

Bulgular: Çalışmaya dahil edilen suşların tümü kolistin hariç tüm antibiyotiklere dirençli idi. İzolatların %76,6'sında blaOXA-48; %16,6'sında NDM-1; %3,3'ünde NDM-1 ve OXA-48, %3,3'ünde KPC tespit edildi. Suşların 20'sinde (%66,6) I/R duyarlı, 18 suşta (%60) M/V duyarlı, 21 suşta (%70) CAZ/AVİ duyarlı bulundu. I/R duyarlı izolatların %90'ı OXA-48 taşırken diğer iki izolatta KPC ve NDM-1 mevcuttu. I/R dirençli izolatların %50'si OXA-48, %40'ı NDM-1 taşırken, bir izolatta ise NDM-1 ve OXA-48 mevcuttu. M/V duyarlı izolatların %88,8'inde OXA-48 mevcutken, diğer izolatlarda NDM-1 ve KPC mevcuttu. Dirençli izolatların %58,3'ünde OXA-48, %33,3'ünde NDM-1, %8,1'inde NDM-1/OXA-48 mevcuttu. CAZ/AVİ duyarlı izolatların %95,2'sinde OXA-48, %4,7'sinde NDM-1 mevcuttu. MİK değeri >8 olan I/R dirençli izolatların ikisinde blaOXA-48, ikisinde ise NDM-1 geni mevcuttu. MİK değeri >8 olan M/V dirençli izolatların dördünde NDM-1 geni mevcuttu. Suşların %56,6'sı antimikrobiyal ajanların tümüne duyarlı, %23,3'ü ise tüm ajanlara dirençli olarak saptandı. Bu suşların dördü (%57,1) NDM genine sahipken üçünde (%42,8) blaOXA-48 geni tespit edildi.

Sonuç: Merkezimizde saptadığımız CPKP suşlarının en sık OXA-48 geni taşıdığı görüldü. Bu sonuçlar Türkiye verileri ile benzerdi. Bu durumun OXA-48 suşlarında duyarlılığının düşük olduğu bilinen I/R ve M/V'nin ülkemizde klinik kullanımı sırasında göz önünde bulundurulması gereklidir. Çalışmamız KPC suşlarında CAZ/AVİ duyarlılığının daha yüksek olduğunu gösterdi.

Anahtar Kelimeler: Seftazidim-avibaktam, meropenem-vaborbaktam, imipenem-relebaktam, OXA-48

Introduction

Carbapenems are bactericidal β-lactam antibiotics recommended for the treatment of infections caused by extended-spectrum β-lactamase (ESBL) and/or AmpC-cephalosporinase-producing *Enterobacterales*[1]. However, infections caused by carbapenemase-producing *Enterobacterales* (CPE) isolates have become an important problem worldwide, including Turkey[2,3]. Mortality rates in these infections vary between 20-50%. Although *Klebsiella pneumoniae* has been accepted as an important pathogen causing a series of infections, including sepsis, pneumonia and urinary tract infections for many years; high healthcare costs, treatment failures and high mortality rates in these infections have been increasingly reported in recent years, especially due to increasing carbapenem resistance rates[4]. Two clinically important mechanisms of resistance to carbapenems have been identified among *Enterobacterales.* The first is the production of carbapenemases such as serine carbapenemases (KPC and OXA) and metallo-β-lactamases (VIM, IMP and NDM), while the other is the production of Ambler class C β-lactamases with porin loss of function[1]. Among the carbapenemase enzymes, especially KPC, NDM and OXA-48 are globally spread.

The limitations of treatments to be used in carbapenem-resistant isolates have led to new searches. Recently, new β-lactam/β-lactamase inhibitor (BLBLI) combinations such as ceftazidime-avibactam (CAZ/AVI), imipenem-relabactam (I/R), and meropenem-vaborbactam (M/V) appear to be promising alternatives to existing agents for the treatment of severe infections caused by carbapenemase-producing *K. pneumoniae* (CPKP) strains[5,6].

Avibactam, a new β-lactamase inhibitor, is a diazabicyclooctane non-β-lactam inhibitor with activity against class A (KPC), class C (AmpC) and certain class D (OXA-48) carbapenemases[7]. However, it is not active against class B metallo-β-lactamases (MBL) such as VIM, IMP and NDM. Avibactam restores the activity of ceftazidime against CPKP strains. It has now been approved by the Food and Drug Administration for complicated intra-abdominal infections, hospital-or ventilator-associated pneumonia (HAP/VAP) and complicated urinary tract infections, including pyelonephritis. However, despite good *in vitro* activity of CAZ/AVI, resistant CPE isolates have been reported during treatment, shortly after its approval[8].

Vaborbactam (formerly RPX7009) is a non-β-lactam, cyclic, boronic acid-based inhibitor of β-lactamases[9]. Although vaborbactam is not active against Metallo-β-lactamase enzymes, it potentiates the *in vitro* activity of meropenem against KPC, ESBL and AmpC producing isolates[10]. M/V is approved for clinical use in Europe in complicated urinary tract infection, complicated intra-abdominal infections, HAP, VAP and associated bacteremia[4]. Although the *in vitro* activity of the drug against carbapenem-resistant *Enterobacterales* isolates has been evaluated by various studies worldwide, new studies are still needed[11,12].

Relebactam is a bicyclic diazabicyclooctane β-lactamase inhibitor structurally related to avibactam, differing by the addition of a piperidine ring to the 2-position carbonyl group. Similar to avibactam, relabactam shows activity against KPC-type carbapenemases, class C β-lactamases (AmpC) and class A β-lactamases, but unlike avibactam, relebactam has been reported to have insufficient effect on OXA-48 carbapenemase-producing isolates[13]. Relebactam was combined with imipenem cilastatin to restore the clinical activity of imipenem against KPC-producing *K. pneumoniae*, other KPC-producing *Enterobacterales*, and *Pseudomonas aeruginosa*, which exhibits carbapenem resistance due to impermeability with AmpC expression[14]. While these novel combinations are effective against KPC isolates producing KPC and other class A carbapenemases, they can also inhibit ESBLs and constitutive or plasmid-derived cephalosporinases. However, it is thought that these BLBLI combinations may be affected by non-enzymatic resistance mechanisms[5]. Various studies have reported that the I/R combination has equivalent efficacy to CAZ/AVI and ceftolozane-tazobactam in multidrug-resistant (MDR) *P. aeruginosa* and *Enterobacterales* isolates[15].

The aim of this study was to investigate the *in vitro* susceptibility of I/R, M/V, CAZ/AVI in CPKP strains.

Materials and Methods

Thirty CPKP strains in the laboratory culture collection were included in our study. Species-level identification of strains was confirmed by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using Vitek MS (BioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions. The sensitivities of amikacin, gentamicin, ciprofloxacin, levofloxacin, cefepime, ceftriaxone, ceftazidime, piperacillin-tazobactam, cefuroxymaksetil, amoxicillin-clavunate, imipenem, meropenem and trimetprim-sulfamethoxazole were studied with the automated system Vitek 2 (BioMérieux, France). Among the *K. pneumoniae* isolates included in the study, strains that were resistant to three or more antimicrobial agents, including pipracillin-tazobactam, cefepime, meropenem, ciprofloxacin, and aminoglycosides, were accepted as MDR[16].

Imipenem-relabactam, M/V, CAZ-AVI sensitivities were investigated by using gradient strip test (BioMérieux). Colistin sensitivity was investigated by liquid microdilution method in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Antimicrobial susceptibility results were interpreted according to the recommendations the EUCAST[17]. Minimal inhibitory concentration (MIC) cut-off values were as follows: For IR, MIC >2 susceptible, MIC ≤2 resistant; for M/V, MIC >8 resistant, MIC ≤8 susceptible; and for CAZ-AVI, MIC >8 resistant, MIC ≤8 susceptible.

Modified Carbapenemase Inactivation Method (mCIM)

A suspension was prepared with the isolate where carbapenemase enzyme production was to be investigated in the sterile distilled water and 10 µl meropenem disc (BioMérieux, France) was put into it. After two hours of incubation, meropenem disc in suspension was placed on Mueller-Hinton (Merck, USA) agar medium inoculated with *E. coli* ATCC 29522 and incubated at 35 °C for six hours. The test result was considered positive if there was growth around the meropenem disc after six hours of incubation[18].

Carbapenemase Nordmann-Poirel (Carba NP) Test

The Rapidec® Carba NP (BioMérieux, France) test was performed in accordance with the manufacturer's recommendations. The bacterial colony to be tested was suspended in lysis buffer and incubated for 30 minutes. This bacterial suspension was centrifuged at 10,000 x g for 5 minutes at room temperature. The solution prepared using supernatant, imipenem monohydrate (Sigma, SaintQuentin Fallavier, France), phenol red and 0.1 mmol/l ZnSO4 (Merck Millipore, Guyancourt, France) was mixed in 96-well plates. Phenol red solution and enzymatic suspension were incubated at 37 °C for two hours. Then the test results were interpreted.

Molecular Tests

The blaIMP-1, blaKPC, blaNDM-1, blaOXA-48 and blaVIM gene regions in all *Klebsiella pneumoniae* isolates included in the study were investigated with Gene-Xpert® system Xpert CARBA-R test kits.

Results

K. pneumoniae isolates included in the study were resistant to three or more antimicrobial agents, including piperacillin-tazobactam, cefepime, meropenem, ciprofloxacin and aminoglycosides. Carbapenemase production was also confirmed by Carba NP and CIM methods and was positive in both tests.

In the analysis with Gene-Xpert of the strains included in the study, blaOXA-48 was detected in 23 (76.6%) strains, NDM-1 in 5 (16.6%), NDM-1 and blaOXA-48 in 1 isolate (3.3%), and KPC in 1 isolate (3.3%).

It was found that 20 strains (66.6%) were susceptible to I/R, 18 strains (60%) were susceptible to M/V, and 21 strains (70%) were susceptible to CAZ/AVI (Table 1).

Eighteen (90%) of 20 I/R susceptible isolates carried blaOXA-48, while the other two isolates had KPC and NDM-1. Of the 10 I/R resistant strains, 5 (50%) had blaOXA-48, four (40%) had NDM-1, and one isolate had NDM-1 and OXA-48 (Table 1).

Of the 18 M/V susceptible isolates, 16 had blaOXA-48, while the other isolates had NDM-1 and KPC. Of the 12 resistant isolates,

Table 1. Gene distributions of all strains according to susceptibility-resistance status

	I/R		M/V		CAZ/AVI	
	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)
OXA-48	5 (50)	18 (90)	7 (58.3)	16 (88.8)	4 (44.4)	19 (90.4)
NDM-1	4 (40)	1 (5)	4 (33.3)	1 (5.5)	4 (44.4)	1 (4.7)
KPC	-	1 (5)	-	1 (5.5)	-	1 (4.7)
OXA-48+NDM-1	1 (10)	-	1 (8.3)		1 (11.1)	
Total	10	20	12	18	9	21

CAZ-AVI: Ceftazidime-avibactam

Table 2. Ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam MIC values of all strains

	<0.5 n (%)	0.5-2 n (%)	2-4 n (%)	4-8 n (%)	>8 n (%)
CAZ/AVI	4 (13.3)	11(36.6)	5 (16.6)	1 (3.3)	9 (30)
I/R	8 (26.6)	12 (40)	6 (20)	0	4 (13.3)
M/V	5 (16.6)	2 (6.6)	9 (30)	2 (6.6)	12 (40)

CAZ-AVI: Ceftazidime-avibactam, MIC: Minimal inhibitory concentration

seven had blaOXA-48, four had NDM-1, and one had NDM-1 and blaOXA-48 (Table 1).

Of the 21 CAZ/AVI susceptible strains, 20 had blaOXA-48, while one strain had NDM-1 (Table 1).

All 17 (56.6%) strains were susceptible to antimicrobial agents. BlaOXA-48 was detected in 15 (70.5%) of these strains, KPC in one (5.8%) and NDM in one (5.8%).

Seven strains were found to be resistant to all agents. While 4 (57.1%) of these strains had NDM gene, blaOXA-48 gene was detected in three (42.8%) strains. When the MIC values of the isolates were examined, MIC >16 in all of the resistant strains carrying NDM.

Of the I/R resistant isolates with MIC >8, two had the blaOXA-48 gene and two had the NDM-1 gene. The NDM-1 gene was present in four of the M/V resistant isolates with MIC >8 (Tables 1, 2).

Discussion

Carbapenemase-producing *K. pneumoniae* isolates are generally resistant to multiple drugs alongwith carbapenem. Therefore, major problems are experienced in the treatment of CPKP infections in clinical practice, and these infections generally progress with high mortality. Studies have shown that in patients with CPKP infection, in the event of treatment delay, nearly 50% of the patients die within 30 days. It has also been proven by studies that the application of combination therapy in these patients increases the survival rates by two or three times. In clinical practice, combination options of carbapenem antibiotics with other antibacterial agents such as tigecycline, polymyxin, amikacin or fosfomycin are generally used, and better results are obtained than carbapenem treatment alone[18]. However, side effects may be encountered with these combination options[19]. In recent years, new combinations have been developed by adding a β-lactamase inhibitor to an existing β-lactam agent. New generation inhibitors such as avibactam, vaborbactam, relebactam, which are preferred in newly developed combinations, are generally active against acquired and intrinsic β-lactamases, which have limited or no hydrolytic activity against carbapenems. Between 2016 and 2018, CAZ/AVI, I/R, M/V susceptibility rates were reported as 100%, 93%, and 93.3%, respectively, in 45 CPE strains, and susceptibility rates of 100%, 90%, and 80%, respectively, were reported in *K. pneumoniae* isolates[19]. These high sensitivity rates obtained in *in vitro* susceptibility studies have increased the interest in these combinations. However, it should be noted that these inhibitors do not protect the β-lactam agent against other resistance mechanisms[20,21]. The efficacy of BLBLI combinations developed for use in such infections varies according to the resistance mechanisms of the isolate and the enzymes it carries. Studies have proven that especially metallobelactamase producing isolates are resistant to these combinations. Maraki et al.[22] found resistance to I/R and CAZ/AVI in all strains, while M/V resistance was found in 95% of the strains in their study on 40 isolates producing metallobetalactamase. Therefore, it is of great importance to determine the enzymes carried by these carbapenem-resistant strains and to understand the resistance mechanisms in terms of treatment effectiveness[22]. The genes carried by CPKP strains show differences throughout the world. For example, while OXA-48 is more common in Turkey, Belgium, France, Romania and Spain, Germany and Italy, KPC is more

common in the United States[23]. For this reason, it is of great importance for clinicians to know the frequency of resistance genes in their region while these combinations are preferred.

Although M/V is not active against isolates carrying MBL, it has limited activity against isolates carrying genes encoding oxacilinases with carbapenemase activity, such as OXA-48. *In vitro* studies have shown that the combination of vaborbactam restores meropenem activity against CPKP isolates[24,25]. While M/V susceptibility in *K. pneumoniae* strains producing KPC varies between 87-100% worldwide, these rates vary between 0-95.1% in OXA-48 producing strains[6,12,21,25-27]. Differences in rates vary with whether the bacteria carry more than one resistance mechanism[6,19,26]. Hackel et al.[12] found in their multicenter study across Europe that the M/V and CAZ/AVI susceptibility rates were very close to each other (98.9% / 98.2%) in 878 KPC-producing *K. pneumoniae* strains and observed that the two combinations could be an alternative to each other in these strains. Bonnin et al.[27] found susceptibility rates of 81.3% for I/R combination and 95.1% for M/V combination in *Enterobacterales* carrying the blaOXA-48 gene, while the susceptibility rate of CAZ/AVI was found to be 99.5% in these isolates, and they reported that CAZ/AVI continued to be the best treatment option. In our study, we found M/V susceptibility in 66.6% of OXA 48-producing strains. However, we could not make an evaluation because there was only one KPC strain in our study. In clinical studies as well as in *in vitro* studies, it was observed that the combination of vaborbactam contributed to the reduction in mortality rates[28]. Although *in vitro* studies show that KPC producers have a low ability to acquire resistance against the M/V combination, M/V resistant strains have been reported in clinical practice in recent years. In a study conducted in Italy, it was reported that M/V resistance rate was 13% and it was observed that this resistance was also responsible for CAZ/AVI cross-resistance[4]. Gaibani et al.[4] found in their study that this resistance was caused by loss-of-function mutations in ompK35 and ompK36 porins.

Imipenem-relabactam susceptibility also varies according to the type of carbapenemase carried by the bacteria. While I/R susceptibility rate is 78.1% in CPKP strains, this rate decreases to 66.7% in those carrying the blaOXA-48 gene. I/R susceptibility rates in blaOXA-48 producing strains range from 0 to 66.7%, while these rates reach 100% in KPC positive isolates[13,20,29]. I/R resistance rates of *K. pneumoniae* isolates producing OXA-48 may differ between regions. The authors attributed the different resistance rates they found in a joint study from Spain and Portugal to the high-risk clones of OXA-48-ST11 and OXA-48-ST147 strains in Spain[30]. High-risk clones of blaOXA-48-producing *K. pneumoniae*, commonly found in hospital settings in Spain, contribute to the spread of I/R resistance among MDR *Enterobacterales* isolates in patients in intensive care units. Galani et al.[13] investigated the susceptibility of I/R and CAZ/AVI in 314 *K. pneumoniae* strains producing carbapenemase in their study between 2015-2016 and reported it to be 98% and 99.6%, respectively, among all isolates. However, I/R susceptibility rates decreased to 10% in OXA 48-carrying strains included in the study, while 80% was found in KPC-positive isolates. In 470 carbapenem resistant *K. pneumoniae* strains obtained from culture collections of 16 different hospitals in Taiwan, I/R combination was shown to have the highest efficacy (88.4%) in strains carrying the KPC enzyme. While this rate was found to be 82.4% in isolates carrying OXA-48 enzyme, no isolates carrying NDM could be detected in this study. However, it was observed that the susceptibility rates decreased to 42.9% in strains carrying the IMP enzyme in the same group as NDM in the Ambler classification[31].

Although other carbapenemases have been reported in Turkey, *K. pneumoniae* strains producing blaOXA-48 are considered endemic[32]. In this study, we found that I/R susceptibility in blaOXA-48 producing strains was higher (78.2%) than in other centers.

New drug combinations containing I/R have recently been introduced as promising combinations against *K. pneumoniae* isolates producing KPC in patients in intensive care units with complicated infections with little or no other treatment options. However, there have been reports of I/R resistance occurring *in vivo* in *K. pneumoniae*, which produces KPCs very rapidly[33]. Findlay ve ark.[33] observed that mutations that caused I/R resistance also caused an increase in CAZ/AVI and M/V MIC values.

Studies show that CAZ/AVI has excellent *in vitro* activity against ESBL-, AmpC, KPC, and OXA-48-producing *Enterobacterales*[34]. CAZ/AVI susceptibility rates range from 92-100% in strains producing BlaOXA-48 and KPC[21,32]. If the strains producing BlaOXA-48 have a different resistance mechanism or a different carbapenemase gene, resistance rates increase[11,22]. While CAZ/AVI susceptibility rate was found to be 92.5% in OXA-48-carrying strains collected from 20 countries between 2012 and 2015, this rate decreased to 21% in those with OXA-48 and MBL coexistence[22]. Jayol et al.[35] found that CAZ/AVI susceptibility rate was 100% in all *K. pneumoniae* isolates producing KPC or OXA-48 carbapenemase, while isolates producing NDM alone or in association with another carbapenemase showed a high level of resistance to CAZ/AVI. Although NDM producing isolates showed high resistance to CAZ/AVI, CAZ/AVI resistance was found in 80% of NDM gene producing strains in our study. In a study examining the *in vitro* activities of CAZ/AVI in MDR *Klebsiella pneumoniae* isolates, CAZ/AVI resistance was found at a rate of 27%. All strains with the NDM-1, NDM-1+OXA-48, OXA-48 and KPC genes detected were susceptible to CAZ/AVI[36]. In our study, the susceptibility of CAZ/AVI was lower than in other studies. These results show that we should be more careful about the

increasing resistance to CAZ/AVI over the years. In comparative clinical efficacy studies, the CAZ/AVI combination was compared with combinations containing colistin and aminoglycosides, and a statistically significant decrease in clinical success and long-term survival was observed. In these studies, renal complications were found to be significantly lower with the combination of CAZ/AVI[28]. However, in recent years, the increase in CAZ/AVI resistance rates has been striking. While CAZ/AVI resistance is <0.6% in *Enterobacterelas*, this rate increases to only 16.7-21% in carbapenemase-producing isolates[37]. In our study conducted in 2018, we found the susceptibility rate of CAZ/AVI to be 72.7% in such strains, while we found the susceptibility rate to be 70% in this study[38].

Study Limitations

Only 30 carbapenem resistant *K. pneumoniae* strains in the laboratory culture collection were included in our study. This relatively small number of strains was a limitation for our study. The number of new combination antibiotics of which susceptibility rate we studied in CPKP isolates could have been higher. In addition, the fact that we did not have the opportunity to study clonal relationships was a limitation of our research. We are planning follow-up studies where these limitations will be resolved.

Conclusion

In conclusion, it should be kept in mind that none of these new combination agents, which have been marketed as promising for resistant *K. pneumoniae* infections, will show activity against all carbapenemase producing strains. Although there are studies reporting the clinical efficacy of these new agents, data are still lacking. In addition, it is an important point to note that resistance rates have started to increase in recent years, especially in CAZ/AVI.

Ethics

Ethics Committee Approval and Informed Consent: Anonymous strains were not used because they were used.

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Authorship Contributions

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