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In Vitro Activity of Ceftolozane/tazobactam and Ceftazidime/avibactam Against Carbapenemase-producing *Pseudomonas aeruginosa*

Karbapenemaz Üreten *Pseudomonas aeruginosa* Kökenlerine Karşı Seftolozan/tazobaktam ve Seftazidim/avibaktam İn Vitro Etkinliğinin Araştırılması

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Abstract

Introduction: The emergence of multidrug-resistant (MDR) and extensively drug-resistant strains of *Pseudomonas aeruginosa* in recent years has become a major issue due to treatment difficulties as well as high morbidity and mortality rates. Treatment options for infections caused by these microorganisms are very limited. Ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (CZA) are recently developed cephalosporin/beta-lactamase inhibitor combinations for the treatment of infections caused by MDR *P. aeruginosa* strains. The aim of this study was to investigate the *in vitro* efficacy of C/T and CZA against MDR *P. aeruginosa* strains and to compare the *in vitro* efficacy of these two drugs.

Materials and Methods: Thirty-two MDR *P. aeruginosa* isolates were included in the study. Identification and antimicrobial susceptibilities of the strains were performed using a VITEK 2® automated system. The efficacy of CZA and C/T was determined by the gradient strip test (Liofilchem MIC strip test, Italy). Modified carbapenemase inactivation method was used to detect carbapenemase production in all strains.

Results: Rates of antibiotic resistance in the isolates were 78% for amikacin, 96.8% for levofloxacin, 90.6% for ciprofloxacin, 71.8% for gentamicin, and 78% for netilmicin. Ceftazidime/avibactam resistance was detected in 7 (21.8%) of the isolates and C/T resistance in 10 (31.2%). All strains with resistance to CZA also had resistance to C/T. Three strains were resistant to C/T but susceptible to CZA. Carbapenemase production was positive in all strains.

Conclusion: The results of this study indicate that CZA and C/T may be an alternative treatment for some of the carbapenem-resistant *P. aeruginosa* infections. Further *in vitro* and *in vivo* studies are needed to evaluate the effectiveness of these new treatment options against the increasing threat of MDR *P. aeruginosa*.

Keywords: Colistin, epidemiology, hospital-acquired infections, salvage therapy, extended-spectrum beta-lactamases

Öz

Giriş: Son yıllarda çok ilaca dirençli (ÇİD) ve yaygın ilaca dirençli (YİD) *Pseudomonas aeruginosa* suşlarının ortaya çıkması; tedavi zorluğu, yüksek morbidite ve mortalite oranları nedeni ile ciddi bir problem haline gelmiştir. Bu karbapeneme dirençli mikroorganizmaların etken olduğu enfeksiyonlarda tedavi seçenekleri oldukça kısıtlıdır. Seftolozan/tazobaktam (C/T) ve seftazidim/avibaktam (CZA) ÇİD *P. aeruginosa* suşlarının neden olduğu enfeksiyonların tedavisi için yeni geliştirilmiş sefalosporin/beta-laktamaz inhibitörü kombinasyonlarıdır. Bu çalışmada, ÇİD *P. aeruginosa* suşlarına karşı C/T ve CZA'nın *in vitro* duyarlılıklarının araştırılması ve etkililiklerinin karşılaştırılması amaçlanmıştır.

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Gereç ve Yöntem: Çok ilaca dirençli olan 32 *P. aeruginosa* kökeni çalışmaya dahil edildi. Kökenlerin tanımlaması ve antimikrobiyal duyarlılıkları VITEK 2® otomatize sistemi ile yapıldı. Seftolozan/tazobaktam (C/T) ve CZA etkinliği gradiyent strip test ile (Liofilchem MIC strip test, İtalya) tespit edildi. Tüm kökenlerde karbapenemaz üretimi tespiti amacıyla modifiye karbapenemaz inaktivasyon metodu kullanıldı.
Bulgular: İncelemeye alınan kökenlerdeki antibiyotik direnç oranları; amikasin %78, levofloksasin %96,8, siprofloksasin %90,6, gentamisin %71,8 ve netilmisin %78 şeklinde saptandı. İncelenen kökenlerin 7'sinde (%21,8) CZA direnci saptanırken, 10'unda (%31,2) C/T direnci olduğu tespit edildi. Seftazidim/avibaktama dirençli olan tüm kökenlerde C/T'ye de direnç vardı. Üç köken C/T'ye dirençli iken CZA'ya karşı duyarlı idi. Tüm kökenlerde karbapenemaz üretimi saptandı.
Sonuç: Bu çalışmadan elde edilen sonuçlar ÇİD ve karbapenem dirençli *P. aeruginosa* enfeksiyonlarında CZA ve C/T'nin tedavi alternatifi olabileceğini göstermektedir. Giderek artan ÇİD *P. aeruginosa* tehdidine karşı bu yeni tedavi seçeneklerinin etkililiğini değerlendirmek için daha fazla *in vitro* ve *in vivo* çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: Kolistin, epidemiyoloji, hastane kaynaklı enfeksiyonlar, kurtarma tedavisi, genişlemiş spektrumlu beta-laktamazlar

Introduction

Pseudomonas aeruginosa is one of the major causes of nosocomial infections including sepsis, hospital-acquired pneumonia, ventilator-associated pneumonia, skin, and urinary tract infections. The recent emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* strains has become a serious problem due to treatment difficulties and high morbidity and mortality rates. Many of these solates show reduced sensitivity to antipseudomonal drugs, including beta-lactam antibiotics[1]. The alternative treatment options are limited and comprise usage of newly developed antibiotics or combination of certain antibiotics to benefit from their synergistic effect[2].

Ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (CZA) are Food and Drug Administration (FDA)-approved cephalosporin/beta-lactamase inhibitor combinations. They are recently developed for the treatment of infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, including MDR *P. aeruginosa* strains and other Gram-negative bacteria[1,3,4]. The introduction of these new antimicrobial agents was promising for the treatment of drug-resistant infections, and the clinical importance of these antibiotics is steadily increasing[1].

Although the efficacy of CZA and C/T against *P. aeruginosa* isolates has been adequately demonstrated, there has not been enough research investigating their efficacy against MDR *P. aeruginosa* infections or comparing their efficacy[5-7]. Our review of the literature yielded no studies on this subject conducted in Turkey. The aims of the present study were to investigate the *in vitro* efficacy of C/T and CZA against MDR *P. aeruginosa* strains as well as to compare their *in vitro* efficacies to provide guidance for clinicians in the therapeutic use of these drugs, which will soon become available in Turkey.

Materials and Methods

A total of 570 *P. aeruginosa* strains isolated from various clinical samples between January 2015 and September 2018 were analyzed. All clinical samples sent to the laboratory were cultured on sheep blood agar and eosin methylene blue agar. After 24-48 hours of incubation, species-level identification and antibiotic sensitivity analyses of the isolates were performed using a VITEK 2® automated system (Biomerieux, France). The clinical sources of the isolates and patient data were obtained retrospectively from the hospital automated records system. Multidrug-resistant was defined as resistance to at least three of the following: antipseudomonal cephalosporin (cefepime), piperacillin-tazobactam, meropenem (MEM), ciprofloxacin, and aminoglycosides[4]. Strains sensitive to only colistin and/or aminoglycoside were considered to be XDR[8]. The 32 *P. aeruginosa* isolates classified as MDR were included in the study. All included strains were resistant to imipenem, MEM, ertapenem, piperacillin-tazobactam, ceftazidime, and cefepime. Efficacy of C/T and CZA was determined using a gradient strip test (Liofilchem MIC strip test, Italy). Antimicrobial sensitivity results were evaluated according to the Clinical and Laboratory Standards Institute criteria[9]. For CZA, a minimum inhibitory concentration (MIC) ≤8 was considered to be susceptible, and ≥16 was considered resistant. For C/T, MIC ≤4 was regarded as susceptible, 8 as intermediate, and ≥16 as resistant. Intermediate strains were also classified as resistant. For all strains, the modified carbapenemase inactivation method was used to detect carbapenemase production[9]. The study was approved by the Sakarya University Faculty of Medicine of Ethics Committee (Protocol number: 71522473/050.01.04/7).

Results

It was determined that all 32 strains included in the study were isolated from patients in the intensive care unit [17 males (53.2%), 15 females (46.8%)]. The clinical sources of the isolates included eight tracheal aspirate (25%), seven urine (22.1%), six surgical site (18.7%), five blood and catheter (15.6%), three sputum (9.3%), two bronchoalveolar lavage (6%), and one sterile body fluid (3.4%) samples.

Antibiotic resistance rates were 78% for amikacin, 96.8% for levofloxacin, 90.6% for ciprofloxacin, 71.8% for gentamicin, and

78% for netilmicin. The lowest resistance was to colistin, with 3% (Table 1). CZA (MIC=1) and C/T (MIC=0.25) susceptibility were detected in the isolates with colistin (MIC ≥16) resistance. Ceftazidime/avibactam resistance was detected in 7 (21.8%) of the strains and C/T resistance in 10 (31.2%). All strains that were resistant to CZA were also resistant to C/T. Three strains were resistant to C/T but sensitive to CZA. Rates of antibiotic resistance among the strains are presented in Table 1. MIC values for CZA and C/T are given in Table 2. Of the C/T-resistant strains, three (30%) were isolated from tracheal aspirate, three (30%) from urine, two (20%) from wound, and one (10%) from sputum, and one (10%) from catheter samples. Of the CZA-resistant strains, two (28.5%) were isolated from urine, two (28.5%) from surgical site, two (28.5%) from tracheal aspirate, and one from catheter. Carbapenemase activity was detected in all strains.

Table 1. Antibiotic resistance rates of the analyzed strains

Antibiotic	Proportion resistant
Levofloxacin	78%
Ciprofloxacin	96.8%
Gentamicin	71.8%
Netilmicin	78%
Ceftazidime	100%
Piperacillin/tazobactam	100%
Ceftriaxone	100%
Colistin	3%
Ceftazidime/avibactam	21.8%
Ceftolozane/tazobactam	31.2%
Imipenem	100%
Meropenem	100%

Table 2. Minimum inhibitory concentration values for ceftazidime/avibactam and ceftolozane/tazobactam

MIC (mg/l)	CZA, n (%)	C/T, n (%)
<1	4 (12.5%)	15 (46.8%)
1	5 (15.6%)	3 (9.3%)
2	4 (12.5%)	3 (9.3%)
3	2 (6.2%)	1 (3.1%)
4	5 (15.6%)	-
6	5 (15.6%)	-
16	2 (6.2%)	1 (3.1%)
64	2 (6.2%)	3 (9.3%)
256	3 (9.3%)	6 (18.7%)

MIC: Minimum inhibitory concentration, CZA: Ceftazidime/avibactam, C/T: Ceftolozane/tazobactam

Discussion

The limited treatment options for resistant *P. aeruginosa* infections are a source of critical clinical problem today[10]. Combination therapies are often used to treat these infections in an effort to find a solution[11]. Treatment regimens for infections caused by resistant strains usually include colistin, aminoglycosides, and/or fosfomycin. However, these agents have adverse effects and spectrum of activity problems that limit their clinical use[2]. Although *in vitro* data show that colistin seems to be the most effective agent against resistant *P. aeruginosa* strains, its pharmacokinetic properties and nephrotoxicity limit its use in the treatment of these types of infections[12].

The beta-lactam/beta-lactamase inhibitor combinations C/T and CZA both have the potential to overcome most of the beta-lactam resistance mechanisms commonly found in *P. aeruginosa* strains[13]. Both drugs first received FDA approval for the treatment of urinary tract and complicated intra-abdominal infections[2]. Ceftolozane is a new aminothiazolyloximino cephalosporin with a structure similar to ceftazidime. Compared to ceftazidime, ceftolozane is less sensitive to hydrolysis by AmpC and less affected by porin loss. While both tazobactam and avibactam inhibit serine beta-lactamase, tazobactam irreversibly binds to the active site of serine beta-lactamases. In addition, avibactam not only inhibits ESBLs, but also effectively inhibits class A carbapenemases such as AmpC beta-lactamases and *Klebsiella pneumoniae* carbapenemase (KPC)[7,14,15]. Avibactam has *in vitro* activity against Ambler class A, C, and some class D serine beta-lactamases, but not against metallo-beta-lactamases[1,4]. Studies have revealed that C/T and CZA are more effective against MDR infections than other cephalosporins and beta-lactamase inhibitors[11,14].

In our study, resistance rates against CZA and C/T in MDR and XDR *P. aeruginosa* strains were 21.8% and 31.2%, respectively, and these agents were the most effective after colistin among the antibiotics studied (Table 1). In *P. aeruginosa* isolates, susceptibility to C/T ranges between 86-97.5% while this rate is 60-80% in carbapenem- and ceftazidime-resistant isolates[10,14,16,17]. Similarly, susceptibility to CZA is 84-97% among all *P. aeruginosa* isolates[16,18-20]. CZA and C/T susceptibility rates can vary depending on resistance mechanisms, which change according to region and time periods[13,20]. Taking this into consideration when making treatment decisions will impact treatment outcomes[13].

The *in vitro* efficacy of C/T and CZA against carbapenem-resistant *P. aeruginosa* isolates depends on the type of dominant carbapenemase, which varies globally[13]. In a multicenter study performed in Spain, the rate of susceptibility to C/T was

approximately 70% in carbapenemase-producing strains, and their resistance levels were correlated with carbapenemase production[8]. Similarly, Evans et al.[13] reported 100% susceptibility to CZA and C/T in carbapenem-susceptible strains. However, in the same study, it was found that CZA and C/T sensitivity was 0% in carbapenem-resistant strains producing VIM and 50% in strains producing KPC, while C/T sensitivity was 73.1% and CZA sensitivity was 77.2% in all strains. In another study, resistance to C/T in strains producing VIM-2 carbapenemase was found to be 55%, and this high resistance rate was attributed to the fact that the antibiotic does not inhibit Ambler class B carbapenemases[2]. Giani et al.[21] detected carbapenemase production in 56.5% of C/T resistant *P. aeruginosa* strains, while 5.1% of all *Pseudomonas* strains produced carbapenemase. Among the carbapenemase-producing strains, the rate of bla_{VIM} production was highest at 66.6%, followed by bla_{IMP} at 25%. Bla_{GES-5} was only detected in four isolates (8.3%). They reported that while all VIM- and IMP-producing strains were resistant to C/T, strains with bla_{GES-5} were susceptible to C/T. They also determined that 9.1% of all isolates were resistant to C/T, but did not mention the proportion of carbapenemase-producing isolates that were C/T-resistant[21]. These studies demonstrate that identifying carbapenemase resistance genes is important for CZA and C/T susceptibility. All of the strains in our study were positive for carbapenemase production. Similar to results reported in the literature, we believe this may be responsible for the high C/T and CZA resistance rates in our study[21-24]. However, the inability to determine carbapenemase type or carbapenemase resistance genes was the major limitation of our study.

Various studies report that susceptibility to C/T in MDR *P. aeruginosa* isolates varies between 57.4% and 88.6% (Table 3)[14,25-27]. In a phase 3 trial by Stone et al.[24], the proportion of CZA sensitivity in MDR *P. aeruginosa* isolates was 66.1% and the authors reported that CZA may be a good alternative to carbapenems. Other than the study of Stone et al.[24] study, our literature search yielded no other studies on this subject. All isolates analyzed in our study were MDR and their susceptibility rates for C/T and CZA were 68.8% and 78.2%, respectively. Therefore, our study will serve as a guide to CZA therapy for MDR *P. aeruginosa* isolates. Our study patients could not be

Table 3. Previous studies on the effectiveness of ceftolozane/tazobactam and ceftazidime/avibactam against *Pseudomonas aeruginosa* strains

References	Year of publication	Characteristics of the *P. aeruginosa* strains	n	C/T susceptibility	CZA susceptibility
Walkty et al.[18]	2013	MEM resistant	401	96.5%	
Farrell et al.[17]	2014	MEM resistant	268	78%	
Sader et al.[16]	2014	MEM and resistant	354	-	87.3%
		CZA resistant	330		82.1%
Sader et al.[29]	2014	MDR	698	57.4%	-
		XDR	538	46.3%	
Tato et al.[32]	2015	MEM resistant	177	85.3%	
Denisuik et al.[33]	2015	Beta-lactam resistant	29		84%
Grupper et al.[6]	2017	MEM resistant	290	91%	81%
Humphries et al.[7]	2017	Beta-lactamase resistant	105	72.5%	61.8%
Buehrle et al.[22]	2016	Carbapenem resistant	38	91%	81%
Gonzalez et al.[23]	2017	Carbapenem resistant	45	87%	82%
Munita et al.[25]	2017	Carbapenem resistant	35	87%	
Escolà-Vergé et al.[26]	2018	XDR	38	79%	
Shortridge et al.[14]	2018	MDR	783	88.6%	
		XDR	348	77.6%	
Wi et al.[10]	2017	Carbapenem resistant	42	95.2%	
Evans et al.[13]	2018	Carbapenem susceptible	83	100%	100%
		VIM (+)	15	0%	0%
		KPC (+)	20	0%	50%
		Carbapenem resistant	79	73.1%	77.1%
Karlowsky et al.[4]	2018	Carbapenem resistant			
Katchanov et al.[2]	2018	VIM (+)		55%	
Stone et al.[24]	2018	XDR	56		66.1%

MEM: Meropenem, MDR: Multidrug-resistant, XDR: Extensively drug-resistant, CZA: Ceftazidime/avibactam, C/T: Ceftolozane/tazobactam

questioned about previous antibiotic use. However, since all patients were being treated in the intensive care unit, a history of antibiotic use was highly probable.

Similar to the current study comparing the efficacy of C/T and CZA against MDR *P. aeruginosa* strains, there are a few other studies in the literature that compare the efficacy of these two drugs[6,7,22,23]. In some of these studies, C/T-resistant *P. aeruginosa* isolates were found to be susceptible to CZA, while most demonstrated that C/T had greater *in vitro* inhibitory activity than CZA (Table 3)[1,7]. In a large study investigating 309 resistant (to ceftazidime, cefepime, meropenem, imipenem and/or piperacillin-tazobactam) *P. aeruginosa* isolates, Humphries et al.[7] reported 52.4% and 27.6% C/T and CZA susceptibility in beta-lactam and carbapenem-resistant strains, respectively. In a study from turkey Aktaş et al.[28] reported 86% CZA susceptibility in *P. aeruginosa* strains producing PER-1 beta-lactamase. In our study, we determined a higher rate of CZA susceptibility among carbapenem-resistant strains. This may be attributable to the strains included in our study being MDR and to probable differences in their carbapenemase genes or their mechanisms of resistance against the two drugs.

Data on the efficacy of C/T in bloodstream and lower respiratory tract infections are limited. However, Farrell et al.[17] reported that C/T had higher *in vitro* efficacy than carbapenems and piperacillin-tazobactam in pneumonia[5,25,26]. There is an ongoing phase 3 study evaluating the efficacy of C/T compared to MEM in the treatment of ventilator-associated pneumonia and hospital-acquired pneumonia caused by *P. aeruginosa*[27]. It was reported that C/T had higher *in vitro* efficacy against bloodstream and urinary tract infections than other beta-lactam antibiotics and carbapenems[14,28-33]. *In vitro* susceptibility to CZA in lower respiratory tract infections caused by resistant *P. aeruginosa* was also reported to be high[34].

Conclusion

In conclusion, our study demonstrated that C/T and CZA are more efficacious than other beta-lactam antibiotics and beta-lactamase inhibitors and carbapenems against MDR *P. aeruginosa* strains. Moreover, we found that some *P. aeruginosa* strains may be susceptible to CZA but resistant to C/T. Based on our review of the literature, ours appears to be the first study performed in Turkey on this subject. 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 CZA and C/T may be promising for the treatment of infections caused by MDR *P. aeruginosa* strains.

Ethics

Ethics Committee Approval: The study was approved by the Sakarya University Faculty of Medicine of Ethics Committee (Protocol number: 71522473/050.01.04/7).

Informed Consent: Retrospective study that did not use any patient data.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ö.A., Design: Ö.A., Data Collection or Processing: Ö.A., H.A.T., Analysis or Interpretation: H.A.T., M.A., Literature Search: Ö.A., M.K., Writing: Ö.A.

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