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In vitro activity of ceftolozane–tazobactam and ceftazidime–avibactam against clinical isolates of meropenem-non-susceptible *Pseudomonas aeruginosa*: A two-centre study

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ABSTRACT

Objectives: This study aimed to compare the activity of ceftazidime–avibactam (C/A), ceftolozane–tazobactam (C/T) and three anti-pseudomonal β-lactams (piperacillin–tazobactam, ceftazidime and cefepime) against a collection of meropenem-non-susceptible *Pseudomonas aeruginosa* (*P. aeruginosa*) clinical isolates recovered from two centres in Turkey.
Methods: A total of 102 unique patient isolates of meropenem-non-susceptible *P. aeruginosa* were included in the study. MICs of antimicrobials were determined by the gradient diffusion method.
Results: Overall susceptibility rates for C/A and C/T were 83.3% and 82.4%, respectively. Both C/A and C/T had better activity than any one of the three anti-pseudomonal β-lactams. According to the MIC_{50} values, C/T was the most potent agent against isolates. Although the susceptibility rates of isolates to C/T and C/A were similar, C/T (MIC_{50}, 1 μg/mL) was four-fold more potent than C/A (MIC_{50}, 4 μg/mL). The MIC_{50} values of C/A and C/T for the isolates that were non-susceptible to three β-lactams were significantly higher than those for isolates that were non-susceptible to zero, one or two β-lactams. Also, the C/A MIC_{50} value for the isolates that were non-susceptible to two β-lactams was higher than that for isolates which were non-susceptible to one β-lactam.
Conclusions: C/A and C/T showed good activity against meropenem-non-susceptible *P. aeruginosa* isolates. However, resistance to these agents was not uncommon among these isolates. The overall β-lactam susceptibility profile of isolates seems to have an effect on the probability of susceptibility to C/A and C/T. Antimicrobial susceptibility testing should be performed for C/A and C/T if these agents are considered for treatment of infections caused by meropenem-non-susceptible *P. aeruginosa*.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a non-fermentative Gram-negative bacilli that is a common cause of nosocomial infections, including hospital-acquired bacterial pneumonia, ventilator-associated pneumonia, skin infections, urinary tract infections, and bacteraemia [1,2]. *P. aeruginosa* presents a therapeutic challenge because of its intrinsically low susceptibility to a range of antimicrobials and its great ability to develop antibiotic resistance [2,3]. Carbapenems remain one of the best therapeutic choices for the treatment of serious infections caused by *P. aeruginosa* [4–6]. However, in recent years, an increase in the prevalence of resistance to carbapenems among *P. aeruginosa* isolates has been reported worldwide [6,7]. Infections caused by carbapenem-resistant *P. aeruginosa* are known to be associated with higher mortality rates, longer hospital stays and increased healthcare costs [7,8]. Resistance to other anti-pseudomonal β-lactam antibiotics is frequently observed among carbapenem-resistant *P. aeruginosa* isolates [9].

Ceftazidime–avibactam (C/A) and ceftolozane–tazobactam (C/T) are novel β-lactam/β-lactamase inhibitor combinations with broad-spectrum activity against Gram-negative bacteria, including *P. aeruginosa* [7,10,11], and may also represent therapeutic options for infections caused by carbapenem-resistant strains of *P. aeruginosa*. C/A recently received U.S. Food and Drug Administration

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(FDA) approval for the treatment of complicated intra-abdominal infections (in combination with metronidazole), complicated urinary infections, hospital-acquired bacterial pneumonia (HABP), and ventilator-associated bacterial pneumonia (VABP) [12]. C/T was approved by the FDA for the same clinical indications with the exception of HABP/VABP. However, C/T is currently being tested in a phase 3 trial for the treatment of HABP/VABP [13].

Limited data exist regarding the comparative activity of C/A and C/T against carbapenem-resistant *P. aeruginosa* isolates [6,10]. It is believed that no study has directly compared the activity of these antimicrobials against carbapenem-resistant *P. aeruginosa* isolates in Turkey. The objective of the current study was to evaluate the in vitro activity of C/A and C/T against a collection of meropenem-non-susceptible *P. aeruginosa* clinical isolates recovered from two centres in Turkey and to compare the activity of C/A, C/T and three traditional anti-pseudomonal β-lactam antibiotics (piperacillin–tazobactam, ceftazidime and cefepime) against these isolates.

2. Material and methods

2.1. Bacterial isolates

A total of 102 non-duplicate meropenem-non-susceptible *P. aeruginosa* clinical isolates from Başkent University Ankara Hospital (centre 1) (n = 82) and Başkent University Adana Medical and Research Center (centre 2) (n = 20) were included in the study. All isolates were recovered between 2014 and 2018. Specimen sources included tracheal aspirate (n = 43), blood (n = 20), wound (n = 14), bronchoalveolar lavage (n = 8), sputum (n = 6), urine (n = 6), pus (n = 4), and catheter (n = 1). All isolates were stored at −80 °C in skim milk and subcultured twice onto 5% sheep's blood agar prior to testing. *P. aeruginosa* ATCC 27853 was used as quality control.

2.2. Antimicrobial susceptibility testing

MICs of meropenem, piperacillin–tazobactam, ceftazidime, cefepime, C/A, and C/T were determined by MIC test strips (Liofilchem, Italy). In brief, a 0.5 McFarland turbidity standard suspension of each isolate was spread onto Mueller–Hinton agar (BD, USA). The surface of the plate was allowed to dry for 15 min before applying the MIC test strip. After the application of the MIC test strip, plates were incubated at 35 °C in ambient air. The MIC results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria in M100-S28 as well as European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables (version 9.0) [14,15]. Both CLSI and EUCAST have defined C/A MIC breakpoints for *P. aeruginosa* as follows: ≤8 μg/mL (susceptible) and ≥16 μg/mL (resistant). The CLSI has defined C/T MIC breakpoints for *P. aeruginosa* as ≤4 μg/mL (susceptible), 8 μg/mL (intermediate) and ≥16 μg/mL (resistant), while EUCAST has defined C/T MIC breakpoints as ≤4 μg/mL (susceptible) and ≥8 μg/mL (resistant).

2.3. Statistical analysis

Mann–Whitney U test was used to compare continuous variables. Pearson χ^2 test was used to compare categorical variables. Data were analysed using SPSS software (version 17, SPSS Inc., Chicago, IL). *P*-values of <0.05 were considered as statistically significant.

3. Results

All isolates (n = 102) were resistant to meropenem (MIC range : 8 - > 32 μg/mL) according to CLSI breakpoints. According to EUCAST, 90.2% (92 of 102) of isolates were resistant to meropenem and the remaining isolates were categorised as intermediate. Among the isolates, 28.4% (29 of 102), 12.8% (13 of 102), 19.6% (20 of 102), and 39.2% (40 of 102) were susceptible to zero, one, two, and three traditional anti-pseudomonal β-lactam agents, respectively, according to both CLSI and EUCAST criteria. Table 1 shows the anti-pseudomonal β-lactam susceptibility patterns of isolates and their susceptibility rates to C/A and C/T. Both C/A and C/T had better activity than any one of the three anti-pseudomonal β-lactams (Table 2). Susceptibility rates of isolates to C/A and C/T were both 86.6% (71 of 82) in centre 1. Isolates from centre 2 exhibited lower susceptibility rates (70% (14 of 20) for C/A and 65% (13 of 20) for C/T). The isolates from centre 2 included a lower proportion of isolates that were non-susceptible to two β-lactams (5.0% vs. 14.6%) but a higher proportion of isolates that were non-susceptible to three β-lactams (55.0% vs. 22.0%) than was observed for centre 1. Overall susceptibility rates for C/A and C/T were 83.3% (85 of 102) and 82.4% (84 of 102), respectively, according to both CLSI and EUCAST criteria.

Fig. 1 depicts the C/A and C/T MIC distributions for 102 meropenem-non-susceptible *P. aeruginosa* isolates. Among 102 isolates, 16 were resistant to both C/A and C/T, according to both CLSI and EUCAST breakpoints. One isolate was susceptible to C/T but resistant to C/A, according to CLSI and EUCAST breakpoints. Two isolates were susceptible to C/A but were intermediate to C/T, according to CLSI breakpoints. These two isolates were resistant to C/T, according to EUCAST breakpoints.

The MIC_{50} values of C/A and C/T were 4 μg/mL and 1 μg/mL, respectively. Using MIC_{50} values, C/T was four-fold more potent than C/A and ceftazidime (MIC_{50}, 4 μg/mL) and was 16-fold more potent than piperacillin-tazobactam and cefepime (MIC_{50}, 16 μg/mL). C/A was equal in potency to that of ceftazidime (MIC_{50}, 4 μg/mL) and was four-fold more potent than piperacillin-tazobactam and cefepime (MIC_{50}, 16 μg/mL) (Table 2). The MIC_{50} values of C/A were 2 μg/mL, 2 μg/mL, 4 μg/mL, and 16 μg/mL for the isolates that were non-susceptible to zero, one, two, and three anti-pseudomonal β-lactams, respectively. The MIC_{50} values of C/T were 1 μg/mL, 1 μg/mL, 1 μg/mL, and 8 μg/mL for the isolates that were non-susceptible to zero, one, two, and three anti-pseudomonal β-lactams, respectively (Fig. 2). The MIC_{50} values of C/A and C/T for the isolates that were non-susceptible to three β-lactams were significantly higher than those for isolates that were non-susceptible to zero, one or two β-lactams (16 μg/mL vs. 2 μg/mL; U = 351, $P < 0.001$ for C/A and 8 μg/mL vs. 1 μg/mL; U = 176.5, $P < 0.001$ for C/T). In addition, the C/A MIC_{50} value for the isolates that were non-susceptible to two β-lactams was significantly higher than that for the isolates which were non-susceptible to one β-lactam agent (4 μg/mL vs. 2 μg/mL; U = 196.5, $P = 0.013$). The

Table 1
Anti-pseudomonal β-lactam susceptibility patterns of isolates and their susceptibility rates to C/A and C/T.

Susceptibility pattern	N (%)	Susceptible to C/A N (%)	Susceptible to C/T N (%)
S to PTZ, CAZ and FEP	40 (39.2)	40 (100)	40 (100)
NS to PTZ only	6 (5.9)	6 (100)	6 (100)
NS to CAZ only	0 (0)	0 (0)	0 (0)
NS to FEP only	14 (13.7)	14 (100)	14 (100)
NS to PTZ and CAZ	3 (2.9)	1 (33.3)	1 (33.3)
NS to PTZ and FEP	10 (9.8)	10 (100)	9 (90)
NS to CAZ and FEP	0 (0)	0 (0)	0 (0)
NS to PTZ, CAZ and FEP	29 (28.4)	14 (48.3)	14 (48.3)

Abbreviations: S, susceptible; NS, non-susceptible; PTZ, piperacillin–tazobactam; CAZ, ceftazidime; FEP, cefepime; C/A, ceftazidime–avibactam; C/T, ceftolozane–tazobactam.

Table 2
Activity of β-lactam agents against 102 clinical isolates of meropenem-non-susceptible *Pseudomonas aeruginosa*.

Antimicrobial agent	MIC (μg/mL)			%S/%I/%R	
	50%	90%	Range	CLSI	EUCAST
Meropenem	>32	>32	8 –> 32	0/0/100.0	0/9.8/90.2
PTZ	16	>256	4 –> 256	53.0/16.6/30.4	53.0/0/47.0
Ceftazidime	4	64	1 –> 256	68.6/3.9/27.5	68.6/0/31.4
Cefepime	16	64	2 –> 256	48.0/26.5/25.5	48.0/0/52.0
C/A	4	64	1 –> 256	83.3/0/16.7	83.3/0/16.7
C/T	1	>256	0.25 –> 256	82.4/2.0/15.6	82.4/0/17.6

Abbreviations: PTZ, piperacillin–tazobactam; C/A, ceftazidime–avibactam; C/T, ceftolozane–tazobactam; CLSI, Clinical and Laboratory Standards Insitute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; I, intermediate; R, resistant.

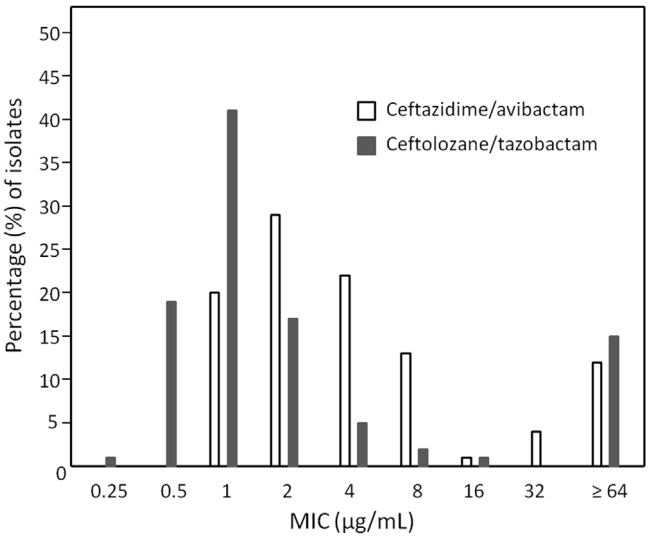

Fig. 1. Ceftazidime/avibactam and ceftolozane/tazobactam MIC distributions for 102 meropenem-non-susceptible *Pseudomonas aeruginosa* isolates.

susceptibility rate for both C/A and C/T was 48.3% among the isolates that were non-susceptible to all three β-lactam agents. Among the C/A susceptible isolates of *P. aeruginosa*, 15.3% (13 of 85) had MICs that were at the susceptibility breakpoint (8 μg/mL). However, 6.0% (five of 84) of isolates that were susceptible to C/T had MICs at the susceptibility breakpoint (4 μg/mL) (15.3% vs. 6.0%; χ^2 = 3.87, P = 0.049).

4. Discussion

The increase in carbapenem resistance among the clinical isolates of *P. aeruginosa* is worrisome, as few antimicrobial agents have been introduced in the last few years to treat infections caused by this organism [7,16]. This study assessed the in vitro activity of two novel β-lactam/β-lactamase inhibitor combinations (C/A and C/T) against 102 clinical isolates of meropenem-non-susceptible *P. aeruginosa* recovered from two hospitals in Turkey. Furthermore, it compared the activities of C/A, C/T, piperacillin–tazobactam, ceftazidime, and cefepime against these isolates. It is believed that this is the first study to directly compare the activities of C/A and C/T against meropenem-non-susceptible *P. aeruginosa* isolates in Turkey.

In previous studies, the susceptibility rates of meropenem-non-susceptible/resistant *P. aeruginosa* isolates ranged from 51.8 to 92% for C/A and 65.4 to 94% for C/T [6,9,10,16–18]. In most studies, higher susceptibility rates were observed for C/T than those for C/A among meropenem-non-susceptible/resistant *P. aeruginosa* isolates [6,7,10,16]. Similar susceptibility rates for C/A (83.3%) and C/T (82.4%) were found in the current study. This finding is consistent with the findings reported by Buehrle et al. They found identical susceptibility rates (92%) for C/A and C/T among meropenem-resistant *P. aeruginosa* isolates. Although overall susceptibility rates for C/A and C/T were higher than 80% in the current study, susceptibility rates of isolates from centre 2 were lower (70% (14 of 20) for C/A and 65% (13 of 20) for C/T). The lower susceptibility rates of isolates to C/A and C/T in centre 2 may be partly due to the low number of tested isolates because only 20 isolates of meropenem-non-susceptible *P. aeruginosa* were available from centre 2. Also, patterns of antibiotic usage and susceptibility rates to different antibiotics may vary by geographical region in the world, as well as Turkey, and could have affected the results.

According to MIC_{50} values, C/T was the most potent agent tested against *P. aeruginosa* isolates. Although the susceptibility rates of isolates to C/T and C/A were similar, C/T (MIC_{50}, 1 μg/mL) was four-fold more potent than C/A (MIC_{50}, 4 μg/mL). This finding is consistent with previous studies that found lower MIC_{50} values for C/T when compared with C/A for meropenem-non-susceptible *P. aeruginosa* isolates [6,7,16].

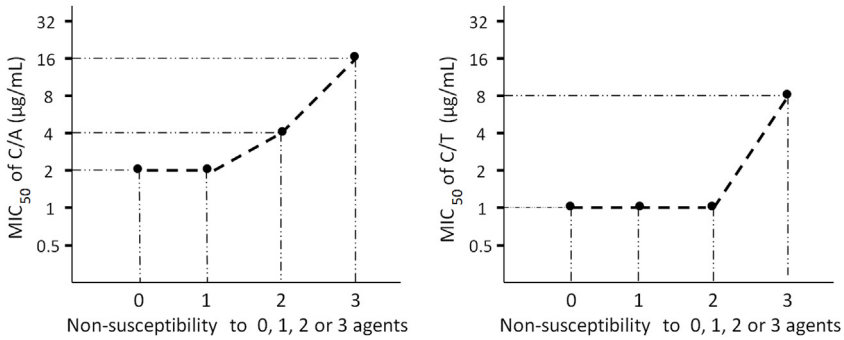

Fig. 2. Ceftazidime/avibactam and ceftolozane/tazobactam MIC50 values for the isolates that were non-susceptible to zero, one, two, or three β-lactam agents.

In the current study, the rate of isolates with MIC at the susceptibility breakpoint was higher for C/A (15.3%) than for C/T (6.0%) among susceptible strains. This finding is consistent with the findings by Buehrle et al. and Grupper et al. [6,9], and considered as a support for higher activity of C/T by some authors [9].

Ceftazidime–avibactam and C/T MIC_{50} values for the isolates that were non-susceptible to all three anti-pseudomonal β-lactams were significantly higher than those for isolates that were non-susceptible to two or less β-lactams. Also, the C/A MIC_{50} value for the isolates that were non-susceptible to two β-lactams was significantly higher than that for isolates which were non-susceptible to one β-lactam agent. However, the C/T MIC_{50} values remained unchanged (1 μg/mL) for the isolates that were non-susceptible to zero, one or two β-lactam agents. According to these data, the MICs of C/T may be relatively less influenced than MICs of C/A by the β-lactam susceptibility profile of isolates. In the current study, the susceptibility rates for C/A and C/T were low (48.3% both for C/A and C/T) among the isolates that were non-susceptible to all three β-lactams. The overall β-lactam susceptibility profile of meropenem-non-susceptible *P. aeruginosa* isolates seems to have an effect on the probability of susceptibility to C/A and C/T. This finding is important because these agents would probably be considered for the isolates that exhibit resistance to traditional anti-pseudomonal β-lactam agents [10].

This study had several limitations. First, all isolates were from two centres in Turkey. The low number of centres included in the study limited the generalisation of results to the whole country. Second, the mechanisms of resistance to C/A and C/T were not explored in this study. Although this was not a study on the mechanisms of antibiotic resistance, determining the mechanisms responsible for resistance to C/A and C/T may be helpful for developing effective strategies to cope with resistance and epidemiological purposes. No studies have explored the resistance mechanisms for C/A and C/T among *P. aeruginosa* isolates in Turkey but studies reporting the main resistance mechanisms for these agents are found in the literature. Resistance to C/A mainly develops because of decreased membrane permeability and overexpressed efflux pumps [19]. Structural modifications in AmpC may also lead to resistance to C/A [20]. Resistance to C/T develops mainly due to overexpression and structural modifications of AmpC and structural modifications of OXA enzymes [21,22]. Also, the metallo-β-lactamase producing isolates of *P. aeruginosa* can lead to resistance to both C/A and C/T, as these agents do not inactivate metallo-β-lactamase enzymes. The vast majority of isolates in the current study that were resistant to C/A were also resistant to C/T, suggesting that there are resistance mechanisms affecting the activity of both agents.

In conclusion, C/A and C/T showed good activity against 102 meropenem-non-susceptible *P. aeruginosa* isolates and may serve as therapeutic options for infections caused by these organisms. However, resistance to these agents is not uncommon among meropenem-non-susceptible *P. aeruginosa*. Susceptibility rates for both C/A and C/T was approximately 50% among the isolates that were non-susceptible to all three anti-pseudomonal β-lactam agents (piperacillin–tazobactam, ceftazidime and cefepime). Antimicrobial susceptibility testing should be performed for C/A and C/T if these agents are to be considered for treating infections caused by meropenem-non-susceptible *P. aeruginosa*.

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Competing interests

None declared.

Ethical approval

This study was approved by Başkent University Institutional Review Board (Project no: KA17/330).

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