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ORIGINAL ARTICLE

Ceftazidime–avibactam and ceftolozane–tazobactam susceptibility of multidrug resistant *Pseudomonas aeruginosa* strains in Hungary

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ABSTRACT

Our objective was to compare the activity ceftazidime-avibactam (C/A) and ceftolozane–tazobactam (C/T) against multidrug (including carbapenem) resistant *Pseudomonas aeruginosa* clinical isolates collected from six diagnostic centers in Hungary and to reveal the genetic background of their carbapenem resistance.

Two hundred and fifty consecutive, non-duplicate, carbapenem-resistant multidrug resistant (MDR) *P. aeruginosa* isolates were collected in 2017. Minimal inhibitory concentration values of ceftazidime, cefepime, piperacillin/tazobactam, C/A and C/T were determined by broth microdilution method and gradient diffusion test. Carbapenem inactivation method (CIM) test was performed on all isolates. Carbapenemase-encoding bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM} genes were identified by multiplex PCR.

Of the isolates tested, 33.6% and 32.4% showed resistance to C/A and C/T, respectively. According to the CIM test results, 26% of the isolates were classified as carbapenemase producers. The susceptibility of *P. aeruginosa* isolates to C/A and C/T without carbapenemase production was 89% and 91%, respectively. Of the CIM-positive isolates, 80% were positive for bla_{VIM} and 11% for bla_{NDM}. The prevalence of Verona integron-encoded metallo-beta-lactamase (VIM)-type carbapenemase was 20.8%. NDM was present in 2.8% of the isolates.

Although the rate of carbapenemase-producing *P. aeruginosa* strains is high, a negative CIM result indicates that either C/A or C/T could be effective even if carbapenem resistance has been observed.

KEYWORDS

Pseudomonas aeruginosa, ceftazidime-avibactam, ceftolozane–tazobactam, carbapenem resistance, carbapenemase

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterial pathogen that is an important cause of multidrug-resistant healthcare-associated infections [1]. *P. aeruginosa* has recently shown an increasing resistance rate to carbapenems, making treatment more challenging [2–4]. To treat carbapenem-resistant *P. aeruginosa*, two new combinations of β-lactams with β-lactamase-

inhibitors have recently become commercially available. Ceftazidime (CAZ), an older third generation cephalosporin, has been re-issued in combination with the new broad-spectrum β-lactamase inhibitor avibactam in 2015 [5, 6]. Ceftolozane, a novel fifth generation cephalosporin, was issued in combination with the older β-lactamase inhibitor tazobactam in late 2014 [7]. Both ceftazidime-avibactam (C/A) and ceftolozane–tazobactam (C/T) have been shown to successfully treat most carbapenem-resistant *P. aeruginosa* infections [8–10].

There are several advantages of the novel drugs avibactam and ceftolozane compared to their older counterparts. Both tazobactam and avibactam inhibit serin β-lactamases. While tazobactam is ineffective against many known β-lactamases and carbapenemases, avibactam is active against ESBL and AmpC β-lactamases as well as the Klebsiella pneumoniae carbapenemase (KPC) and OXA-48 carbapenemases [11]. Additionally, avibactam binds to β-lactamases reversibly, allowing it to be recycled and inhibit additional β-lactamases [11]. Likewise, when comparing ceftolozane to CAZ, the newer drug ceftolozane has further advantages including higher penetration despite porin downregulation, resistance to efflux pump mechanisms, and greater stability to AmpC β-lactamases [11–13].

Regardless of the advantages of the new drugs avibactam and ceftolozane, neither are effective against most *P. aeruginosa* strains that produce carbapenemases. [3, 13, 14]. The metallo-β-lactamase (MBL)-type carbapenemase *P. aeruginosa* strains are particularly concerning due to their strong capacity to hydrolyze β-lactams, co-resistance with other antimicrobial classes, and their accelerating spread worldwide [3, 13–17]. Early laboratory detection of carbapenemase production can be critical to provide optimal antimicrobial treatment. Carbapenemase production can be evaluated by the carbapenem inactivation method (CIM), a simple and cost-effective test [18]. Multiplex PCR is used to detect various carbapenemase-encoding genes, including the carbapenem-hydrolyzing class D β-lactamase OXA-48, Ambler class A KPC, and the MBL Verona integron-encoded metallo-beta-lactamase (VIM), IMP, and NDM [19].

The purpose of the study was threefold. First, we evaluated the susceptibility of carbapenem-resistant multidrug resistant (MDR) *P. aeruginosa* isolates to C/A and C/T. Second, we examined the genotype of carbapenemase-producing *P. aeruginosa* isolates and how carbapenemase production influences C/A and C/T resistance. Third, we sought to determine the clinical value of the CIM test as a tool for determining antibiotic susceptibility.

MATERIALS AND METHODS

A total of 250 consecutive, non-duplicate, multidrug (including carbapenem) resistant *P. aeruginosa* isolates from six diagnostic centers in Hungary (representing the country) were collected in 2017. The criteria of collection were multidrug resistance (non-susceptible to at least one agent in ≥3 antimicrobial groups: aminoglycosides (testing gentamycin, tobramycin, amikacin), fluoroquinolones (testing ciprofloxacin and levofloxacin) and β-lactams (testing CAZ, cefepime (FEP), piperacillin/tazobactam (P/T)), including both meropenem and imipenem resistance, based on disk diffusion method according to EUCAST guidelines [20]. Bacterial identification was performed by MALDI-TOF MS.

Susceptibility patterns of CAZ, FEP and P/T were also tested by broth microdilution method [21]. Minimal inhibitory concentration (MIC) values of C/A and C/T were determined by gradient diffusion test (Liofilchem). *Escherichia coli* ATCC 25922 and *P. aeruginosa* American Tissue Type Collection (ATCC) 27853 were used as control strains.

CIM test was performed on all isolates in the manner described by Zwalul [18]. Briefly, a full 10 μL inoculation loop of bacteria was suspended in 400 μL water, then a disk containing 10 μg meropenem was immersed in the suspension and incubated for 3 hours at 35 °C. After incubation, the disk was removed from the suspension and placed on a Mueller-Hinton agar plate that had already been inoculated with ATCC 29522 *E. coli* indicator strain. The plate was subsequently incubated at 35 °C overnight. Isolates showing an inhibition zone ≤15 mm around the meropenem disk were defined as carbapenemase producers [2]. The carbapenemase-encoding genes bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM} were tested among CIM-positive isolates by multiplex PCR according to Poirel et al. [19].

RESULTS

Results of antimicrobial susceptibility testing are summarized in Table 1. Of the 250 tested isolates, 33.6% (n = 84) were resistant to C/A and 32.4% (n = 81) were resistant to C/T. Most of the isolates (97%, n = 243) showed categorical

Table 1. MIC values and resistance rates of investigated β-lactams among carbapenem-resistant *Pseudomonas aeruginosa* isolates

Antimicrobial agent	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	EUCAST susceptibility breakpoint (mg/L)	Resistance (%)
Ceftazidime–avibactam	1 to ≥256	8	≥256	8	33.6
Ceftolozane-tazobactam	0.5 to ≥256	4	≥256	4	32.4
Ceftazidime	2 to >256	64	256	8	93.2
Cefepime	8 to >256	64	>256	8	99.2
Piperacillin-tazobactam	4 to >256	128	>256	16	97.2

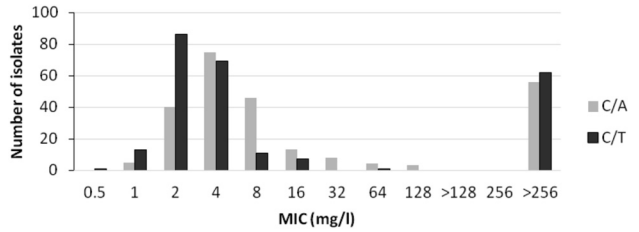

Figure 1. Distribution of MIC values of ceftazidime-avibactam and ceftolozane–tazobactam among carbapenem- and multidrug-resistant *Pseudomonas aeruginosa* isolates, gray column: C/A: ceftazidime-avibactam, black column: C/T: ceftolozane–tazobactam

agreement to C/A and C/T. Two isolates showed susceptibility to C/A but resistance to C/T, and five isolates showed resistance to C/A yet susceptibility to C/T. All strains with discordant susceptibility results were retested to minimize methodical error. Distribution of MIC values of C/A and C/T are shown in Fig. 1.

Only 17 of the 250 isolates (7%) were susceptible to CAZ without avibactam. Sixty-four percent of the CAZ-resistant isolates (149/233) retained susceptibility to C/A. Only seven (2.8%) strains showed susceptibility to P/T. All of these susceptible isolates also showed susceptibility to C/T. Of the 243 P/T resistant isolates, 162 (66%) showed susceptibility to C/T.

Sixty-five of the 250 isolates were classified as carbapenemase producers according to the CIM test results. All but one CIM-positive isolate showed resistance to both C/A and C/T. CIM positivity showed strong correlation with resistance to both tested cephalosporin/β-lactamase inhibitor combinations. For both C/A and C/T resistance, the CIM test showed a positive predictive value (PPV) of 98% and a specificity of 99%. For C/A resistance, the CIM test showed a sensitivity of 76% and negative predictive value (NPV) of 89%. For C/T resistance alone, the CIM test showed a sensitivity of 79% and NPV of 91%.

Of the 65 isolates that tested positive in the CIM test, 80% (n = 52) were positive for the VIM gene and 11% (n = 7) were positive for the NDM gene. All seven isolates found to contain NDM genes were resistant to C/A and C/T, with minimal inhibitory concentrations ≥256 mg/L. All VIM-positive isolates conferred resistance to C/A and C/T with MIC values 16 to ≥256 mg/L and ≥256 mg/L, respectively. The remaining six CIM-positive isolates did not contain any of the genes tested.

DISCUSSION

In this study the susceptibility rates of carbapenem-resistant MDR *P. aeruginosa* to both C/A and C/T are comparable to one another, at 66.4% and 67.6% respectively. Of note, other studies reported higher susceptibility to C/T than C/A: Grupper et al. observed 91% C/T susceptibility and 81% C/A susceptibility among 290 meropenem-non-susceptible isolates from 34 hospitals in the United States in 2013 and 2014 [22]. Humphries et al. observed 61.8% susceptibility to C/A and 72.5% susceptibility to C/T in a similar study of β-lactam resistant *P. aeruginosa*, with 36.4% of C/A-resistant isolates still susceptible to C/T [7]. Ceftolozane is believed to have several advantages over CAZ regarding resistance: greater affinity for penicillin-binding proteins produced by *P. aeruginosa*, better membrane permeability, greater stability against AmpC β-lactamases, and more potency against *P. aeruginosa* isolates with up-regulated efflux pumps and loss of porins [9, 12]. Our results support only modest advantages of C/T over C/A. Of the tested 250 isolates just five were susceptible to C/T but not to C/A. Additionally, two isolates were susceptible to C/A but not to C/T. The previously-mentioned advantageous mechanisms of ceftolozane may be responsible for the strains that were susceptible to C/T but resistant to C/A. For the strains that were susceptible to C/A but resistant to C/T, differences between the carbapenemase inhibitor potency of avibactam and tazobactam may have an impact. The susceptibility to CAZ among our isolates increased from 7% to 66% when avibactam is included, indicating strong potency of avibactam as a β-lactamase inhibitor. We do not have a similar comparison to make with ceftolozane and tazobactam, but P/T showed an even higher rate of resistance than that was seen in CAZ without a β-lactamase inhibitor. Prior use of tazobactam as a β-lactamase inhibitor has allowed several decades for resistance mechanisms to develop, and this may be a contributing factor to resistance to C/T but not C/A.

The potency of C/A and C/T in *P. aeruginosa* isolates that do not produce some form of carbapenemase is consistent with previous studies [8]. For strains that did not produce carbapenemase (tested negative in the CIM test), susceptibility to C/A and to C/T was 89% and 91%, respectively. This susceptibility rate among CIM-negative isolates is much higher than the overall susceptibility rates of 66–68%. Buehrle et al. reported similar patterns of 92% susceptibility to both C/A and C/T among 38 meropenem-resistant *P. aeruginosa* isolates without carbapenemase production [8]. This indicates that the CIM test could be valuable in routine clinical antibiotic susceptibility testing. CIM is a simple and low-cost test that can be completed in less than one day [2, 18]. A negative CIM result indicates that either C/A or C/T would likely be effective treatments, even if carbapenem resistance has already been established.

The rate of spread of the VIM-type MBL is alarming, appearing in 20.8% of the total number of isolates. The presence of the NDM in 2.8% of our *P. aeruginosa* isolates is also a cause for concern, since the first $bla_{\text{NDM-1}}$ positive *P. aeruginosa* in Hungary was reported in 2019 [23]. While the sample size is small (n = 7), the isolates expressing NDM showed the strongest possible resistance to all β-lactam antibiotics that were tested. The rapid spread of VIM and NDM carbapenemases among many species of Gram-negative bacteria over the last decade could soon end many of the currently-available antimicrobial therapeutic options, including the newer treatments C/A and C/T. Only more toxic antimicrobials such as colistin appear to

maintain potency against these multidrug or extensively drug-resistant *P. aeruginosa* isolates, although resistance against even these antimicrobial classes is beginning to develop [15, 17].

Although the rate of carbapenemase-producing *P. aeruginosa* strains is high according to our study, a negative CIM result indicates that either C/A or C/T could be effective even if carbapenem resistance has been observed. As *P. aeruginosa* is known to quickly gain antibiotic resistance via several mechanisms besides carbapenemase production, further testing of susceptibility patterns to C/A and C/T should be performed regularly. As the rate of resistance rises, there is an urgent need to develop new and safe classes of antimicrobial therapy. Until novel agents have been developed, strong infection control measurements are essential to protect patients from MDR *P. aeruginosa* infections.

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Authors' contributions: KK, EJ conceived the experiments. DO, EJ, KK performed the testes, analyzed the data. DO wrote the manuscript. DO, EJ, KK corrected the manuscript. ÁT, KK, EU, SzM, JSz collected the strains according to certain criteria. All authors read and approved the final manuscript.

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