Letter to the Editor
Clinical Microbiology

Ann Lab Med 2017;37:174-176
https://doi.org/10.3343/alm.2017.37.2.174
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Susceptibility of Ceftolozane-Tazobactam and Ceftazidime-Avibactam Against a Collection of β-Lactam-Resistant Gram-Negative Bacteria

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Dear Editor,

Ceftolozane-tazobactam (C/T) and ceftazidime-avibactam (CZA) were recently approved for the treatment of complicated intra-abdominal infections and complicated urinary tract infections (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427534.htm, http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm435629.htm, both accessed February 24, 2016). To date, only one study has simultaneously evaluated the activities of C/T and CZA *in vitro* against *Pseudomonas aeruginosa*, and few studies have evaluated the effects of these antibiotics on multi-drug resistant (MDR) gram-negative bacteria [1-3]. This study aimed to examine the activities of C/T and CZA against β-lactam-resistant *Enterobacteriaceae* and *P. aeruginosa* clinical isolates.

The isolates were recovered from clinical specimens at Barnes-Jewish Hospital (St. Louis, MO, USA) from September to December 2014. Specimen sources included respiratory, blood, urine, and wound samples. Isolates of *Enterobacteriaceae* were included if they tested non-susceptible to cefepime and/or had an extended-spectrum β-lactamase (ESBL)-producing phenotype. *P. aeruginosa* isolates were included if they tested non-susceptible to meropenem. We included carbapenem-resistant *Enterobacteriaceae* isolates recovered from August 2012 to December 2014; these strains were tested for the bla_{KPC} and bla_{NDM} genes by real-time PCR [4, 5]. All isolates were negative for the bla_{NDM} gene.

Frozen stocks of all isolates were subcultured twice consecutively on 5% sheep's blood agar (Hardy Diagnostics, Santa Maria, CA, USA) prior to antimicrobial susceptibility testing (AST). Species-level identification was confirmed by using the VITEK MS system (IVD v2.3.3, bioMérieux, Durham, NC, USA) [6, 7]. AST was performed by using gradient diffusion (Etest, bioMérieux). In brief, a 0.5 McFarland standard suspension of each isolate was inoculated onto Mueller–Hinton agar (Hardy Diagnostics), and Etest strips were applied. Plates were incubated overnight at 35°C in ambient air. Each day, QC strains (*Escherichia coli* ATCC 25922 and ATCC 35218, and *P. aeruginosa* ATCC 27853) were tested. C/T and CZA results and QC were interpreted by using Food and Drug Administration breakpoints. The categorical interpretation and QC ranges for all other antibiotics (BD BBL and Co., Sparks, MD, USA) were based on standardized disk diffusion criteria [8].

We evaluated 120 clinical isolates comprising 45 *P. aeruginosa*

Received: March 14, 2016
Revision received: August 10, 2016
Accepted: November 17, 2016

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Table 1. β-Lactam susceptibility profiles of the study isolates (N=120)

Bacterial group (N)	% Susceptible*					
	Ceftazidime	Pip-Tazo	Cefepime	Meropenem	Ertapenem	Imipenem
Pseudomonas aeruginosa (45)	73	67	71	13	NA	22
BLR *P. aeruginosa*† (10)	10	0	0	0	NA	0
Enterobacteriaceae (75)	27	40	21	64	57	73
Enterobacter spp. (17)	6	6	12	35	12	47
Escherichia coli (29)	59	86	28	100	100	100
Klebsiella pneumoniae (24)	8	17	8	42	42	63
ESBL *K. pneumoniae* (8)	13	50	25	100	100	100
CRE *K. pneumoniae*‡ (16)	6	0	0	13	13	44
Remaining isolates§ (5)	0	0	80	60	40	60
Enterobacteriaceae bla_{KPC} status‖						
bla_{KPC} positive¶ (10)	0	0	10	0	10	0
bla_{KPC} negative** (65)	31	46	23	74	65	85

*Based on CLSI M100-S25 antibiotic disk diffusion criteria; †BLR *P. aeruginosa* isolates were not susceptible to piperacillin-tazobactam, cefepime, meropenem, and imipenem; ‡CRE *K. pneumoniae*, carbapenem-resistant *K. pneumoniae* isolates that either tested positive for bla_{KPC} (n=8) or were negative for bla_{KPC} and bla_{NDM} (n=8) by real-time PCR; §Remaining isolates, including *Citrobacter freundii* complex (n=3), *Klebsiella oxytoca* (n=1), and *Morganella morganii* (n=1); ‖*Enterobacteriaceae* bla_{KPC} status, all of the above *Enterobacteriaceae* isolates identified only by bla_{KPC} status; ¶bla_{KPC}-positive, isolates that tested positive for bla_{KPC} by real-time PCR; **bla_{KPC}-negative, isolates that either tested negative for bla_{KPC} and bla_{NDM} by real-time PCR (n=20) or lacked a phenotype (n=45) that is consistent with a bla_{KPC}-positive organism (i.e., lack of resistance to meropenem).
Abbreviations: C/T, ceftolozane-tazobactam; CZA, ceftazidime-avibactam; MIC, minimum inhibitory concentration; NA, not applicable; ESBL, extended spectrum β-lactamase; CRE, carbapenem-resistant *Enterobacteriaceae*.

Table 2. Ceftolozane-tazobactam (C/T) and ceftazidime-avibactam (CZA) activity against BLR gram-negative bacteria

Bacterial group (N)	C/T MIC Range μg/mL	C/T MIC_{50}	C/T MIC_{90}	C/T % Sus*	CZA MIC Range μg/mL	CZA MIC_{50}	CZA MIC_{90}	CZA % Sus*
Pseudomonas aeruginosa (45)	0.25–16	1	8	87	0.5–64	2	16	82
BLR *P. aeruginosa*† (10)	2–16	4	8	60	2–64	8	64	50
Enterobacteriaceae (75)	0.125–≥256	2	32	56	0.032–32	0.5	2	99
Enterobacter spp. (17)	0.5–64	4	64	18	0.125–32	1	4	94
Escherichia coli (29)	0.125–4	0.25	0.5	97	0.032–2	0.125	0.5	100
Klebsiella pneumoniae (24)	0.25–≥256	4	128	42	0.125–8	1	2	100
ESBL *K. pneumoniae* (8)	0.25–4	0.25	4	88	0.125–1	0.25	1	100
CRE *K. pneumoniae*‡ (16)	1–≥256	8	≥256	19	1–8	2	4	100
Remaining isolates§ (5)	2–16	16	16	20	0.5–2	1	2	100
Enterobacteriaceae bla_{KPC} status‖								
bla_{KPC} positive¶ (10)	2–128	8	128	20	0.5–4	1	2	100
bla_{KPC} negative** (65)	0.125–≥256	0.5	16	62	0.032–32	0.25	2	99

*% Sus, % Susceptible based on Food and Drug Administration interpretative criteria for ceftolozane-tazobactam and ceftazidime-avibactam; †BLR *P. aeruginosa* isolates that were not susceptible to piperacillin-tazobactam, cefepime, meropenem, and imipenem; ‡CRE *K. pneumoniae*, carbapenem-resistant *K. pneumoniae* isolates that either tested positive for bla_{KPC} (n=8) or were negative for bla_{KPC} and bla_{NDM} (n=8) by real-time PCR; §Remaining isolates, including *Citrobacter freundii* complex (n=3), *Klebsiella oxytoca* (n=1), and *Morganella morganii* (n=1); ‖*Enterobacteriaceae* bla_{KPC} status, all of the above *Enterobacteriaceae* isolates identified only by bla_{KPC} status; ¶bla_{KPC}-positive, isolates that tested positive for bla_{KPC} by real-time PCR; **bla_{KPC}-negative, isolates that either tested negative for bla_{KPC} and bla_{NDM} by real-time PCR (n=20) or lacked a phenotype (n=45) that is consistent with a bla_{KPC}-positive organism (i.e., lack of resistance to meropenem).
Abbreviations: C/T, ceftolozane-tazobactam; CZA, ceftazidime-avibactam; MIC, minimum inhibitory concentration; ESBL, extended spectrum β-lactamase; CRE, carbapenem-resistant *Enterobacteriaceae*.

strains and 75 *Enterobacteriaceae* strains. Table 1 shows the overall β-lactam susceptibility profile. A subset of *P. aeruginosa* isolates (n=10, 22%), termed "β-lactam-resistant (BLR)", were resistant to piperacillin/tazobactam, cefepime, meropenem, and imipenem.

The 50% minimum inhibitory concentration (MIC_{50}, 1 μg/mL) and 90% minimum inhibitory concentration (MIC_{90}, 8 μg/mL) of C/T were lower for the *P. aeruginosa* isolates than for the *Enterobacteriaceae* isolates (2 μg/mL and 32 μg/mL, respectively; Table 2). Furthermore, 87% (n=39) of all *P. aeruginosa* isolates and 60% (n=6) of the BLR *P. aeruginosa* isolates were C/T-susceptible (Table 2).

Within the *Enterobacteriaceae*, the C/T data showed group-dependent differences. For example, the *E. coli* isolates had a low MIC_{90} (0.5 μg/mL), whereas the *Enterobacter* spp. had a higher MIC_{90} (64 μg/mL). Overall, 56% (n=42) of all *Enterobacteriaceae* isolates were C/T-susceptible (Table 2).

In contrast to the C/T results, the *Enterobacteriaceae* had lower MIC_{50} (0.5 μg/mL) and MIC_{90} (2 μg/mL) values for CZA compared to the *P. aeruginosa* isolates (2 μg/mL and 16 μg/mL, respectively; Table 2). Notably, 82% (n=37) of all *P. aeruginosa* isolates were CZA-susceptible (Table 2), whereas 99% (n=74) of *Enterobacteriaceae* isolates were CZA-susceptible. An *Enterobacter* spp. isolate was CZA-resistant (MIC of 32 μg/mL), but was found to be negative for the bla_{KPC} and bla_{NDM} genes by real-time PCR.

Comparison of the *P. aeruginosa* C/T and CZA results showed 87% (n=39) concordance with 35 isolates testing susceptible and four isolates testing non-susceptible to both antibiotics. For the *Enterobacteriaceae*, there was only a 57% concordance between the C/T and CZA results; 42 isolates tested susceptible and one isolate tested non-susceptible to both antibiotics. All remaining isolates were only CZA-susceptible.

The availability of new agents with anti-gram-negative activity holds promise for treating MDR organisms. CZA provides an alternative treatment for bla_{KPC}-positive organisms, which are otherwise treated by agents with less desirable safety or efficacy profiles. Ceftolozane is a new cephalosporin with activity against *P. aeruginosa*, and in combination with tazobactam shows activity against ESBL-producing *Enterobacteriaceae* [9]. Notably, although two of the bla_{KPC}-positive isolates were C/T-susceptible, we would not expect C/T to be clinically effective.

In summary, we report the activities of C/T and CZA against a collection of BLR *Enterobacteriaceae* and *P. aeruginosa* isolates. Our results suggest that C/T and CZA are active against and represent possible therapeutic options for infections with BLR gram-negative bacteria.

Acknowledgments

Cubist supplied the C/T Etest strips, and Actavis supplied the CZA Etest strips. There was no funding for this study.

Authors' Disclosures of Potential Conflicts of Interest

MDG has none to declare. CAB has received research support from bioMerieux, Cepheid, and Accelerate Diagnostics.

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