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In Vitro Activity of Ceftolozane-Tazobactam against *Pseudomonas aeruginosa* Isolates Obtained from Patients in Canadian Hospitals in the CANWARD Study, 2007 to 2012

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The *in vitro* activity of ceftolozane in combination with tazobactam (fixed concentration of 4 μg/ml) was evaluated against 2,435 *Pseudomonas aeruginosa* clinical isolates obtained from across Canada using Clinical and Laboratory Standards Institute broth microdilution methods. The MIC_{50} and MIC_{90} values for ceftolozane-tazobactam were 0.5 μg/ml and 1 μg/ml, respectively (a 32-fold-lower MIC_{90} than that for ceftazidime). Eighty-nine percent (141/158) of multidrug-resistant isolates were inhibited by ≤8 μg/ml of ceftolozane-tazobactam.

Pseudomonas aeruginosa is an important cause of nosocomial bloodstream, respiratory, urinary tract, and wound infections (1–4). Clinical isolates of *P. aeruginosa* may demonstrate resistance to multiple classes of antimicrobials, leaving clinicians with few therapeutic options from which to choose (5). Not surprisingly, multidrug resistance among *P. aeruginosa* has been associated with adverse clinical outcomes, including increased mortality (6–9). Of concern, there are few novel antimicrobials on the horizon with significant *in vitro* activity versus *P. aeruginosa* (10).

Ceftolozane (formerly CXA-101) is a novel antibacterial currently under development that could prove useful in the treatment of infections caused by multidrug-resistant (MDR) *P. aeruginosa* (11–13). Resistance to β-lactams among *P. aeruginosa* strains may be mediated by the overproduction of an AmpC β-lactamase, reduction in cell permeability (OprD loss), upregulation of efflux pumps, and/or the acquisition of extended-spectrum or metallo-β-lactamases (14). Ceftolozane demonstrates low affinity for the AmpC β-lactamase of *P. aeruginosa* and improved binding to *P. aeruginosa* penicillin-binding proteins relative to ceftazidime (15, 16). Furthermore, the *in vitro* activity of ceftolozane does not appear to be significantly compromised by common efflux pumps found in *P. aeruginosa* or by reduced permeability related to OprD loss (17). The purpose of this study was to evaluate the *in vitro* activity of ceftolozane in combination with tazobactam against *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals.

Between 2007 and 2012, 10 to 15 tertiary care medical centers (depending on the study year) representing 8 of the 10 Canadian provinces submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units (CANWARD Study). The sites were geographically distributed in a population-based fashion. Annually, each study site was asked to submit clinical isolates (consecutively, one per patient per infection site) from inpatients and outpatients with bloodstream, respiratory, urine, and wound or intravenous (i.v.) infections. The medical centers submitted clinically significant isolates, as defined by their local site criteria. Isolate identification was performed by the submitting site and confirmed at the reference site as required (i.e., when morphological characteristics and antimicrobial susceptibility patterns did not fit the reported identification). Isolates were shipped on Amies semisolid transport medium to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto the appropriate medium, and stocked in skim milk at −80°C until MIC testing was carried out.

Following 2 subcultures from frozen stock, the *in vitro* activities of commonly used antipseudomonal antimicrobials were determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (18, 19). For testing of ceftolozane-tazobactam, doubling concentrations of ceftolozane were evaluated in combination with a fixed concentration of tazobactam (4 μg/ml). Antimicrobial MIC interpretive standards were defined according to CLSI breakpoints (18). At present, no breakpoints have been set for the combination ceftolozane-tazobactam. MDR *P. aeruginosa* isolates were defined as those isolates demonstrating resistance to at least one antimicrobial from 3 or more different classes. For the purpose of this report, the five antimicrobial classes considered were aminoglycosides (tobramycin), fluoroquinolones (ciprofloxacin), antipseudomonal cephalosporins (ceftazidime), antipseudomonal penicillins (piperacillin-tazobactam), and carbapenems (meropenem). Colistin was not used in the classification of MDR isolates.

In total, 2,435 *P. aeruginosa* isolates were obtained as part of the CANWARD Study, grouped as follows: (i) by year, 623 from 2007, 373 from 2008, 470 from 2009, 376 from 2010, 329 from 2011, and 264 from 2012; (ii) by specimen source, 61.6% respiratory, 21.3% blood, 11.4% wound, and 5.7% urine; and (iii) by ward type, 32.1% medical, 25.2% clinics, 21.9% intensive care units (ICUs), 13.0% emergency rooms (ERs), and 7.8% surgical. The antimicrobial susceptibility profile of these isolates is presented in Table 1. Ceftolozane-tazobactam demonstrated the lowest MIC_{90} value of all antipseudomonal antimicrobials evaluated, including colistin.

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TABLE 1 Antimicrobial susceptibility of 2,435 *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals from 2007 to 2012

	All isolates (n = 2,435)								
	MIC (μg/ml)[a]		Range of MIC values (μg/ml)		Breakpoint interpretation[b]			MDR isolates (n = 158)[c]	
Antimicrobial(s)	50%	90%	Minimum	Maximum	% S	% I	% R	MIC_{50}/MIC_{90} (μg/ml)	% S
Ceftazidime	4	32	≤0.25	>32	83.7	5.6	10.8	>32/>32	11.4
Ceftolozane-tazobactam	0.5	1	≤0.12	>64	ND[d]	ND	ND	2/16	ND
Ciprofloxacin	0.25	4	≤0.06	>16	77.9	7.9	14.2	4/>16	18.4
Colistin	1	2	0.12	>16	97.5	1.6	0.9	1/2	98.1
Meropenem	0.5	8	≤0.03	>32	83.5	6.4	10.1	8/>32	16.5
Piperacillin-tazobactam	4	32	≤1	>512	85.1	8.5	6.5	128/512	13.4
Tobramycin	≤0.5	2	≤0.5	>64	92.6	1.3	6.0	4/64	51.3

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] S, susceptible; I, intermediate; R, resistant. Breakpoint interpretations: ceftazidime, S ≤ 8 μg/ml, I = 16 μg/ml, R ≥ 32 μg/ml; ciprofloxacin, S ≤ 1 μg/ml, I = 2 μg/ml, R ≥ 4 μg/ml; colistin, S ≤ 2 μg/ml, I = 4 μg/ml, R ≥ 8 μg/ml; meropenem, S ≤ 2 μg/ml, I = 4 μg/ml, R ≥ 8 μg/ml; piperacillin-tazobactam, S ≤ 16/4 μg/ml, I = 32/4 to 64/4 μg/ml, R ≥ 128/4 μg/ml; tobramycin, S ≤ 4 μg/ml, I = 8 μg/ml, R ≥ 16 μg/ml.
[c] MDR, multidrug-resistant (resistant to at least one antimicrobial from 3 or more different classes).
[d] ND, breakpoints not defined.

The *in vitro* activity of ceftolozane-tazobactam against *P. aeruginosa* isolates susceptible or nonsusceptible to various antimicrobials is presented in Table 2.

One hundred fifty-eight isolates (6.5%) were MDR. The MIC_{50} and MIC_{90} values for ceftolozane-tazobactam and ceftazidime versus the MDR isolates were 2 μg/ml and 16 μg/ml, and >32 μg/ml and >32 μg/ml, respectively (Table 1). The most common MDR phenotypes were resistance to ceftazidime, piperacillin-tazobactam, and meropenem (phenotype 1; n = 35), resistance to meropenem, tobramycin, and ciprofloxacin (phenotype 2; n = 25), and resistance to ceftazidime, piperacillin-tazobactam, meropenem, and ciprofloxacin (phenotype 3; n = 21). The ceftolozane-tazobactam MIC_{50} and MIC_{90} values for MDR isolates demonstrating resistance phenotypes 1, 2, and 3 were 2 and 8 μg/ml, 1 and 2 μg/ml, and 2 and 4 μg/ml, respectively.

Previously published studies have demonstrated excellent *in vitro* activity of ceftolozane, with or without tazobactam, against *P. aeruginosa*. In general, MIC_{90} values for ceftolozane-tazobac-

TABLE 2 *In vitro* activity of ceftolozane-tazobactam against antimicrobial-susceptible and nonsusceptible *P. aeruginosa* isolates

	No. of isolates with ceftolozane-tazobactam MIC (μg/ml) of[a]:								Total no. of isolates
Category of isolates (n)	≤0.25	0.5	1	2	4	8	16	>16	
All isolates (2,435)	204 (8.4)	1,414 (66.4)	584 (90.4)	137 (96.1)	56 (98.4)	16 (99.0)	9 (99.4)	15 (100.0)	2,435
Ceftazidime									
Susceptible (2,037)	203 (10.0)	1,380 (77.7)	404 (97.5)	44 (99.7)	2 (99.8)	2 (99.9)	1 (99.9)	1 (100.0)	2,037
Nonsusceptible (398)	1 (0.3)	34 (8.8)	180 (54.0)	93 (77.4)	54 (91.0)	14 (94.5)	8 (96.5)	14 (100.0)	398
Ciprofloxacin									
Susceptible (1,898)	186 (9.8)	1,237 (75.0)	376 (94.8)	68 (98.4)	23 (99.6)	6 (99.9)	1 (99.9)	1 (100.0)	1,898
Nonsusceptible (537)	18 (3.4)	177 (36.3)	208 (75.0)	69 (87.9)	33 (94.0)	10 (95.9)	8 (97.4)	14 (100.0)	537
Colistin									
Susceptible (2,375)	200 (8.4)	1,378 (66.4)	570 (90.4)	133 (96.0)	56 (98.4)	16 (99.1)	9 (99.5)	13 (100.0)	2,375
Nonsusceptible (60)	4 (6.7)	36 (66.7)	14 (90.0)	4 (96.7)	0 (96.7)	0 (96.7)	0 (96.7)	2 (100.0)	60
Meropenem									
Susceptible (2,034)	197 (9.7)	1,308 (74.0)	420 (94.6)	68 (98.0)	22 (99.1)	9 (99.5)	2 (99.6)	8 (100.0)	2,034
Nonsusceptible (401)	7 (1.7)	106 (28.2)	164 (69.1)	69 (86.3)	34 (94.8)	7 (96.5)	7 (98.3)	7 (100.0)	401
Piperacillin-tazobactam									
Susceptible (2,071)	203 (9.8)	1,383 (76.6)	419 (96.8)	51 (99.3)	4 (99.5)	3 (99.6)	2 (99.7)	6 (100.0)	2,071
Nonsusceptible (364)	1 (0.3)	31 (8.8)	165 (54.1)	86 (77.7)	52 (92.0)	13 (95.6)	7 (97.5)	9 (100.0)	364
Tobramycin									
Susceptible (2,256)	198 (8.8)	1,386 (70.2)	515 (93.0)	94 (97.2)	44 (99.2)	13 (99.7)	3 (99.9)	3 (100.0)	2,256
Nonsusceptible (179)	6 (3.4)	28 (19.0)	69 (57.5)	43 (81.6)	12 (88.3)	3 (89.9)	6 (93.4)	12 (100.0)	179
MDR (158)	0 (0.0)	3 (1.9)	48 (32.3)	46 (61.4)	38 (85.4)	6 (89.2)	7 (93.7)	10 (100.0)	158

[a] MICs were determined by broth microdilution. Values in parentheses represent the cumulative percentage for all isolates tested.

tam have been reported to be 8- to 16-fold lower than those for ceftazidime, irrespective of whether ceftazidime-susceptible or ceftazidime-resistant isolates were evaluated (11, 20). The data presented here are in agreement with these results. This report serves to expand on the previous literature by providing further evaluation of ceftolozane-tazobactam versus a random collection of *P. aeruginosa* clinical isolates obtained across a large geographic area (the country Canada).

There are several limitations to this study that deserve attention. Ceftolozane was not tested in the absence of tazobactam, because of limited space on the antimicrobial susceptibility panels. Hence, the value of adding tazobactam to ceftolozane could not be ascertained from these data. Previous publications suggest that the contribution of tazobactam to the activity of ceftolozane against *P. aeruginosa*, if any, is minimal (11, 20). The mechanisms conferring resistance to the antipseudomonal antimicrobials evaluated here were also not investigated.

In summary, the combination of ceftolozane and tazobactam demonstrated excellent *in vitro* activity (low MIC_{50} and MIC_{90} values) against a large collection of 2,435 *P. aeruginosa* clinical isolates obtained from across Canada. The MIC_{90} value for the combination ceftolozane-tazobactam was 32-fold lower than that for ceftazidime. Furthermore, 89.2% of MDR *P. aeruginosa* isolates were inhibited by ≤8 μg/ml of ceftolozane-tazobactam (Table 2). These *in vitro* data suggest that ceftolozane-tazobactam may prove useful in the future treatment of infections caused by *P. aeruginosa*, including MDR strains resistant to piperacillin-tazobactam, antipseudomonal cephalosporins, and carbapenems.

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