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In Vitro Activity of Ceftolozane/Tazobactam (CXA-201) versus *Pseudomonas aeruginosa* Isolates Obtained from Patients in Canadian Hospitals: CANWARD 2011

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Abstract

Background: Cephalosporin resistance among *P. aeruginosa* isolates is mediated, in part, by production of an AmpC beta-lactamase. Ceftolozane, a novel anti-pseudomonal cephalosporin, demonstrates reduced affinity for AmpC beta-lactamases and improved binding to *P. aeruginosa* penicillin binding proteins relative to ceftazidime. The purpose of this study was to evaluate the *in vitro* activity of ceftolozane in combination with tazobactam (ceftolozane/tazobactam: formerly CXA-201) versus *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals.
Methods: From January through October 2011, 15 sentinel Canadian hospitals submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units (CANWARD). Each centre was asked to submit pathogens (consecutive, one per patient/infection site) from blood (100), respiratory (100), urine (25), and wound/IV (25) infections. Susceptibility testing was performed using CLSI broth microdilution. Doubling concentrations of ceftolozane were evaluated in combination with a fixed concentration of tazobactam (4 µg/mL).
Results: In total, 330 *P. aeruginosa* isolates were obtained as a part of CANWARD. The antimicrobial susceptibility profile of these isolates is presented below.

Antimicrobial	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range	Susceptibility Breakpoint	% Susceptible
Amikacin	4	8	≤1 to >64	≤16	94.6
Cefepime	4	16	≤0.25 to >64	≤8	86.1
Ceftazidime	2	32	≤0.25 to >32	≤8	85.2
Ceftolozane/ Tazobactam	0.5	1	≤0.12 to >64	Not defined	Not defined
Ciprofloxacin	0.25	4	≤0.06 to >16	≤1	78.2
Colistin	1	2	0.25 to 16	≤2	95.5
Gentamicin	1	8	≤0.5 to >32	≤4	88.2
Meropenem	0.5	4	≤0.03 to >32	≤2	84.2
Piperacillin/ Tazobactam	4	64	≤1 to >512	≤16/4	84.6

Twenty-three isolates (7.0%) were multidrug-resistant (MDR = resistant to at least 3 different antimicrobial classes). The MIC_{90} values for ceftolozane/tazobactam, cefepime, and ceftazidime versus the MDR isolates were 8 µg/mL, 32 µg/mL, and >32 µg/mL, respectively.
Conclusion: Ceftolozane/tazobactam demonstrated improved *in vitro* activity (16-32 fold lower MIC_{90} value) over ceftazidime and cefepime versus a collection of 330 *P. aeruginosa* clinical isolates. Over 90% of MDR *P. aeruginosa* isolates were inhibited by ≤8 µg/mL of ceftolozane/tazobactam.

Background

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 oxyimino-aminothiazolyl cephalosporin currently under development that could prove useful in the treatment of infections caused by multidrug-resistant (MDR) *P. aeruginosa* [11-13]. Resistance to beta-lactams among *P. aeruginosa* may be mediated by the overproduction of an AmpC beta-lactamase, reduction in cell permeability (OprD loss), up-regulation of efflux pumps, and/or the acquisition of extended-spectrum or metallo-beta-lactamses [14]. Ceftolozane demonstrates reduced affinity for the AmpC beta-lactamase of *P. aeruginosa* and improved binding to *P. aeruginosa* penicillin binding proteins relative to ceftazidime [15, 16]. Further, 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 (ceftolozane/tazobactam, formerly CXA-201) versus *P. aeruginosa* clinical isolates obtained from patients in Canadian hospitals.

Materials and Methods

Bacterial Isolates: Fifteen tertiary-care medical centers 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). The sites were geographically distributed in a population-based fashion. From January through October 2011, inclusive, each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with bloodstream (n = 100), respiratory (n = 100), urine (n = 25), and wound/IV (n = 25) 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 semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at -80°C until minimum inhibitory concentration (MIC) testing was carried out.
Antimicrobial Susceptibilities: Following 2 subcultures from frozen stock, the *in vitro* activity of commonly used antipseudomonal antimicrobials was 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 minimum inhibitory concentration (MIC) interpretive standards were defined according to CLSI breakpoints [18]. At present, no breakpoints have been set for the combination of ceftolozane and tazobactam. Multidrug-resistant (MDR) *P. aeruginosa* isolates were defined as 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 (amikacin, gentamicin), fluoroquinolones (ciprofloxacin), antipseudomonal cephalosporins (ceftazidime, cefepime), antipseudomonal penicillins (piperacillin/tazobactam), and carbapenems (meropenem). Colistin was not used in the classification of MDR isolates.

Results

In total, 330 *P. aeruginosa* isolates were obtained as a part of CANWARD. The antimicrobial susceptibility profile of these isolates is presented in Table 1. Figure 1 contrasts the *in vitro* activity of ceftolozane/tazobactam with that of other antipseudomonal cephalosporins (cefepime, ceftazidime). The *in vitro* activity of ceftolozane/tazobactam versus *P. aeruginosa* isolates resistant to various antimicrobials is presented in Table 2.
Twenty-three isolates (7.0%) were multi-drug resistant (MDR = resistant to at least 3 different antimicrobial classes). The MIC_{90} values for ceftolozane/tazobactam, cefepime, and ceftazidime versus the MDR isolates were 8 µg/mL, 32 µg/mL, and >32 µg/mL, respectively.

Table 1. Antimicrobial Susceptibility of 376 *P. aeruginosa* Clinical Isolates Obtained from Patients in Canadian Hospitals (2011)

	All Isolates (n = 330)							MDR (n = 23)	
	MIC (µg/mL)		Range of Values		Breakpoint Interpretations				
Antimicrobial	MIC_{50}	MIC_{90}	Min	Max	% S	% I	% R	MIC_{90} (µg/mL)	% S
Amikacin	4	8	</=1	>64	94.6	2.7	2.7	64	78.3
Cefepime	4	16	</=0.25	>64	86.1	11.8	2.1	32	26.1
Ceftazidime	2	32	</=0.25	>32	85.2	4.2	10.6	>32	17.4
Ceftolozane/Tazobactam	0.5	1	</=0.12	>64	n.d.	n.d.	n.d.	8	n.d.
Ciprofloxacin	0.25	4	</=0.06	>16	78.2	8.5	13.3	>16	13.0
Colistin	1	2	0.25	16	95.5	1.5	3.0	2	95.7
Gentamicin	1	8	</=0.5	>32	88.2	4.6	7.3	>32	39.1
Meropenem	0.5	4	</=0.03	>32	84.2	6.1	9.7	>32	17.4
Piperacillin/ Tazobactam	4	64	</=1	>512	84.6	8.8	6.7	512	17.4

a: % S = % susceptible, % I = % intermediate, % R = % resistant; Breakpoint Interpretation: Amikacin S ≤16 µg/mL, I = 32 µg/mL, R ≥64 µg/mL, Cefepime S ≤8 µg/mL, I = 16 µg/mL, R ≥32 µg/mL, 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, Gentamicin S ≤4 µg/mL, I = 8 µg/mL, R ≥16 µg/mL, Meropenem S ≤2 µg/mL, I = 4 µg/mL, R ≥8 µg/mL, Piperacillin/Tazobactam S ≤16/4 µg/mL, I = 32/4-64/4 µg/mL, R ≥128/4 µg/mL
b: MDR = multi-drug resistant – resistant to at least one antimicrobial from 3 or more different classes
n.d. = Breakpoints not defined

Figure 1. *In vitro* Susceptibility of 330 *P. aeruginosa* Clinical Isolates to Ceftolozane/Tazobactam in Comparison with Ceftazidime and Cefepime

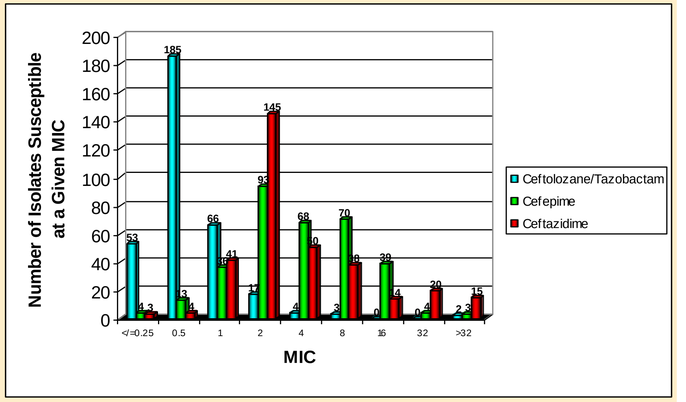

Table 2. In vitro Activity of Ceftolozane/Tazobactam Versus Antimicrobial Resistant *P. aeruginosa* Isolates

	Ceftolozane/Tazobactam - Microbroth Dilution Value (µg/mL) Number of isolates with MIC (Cumulative % of all Isolates Tested)								
***P. aeruginosa* (number of isolates)**	</=0.25	0.5	1	2	4	8	16	>16	Total
All Isolates (n = 330)	53 (16.1)	185 (72.1)	66 (92.1)	17 (97.3)	4 (98.5)	3 (99.4)	0 (99.4)	2 (100.0)	330
Amikacin Resistant (n = 9)	3 (33.3)	0 (33.3)	1 (44.4)	3 (77.8)	0 (77.8)	0 (77.8)	0 (77.8)	2 (100.0)	9
Cefepime Resistant (n = 7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)	2 (57.1)	2 (85.7)	0 (85.7)	1 (100.0)	7
Ceftazidime Resistant (n = 35)	0 (0.0)	1 (2.9)	15 (45.7)	11 (77.1)	3 (85.7)	3 (94.3)	0 (94.3)	2 (100.0)	35
Ciprofloxacin Resistant (n = 44)	2 (4.5)	18 (45.5)	14 (77.3)	5 (88.6)	2 (93.2)	1 (95.5)	0 (95.5)	2 (100.0)	44
Gentamicin Resistant (n = 24)	4 (16.7)	4 (33.3)	8 (66.6)	5 (87.5)	1 (91.7)	1 (95.8)	0 (95.8)	1 (100.0)	24
Meropenem Resistant (n = 32)	0 (0.0)	4 (12.5)	16 (62.5)	8 (87.5)	2 (93.8)	1 (96.9)	0 (96.9)	1 (100.0)	32
Piperacillin/Tazobactam Resistant (n = 22)	0 (0.0)	0 (0.0)	7 (31.8)	10 (77.3)	3 (90.9)	2 (100.0)	0 (100.0)	0 (100.0)	22
MDR Isolates[a] (n = 23)	0 (0.0)	2 (8.7)	6 (34.8)	8 (69.6)	3 (82.6)	2 (91.3)	0 (91.3)	2 (100.0)	23

a: MDR = multi-drug resistant – resistant to at least one antimicrobial from 3 or more different classes

Conclusions

- Ceftolozane/tazobactam demonstrated improved *in vitro* activity (16-32 fold lower MIC_{90} value) over ceftazidime and cefepime versus a collection of 330 *P. aeruginosa* clinical isolates. Further, ceftazidime/tazobactam had the lowest MIC_{90} value (1 µg/mL) of all antipseudomonal antimicrobials evaluated.
- 94.3% and 85.7% of ceftazidime and cefepime resistant isolates were inhibited by ≤8 µg/mL of ceftolozane/tazobactam, respectively.
- Over 90% of MDR *P. aeruginosa* isolates were inhibited by ≤8 µg/mL of ceftolozane/tazobactam.

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