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Ceftolozane-Tazobactam Combined with Colistin or Tobramycin Produces Synergistic Inhibition of Multidrug-Resistant (MDR) Clinical Isolates of *Pseudomonas aeruginosa* Obtained from the CANWARD Surveillance Study 2007-2017

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Introduction

Ceftolozane-tazobactam is a novel β-lactam/β-lactamase inhibitor combination with excellent *in vitro* activity against a diverse range of Gram-negative bacilli, including *Pseudomonas aeruginosa* (1). This antimicrobial is able to circumvent many of the common resistance mechanisms present in *P. aeruginosa*, supporting its role in the treatment of infections caused by antimicrobial-resistant *P. aeruginosa* isolates, including multidrug-resistant (MDR) and extremely-resistant (XDR) strains (1-3). We recently reported that ceftolozane-tazobactam demonstrated excellent *in vitro* activity versus antimicrobial non-susceptible *P. aeruginosa* clinical isolates, including MDR and XDR subsets from our national, Canadian Antimicrobial Resistance Alliance-CARA/Health Canada partnered surveillance study CANWARD (2-4). The microbiological/clinical efficacy of ceftolozane-tazobactam in treating antimicrobial non-susceptible *P. aeruginosa* infections is further supported by limited published clinical series, although further data are needed (2, 3).

Infections caused by MDR/XDR *P. aeruginosa* are frequently treated with ceftolozane-tazobactam in combination with either colistin or an aminoglycoside such as tobramycin or amikacin (5-7). The purpose of this study was to evaluate *in vitro* combinations of colistin or tobramycin with ceftolozane-tazobactam against XDR *P. aeruginosa* clinical isolates across Canada.

Materials and Methods

Participating Sites: CANWARD (a collaboration between the Canadian Antimicrobial Resistance Alliance-CARA and the National Microbiology Laboratory-NML of Health Canada) is a national ongoing surveillance study which assess pathogens and associated antimicrobial resistance patterns in Canadian hospitalized patients. From January 2007 to October 2017, sentinel hospital sites (12 in 2007, 10 in 2008, 15 in 2009, 14 in 2010, 15 in 2011, 12 in 2012, 15 in 2013, 13 in 2014, 13 in 2015, 13 in 2016, 14 in 2017) in major population centres in 8 of the 10 provinces in Canada were recruited (1,2,4). These sites were geographically distributed in a population based fashion: (BC [1 site], Alberta [1 site], Saskatchewan [1 site], Manitoba [1 site], Ontario [3-5 sites], Quebec [2-4 sites], Maritimes [1-2 sites]).

Bacterial Isolates: Tertiary-care medical centres submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. From January 2007 through October 2017, inclusive, each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with respiratory, urine, wound, and bloodstream infections. The medical centres submitted "clinically significant" isolates from patients with a presumed infectious disease. Surveillance swabs, eye, ear, nose and throat swabs were excluded. We also excluded anaerobic organisms. Isolate identification was performed by the submitting site and confirmed at the reference site as required, based on morphological characteristics and antimicrobial susceptibility patterns. 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 -80ºC until minimum inhibitory concentration (MIC) testing was carried out. From the 46,356 total isolates collected, only *P. aeruginosa* deemed to be XDR (concomitant non-susceptibility to ceftazidime, ciprofloxacin, meropenem and piperacillin/tazobactam) were selected. Forty-one such isolates were available for this study.

Antimicrobial Susceptibilities: The *in vitro* activity of selected antimicrobials was determined by broth microdilution in accordance with CLSI guidelines (8) using custom- designed microtitre plates. Antimicrobial agents were obtained as laboratory grade powders from their respective manufacturers or commercial sources. Minimum inhibitory concentration (MIC) interpretive standards were defined according to CLSI breakpoints (9).

Combination MIC: Testing was performed with ceftolozane-tazobactam, colistin and tobramycin alone and with combinations of ceftolozane-tazobactam and fixed concentrations of colistin (2 μg/mL) or tobramycin (8 μg/mL). Combinations of ceftolozane-tazobactam and colistin or tobramycin were tested using checkerboard assays with fractional inhibitory concentration indices (FICIs) defined as synergistic ≤0.5, no interaction/indifferent FICI >0.5 - ≤4.0, and antagonistic FICI >4.0.

Results

Table 1. Activity of ceftolozane-tazobactam and comparators versus 41 (XDR) *Pseudomonas aeruginosa* obtained from the CANWARD study

Isolate #	MIC (μg/mL)											
	CEFTOL-TAZO	AMK	TOB	AZT	CAZ	CEF	BPR	CIP	COL	IMI	MER	PIP-TAZO
CW-1	1	8	> 64	32	> 32	16	16	> 16	2	32	32	128
CW-2	1	8	≤ 0.5	32	32	16	8	4	1	16	16	128
CW-3	1	8	1	16	16	8	16	16	1	32	8	64
CW-4	1	4	≤ 0.5	16	32	8	4	16	2	> 32	8	64
CW-5	2	16	2	64	32	32	32	8	1	4	16	128
CW-6	2	32	2	16	> 32	8	8	2	2	32	16	256
CW-7	2	16	1	32	> 32	16	16	4	1	16	16	256
CW-8	2	16	1	32	> 32	16	8	2	2	16	16	128
CW-9	2	16	1	32	> 32	16	> 32	2	1	32	16	256
CW-10	2	16	1	16	> 32	16	8	> 16	1	32	8	128
CW-11	2	16	2	> 64	> 32	32	16	> 16	1	16	32	256
CW-12	2	32	2	32	> 32	16	8	8	1	16	16	256
CW-13	2	16	> 64	32	> 32	16	16	> 16	1	16	32	128
CW-14	2	4	≤ 0.5	32	> 32	16	16	4	2	16	16	256
CW-15	2	4	≤ 0.5	64	> 32	16	8	4	1	2	4	256
CW-16	2	32	2	16	> 32	16	16	2	2	32	16	128
CW-17	2	8	1	32	> 32	16	16	16	1	16	32	256
CW-18	2	16	1	32	> 32	32	16	> 16	1	16	16	256
CW-19	2	4	≤ 0.5	64	> 32	16	8	4	2	16	32	256
CW-20	2	16	1	64	32	16	16	16	1	16	16	256
CW-21	2	16	> 64	64	> 32	32	32	> 16	1	32	32	256
CW-22	2	16	> 64	64	> 32	16	32	8	1	32	32	256
CW-23	2	16	> 64	64	> 32	32	32	> 16	1	32	32	256
CW-24	2	32	4	16	32	16	16	2	1	32	8	64
CW-25	2	32	2	32	> 32	16	16	2	2	16	8	256
CW-26	2	4	≤ 0.5	64	> 32	16	8	2	1	16	8	256
CW-27	2	≤ 1	8	16	> 32	16	8	16	1	32	16	128
CW-28	2	8	≤ 0.5	64	> 32	16	32	8	0.5	32	32	256
CW-29	2	16	64	32	> 32	32	32	> 16	4	8	16	128
CW-30	2	32	> 64	32	> 32	32	32	> 16	1	16	32	128
CW-31	4	> 64	> 64	2	> 32	32	32	2	1	32	16	256
CW-32	4	16	1	> 64	> 32	64	32	> 16	1	16	> 32	512
CW-33	4	8	≤ 0.5	64	> 32	16	16	16	1	4	4	256
CW-34	4	2	≤ 0.5	64	> 32	16	16	4	1	16	32	256
CW-35	4	16	2	64	> 32	32	32	2	1	16	16	512
CW-36	4	8	1	32	> 32	16	8	4	1	16	8	256
CW-37	4	> 64	8	> 64	> 32	32	> 32	4	4	> 32	> 32	32
CW-38	8	16	1	64	> 32	16	8	4	1	8	8	256
CW-39	8	4	≤ 0.5	> 64	> 32	32	8	2	1	4	8	512
CW-40	32	> 64	> 64	> 64	> 32	> 64	> 32	4	0.5	32	> 32	> 512
CW-41	> 64	64	> 64	8	> 32	64	> 32	> 16	1	> 32	> 32	256

Yellow color designates intermediate susceptibility (non-susceptible); Orange color designates resistance

Table 2. Activity of combinations of ceftolozane-tazobactam and colistin or ceftolozane-tazobactam and tobramycin versus 41 XDR *Pseudomonas aeruginosa* obtained from the CANWARD study

Phenotype/ Isolate #	Ceftol-Tazo MIC	Colistin MIC	Ceftol-Tazo/ Colistin[a] MIC	Interpretation	Ceftol-Tazo MIC	Tobramycin MIC	Ceftol-Tazo/ Tobramycin[b] MIC	Interpretation
Ceftol-Tazo Susceptible								
CW-1	1	2	≤ 0.12	S	1	> 64	2	I
CW-2	1	1	≤ 0.12	S	1	≤ 0.5	≤ 0.12	S
CW-3	1	1	≤ 0.12	S	1	1	≤ 0.12	S
CW-4	1	2	≤ 0.12	S	1	≤ 0.5	≤ 0.12	S
CW-5	2	1	≤ 0.12	S	2	2	≤ 0.12	S
CW-6	2	2	≤ 0.12	S	2	2	≤ 0.12	S
CW-7	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-8	2	2	≤ 0.12	S	2	1	≤ 0.12	S
CW-9	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-10	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-11	2	1	≤ 0.12	S	2	2	≤ 0.12	S
CW-12	2	1	≤ 0.12	S	2	2	≤ 0.12	S
CW-13	2	1	≤ 0.12	S	2	> 64	2	I
CW-14	2	2	≤ 0.12	S	2	≤ 0.5	≤ 0.12	S
CW-15	2	1	≤ 0.12	S	2	≤ 0.5	≤ 0.12	S
CW-16	2	2	≤ 0.12	S	2	2	≤ 0.12	S
CW-17	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-18	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-19	2	2	≤ 0.12	S	2	≤ 0.5	≤ 0.12	S
CW-20	2	1	≤ 0.12	S	2	1	≤ 0.12	S
CW-21	2	1	≤ 0.12	S	2	> 64	4	I
CW-22	2	1	≤ 0.12	S	2	> 64	4	I
CW-23	2	1	≤ 0.12	S	2	> 64	4	I
CW-24	2	1	≤ 0.12	S	2	4	≤ 0.12	S
CW-25	2	2	≤ 0.12	S	2	2	≤ 0.12	S
CW-26	2	1	≤ 0.12	S	2	≤ 0.5	≤ 0.12	S
CW-27	2	1	≤ 0.12	S	2	8	≤ 0.12	S
CW-28	2	0.5	≤ 0.12	S	2	≤ 0.5	≤ 0.12	S
CW-29	2	4	≤ 0.12	S	2	64	1	I
CW-30	2	1	≤ 0.12	S	2	> 64	2	I
CW-31	4	1	≤ 0.12	S	4	> 64	2	I
CW-32	4	1	≤ 0.12	S	4	1	≤ 0.12	S
CW-33	4	1	≤ 0.12	S	4	≤ 0.5	≤ 0.12	S
CW-34	4	1	≤ 0.12	S	4	≤ 0.5	≤ 0.12	S
CW-35	4	1	≤ 0.12	S	4	2	≤ 0.12	S
CW-36	4	1	0.25	S	4	1	≤ 0.12	S
CW-37	4	4	≤ 0.12	S	4	8	≤ 0.12	S
Ceftol-Tazo Intermediate								
CW-38	8	1	≤ 0.12	S	8	1	≤ 0.12	S
CW-39	8	1	≤ 0.12	S	8	≤ 0.5	≤ 0.12	S
Ceftol-Tazo Resistant								
CW-40	32	0.5	≤ 0.12	S	32	> 64	32	I
CW-41	> 64	1	≤ 0.12	S	> 64	> 64	> 64	I
Colistin Resistant								
CW-29	2	4	≤ 0.12	S	2	64	0.5	I
CW-37	4	4	≤ 0.12	S	4	8	2	I
Tobramycin Intermediate								
CW-27	2	1	≤ 0.12	S	2	8	≤ 0.12	S
CW-37	2	1	≤ 0.12	S	2	8	2	I
Tobramycin Resistant								
CW-1	1	2	≤ 0.12	S	1	> 64	2	I
CW-13	2	1	≤ 0.12	S	2	> 64	2	I
CW-21	2	1	≤ 0.12	S	2	> 64	4	I
CW-22	2	1	≤ 0.12	S	2	> 64	4	I
CW-23	2	1	≤ 0.12	S	2	> 64	4	I
CW-29	2	4	≤ 0.12	S	2	64	1	I
CW-30	2	1	≤ 0.12	S	2	> 64	2	I
CW-31	4	1	≤ 0.12	S	4	> 64	2	I
CW-40	32	0.5	≤ 0.12	S	32	> 64	32	I
CW-41	> 64	1	≤ 0.12	S	> 64	> 64	> 64	I

Yellow color designates intermediate susceptibility (non-susceptible); Orange color designates resistance; S = synergistic; I = intermediate or additive;
[a]Colistin constant at 2 μg/mL; [b]Tobramycin constant at 8 μg/mL

Conclusions

1. Against colistin susceptible isolates, colistin combined with ceftolozane-tazobactam demonstrated synergy versus XDR *P. aeruginosa*.
2. Against two colistin resistant isolates, colistin combined with ceftolozane-tazobactam demonstrated synergy versus XDR *P. aeruginosa.*
3. Against tobramycin susceptible isolates, tobramycin combined with ceftolozane-tazobactam demonstrated synergy versus XDR *P. aeruginosa.*
4. Indifference was achieved between tobramycin and ceftolozane-tazobactam versus XDR *P. aeruginosa* when the isolate was resistant to tobramycin.

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