E – 1689

In Vitro Activity of Ceftolozane/Tazobactam Tested Against 5715 Gram-negative and Gram-positive Pathogens Isolated From Patients in Canadian Hospitals in 2011 and 2012: CANWARD Surveillance Study

G. G. ZHANEL[1], H. J. ADAM[1,2], M. BAXTER[1], A. WALKTY[1,2], P. R. S. LAGACÉ-WIENS[1,3], A. DENISUIK[1], B. WESHNOWESKI[2], R. VASHISHT[1], D. J. HOBAN[1,2] and J. A. KARLOWSKY[1,2]

University of Manitoba[1], Health Sciences Centre[2], and St. Boniface Hospital[3], Winnipeg, Manitoba, Canada

Dr. G. G. Zhanel
Health Sciences Centre
MS673 - 820 Sherbrook St.
Winnipeg, MB, Canada R3A 1R9
(204) 787-4902
ggzhanel@pcs.mb.ca

ABSTRACT (REVISED)

Background: Ceftolozane/tazobactam is a novel cephalosporin and β-lactamase inhibitor with activity against *Pseudomonas aeruginosa*, including drug-resistant strains, and other common Gram-negative pathogens including most ESBL-producing Enterobacteriaceae. Ceftolozane/tazobactam is currently in Phase 3 trials for the treatment of cUTI and cIAI. Studies in nosocomial pneumonia are also planned. We determined the in vitro activity of ceftolozane/tazobactam against Gram-negative and Gram-positive pathogens isolated from patients at Canadian hospitals from January 2011 to December 2012.

Methods: Antimicrobial susceptibility testing was performed using in-house broth microdilution panels following the method recommended by CLSI. Tazobactam was tested at a fixed concentration of 4 μg/mL in combination with doubling dilutions of ceftolozane.

Results: The activity of ceftolozane/tazobactam and comparators versus Gram-negative bacilli and select Gram-positive cocci is summarized below.

Organism (n)	MIC_{50}/MIC_{90} (μg/mL)						
	TOL/TAZ	TZP	CAZ	IPM	CST	CIP	TGC
P. aeruginosa (593)	0.5/1	4/64	4/32	2/16	2/2	0.25/8	16/>16
E. coli-All (1146)	≤0.12/0.25	2/4	≤0.5/1	0.12/0.25	0.25/0.5	≤0.06/>16	0.25/0.5
E. coli-ESBL (84)	0.25/1	4/16	16/>32	0.12/0.25	0.5/1	>16/>16	0.5/1
K. pneumoniae (395)	0.25/0.5	2/4	≤0.5/1	0.25/0.25	0.52/1	≤0.06/0.5	1/2
E. cloacae (173)	0.25/8	2/64	0.5/>32	0.25/0.5	0.25/>16	≤0.06/0.25	1/1
S. marcescens (109)	0.5/1	≤1/4	≤0.5/1	0.5/1	>16/>16	≤0.06/1	2/4
P. mirabilis (85)	0.25/0.5	≤1/≤1	≤0.5/≤0.5	2/4	>16/>16	≤0.06/2	8/16
K. oxytoca (113)	≤0.12/0.5	2/128	≤0.5/0.5	0.25/0.25	0.5/1	≤0.06/0.12	0.5/1
E. aerogenes (55)	0.25/2	4/32	0.5/>32	0.25/1	0.5/1	≤0.06/0.5	1/2
C. freundii (24)	≤0.12/≤0.12	2/16	0.5/>32	0.25/0.5	0.25/0.5	≤0.06/0.5	0.5/1
M. morganii (21)	≤0.12/0.25	≤1/≤1	≤0.5/4	2/4	>16/>16	≤0.06/2	2/8
S. maltophilia (104)	32/>64	256/>512	>32/>32	>32/>32	8/>16	2/16	2/8
A. baumannii (26)	0.5/2	2/128	8/32	0.25/0.5	1/2	0.25/2	0.5/1
H. influenzae (111)	≤0.12/≤0.12	≤1/≤1	NA	0.25/1	NA	≤0.015/≤0.015	0.25/2
S. aureus (1208)	16/32	≤1/≤1	16/16	≤0.03/≤0.03	>16/>16	0.5/8	0.12/0.5
S. pneumoniae (321)	≤0.12/1	≤1/≤1	NA	≤0.03/≤0.03	NA	1/2	0.03/0.06

NA, not available; TZP, piperacillin/tazobactam; CAZ, ceftazidime; IPM, imipenem; CST, colistin; CIP, ciprofloxacin; TGC, tigecycline.

Conclusions: Ceftolozane/tazobactam demonstrated potent in vitro activity against recent clinical isolates of Enterobacteriaceae, nonfermentative Gram-negative bacilli, *Haemophilus influenzae* and *S. pneumoniae*. Ceftolozane/tazobactam was the most potent anti-pseudomonal agent tested followed by colistin. Ceftolozane/tazobactam demonstrates potential for the treatment of infections caused by resistant Gram-negative bacilli.

INTRODUCTION

Ceftolozane/tazobactam consists of a novel cephalosporin and a well established β-lactamase inhibitor, and has activity against *Pseudomonas aeruginosa*, including drug-resistant strains, and other common Gram-negative pathogens, including most extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae [1]. There are currently 2 ongoing Phase III clinical trials evaluating ceftolozane/tazobactam in the settings of complicated urinary tract infections (cUTIs) and complicated intra-abdominal infections (cIAI) (http://clinicaltrials.gov, identifiers NCT01345929, NCT01345955, NCT01445665, and NCT01445678). Additional Phase 3 clinical trials are scheduled to be conducted to evaluate the role of ceftolozane/tazobactam in nosocomial pneumonia and ventilator-associated pneumonia (VAP).

PURPOSE

To determine the in vitro activity of ceftolozane/tazobactam along with comparators versus Gram-negative pathogens isolated from patients in Canadian hospitals from January 2011 to December 2012.

MATERIALS & METHODS

Study Background and Bacterial Isolates:
The isolates tested in this study were obtained from January 2011 to December 2012, inclusive, from an ongoing cross-Canada surveillance study (CANWARD; www.can-r.ca) organized by the investigators [2,3].
The goal of the CANWARD study was to assess pathogens and antimicrobial resistance patterns associated with lower respiratory tract, skin/skin structure, urinary, and bacteremic infections in Canadian patients on medical wards, surgical wards, intensive care units, and presenting to emergency rooms and hospital clinics [2,3].

Antimicrobial Susceptibility Testing Methodology:
Isolates were tested for antimicrobial susceptibilities using in-house prepared (Department of Clinical Microbiology, Health Sciences Centre, Winnipeg, Canada) 96-well broth microdilution panels according to Clinical Laboratory Standards Institute (CLSI) M100-S21 (2011) guidelines [2,3]. The antimicrobial agents tested were obtained as laboratory-grade powders from their respective manufacturers. Tazobactam was tested at a fixed concentration of 4 μg/mL in combination with doubling dilutions of ceftolozane. Stock solutions were prepared and dilutions made, as described by the CLSI (M07-A8, 2009), in cation-adjusted Mueller-Hinton broth (MHB). Following 2 subcultures from frozen stock, the minimum inhibitory concentrations (MICs) of the antimicrobial agents for the isolates were determined by the CLSI broth microdilution method. Colony counts were performed periodically to confirm inocula. Quality control was performed using CLSI recommended (M100-S21) American Type Culture Collection (ATCC) organisms, including: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

ACKNOWLEDGMENTS

The authors would like to thank the investigators and laboratory site staff at each medical centre that participated in the CANWARD study. The medical centres (investigators) were: Vancouver Hospital, Vancouver, BC (Dr. D. Roscoe); University of Alberta Hospital, Edmonton, AB (Dr. J. Fuller); Royal University Hospital, Saskatoon, SK (Dr. J. Blondeau); Health Sciences Centre, Winnipeg, MB (Dr. D. Hoban/Dr. G. Zhanel); London Health Sciences Centre, London, ON (Dr. Z. Hussain); Mount Sinai Hospital, Toronto, ON (Dr. S. Poutanen); St. Michael's Hospital, Toronto, ON (Dr. L. Matukas); Children's Hospital of Eastern Ontario, Ottawa, ON (Dr. F. Chan); The Ottawa Hospital, Ottawa, ON (Dr. M. Desjardins); Royal Victoria Hospital, Montreal, QC (Dr. V. Loo); Montreal General Hospital, Montreal, QC (Dr. V. Loo); Hôpital Maisonneuve-Rosemont, Montreal, QC (Dr. M. Laverdière) ; CHRTR Pavilion Ste. Marie, Trois-Rivières, QC (Dr. M. Goyette); South East Regional Health Authority, Moncton, NB (Dr. M. Kuhn); Queen Elizabeth II HSC, Halifax, NS (Dr. R. Davidson).
Financial support for the CANWARD study was provided in part by the University of Manitoba, National Microbiology Laboratory and Cubist Pharmaceuticals, Inc.

REFERENCES

1. Miller B, Hershberger E, Benziger D, et al.. Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. *Antimicrob Agents Chemother* 2012;56(6):3086-3091.
2. Zhanel GG, DeCorby M, Adam H, et al. Prevalence of antimicrobial resistant pathogens in Canadian hospitals: Results of the Canadian ward surveillance study (CANWARD 2008). *Antimicrob Agents Chemother* 2010;54(11):4684-4693.
3. Zhanel GG, Adam HJ, Baxter MR, et al. Antimicrobial Susceptibility of 22,746 Pathogens from Canadian Hospitals: Results of the CANWARD 2007-2011 Study. *J Antimicrob Chemother* 2013;68 (Suppl 1):7-22.

RESULTS

Bacterial Isolates Collected
6365 clinical isolates were collected from 2011 to 2012.

- 2631 (41.3%), 2438 (38.3%), 672 (10.6%), 624 (9.8%) were from blood, respiratory sources, urine, and wounds, respectively.
- 3515 (55.2%) were collected from male patients; 895 (14%) were from patients aged ≤17 years, 2736 (43.0%) from patients aged 18-64 years, and 2734 (43%) from patients aged ≥65 years
- 1805 (28.3%) were from patients on medical wards, 1557 (24.5%) from emergency rooms, 1423 (22.4%) from intensive care units, 1122 (17.6%) from hospital clinics, and 458 (7.2%) from surgical wards

Table 1. ***In vitro*** **activity of Ceftolozane/tazobactam and comparators versus Gram-negative bacilli**

Organism (n)	MIC_{50} / MIC_{90} / Range / %S							
	Ceftolozane-Tazobactam	Piperacillin-Tazobactam	Ceftazidime	Imipenem	Colistin	Ciprofloxacin	Tigecycline[a]
E. coli-All (1146)	≤0.12 / 0.25 / ≤0.12-32 / NA	≤1 / 4 / ≤1->512 / 97.7	≤0.25 / 2 / ≤0.25->32 / 92.9	0.12 / 0.25 / ≤0.03-2 / 99.9	0.25 / 0.5 / ≤0.06->16 / NA	≤0.06 / >16 / ≤0.06->16 / 73.4	0.25 / 0.5 / 0.12-2 / 100
E. coli-ESBL pos (84)	0.25 / 1 / ≤0.12-4 / NA	4 / 16 / ≤1-256 / 94	16 / >32 / 1->32 / 29.8	0.12 / 0.25 / 0.12-0.5 / 100	0.25 / 0.5 / 0.12->16 / NA	>16 / >16 / ≤0.06->16 / 7.1	0.5 / 0.5 / 0.12-1 / 100
E. coli-ESBL neg	≤0.12 / 0.25 / ≤0.12-32 / NA	≤1 / 4 / ≤1->512 / 98.2	≤0.25 / 0.5 / ≤0.25->32 / 99.6	0.12 / 0.25 / ≤0.03-2 / 99.9	0.25 / 0.5 / ≤0.06->16 / NA	≤0.06 / >16 / ≤0.06->16 / 78.9	0.25 / 0.5 / 0.12-2 / 100
K. pneumoniae (395)	≤0.12 / 0.5 / ≤0.12-64 / NA	2 / 4 / ≤1->512 / 97.7	≤0.25 / 0.5 / ≤0.25->32 / 96.5	0.25 / 0.25 / 0.12-4 / 99.5	0.25 / 0.5 / ≤0.06->16 / NA	≤0.06 / 0.25 / ≤0.06->16 / 94.7	0.5 / 1 / 0.06-8 / 96.5
K. pneumoniae-ESBL + (15)	0.5 / 2 / ≤0.12-32 / NA	8 / 128 / 2-512 / 80	32 / >32 / 0.25->32 / 33.3	0.12 / 0.25 / 0.12-0.5 / 100	0.5 / 0.5 / 0.25-2 / NA	0.5 / >16 / ≤0.06->16 / 53.3	1 / 2 / 0.5-2 / 100
E. cloacae (173)	0.25 / 8 / ≤0.12-16 / NA	2 / 128 / ≤1-256 / 82.1	0.5 / >32 / ≤0.25->32 / 72.3	0.25 / 0.5 / 0.12-8 / 98.3	0.25 / >16 / 0.12->16 / NA	≤0.06 / 0.25 / ≤0.06->16 / 93.1	0.5 / 1 / 0.25-8 / 96.5
S. marcescens (109)	0.5 / 1 / 0.25-1 / NA	≤1 / 4 / ≤1-256 / 94.5	≤0.25 / 1 / ≤0.25-2 / 100	0.5 / 1 / 0.06-1 / 100	>16 / >16 / 0.5->16 / NA	≤0.06 / 1 / ≤0.06->16 / 86.2	1 / 2 / 1-8 / 95.4
P. mirabilis (85)	0.25 / 0.5 / 0.25-2 / NA	≤1 / ≤1 / ≤1->512 / 100	≤0.25 / ≤0.25 / ≤0.25-4 / 100	2 / 4 / 0.12-4 / 49.4	>16 / >16 / ≤0.06->16 / NA	≤0.06 / 4 / ≤0.06->16 / 87.1	4 / 8 / 1-8 / 21.2
K. oxytoca (113)	≤0.12 / 0.5 / ≤0.12-2 / NA	≤1 / 128 / ≤1->512 / 87.6	≤0.25 / 0.5 / ≤0.25->32 / 99.1	0.25 / 0.25 / 0.12-0.5 / 100	0.25 / 0.5 / ≤0.06->16 / NA	≤0.06 / ≤0.06 / ≤0.06->16 / 99.1	0.5 / 1 / 0.12-1 / 100
E. aerogenes (55)	0.25 / 2 / ≤0.12-8 / NA	2 / 16 / ≤1-128 / 92.7	≤0.25 / 32 / ≤0.25->32 / 78.2	0.25 / 1 / 0.12-2 / 98.2	0.25 / 0.5 / 0.25-4 / NA	≤0.06 / 0.5 / ≤0.06-8 / 96.4	1 / 1 / 0.25-4 / 96.3
C. freundii (24)	≤0.12 / ≤0.12 / ≤0.12-0.5 / NA	≤1 / 2 / ≤1-4 / 100	0.5 / 0.5 / ≤0.25-32 / 95.8	0.25 / 0.5 / 0.12-1 / 100	0.25 / 0.25 / 0.12-0.5 / NA	≤0.06 / 0.12 / ≤0.06-0.5 / 100	0.5 / 0.5 / 0.25-1 / 100
M. morganii (21)	≤0.12 / 0.25 / ≤0.12-0.5 / NA	≤1 / ≤1 / ≤1->512 / 100	≤0.25 / 1 / ≤0.25-8 / 95.2	2 / 4 / 0.5-4 / 14.3	>16 / >16 / 8->16 / NA	≤0.06 / 0.5 / ≤0.06-1 / 100	2 / 4 / 1-4 / 85.7
S. maltophilia (104)	32 / >64 / ≤0.12-64 / NA	256 / >512 / ≤1->512 / NA	>32 / >32 / 1->32 / 23.1	>32 / >32 / >32->32 / NA	8 / >16 / 0.25->16 / NA	2 / 8 / 0.5->16 / NA	1 / 4 / 0.25-8 / NA
A. baumannii (26)	0.5 / 2 / ≤0.12-16 / NA	4 / 256 / ≤1-512 / 84.6	4 / 16 / 2->32 / 84.6	0.25 / 0.5 / 0.12->32 / 96.2	0.5 / 1 / 0.25-1 / 100	0.25 / 1 / 0.12->16 / 96.2	0.25 / 1 / 0.12-2 / NA
H. influenzae (111)	≤0.12 / ≤0.12 / ≤0.12-1 / NA	≤1 / ≤1 / ≤1->512 / 100	not tested	0.25 / 1 / ≤0.06-4 / 100	not tested	≤0.015 / ≤0.015 / ≤0.015-0.06 / 100	not tested

NA = Not available
[a] = Interpretive breakpoints defined by FDA

Table 2. ***In vitro*** **activity of ceftolozane/tazobactam and comparators versus Gram-positive cocci**

Organism (no. tested)/antimicrobial agent	% S	% I	% R	MIC_{50}	MIC_{90}	Range
S. aureus (1208)						
Ceftolozane/tazobactam	NA	NA	NA	16	32	0.25-64
Ceftazidime	NA	NA	NA	16	16	2->32
Imipenem	NA	NA	NA	≤0.03	≤0.03	≤0.03-1
Piperacillin/tazobactam	NA	NA	NA	≤1	≤1	≤1-32
Colistin	NA	NA	NA	>16	>16	≤0.06->16
Ciprofloxacin	87.6	2	10.4	0.5	4	≤0.06->16
Tigecycline[b]	100			0.12	0.25	0.06-0.5
S. pneumoniae (321)						
Ceftolozane/tazobactam	NA	NA	NA	≤0.12	1	≤0.12-32
Ceftazidime					not tested	
Imipenem	94.4	4	1.6	≤ 0.03	≤0.03	≤0.03-1
Piperacillin/tazobactam	NA	NA	NA	≤1	≤1	≤1-8
Colistin					not tested	
Ciprofloxacin	98.4		1.6	1	2	0.12->16
Tigecycline	100			≤0.015	0.03	≤0.015-0.06

%S = % susceptible; %I = % intermediate; %R = % resistant; NA = not available, [a] - Interpretive breakpoints defined by FDA.

Table 3. ***In vitro*** **activity of Ceftolozane/tazobactam and comparators versus *P. aeruginosa***

	All Isolates (n = 593)							MDR [b] (n=30)		
	MIC (μg/mL)		Range of Values		Breakpoint Interpretations [a]			MIC (μg/mL)		
Antimicrobial	MIC_{50}	MIC_{90}	Min	Max	% S	% I	% R	MIC_{50}	MIC_{90}	% S
Ceftolozane/tazobactam	0.5	1	≤0.12	>64	n.d.	n.d.	n.d.	2	8	n.d.
Ceftazidime	2	16	≤0.25	>32	85.7	4.6	9.8	32	>32	16.7
Ciprofloxacin	0.25	4	≤0.06	>16	80.4	7.6	12	4	>16	13.3
Colistin	1	2	0.25	>16	96.8	1.0	2.2	1	2	93.3
Meropenem	0.5	4	≤0.03	>32	83.0	7.6	9.4	8	>32	13.3
Piperacillin/tazobactam	4	32	≤1	>512	85.8	8.3	5.9	128	256	13.3
Tobramycin	≤0.5	2	≤0.5	>64	94.4	1.0	4.6	2	>64	53.3

% S = % susceptible, % I = % intermediate, % R = % resistant.
[a] Breakpoint Interpretation: 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 = 32/4-64/4 μg/mL, R ≥128/4 μg/mL; Tobramycin S ≤4 μg/mL, I = 8 μg/mL, R ≥16 μg/mL.; MDR = multi-drug resistant – acquired nonsusceptibility to at least 1 agent in 3 or more antimicrobial categories; . n.d. = Breakpoints not defined.

Table 4. ***In vitro*** **activity of Ceftolozane/tazobactam and comparators versus antimicrobial-susceptible and non-susceptible *P. aeruginosa* isolates**

P. aeruginosa (number of isolates)	Ceftolozane/tazobactam - Microbroth Dilution Value (μg/mL) Number of isolates with MIC (Cumulative % of all Isolates Tested)								
	≤0.25	0.5	1	2	4	8	16	>16	Total
All isolates (n = 593)	74 (12.5)	343 (70.3)	133 (92.7)	28 (97.5)	8 (98.8)	4 (99.5)		3 (100)	593
Ceftazidime susceptible (n = 508)	74 (14.6)	332 (79.9)	95 (98.6)	6 (99.8)		1 (100)			508
Ceftazidime nonsusceptible (n = 85)		11 (12.9)	38 (57.6)	22 (83.5)	8 (92.9)	3 (96.5)		3 (100)	85
Ciprofloxacin susceptible (n = 477)	71 (1.5)	299 (14.9)	86 (77.6)	14 (95.6)	4 (98.5)	3 (100)			477
Ciprofloxacin nonsusceptible (n = 116)	3 (2.6)	44 (40.5)	47 (81)	14 (93.1)	4 (96.5)	1 (97.4)		3 (100)	116
Colistin susceptible (n = 574)	73 (12.7)	333 (70.7)	128 (93.0)	25 (97.4)	8 (98.8)	4 (99.5)		3 (100)	574
Colistin nonsusceptible (n = 19)	1 (5.3)	10 (57.9)	5 (84.2)	3 (100)					19
Meropenem susceptible (n = 492)	70 (14.2)	309 (77.0)	88 (94.9)	14 (97.8)	6 (98.9)	3 (99.6)		2 (100)	492
Meropenem nonsusceptible (n = 101)	4 (4.0)	34 (37.6)	45 (82.2)	14 (96.0)	2 (98.0)	1 (99.0)		1 (100)	101
Piperacillin/tazobactam susceptible (n = 509)	74 (14.5)	332 (79.8)	94 (98.2)	7 (99.6)	1 (99.8)	1 (100)			509
Piperacillin/tazobactam nonsusceptible (n = 84)		11 (13.1)	39 (59.5)	21 (84.5)	7 (92.8)	3 (96.4)		3 (100)	84
Tobramycin susceptible (n = 560)	70 (12.5)	337 (72.7)	119 (93.9)	23 (98.0)	7 (99.3)	4 (100)			560
Tobramycin nonsusceptible (n = 33)	4 (12.1)	6 (30.3)	14 (72.7)	5 (87.9)	1 (90.9)			3 (100)	33
MDR (n = 30)		1 (3.3)	11 (40.0)	11 (76.7)	3 (86.7)	1 (90.0)		3 (100)	30

CONCLUSIONS

- Ceftolozane/tazobactam demonstrated potent in vitro activity against recent clinical isolates of *Enterobacteriaceae*, with greater activity than piperacillin/tazobactam, ceftazidime, ciprofloxacin, and tigecycline.
- Potent in vitro activity against ESBL-producing *E. coli* and *K. pneumoniae*.
- Potent in vitro activity against recent clinical isolates of *Haemophilus influenzae, S. pneumoniae* and *Acinetobacter baumannii*.
- Ceftolozane/tazobactam was the most potent antipseudomonal agent tested followed by colistin.
- A very high level of activity against *P. aeruginosa* resistant to other antimicrobial classes was observed.
- This novel antibacterial is a promising candidate for the treatment of infections caused by resistant Gram-negative bacilli.

53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, September 10-13, 2013