SUNDAY - 3

In Vitro Activity of Plazomicin Against Gram-Negative and Gram-Positive Pathogens Isolated from Patients in Canadian Hospitals in 2011-2015: CANWARD Surveillance Study

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ABSTRACT

Background: Plazomicin is a next-generation aminoglycoside active against Gram-negative and Gram-positive organisms and currently in Phase 3 development. We determined the *in vitro* activity of plazomicin along with comparators versus Gram-negative and Gram-positive pathogens isolated from January 2011 to December 2015 from patients at 12-15 Canadian hospitals (CANWARD surveillance study).

Methods: Antimicrobial susceptibility testing was performed using in-house broth microdilution panels following the method recommended by CLSI.

Results: The activity of plazomicin and comparators is summarized below:

Organism (n)	MIC$_{50}$/MIC$_{90}$ (µg/mL)				
	PLAZOMICIN	GENTAMICIN	MEROPENEM	TIGECYCLINE	COLISTIN
E. coli All (2976)	0.5/1 (≤0.12-4)*	≤0.5/8	≤0.03/≤0.03	0.25/0.5	0.25/0.5
E. coli Gent-R (288)	0.5/1 (≤0.12-2)	32/>32	≤0.03/≤0.03	0.25/0.5	0.25/0.5
ESBL *E. coli* (286)	0.5/1 (≤0.12-2)	≤0.5/>32	≤0.03/≤0.03	0.25/1	0.25/0.5
K. pneumoniae All (1006)	0.25/0.5 (≤0.12->64)	≤0.5/≤0.5	≤0.03/≤0.03	0.5/1	0.25/1
K. pneumoniae Gent-R (28)	0.25/2 (0.25->64)	>32/>32	≤0.03/2	1/2	0.5/1
ESBL *K. pneumoniae* (49)	0.25/1 (≤0.12->64)	≤0.5/>32	≤0.03/0.25	1/2	0.5/4
E. cloacae All (434)	0.25/0.5 (≤0.12->64)	≤0.5/≤0.5	≤0.03/0.12	0.5/1	0.25/>16
E. cloacae Gent-R (14)	0.25/1 (0.25->64)	>32/>32	0.06/0.5	1/4	0.5/>16
S. marcescens All (242)	0.5/1 (≤0.12-4)	≤0.5/≤0.5	0.06/0.06	2/2	>16/>16
S. marcescens Gent-R (1)#	1	>32	0.12	2	>16
K. oxytoca All (253)	0.25/0.5 (≤0.12-32)	≤0.5/≤0.5	≤0.03/≤0.03	0.5/0.5	0.25/0.5
K. oxytoca Gent-R (1)#	0.25	32	≤0.03	0.25	0.5
P. mirabilis All (237)	2/4 (0.25-32)	≤0.5/1	0.06/0.12	4/8	16/>16
P. mirabilis Gent-R (10)#	4/4 (2-32)	16/>32	0.06/0.06	4/8	>16/>16
P. aeruginosa All (1685)	4/16 (≤0.12->64)	1/8	0.5/8	16/>16	1/2
P. aeruginosa Gent-R (110)	16/>64 (0.25->64)	>32/>32	4/32	16/>16	1/2
S. maltophilia All (289)	>64/>64 (≤0.12->64)	32/>32	>32/>32	1/4	4/>16
S. maltophilia Gent-R (190)	64/>64 (8->64)	>32/>32	>32/>32	1/4	8/>16
A. baumannii All (63)	1/2 (0.25-16)	≤0.5/1	0.5/1	0.25/1	0.5/1
A. baumannii Gent-R (1)#	8	>32	>32	2	1
MRSA All (738)	0.5/1 (≤0.12-64)	≤0.5/≤0.5	4/32	0.12/0.25	>16/>16
MRSA Gent-R (22)	1/1 (0.25-2)	>32/>32	4/>32	0.25/0.5	>16/>16
MRSE All (38)	0.25/0.5 (≤0.12-4)	>32/>32	32/>32	0.12/0.25	>16/>16
MRSE Gent-R (31)	0.25/0.5 (≤0.12-4)	>32/>32	32/32	0.12/0.25	>16/>16

Gent-R, gentamicin-resistant (MIC ≥16µg/mL); #, median MIC; *, range; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*

Conclusions: Plazomicin demonstrated potent *in vitro* activity against clinical isolates of *Enterobacteriaceae* and staphylococci including strains resistant to gentamicin as well as other antimicrobials. Plazomicin activity against *P. aeruginosa* and *A. baumannii* was similar to gentamicin. Plazomicin demonstrates promise for the treatment of bacterial infections caused by resistant organisms.

INTRODUCTION

Plazomicin (formerly ACHN-490) is a next-generation aminoglycoside that was synthetically derived from sisomicin by appending a hydroxy-aminobutryic acid (HABA) substituent at position 1′ and a hydroxyethyl substituent at position 6′ [1]. Plazomicin inhibits bacterial protein synthesis and exhibits dose-dependent bactericidal activity. Plazomicin demonstrates activity against both Gram-negative and Gram-positive bacterial pathogens, including isolates harboring all clinically relevant aminoglycoside-modifying enzymes. However, like other aminoglycosides, plazomicin is not active against bacterial isolates expressing ribosomal methyltransferases conferring aminoglycoside resistance. Plazomicin has been reported to demonstrate *in vitro* synergistic activity when combined with daptomycin or ceftobiprole versus methicillin-resistant *Staphylococcus aureus* (MRSA), heteroresistant vancomycin-intermediate *S. aureus* (hVISA), VISA, and vancomycin-resistant *S. aureus* (VRSA) and against *Pseudomonas aeruginosa* when combined with cefepime, doripenem, imipenem, or piperacillin-tazobactam. Two Phase 3 trials on plazomicin were recently completed: EPIC (Evaluating Plazomicin In cUTI) and CARE (Combating Antibiotic Resistant Enterobacteriaceae). In the EPIC study, plazomicin demonstrated superior efficacy and microbial eradication rates at test of cure compared to meropenem in patients with cUTI, including pyelonephritis. Additionally, in the CARE study, plazomicin demonstrated reduced all-cause mortality at day 28 in patients with CRE infections relative to those treated with colistin. Given reported increases in bacterial resistance to current antimicrobial agents and the lack of availability of new agents with novel mechanisms, plazomicin may become a welcomed addition to the antibacterial armamentarium.

PURPOSE

To determine the *in vitro* activity of plazomicin, along with aminoglycoside and non-aminoglycoside comparators, versus Gram-negative and Gram-positive pathogens isolated from patients in medical and surgical wards, intensive care units, clinics, and emergency rooms at 15 Canadian hospitals (CANWARD 2011-2015).

MATERIALS AND METHODS

Study Background - CANWARD

The isolates tested in this study were obtained from January to December 2011-2015, inclusive, from an ongoing cross-Canada surveillance study (CANWARD study; 12-15 participant sites, www.can-r.ca) [2]. The goal of the CANWARD 2011-2015 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].

Bacterial Isolates

From January 2011 through December 2015, 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 -80°C until minimum inhibitory concentration (MIC) testing was carried out.

Antimicrobial Susceptibilities

Following 2 subcultures from frozen stock, the *in vitro* activity of selected antimicrobials was determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015 M7-A10). Antimicrobial minimum inhibitory concentration (MIC) interpretive standards were defined according to CLSI breakpoints (M100S, 2015). Antimicrobial agents were obtained as laboratory grade powders from their respective manufacturers. Stock solutions were prepared and dilutions made as described by CLSI (M7-A10, 2015). The MICs of the antimicrobial agents for the isolates were determined using 96-well custom designed microtitre plates. These plates contained doubling antimicrobial dilutions in 100 µL/well of cation adjusted Mueller-Hinton broth and inoculated to achieve a final concentration of approximately 5 x 10^5 CFU/mL then incubated in ambient air for 24 hours prior to reading. Colony counts were performed periodically to confirm inocula. Quality control was performed using ATCC QC organisms including; *S. pneumoniae* 49619, *S. aureus* 29213, *E. faecalis* 29212, *E. coli* 25922, and *P. aeruginosa* 27853.

CONCLUSION

- Plazomicin demonstrated potent *in vitro* activity against recent clinical isolates of *Enterobacteriaceae*, including strains resistant to gentamicin as well as other antimicrobials including colistin.
- Plazomicin activity against *P. aeruginosa* and *A. baumannii* was similar to amikacin.
- Plazomicin was active against staphylococci, including gentamicin-resistant strains.
- Versus MRSA and MRSE, plazomicin and tigecycline were the most active agents.
- Plazomicin demonstrates promise for the treatment of bacterial infections caused by resistant organisms.

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RESULTS

Table 1. *In vitro* activities of plazomicin and comparative agents against Gram-negative bacilli

Organism (no. tested)/antimicrobial agent	MIC (µg/mL)					
	50%	90%	Range	% S	% I	% R
Escherichia coli (2976)						
Plazomicin	0.5	1	≤0.12-4	-[a]	-	-
Amikacin	2	4	≤1->64	99.7	0.2	0.1
Colistin	0.25	0.5	≤0.06-8	-	-	-
Gentamicin	≤0.5	8	≤0.5->32	89.8	0.6	9.6
Meropenem	≤0.03	≤0.03	≤0.03-32	99.97	0	0.03
Tigecycline	0.25	0.5	0.12-4	99.9	0.1	0
Gentamicin-Resistant *Escherichia coli* (288)						
Plazomicin	0.5	1	≤0.12-2	-	-	-
Amikacin	2	4	≤1-64	99.4	0.3	0.3
Colistin	0.25	0.5	≤0.06-4	-	-	-
Gentamicin	32	>32	16->32	0	0	100
Meropenem	≤0.03	≤0.03	≤0.03-32	99.7	0	0.3
Tigecycline	0.25	0.5	0.12-2	100	0	0
Klebsiella pneumoniae (1006)						
Plazomicin	0.25	0.5	≤0.12->64	-	-	-
Amikacin	≤1	2	≤1->64	99.8	0	0.2
Colistin	0.25	1	≤0.06->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5->32	97.2	0	2.8
Meropenem	≤0.03	≤0.03	≤0.03-16	99.5	0.1	0.4
Tigecycline	0.5	1	0.06-16	95.9	3.5	0.6
Gentamicin-Resistant *Klebsiella pneumoniae* (28)						
Plazomicin	0.25	2	0.25->64	-	-	-
Amikacin	2	16	≤1->64	92.9	0	7.1
Colistin	0.5	1	0.25-16	-	-	-
Gentamicin	>32	>32	16->32	0	0	100
Meropenem	≤0.03	2	≤0.03-16	89.3	3.6	7.1
Tigecycline	1	2	0.5-2	100	0	0
Enterobacter cloacae (434)						
Plazomicin	0.25	0.5	≤0.12->64	-	-	-
Amikacin	≤1	2	≤1->64	99.8	0	0.2
Colistin	0.25	>16	≤0.06->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5->32	96.8	0	3.2
Meropenem	≤0.03	0.12	≤0.03->32	98.6	0.7	0.7
Tigecycline	0.5	1	0.12-8	95.4	1.8	2.8
Gentamicin-Resistant *Enterobacter cloacae* (14)						
Plazomicin	n/a[b]	n/a	0.25->64	-	-	-
Amikacin	n/a	n/a	≤1->64	92.9	0	7.1
Colistin	n/a	n/a	0.25->16	-	-	-
Gentamicin	n/a	n/a	32->32	0	0	100
Meropenem	n/a	n/a	≤0.03->32	92.9	0	7.1
Tigecycline	n/a	n/a	0.5-8	78.6	14.3	7.1
Serratia marcescens (242)						
Plazomicin	0.5	1	≤0.12-4	-	-	-
Amikacin	2	2	≤1-16	100	0	0
Colistin	>16	>16	0.5->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5->32	99.2	0.4	0.4
Meropenem	0.06	0.06	≤0.03-8	99.6	0	0.4
Tigecycline	2	2	0.5-8	92.1	7.1	0.8
Gentamicin-Resistant *Serratia marcescens* (1)						
Plazomicin	n/a	n/a	1	-	-	-
Amikacin	n/a	n/a	16	100	0	0
Colistin	n/a	n/a	>16	-	-	-
Gentamicin	n/a	n/a	>32	0	0	100
Meropenem	n/a	n/a	0.12	100	0	0
Tigecycline	n/a	n/a	2	100	0	0
Klebsiella oxytoca (253)						
Plazomicin	0.25	0.5	≤0.12-32	-	-	-
Amikacin	≤1	2	≤1-8	100	0	0
Colistin	0.25	0.5	≤0.06->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5-32	98.8	0.8	0.4
Meropenem	≤0.03	≤0.03	≤0.03-0.5	100	0	0
Tigecycline	0.5	0.5	0.12-4	99.6	0.4	0

[a] –, no breakpoints
[b] n/a, isolate count insufficient for calculation of MIC$_{50/90}$
S, susceptible; I, intermediate; R, resistant

Organism (no. tested)/antimicrobial agent	MIC (µg/mL)					
	50%	90%	Range	% S	% I	% R
Proteus mirabilis (237)						
Plazomicin	2	4	0.25-32	-	-	-
Amikacin	2	4	≤1-32	99.2	0.8	0
Colistin	>16	>16	≤0.06->16	-	-	-
Gentamicin	≤0.5	1	≤0.5->32	94.5	1.3	4.2
Meropenem	0.06	0.12	≤0.03-0.25	100	0	0
Tigecycline	4	8	0.5-8	18.1	53.2	28.7
Gentamicin-Resistant *Proteus mirabilis* (10)						
Plazomicin	n/a	n/a	2-32	-	-	-
Amikacin	n/a	n/a	2-16	100	0	0
Colistin	n/a	n/a	>16->16	-	-	-
Gentamicin	n/a	n/a	16->32	0	0	100
Meropenem	n/a	n/a	≤0.03-0.12	100	0	0
Tigecycline	n/a	n/a	2-8	40	20	40
Pseudomonas aeruginosa (1685)						
Plazomicin	4	16	≤0.12->64	-	-	-
Amikacin	4	16	≤1->64	94.6	2.4	3
Colistin	1	2	0.12->16	96.4	1.8	1.8
Gentamicin	1	8	≤0.5->32	89.2	4.3	6.5
Meropenem	0.5	8	≤0.03->32	79.2	8	12.8
Tigecycline	16	>16	0.25->16	-	-	-
Gentamicin-Resistant *Pseudomonas aeruginosa* (110)						
Plazomicin	16	>64	0.25->64	-	-	-
Amikacin	16	>64	≤1->64	52.7	7.3	40
Colistin	1	2	0.25->16	93.6	2.7	3.6
Gentamicin	>32	>32	16->32	0	0	100
Meropenem	4	>32	≤0.03->32	36.4	17.3	46.4
Tigecycline	16	>16	0.5->16	-	-	-
Stenotrophomonas maltophilia (289)						
Plazomicin	>64	>64	≤0.12->64	-	-	-
Amikacin	>64	>64	≤1->64	-	-	-
Colistin	4	>16	0.25->16	-	-	-
Gentamicin	32	>32	≤0.5->32	-	-	-
Meropenem	>32	>32	0.06->32	-	-	-
Tigecycline	1	4	0.25-16	-	-	-
Gentamicin-Resistant *Stenotrophomonas maltophilia* (190)						
Plazomicin	>64	>64	8->64	-	-	-
Amikacin	>64	>64	16->64	-	-	-
Colistin	8	>16	0.25->16	-	-	-
Gentamicin	>32	>32	16->32	-	-	-
Meropenem	>32	>32	1->32	-	-	-
Tigecycline	1	4	0.25-16	-	-	-
Acinetobacter baumannii (63)						
Plazomicin	1	2	0.25-16	-	-	-
Amikacin	≤1	2	≤1->64	98.4	0	1.6
Colistin	0.5	1	0.25-2	100	0	0
Gentamicin	≤0.5	1	≤0.5->32	95.2	3.2	1.6
Meropenem	0.5	1	0.06->32	96.8	1.6	1.6
Tigecycline	0.25	1	0.12-4	-	-	-
Gentamicin-Resistant *Acinetobacter baumannii* (1)						
Plazomicin	n/a	n/a	8	-	-	-
Amikacin	n/a	n/a	>64	0	0	100
Colistin	n/a	n/a	1	100	0	0
Gentamicin	n/a	n/a	>32	0	0	100
Meropenem	n/a	n/a	>32	0	0	100
Tigecycline	n/a	n/a	2	-	-	-

Bacterial Isolates Collected

16,478 clinical isolates were collected for CANWARD 2011-2015.

- 6861 (41.7%) were from blood, 6342 (38.5%) from respiratory sources, 1688 (10.2%) were from urine, and 1587 (9.6%) were from wounds
- 9055 (55.0%) collected from male patients; 7420 (45%) female patients (gender unknown – 3 isolates)
- 2199 (13.3%) from patients ≤ 17 years of age, 7091 (43.1%) 18-64 years, and 7185 (43.6%) ≥ 65 years (age unknown – 3 isolates)
- 5098 (31.0%) were from patients on medical wards, 3823 (23.2%) from emergency rooms, 3410 (20.7%) from intensive care units, 2953 (17.9%) from hospital clinics, and 1187 (7.2%) from surgical wards (ward unknown – 7 isolates)

Table 2. *In vitro* activities of plazomicin and comparative agents against Gram-positive cocci

Organism (no. tested) / antimicrobial agent	MIC (µg/mL)					
	50%	90%	Range	% S	% I	% R
Methicillin-Susceptible *Staphylococcus aureus* (3057)						
Plazomicin	0.5	1	≤0.12-16	-[a]	-	-
Amikacin	2	4	≤1->64	99.6	0.2	0.2
Colistin	>16	>16	≤0.06->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5->32	98.4	0.1	1.5
Meropenem	0.12	0.25	≤0.03-16	-	-	-
Tigecycline	0.12	0.25	≤0.03-1	99.9	-	-
Methicillin-Resistant *Staphylococcus aureus* [MRSA] (738)						
Plazomicin	0.5	1	≤0.12-64	-	-	-
Amikacin	8	32	≤1->64	84.3	13	2.7
Colistin	>16	>16	2->16	-	-	-
Gentamicin	≤0.5	≤0.5	≤0.5->32	96.3	0.7	3
Meropenem	4	32	0.12->32	-	-	-
Tigecycline	0.12	0.25	≤0.03-2	98.1	-	-
Gentamicin-Resistant MRSA (22)						
Plazomicin	1	1	0.25-2	-	-	-
Amikacin	16	>64	4->64	59.1	0	40.9
Colistin	>16	>16	>16->16	-	-	-
Gentamicin	>32	>32	16->32	0	0	100
Meropenem	4	>32	0.5->32	-	-	-
Tigecycline	0.25	0.5	0.12-0.5	100	-	-
Methicillin-Susceptible *Staphylococcus epidermidis* (306)						
Plazomicin	≤0.12	0.25	≤0.12-0.5	-	-	-
Amikacin	≤1	4	≤1-64	99.4	0.3	0.3
Colistin	>16	>16	≤0.06->16	-	-	-
Gentamicin	≤0.5	32	≤0.5->32	65.7	8.5	25.8
Meropenem	1	8	≤0.03->32	-	-	-
Tigecycline	0.12	0.25	≤0.03-1	-	-	-
Methicillin-Resistant *Staphylococcus epidermidis* [MRSE] (38)						
Plazomicin	0.25	0.5	≤0.12-4	-	-	-
Amikacin	16	32	≤1->64	89.4	5.3	5.3
Colistin	>16	>16	>16->16	-	-	-
Gentamicin	>32	>32	≤0.5->32	13.1	5.3	81.6
Meropenem	32	>32	8->32	-	-	-
Tigecycline	0.12	0.25	0.06-0.25	-	-	-
Gentamicin-Resistant MRSE (31)						
Plazomicin	0.25	0.5	≤0.12-4	-	-	-
Amikacin	16	16	≤1->64	90.3	3.2	6.5
Colistin	>16	>16	>16->16	-	-	-
Gentamicin	>32	>32	16->32	0	0	100
Meropenem	32	32	8->32	-	-	-
Tigecycline	0.12	0.25	0.06-0.25	-	-	-

[a] –, no breakpoints
S, susceptible; I, intermediate; R, resistant

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