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Antimicrobial Activity of GT-1, a Novel Siderophore Cephalosporin, Tested against Multidrug-Resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. Isolates

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Introduction

- New strategies are required to successfully treat multidrug-resistant gram-negative pathogens
- The antimicrobial Trojan horse strategy
  - Siderophores are small molecules that are secreted by bacteria and bind extracellular iron
  - The resulting siderophore-Fe^{3+} complexes are then taken up by bacterial cells that use specific transport mechanisms
  - Synthetic antimicrobial-siderophore conjugates can also be transported into bacterial cells by the same iron uptake systems
  - This process allows efficient delivery of antimicrobials into cells of gram-negative species that possess less permeable outer membranes
- GT-1 (previously LCB10-0200) is a novel siderophore cephalosporin (Figure 1) with broad-spectrum activity against gram-negative bacteria
- This study reports on the *in vitro* antimicrobial activity of GT-1 against sets of *Pseudomonas aeruginosa* and *Acinetobacter* spp. clinical isolates with specific resistance phenotypes or genotypes

Materials and Methods

Organism collection

- *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates were collected from 110 medical centers in 34 countries
- *P. aeruginosa* (108 isolates)
  - Wild-type isolates (susceptible to ceftazidime-avibactam and meropenem; 22 isolates)
  - Class A β-lactamase-producing isolates (6 isolates, including *Klebsiella pneumoniae* carbapenemase [KPC; 3] and Guiana extended-spectrum [GES; 3] producers)
  - Metallo-β-lactamase-producing isolates (MBLs; 36 isolates; see Figure 2 for specific enzyme subclasses tested)
  - Colistin-resistant isolates (10 isolates)
  - Isolates with OprD porin loss (34 isolates)

Figure 1. Structure of GT-1

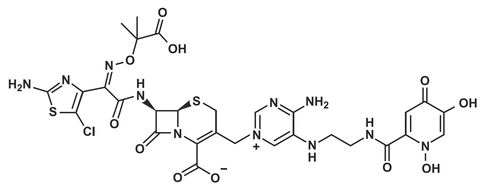
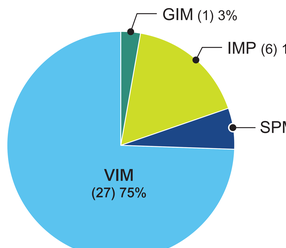


Figure 2. Carbapenemases produced by the *Pseudomonas aeruginosa* metallo-β-lactamase subset isolates (n = 36)



GIM, German imipenemase; IMP, imipenemase; SPM, São Paulo metallo-β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase

- *Acinetobacter* spp. (99 isolates) included: *Acinetobacter baumannii-calcoaceticus* species complex (95), *Acinetobacter berezinae* (1), *Acinetobacter haemolyticus* (1), *Acinetobacter lwoffii* (1), and unspeciated *Acinetobacter* (1)
  - Wild-type isolates (susceptible to ceftazidime and meropenem; 18 isolates)
  - Ceftazidime-resistant and meropenem-susceptible isolates (9 isolates)
  - Meropenem-resistant isolates (51 isolates)
  - Colistin-resistant isolates (21 isolates)
- Where indicated, the presence of specific β-lactamase genes was confirmed by PCR analysis or whole genome sequencing

Susceptibility testing

- Unless otherwise indicated, susceptibility testing followed current CLSI guidelines and interpretive criteria, where applicable
- Bacterial inoculum densities were monitored by colony counts
- MIC values were validated by concurrently tested CLSI-recommended quality control reference strains (*Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853)
- Isolates were tested against GT-1 by the broth microdilution method using iron-depleted (ID) cation-adjusted Mueller-Hinton broth (CAMHB)
  - ID-CAMHB was produced using Chelex® resin
- Comparators (including ceftazidime-avibactam, ceftolozane-tazobactam, colistin, levofloxacin, meropenem, sulbactam, and tigecycline) were tested in CAMHB
- Because some isolates (particularly *Acinetobacter* spp.) exhibited a "trailing inhibition of growth" phenotype in the presence of GT-1 (a known property of siderophore cephalosporins), GT-1 MIC values were read as the lowest concentration of compound that exhibited a significant reduction in growth relative to the growth control
  - This criterion was based on the alternative MIC methodology presented by Shionogi & Co. in January 2016 to the CLSI Methods Development and Standardization Working Group concerning their siderophore cephalosporin

Results

- GT-1 exhibited potent antimicrobial activity against each of the *P. aeruginosa* isolate subsets (Table 1)
  - GT-1 was the most potent antimicrobial tested against the wild-type isolate subset ($MIC_{50/90}$, 0.12/0.5 µg/mL; Table 2)
  - As shown in Table 2, GT-1 maintained excellent potency against carbapenem-resistant isolates that produced class A enzymes (KPC or GES; MIC_{50}, 0.5 µg/mL) or MBL enzymes ($MIC_{50/90}$, 1/4 µg/mL) and that were largely resistant to other tested antimicrobials except colistin
  - GT-1 also exhibited potent activity against a set of phenotypically colistin-resistant isolates with poor susceptibilities to other tested antimicrobials (Table 2; $MIC_{50/90}$, 0.25/1 µg/mL)
  - GT-1 was similarly active against a large set of meropenem-nonsusceptible isolates containing *oprD* mutations (Table 2, $MIC_{50/90}$, 0.25/0.5 µg/mL)
- GT-1 also displayed good antimicrobial activity against most *Acinetobacter* spp. isolates (Table 1)
  - GT-1 was among the most potent antimicrobials tested against the wild-type isolate subset ($MIC_{50/90}$, 0.25/1 µg/mL; Table 3)
  - Against the ceftazidime-resistant/meropenem-susceptible, meropenem-resistant/colistin-susceptible, and colistin-resistant isolate subsets, the GT-1 MIC_{50} values were 2 µg/mL; however, the MIC_{90} values were >64 µg/mL, which reflects the presence of a small number of resistant isolates (Table 1)
  - By MIC_{50} value, GT-1 and tigecycline were the most potent agents tested against the colistin-resistant isolate subset (Table 3)

Table 1. GT-1 MIC values for isolates tested in ID-CAMHB

Organism/organism group (no. of isolates)	No. of isolates at MIC (µg/mL; cumulative %)													MIC$_{50}$	MIC$_{90}$
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64*			
Acinetobacter spp. (99)	5 5.1	10 15.2	16 31.3	20 51.5	6 57.6	11 68.7	5 73.7	0 73.7	1 74.7	0 74.7	0 74.7	25 100.0		0.5	>64
Wild type (18)	3 16.7	4 38.9	5 66.7	4 88.9	2 100.0									0.25	1
Ceftazidime-resistant/ meropenem-susceptible (9)	0 0.0	2 22.2	2 44.4	0 44.4	0 44.4	1 55.6	0 55.6	0 55.6	0 55.6	0 55.6	0 55.6	4 100.0		2	—
Meropenem-resistant (51)	1 2.0	3 7.8	4 15.7	14 43.1	3 49.0	4 56.9	4 64.7	0 64.7	4 64.7	0 64.7	0 64.7	18 100.0		2	>64
Colistin-resistant (21)	1 4.8	1 9.5	5 33.3	2 42.9	1 47.6	6 76.2	1 81.0	0 81.0	1 85.7	0 85.7	0 85.7	3 100.0		2	>64
Pseudomonas aeruginosa (108)	8 7.4	12 18.5	28 44.4	23 65.7	19 83.3	12 94.4	3 97.2	2 99.1	0 99.1	0 99.1	0 99.1	1 100.0		0.5	2
Wild type (22)	7 31.8	5 54.5	5 81.8	3 95.5	1 100.0									0.12	0.5
Class A β-lactamase producer (6)		0 0.0	2 33.3	1 50.0	2 83.3	1 100.0								0.5	—
Metallo-β-lactamase producer (36)			0 0.0	5 13.9	15 55.6	11 86.1	2 91.7	2 97.2	0 97.2	0 97.2	0 97.2	1 100.0		1	4
OprD loss (34)	0 0.0	5 14.7	17 64.7	12 100.0										0.25	0.5
Colistin-resistant (10)	1 10.0	2 30.0	3 60.0	2 80.0	1 90.0	0 90.0	1 100.0							0.25	1

* Greater than the highest concentration tested.

Table 2. Activity of GT-1 and comparator agents tested against phenotypically and genotypically characterized *Pseudomonas aeruginosa* clinical isolates

Organism group/ antimicrobial agent	Antibacterial activity (µg/mL)			CLSI[a]		
	MIC$_{50}$	MIC$_{90}$	Range	%S	%I	%R
Wild type (22)						
GT-1 (ID-CAMHB)	0.12	0.5	≤0.06 — 1			
Ceftazidime-avibactam	2	2	0.5 — 4	100.0		0.0
Ceftolozane-tazobactam	0.5	1	0.25 — 1	100.0	0.0	0.0
Colistin	1	1	0.5 — 2	100.0		0.0
Levofloxacin	0.5	2	0.06 — >4	90.9	0.0	9.1
Meropenem	0.5	2	≤0.06 — 2	100.0	0.0	0.0
Class A β-lactamase producers (6)						
GT-1 (ID-CAMHB)	0.5	—	0.25 — 2			
Ceftazidime-avibactam	4	—	4 — 16	66.7		33.3
Ceftolozane-tazobactam	64	—	16 — >64	0.0	0.0	100.0
Colistin	1	—	1 — 1	100.0		0.0
Levofloxacin	>4	—	>4 — >4	0.0	0.0	100.0
Meropenem	64	—	16 — >64	0.0	0.0	100.0
Metallo-β-lactamase producers (36)						
GT-1 (ID-CAMHB)	1	4	0.5 — >64			
Ceftazidime-avibactam	64	>64	4 — >64	2.8		97.2
Ceftolozane-tazobactam	>64	>64	1 — >64	2.8	0.0	97.2
Colistin	1	2	0.5 — 2	100.0		0.0
Levofloxacin	>4	>4	0.5 — >4	8.3	0.0	91.7
Meropenem	>64	>64	8 — >64	0.0	0.0	100.0
Colistin-resistant (10)						
GT-1 (ID-CAMHB)	0.25	1	≤0.06 — 4			
Ceftazidime-avibactam	4	16	0.5 — >64	80.0		20.0
Ceftolozane-tazobactam	1	32	0.5 — >64	80.0	0.0	20.0
Colistin	8	>8	4 — >8	0.0		100.0
Levofloxacin	>4	>4	1 — >4	10.0	20.0	70.0
Meropenem	4	32	0.12 — 64	30.0	20.0	50.0
OprD loss (34)						
GT-1 (ID-CAMHB)	0.25	0.5	0.12 — 0.5			
Ceftazidime-avibactam	4	16	2 — 32	82.4		17.6
Ceftolozane-tazobactam	2	4	0.5 — 8	91.2	8.8	0.0
Colistin	1	2	≤0.25 — 2	100.0		0.0
Levofloxacin	4	>4	0.5 — >4	32.4	17.6	50.0
Meropenem	16	32	4 — 64	0.0	2.9	97.1

[a] Criteria as published by CLSI 2018.

Table 3. Activity of GT-1 and comparator agents tested against phenotypically and genotypically characterized *Acinetobacter* spp. clinical isolates

Organism group/ antimicrobial agent	Antibacterial activity (µg/mL)			CLSI[a]		
	MIC$_{50}$	MIC$_{90}$	Range	%S	%I	%R
Wild type (18)						
GT-1 (ID-CAMHB)	0.25	1	≤0.06 — 1			
Ceftazidime-avibactam	8	16	4 — 16			
Colistin	0.5	2	≤0.25 — 2	100.0		0.0
Levofloxacin	0.12	4	0.06 — >4	88.9	5.6	5.6
Meropenem	0.5	1	0.25 — 1	100.0	0.0	0.0
Sulbactam	1	2	0.5 — 16			
Tigecycline	0.25	0.5	≤0.12 — 1			
Ceftazidime-resistant/meropenem-susceptible (9)						
GT-1 (ID-CAMHB)	2	—	0.12 — >64			
Ceftazidime-avibactam	32	—	16 — >64			
Colistin	0.5	—	0.5 — 1	100.0		0.0
Levofloxacin	>4	—	2 — >4	11.1	0.0	88.9
Meropenem	2	—	1 — 2	100.0	0.0	0.0
Sulbactam	16	—	4 — >32			
Tigecycline	1	—	0.25 — 2			
Meropenem-resistant (51)						
GT-1 (ID-CAMHB)	2	>64	≤0.06 — >64			
Ceftazidime-avibactam	32	>64	8 — >64			
Colistin	0.5	1	≤0.25 — 2	100.0		0.0
Levofloxacin	>4	>4	0.5 — >4	2.0	2.0	96.1
Meropenem	>64	>64	8 — >64	0.0	0.0	100.0
Sulbactam	32	>32	4 — >32			
Tigecycline	1	2	0.12 — 4			
Colistin-resistant (21)						
GT-1 (ID-CAMHB)	2	>64	≤0.06 — >64			
Ceftazidime-avibactam	64	>64	2 — >64			
Colistin	>8	>8	4 — >8	0.0		100.0
Levofloxacin	>4	>4	0.06 — >4	19.0	0.0	81.0
Meropenem	64	>64	0.25 — >64	23.8	0.0	76.2
Sulbactam	16	>32	0.5 — >32			
Tigecycline	2	4	0.12 — 4			

[a] Criteria as published by CLSI 2018.

Conclusions

- GT-1 exhibited potent *in vitro* activity against a diverse global collection of multidrug-resistant *P. aeruginosa* and *Acinetobacter* spp. isolates
- Importantly, GT-1 maintained good activity against *P. aeruginosa* isolates that were carbapenem-resistant due to the expression of class A enzymes, class B enzymes, and/or the presence of *oprD* mutations
- An accompanying poster (Sunday-571) presents corresponding positive *in vitro* results for GT-1 tested against resistant *Enterobacteriaceae* isolates
- These results support the further clinical development of the novel siderophore cephalosporin GT-1

Acknowledgements

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