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Antimicrobial Activity of the Novel Siderophore Cephalosporin GT-1 Tested Alone and Combined with the β-Lactamase Inhibitor GT-055 against Molecularly Characterized *Enterobacteriaceae* Clinical Isolates

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Introduction

- The development of new β-lactam/β-lactamase inhibitor combinations represents an important strategy to overcome resistance in the major gram-negative pathogens
- GT-1 (previously LCB10-0200) is a novel siderophore cephalosporin with broad-spectrum activity against gram-negative bacteria, and GT-055 (previously LCB18-055) is a novel inhibitor of serine β-lactamases and some metallo-β-lactamases (MBL) (Figure 1)
- Siderophores are small iron chelators secreted by bacteria for iron uptake, and iron-bound siderophores are transported into bacterial cells; thus, siderophore-antibiotic complexes can be used as a Trojan horse for treating multidrug-resistant (MDR) pathogens
- The purpose of this study was to assess the *in vitro* activity of GT-1 with and without GT-055 against a collection of resistant *Enterobacteriaceae* clinical isolates

Materials and Methods

Organism collection
- A total of 334 *Enterobacteriaceae* were collected from 132 medical centers in 31 countries
- The organism collection included
  - *E. coli* (117 isolates)
    - CTX-M-15 producers (33)
    - Isolates producing other extended-spectrum β-lactamases (ESBL; 24; including SHV [13] and TEM [11] producers)
    - *Klebsiella pneumoniae* carbapenemase (KPC) producers (10)
    - MBL producers (10; including NDM-1 [5], NDM-5 [2], NDM-7 [1], IMP-1 [1], and VIM-2 [1])
    - Isolates with porin loss and/or hyperexpression of efflux pumps (4)
    - Colistin-resistant isolates (14; including *mcr-1* positive [7] and β-lactamase-producing isolates [5; including 1 VIM-1, 1 OXA-35/101, 2 CTX-M-15-like, and 1 CMY-2-like+SHV-ESBL])
    - Wild-type isolates (susceptible to ceftazidime, ceftriaxone, and meropenem; 22)
  - *K. pneumoniae* (117 isolates)
    - ESBL producers (20)
    - KPC producers (30; including KPC-2 [15] and KPC-3 [15])
    - MBL producers (13; including NDM-1 [9] and VIM-like [4])
    - Plasmidic AmpC producers (11; including CMY-2-like [2], DHA [5], FOX-like [4])
    - Isolates with porin loss and/or hyperexpression of efflux pumps (12)
    - Colistin-resistant isolates (10)
    - Wild-type isolates (susceptible to ceftazidime, ceftriaxone, and meropenem; 21)
  - Other *Enterobacteriaceae* species (100 isolates)
    - Antimicrobial-resistant isolates (n = 58) producing the following β-lactamases: KPC-2/3 (18), VIM-1 (10), other VIM enzymes (4), CTX-M-15 (6), SHV ESBL (4), TEM ESBL (3), OXA-48 (3), SME-like (2), and others (8)
    - Wild-type isolates (susceptible to ceftazidime, ceftriaxone, and meropenem; 42)
    - This subset included the following species: *C. freundii* species complex (15), *C. koseri* (2), *Enterobacter aerogenes* (6), *E. cloacae* species complex (13), *E. gergoviae* (1), *Klebsiella oxytoca* (17), *Morganella morganii* (6), *Proteus mirabilis* (13), *P. penneri* (1), *P. vulgaris* (1), *Providencia rettgeri* (2), *P. stuartii* (8), and *Serratia marcescens* (15)
- Resistant isolates were characterized by whole genome sequencing or PCR plus sequencing

Susceptibility testing
- Isolates were tested against GT-1 alone and in combination with GT-055 (2:1 ratio) by the broth microdilution method using iron-depleted (ID) cation-adjusted Mueller-Hinton broth (CAMHB)
- ID-CAMHB was produced as follows: 15 g of Chelex® resin (Bio-Rad, catalog #142-2842) was added to each 150 mL of CAMHB, the slurry was mixed for 2 hours at room temperature, the resin was removed by filtration through a 0.2-micron filter, and the pH was adjusted to 7.2–7.4. Next, Ca^{2+} (20–25 mg/L), Mg^{2+} (10–12.5 mg/L), and Zn^{2+} (0.65 mg/L) cations were replenished by adding appropriate volumes of stock solutions of $CaCl_2$, $MgCl_2$, and $ZnSO_4$ to the iron-depleted Mueller-Hinton broth
- The MIC endpoints for GT-1 and GT-1+GT-055 were read at 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 that was presented by Shionogi & Co. in January 2016 to the CLSI Methods Development and Standardization Working Group concerning their siderophore cephalosporin
- Comparator agents included ceftazidime-avibactam, ceftolozane-tazobactam, meropenem, levofloxacin, colistin, and tigecycline

Results

- GT-1 exhibited potent *in vitro* activity against *Escherichia coli* ($MIC_{50/90}$, 0.5/8 μg/mL), including against isolates producing CTX-M-15 (n=33; $MIC_{50/90}$, 2/4 μg/mL), other ESBLs (n=24; $MIC_{50/90}$, 0.25/0.5 μg/mL), and KPC (n=10; $MIC_{50/90}$, 0.5/2 μg/mL), but showed limited activity against MBL-producing strains (MIC_{50}, >64 μg/mL; Table 1)
- GT-1 was also active against *K. pneumoniae* producing ESBL (n=20; $MIC_{50/90}$, 1/4 μg/mL), plasmid AmpC (n=11; $MIC_{50/90}$, 0.5/2 μg/mL), and KPC (n=30; $MIC_{50/90}$, 2/4 μg/mL), but showed limited activity against MBL-producing isolates (MIC_{50}, >64 μg/mL; Table 1)
- The addition of GT-055 markedly improved GT-1 activity against MBL-producing *E. coli* and *K. pneumoniae* ($MIC_{50/90}$, 4/8 μg/mL for both subsets; Table 1)
- The addition of GT-055 also improved GT-1 activity against *E. coli* and *K. pneumoniae* with porin/efflux alterations; GT-1 and GT-1+GT-055 $MIC_{50/90}$ values were 4/64 μg/mL and 2/4 μg/mL, respectively, when tested against *K. pneumoniae* isolates with porin/efflux alterations (Table 1)
- GT-1 was also active against colistin-resistant *E. coli* (n=14; $MIC_{50/90}$, 0.25/2 μg/mL) and *K. pneumoniae* (n=10; $MIC_{50/90}$, 2/2 μg/mL), and against *Enterobacteriaceae* species other than *E. coli* and *K. pneumoniae* (n=100; $MIC_{50/90}$, 0.25/4 μg/mL; Table 1)
- Ceftazidime-avibactam was active against all resistant subsets except MBL-producing strains, whereas ceftolozane-tazobactam exhibited limited activity against isolates producing KPC, MBL, and some ESBLs (data not shown)
- GT-055 alone exhibited *in vitro* activity against *E. coli* ($MIC_{50/90}$, 2/4 μg/mL) and *K. pneumoniae* ($MIC_{50/90}$, 4/>32 μg/mL) isolates (Table 2)

Figure 1 Chemical structure of GT-1 and GT-055

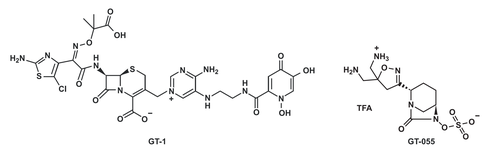

Table 1 Antimicrobial activity of GT-1 and GT-1+GT-055 2:1 ratio tested in ID-CAMHB against resistant subsets of *Enterobacteriaceae* isolates

Organisms/organism groups	No. of isolates at MIC (μg/mL) and cumulative %													MIC_{50}	MIC_{90}
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>			
***Escherichia coli* (117)**															
GT-1	22 18.8	15 31.6	12 41.9	17 56.4	10 65.0	22 83.8	7 89.7	2 91.5	1 92.3	0 92.3	0 92.3	9 100.0		0.5	8
GT-1+GT-055 2:1 ratio	22 18.8	16 32.5	16 46.2	25 67.5	20 84.6	7 90.6	8 97.4	2 99.1	0 99.1	0 99.1	0 99.1	1 100.0		0.5	2
CTX-M-15 producers (33)															
GT-1		0 0.0	2 6.1	4 18.2	6 36.4	14 78.8	4 90.9	1 93.9	0 93.9	0 93.9	0 93.9	2 100.0		2	4
GT-1+GT-055 2:1 ratio	0 0.0	1 3.0	5 18.2	11 51.5	12 87.9	2 93.9	2 100.0							0.5	2
Other ESBL producers (24)															
GT-1	5 20.8	6 45.8	4 62.5	7 91.7	1 95.8	0 95.8	1 100.0							0.25	0.5
GT-1+GT-055 2:1 ratio	6 25.0	4 41.7	6 66.7	7 95.8	0 95.8	1 100.0								0.25	0.5
KPC-like producers (10)															
GT-1	0 0.0	4 40.0	0 40.0	3 70.0	1 80.0	1 90.0	1 100.0							0.5	2
GT-1+GT-055 2:1 ratio	0 0.0	4 40.0	0 40.0	3 70.0	2 90.0	1 100.0								0.5	1
MBL producers (10)															
GT-1				0 0.0	1 10.0	1 20.0	0 20.0	0 20.0	1 30.0	0 30.0	0 30.0	7 100.0		>64	>64
GT-1+GT-055 2:1 ratio				0 0.0	1 10.0	1 20.0	5 70.0	2 90.0	0 90.0	0 90.0	0 90.0	1 100.0		4	8
Porin/efflux alterations (4)															
GT-1			0 0.0	1 25.0	0 25.0	2 75.0	1 100.0							2	—
GT-1+GT-055 2:1 ratio			0 0.0	1 25.0	2 75.0	1 100.0								1	—
Colistin-resistant (14)															
GT-1	6 42.9	0 42.9	1 50.0	2 64.3	1 71.4	3 92.9	0 92.9	1 100.0						0.25	2
GT-1+GT-055 2:1 ratio	5 35.7	1 42.9	1 50.0	3 71.4	2 85.7	1 92.9	1 100.0							0.25	2
Wild type (22)															
GT-1	11 50.0	5 72.7	5 95.5	0 95.5	0 95.5	1 100.0								≤0.06	0.25
GT-1+GT-055 2:1 ratio	11 50.0	6 77.3	4 95.5	0 95.5	1 100.0									≤0.06	0.25
***Klebsiella pneumoniae* (117)**															
GT-1	14 12.0	6 17.1	6 22.2	18 37.6	22 56.4	16 70.1	17 84.6	4 88.0	0 88.0	1 88.9	1 89.7	12 100.0		1	>64
GT-1+GT-055 2:1 ratio	14 12.0	9 19.7	11 29.1	22 47.9	20 65.0	20 82.1	15 94.9	4 98.3	2 100.0					1	4
ESBL producers (20)															
GT-1		0 0.0	1 5.0	4 25.0	9 70.0	3 85.0	3 100.0							1	4

Organisms/organism groups	No. of isolates at MIC (μg/mL) and cumulative %												MIC_{50}	MIC_{90}
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>		
GT-1+GT-055 2:1 ratio		0 0.0	3 15.0	9 60.0	4 80.0	1 100.0							0.5	2
KPC-like producers (30)														
GT-1	0 0.0	2 6.7	0 6.7	8 33.3	4 46.7	4 60.0	9 90.0	1 93.3	0 93.3	0 93.3	0 93.3	2 100.0	2	4
GT-1+GT-055 2:1 ratio	0 0.0	1 3.3	4 16.7	6 36.7	7 60.0	5 76.7	7 100.0						1	4
MBL producers (13)														
GT-1				0 0.0	1 7.7	1 15.4	2 30.8	0 30.8	0 30.8	1 38.5	0 38.5	8 100.0	>64	>64
GT-1+GT-055 2:1 ratio				0 0.0	1 7.7	3 23.1	6 69.2	3 92.3	1 100.0				4	8
Plasmidic AmpC producers (11)														
GT-1	2 18.2	0 18.2	3 45.5	2 63.6	2 81.8	1 90.9	1 100.0						0.5	2
GT-1+GT-055 2:1 ratio	3 27.3	2 45.5	3 72.7	1 81.8	1 90.9	1 100.0							0.25	1
Porin/efflux alterations (12)														
GT-1			0 0.0	1 8.3	3 33.3	1 41.7	2 58.3	3 83.3	0 83.3	0 83.3	1 91.7	1 100.0	4	64
GT-1+GT-055 2:1 ratio			0 0.0	1 8.3	3 33.3	5 75.0	2 91.7	0 91.7	1 100.0				2	4
Colistin-resistant (10)														
GT-1			0 0.0	1 10.0	2 30.0	6 90.0	0 90.0	0 90.0	0 90.0	0 90.0	0 90.0	1 100.0	2	2
GT-1+GT-055 2:1 ratio			0 0.0	2 20.0	4 60.0	3 90.0	0 90.0	1 100.0					1	2
Wild type (21)														
GT-1	12 57.1	4 76.2	2 85.7	2 95.2	1 100.0								≤0.06	0.5
GT-1+GT-055 2:1 ratio	11 52.4	6 81.0	1 85.7	3 100.0									≤0.06	0.5
Other *Enterobacteriaceae* (100)														
GT-1	21 21.0	21 42.0	11 53.0	14 67.0	10 77.0	11 88.0	5 93.0	3 96.0	0 96.0	3 99.0	0 99.0	1 100.0	0.25	4
GT-1+GT-055 2:1 ratio	22 22.0	21 43.0	18 61.0	12 73.0	10 83.0	12 95.0	2 97.0	2 99.0	1 100.0				0.25	2
Antimicrobial-resistant (58)														
GT-1	3 5.2	11 24.1	6 34.5	8 48.3	8 62.1	10 79.3	5 87.9	3 93.1	1 93.1	3 98.3	0 98.3	1 100.0	1	8
GT-1+GT-055 2:1 ratio	5 8.6	10 25.9	10 43.1	8 56.9	9 72.4	11 91.4	2 94.8	2 98.3	1 100.0				0.5	2
Wild type (42)														
GT-1	18 42.9	10 66.7	5 78.6	6 92.9	2 97.6	1 100.0							0.12	0.5
GT-1+GT-055 2:1 ratio	17 40.5	11 66.7	8 85.7	4 95.2	1 97.6	1 100.0							0.12	0.5

Table 2 Activity of GT-1 with and without GT-055 and comparator agents tested against molecularly characterized clinical isolates of *Enterobacteriaceae* collected worldwide

Antimicrobial agent	MIC_{50}	MIC_{90}	CLSI[a]		
			%S	%I	%R
Escherichia coli (117)					
GT-1	0.5	8	—	—	—
GT-1+GT-055 (2:1)	0.5	2	—	—	—
GT-055	2	4	—	—	—
Ceftazidime-avibactam	0.25	16	89.7	—	10.3
Ceftolozane-tazobactam	0.5	>64	76.9	0.9	22.2
Meropenem	≤0.06	4	81.2	6.0	12.8
Levofloxacin	>4	>4	27.4	5.1	67.5
Colistin	≤0.25	4	—	—	—
Tigecycline[b]	0.12	0.25	99.1	0.9	0.0
Klebsiella pneumoniae (117)					
GT-1	1	>64	—	—	—
GT-1+GT-055 (2:1)	1	4	—	—	—
GT-055	4	>32	—	—	—
Ceftazidime-avibactam	1	>64	85.5	—	14.5
Ceftolozane-tazobactam	64	>64	40.2	1.7	58.1
Meropenem	4	>64	46.2	2.6	51.3
Levofloxacin	>4	>4	38.5	4.3	57.3
Colistin	≤0.25	>8	—	—	—
Tigecycline[b]	0.5	2	99.1	0.9	0.0
Other *Enterobacteriaceae* species (100)[c]					
GT-1	0.25	4	—	—	—
GT-1+GT-055 (2:1)	0.25	2	—	—	—
GT-055	>32	>32	—	—	—
Ceftazidime-avibactam	0.25	64	83.0	—	17.0
Ceftolozane-tazobactam	1	>64	59.0	1.0	40.0
Meropenem	≤0.06	16	68.0	4.0	28.0
Levofloxacin	0.5	>4	68.0	5.0	27.0
Colistin	0.5	>8	—	—	—
Tigecycline[b]	0.5	2	91.0	8.0	1.0

[a] Criteria as published by CLSI 2018.
[b] Breakpoints from FDA Package Insert.
[c] Organisms include: *C. freundii* species complex (7), *C. koseri* (2), *Enterobacter aerogenes* (6), *E. cloacae* species complex (13), *E. gergoviae* (1), *Klebsiella oxytoca* (17), *Morganella morganii* (6), *Proteus mirabilis* (13), *P. penneri* (1), *P. vulgaris* (1), *Providencia rettgeri* (2), *P. stuartii* (8), *Serratia marcescens* (15).

Conclusions

- GT-1 and the GT-1+GT-055 combination demonstrated potent *in vitro* activity against a diverse worldwide collection of multidrug-resistant *Enterobacteriaceae* isolates
- The addition of GT-055 markedly improved the activity of GT-1 against MBL producers and all other resistant subsets due to the potent *in vitro* activity of GT-055 against these organisms
- The results of this investigation support further clinical development of GT-1 and GT-055

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