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In Vitro Activity of LYS228 against *Enterobacteriaceae*, Including Molecularly Characterized Multidrug-Resistant Isolates

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Abstract

Background: *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases have become prevalent in both nosocomial and community settings. The *in vitro* activity of a new agent (LYS228) was assessed against molecularly characterized multidrug-resistant (MDR) *Enterobacteriaceae*.

Methods: Eighty-four molecularly characterized ESBL (CTX-M, OXA-1, and SHV)-, AmpC (FOX, DHA ,and CMY)-, and/or carbapenemase (KPC, SME, OXA-48, IMP, VIM, and NDM)-producing isolates were selected. A subset of wild-type (control) isolates were also included. Susceptibility testing was performed according to CLSI (M07-A10). MIC interpretation for comparators applied CLSI or FDA (ie, tigecycline) criteria.

Results: LYS228 had MIC_{50}, MIC_{90} and MIC_{100} values of 0.06, 0.25 and 0.25 µg/mL, respectively, against wild-type *Enterobacteriaceae*. Comparators showed MIC_{90} values that were within ±1 doubling dilution compared to that of LYS228 against wild-type isolates. LYS228 inhibited all tested AmpC/ESBL producers at ≤1 µg/mL, and the MIC_{90} value was similar (2-fold higher than) to that obtained against wild-type isolates. Among comparators, only meropenem, amikacin (MIC_{50}/MIC_{90}, 2/8 µg/mL; 96.3% susceptible) and tigecycline were active against AmpC/ESBL isolates. LYS228 inhibited all carbapenemase producers at ≤4 µg/mL, except for 2 MDR isolates producing NDM-5 (LYS228 MIC, 16-32 µg/mL). LYS228 and tigecycline were the most potent agents and similarly active against carbapenemase-producing isolates. Other agents had susceptibility rates of 3.7-77.8%.

Conclusions: LYS228 showed potent *in vitro* activity against wild-type isolates. The activity of this novel compound was not affected by the presence of ESBL and/or AmpC and showed potency similar or greater than other comparators against carbapenemase-producing MDR *Enterobacteriaceae*. These results warrant further development of this investigational compound.

Introduction

- *Enterobacteriaceae* isolates are common causes of community-acquired and health care-associated infections (HAI) and the latter is particularly evident among intensive care unit patients
- In addition to the high prevalence rates, antimicrobial resistance reports have increased among gram-negative pathogens causing community-acquired and HAI
- Carbapenem- (CRE) and ceftriaxone-resistant *Enterobacteriaceae* accounted for 35,000 HAI in US hospitals, and the percentage of CRE among US medical centers increased from 1.2% in 2001 to 4.2% in 2011
- A combined prevalence of 2% of carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* from US, European, and Latin American centers was previously reported or 6% among *K. pneumoniae* isolates in the US alone
- The described scenario, lack of new antimicrobial agents approved in the last decades, clinical challenges faced when managing infections caused by multidrug-resistant (MDR) organisms, and limited therapeutic options have recently prompted several agencies to promote the development of new antimicrobial agents
- LYS228 is a novel monobactam being developed to treat human infections caused by *Enterobacteriaceae* clinical isolates (Figure 1)
- LYS228 is stable against metallo-β-lactamases (e.g. New Delhi metallo-β-lactamase-1 [NDM-1]), serine carbapenemases (eg, *Klebsiella pneumoniae* carbapenemases [KPC]), and most ESBLs, resulting in retained potency against the majority of extended-spectrum β-lactamase (ESBL) and CRE
- The *in vitro* activity of LYS228 was assessed against wild-type and molecularly characterized MDR *Enterobacteriaceae*

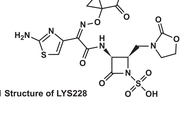

Figure 1 Structure of LYS228

Materials and Methods

Organism collection

- This study used an organism collection of 145 geographically diverse *Enterobacteriaceae* clinical isolates collected worldwide from patients with documented infections
- Wild-type susceptible pathogens (61 isolates) were included as control organisms, as well as a challenge set (84 isolates) consisting of isolates exhibiting resistance phenotypes to broad-spectrum β-lactam agents, such as oxyimino-cephalosporins, aztreonam, and/or carbapenems
- β-lactam resistance mechanisms were characterized by molecular (PCR and sequencing) methods
- *Enterobacteriaceae* pathogens that produced extended-spectrum β-lactamase (ESBL) and/or carbapenemase enzymes were as follows:

Carbapenemases:
- KPC (21 isolates)
- SME (2)
- OXA-48/181 (8)
- IMP (7)
- VIM (8)
- NDM (8)

ESBL:
- Plasmid AmpC (CMY, FOX, DHA; 6)
- CTX-M-Group 1 (CTX-M-15 alone; 7)
- CTX-M-Group 1 (CTX-M-15 plus OXA-1/30; 6)
- CTX-M-Group 9 (CTX-M-9/14; 6)
- SHV variants (5)

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07-A10 document
- Bacterial inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event
- MIC values were validated by concurrently testing CLSI-recommended quality control (QC) reference strains
- MIC interpretations were based on the CLSI (M100-S25) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2015) breakpoint criteria, as available

Results

- Overall, LYS228 showed MIC_{50} and MIC_{90} values of 0.06 and 0.25 µg/mL, respectively, against wild-type *Enterobacteriaceae* isolates (Tables 1 and 2)
- Compound LYS228 exhibited MIC_{90} values against different species of wild-type *Enterobacteriaceae* that varied from ≤0.03 to 0.25 µg/mL (Table 1)
- The overall LYS228 MIC_{50} and MIC_{90} values obtained against carbapenemase-producing *Enterobacteriaceae* were 0.5 and 4 µg/mL, respectively (Tables 1 and 2)
- LYS228 inhibited carbapenemase-producing isolates at ≤4 µg/ml, except for 2 NDM-producing strains (Table 1)
  - LYS228 had an MIC_{100} of 2 µg/mL against the KPC-producing subset (Table 1)
- Overall, only LYS228 (MIC_{50}/MIC_{90}, 0.5/4 µg/mL) and tigecycline (MIC_{50}/MIC_{90}, 0.25/2 µg/mL) demonstrated *in vitro* activity against the carbapenemase-producing isolates (Table 2)
- When LYS228 was tested against AmpC/ESBL (MIC_{50}/MIC_{90}, 0.25/0.5 µg/mL) producers, the MIC_{90} values were only 2-fold higher than those obtained against wild-type *Enterobacteriaceae* (MIC_{50}/MIC_{90}, 0.06/0.25 µg/mL; Table 2)
- MIC_{50} results of 0.25–0.5 µg/mL were observed for LYS228 against AmpC- and ESBL-producing *Enterobacteriaceae* isolates, except for isolates expressing CTX-M-9 group (MIC_{50}, 0.06 µg/mL) (Table 1)
- LYS228 (MIC_{50}/MIC_{90}, 0.25/0.5 µg/mL), meropenem (MIC_{50}/MIC_{90}, ≤0.12/≤0.12 µg/mL), and tigecycline (MIC_{50}/MIC_{90}, 0.25/0.5 µg/mL) were similarly active against AmpC-/ESBL-producing organisms (Table 2)

Table 1 Cumulative frequency distribution of LYS228 MIC results when tested against *Enterobacteriaceae*

		MIC in µg/mL (cumulative %)											MIC (µg/mL)	
Group/organism	No.	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	50%	90%
Wild type														
All	61	11 (18.3)	20 (50.8)	17 (78.7)	13 (100.0)								0.06	0.25
E. coli	10	0 (0.0)	5 (50.0)	2 (70.0)	3 (100.0)								0.06	0.25
K. pneumoniae	10	2 (20.0)	3 (50.0)	2 (70.0)	3 (100.0)								0.06	0.25
P. mirabilis	10	9 (90.0)	1 (100.0)										≤0.03	≤0.03
E. cloacae	10	0 (0.0)	4 (40.0)	5 (90.0)	1 (100.0)								0.12	0.12
C. freundii	11	0 (0.0)	5 (45.5)	4 (81.8)	2 (100.0)								0.12	0.25
S. marcescens	10	0 (0.0)	2 (20.0)	4 (60.0)	4 (100.0)								0.12	0.25
Carbapenemases														
All	54	2 (3.7)	2 (7.4)	5 (16.7)	6 (27.8)	22 (68.5)	9 (85.2)	2 (88.9)	4 (96.3)	0 (96.3)	1 (98.1)	1 (100.0)	0.5	4
KPC	21	0 (0.0)	1 (4.8)	1 (9.5)	3 (23.8)	11 (76.2)	4 (95.2)	1 (100.0)					0.5	1
SME	2	0 (0.0)	0 (0.0)	2 (100.0)									0.12	–[a]
OXA	8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (62.5)	3 (100.0)						0.5	–
IMP	7	0 (0.0)	1 (14.3)	1 (28.6)	0 (28.6)	2 (57.1)	0 (57.1)	0 (57.1)	3 (100.0)				0.5	–
VIM	8	2 (25.0)	0 (25.0)	0 (25.0)	3 (62.5)	1 (75.0)	1 (87.5)	1 (100.0)					0.25	–
NDM	8	0 (0.0)	0 (0.0)	1 (12.5)	0 (12.5)	3 (50.0)	1 (62.5)	0 (62.5)	1 (75.0)	0 (75.0)	1 (87.5)	1 (100.0)	0.5	–
AmpC or ESBL														
All	30	2 (6.7)	2 (13.3)	6 (33.3)	11 (70.0)	6 (90.0)	3 (100.0)						0.25	0.5
AmpC	6	1 (16.7)	0 (16.7)	1 (33.3)	4 (100.0)								0.25	–
CTX-M Group 1	7	0 (0.0)	0 (0.0)	1 (14.3)	3 (57.1)	3 (100.0)							0.25	–
CTX-M Group 1/OXA-1	6	0 (0.0)	0 (0.0)	1 (16.7)	1 (33.3)	2 (66.7)	2 (100.0)						0.5	–
CTX-M Group 9	6	1 (16.7)	2 (50.0)	2 (83.3)	1 (100.0)								0.06	–
SHV	5	0 (0.0)	0 (0.0)	1 (20.0)	2 (60.0)	1 (80.0)	1 (100.0)						0.25	–

[a] MIC_{90} values were not calculated for groups <10 isolates

Table 2 Activity of LYS228 and comparator antimicrobial agents when tested against *Enterobacteriaceae*

	MIC			CLSI[a]			EUCAST[a]		
Phenotype/genotype (No.) antimicrobial agent	50%	90%	Range	%S	%I	%R	%S	%I	%R
Wild type (61)									
LYS228	0.06	0.25	≤0.03 — 0.25	—	—	—	—	—	—
Cefepime	≤0.5	≤0.5	≤0.5	100.0	0.0	0.0	100.0	0.0	0.0
Ceftazidime	0.25	0.5	≤0.06 — 1	100.0	0.0	0.0	100.0	0.0	0.0
Ceftriaxone	0.12	0.25	≤0.06 — 0.5	100.0	0.0	0.0	100.0	0.0	0.0
Colistin	1	>8	≤0.5 — >8	—	—	—	62.3	—	37.7
Meropenem	≤0.12	≤0.12	≤0.12	100.0	0.0	0.0	100.0	0.0	0.0
Amikacin	1	4	0.5 — 8	100.0	0.0	0.0	100.0	0.0	0.0
Aztreonam	≤0.12	0.25	≤0.12 — 0.5	100.0	0.0	0.0	100.0	0.0	0.0
Levofloxacin	≤0.12	>4	≤0.12 — >4	88.5	0.0	11.5	85.2	3.3	11.5
Tigecycline	0.25	1	0.06 — 4	—	—	—	91.8	6.6	1.6
Piperacillin-tazobactam	2	4	≤0.5 — 8	100.0	0.0	0.0	100.0	0.0	0.0
Carbapenemase (54)									
LYS228	0.5	4	≤0.03 — 32	—	—	—	—	—	—
Cefepime	>64	>64	≤0.5 — >64	7.4	7.4	85.2	3.7	5.6	90.7
Ceftazidime	>128	>128	0.5 — >128	3.7	5.6	90.7	3.7	0.0	96.3
Ceftriaxone	>128	>128	0.25 — >128	3.7	0.0	96.3	3.7	0.0	96.3
Colistin	1	>8	≤0.5 — >8	—	—	—	77.8	—	22.2
Meropenem	8	64	0.25 — >64	3.7	1.9	94.4	5.6	44.4	50.0
Amikacin	4	>32	0.5 — >32	77.8	3.7	18.5	68.5	9.3	22.2
Aztreonam	>16	>16	≤0.12 — >16	14.8	3.7	81.5	14.8	0.0	85.2
Levofloxacin	>4	>4	≤0.5 — >4	31.5	7.4	61.1	29.6	1.9	68.5
Tigecycline	0.25	2	0.06 — 4	—	—	—	87.0	11.1	1.9
Piperacillin-tazobactam	>64	>64	2 — >64	9.3	7.4	83.3	7.4	1.9	90.7
ESBL/AmpC (30)									
LYS228	0.25	0.5	≤0.03 — 1	—	—	—	—	—	—
Cefepime	4	>64	≤0.5 — >64	36.7	23.3	40.0	30.0	20.0	50.0
Ceftazidime	16	>128	0.5 — >128	20.0	6.7	73.3	6.7	13.3	80.0
Ceftriaxone	64	>128	1 — >128	3.3	3.3	93.3	3.3	3.3	93.3
Colistin	1	1	≤0.5 — >8	—	—	—	93.3	—	6.7
Meropenem	≤0.12	≤0.12	≤0.12 — 4	90.0	3.3	6.7	93.3	6.7	0.0
Amikacin	2	8	0.5 — 32	96.3	3.7	0.0	92.6	3.7	3.7
Aztreonam	>16	>16	≤0.12 — >16	30.0	6.7	63.3	13.3	16.7	70.0
Levofloxacin	>4	>4	≤0.5 — >4	21.4	0.0	78.6	21.4	0.0	78.6
Tigecycline	0.25	0.5	0.06 — 1	—	—	—	100.0	0.0	0.0
Piperacillin-tazobactam	8	>64	2 — >64	73.3	10.0	16.7	60.0	13.3	26.7

[a] Criteria as published by CLSI (2015) and EUCAST (2015)

Conclusions

- LYS228 exhibited potent activity against wild-type and β-lactamase-producing *Enterobacteriaceae*, regardless of β-lactamase enzymes produced
- LYS228 (MIC_{50}/MIC_{90}, 0.5/4 µg/mL) demonstrated similar potency as tigecycline (MIC_{50}/MIC_{90}, 0.25/2 µg/mL) against selected carbapenemase-producing isolates
- The activity of LYS228 against AmpC and ESBL producers (MIC_{50}/MIC_{90}, 0.25/0.5 µg/mL) was comparable to the activity observed versus wild-type *Enterobacteriaceae* (MIC_{50}/MIC_{90}, 0.06/0.25 µg/mL)
- In summary, LYS228 demonstrated potent activity against *Enterobacteriaceae*, and its potency was not adversely affected by isolates producing different β-lactamase enzymes, warranting further development of LYS228

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