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Potentiation of Clarithromycin When Combined with a New Cationic Peptide against Gram-negative Clinical Isolates and Molecular Analysis of Macrolide Resistance Mechanisms by Next-Generation Sequencing

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Abstract

Background: *A. baumannii* and *Enterobacteriaceae* can express a diverse array of antimicrobial resistance mechanisms, which can compromise therapy. The activity of clarithromycin (CLA) combined with SPR741 was assessed against a recent collection of clinical pathogens.

Methods: A total of 134 *A. baumannii* and 342 *Enterobacteriaceae* (3 species) isolates were selected. Isolates were tested for susceptibility by CLSI methods. CLA was tested in combination with SPR741 at a fixed concentration of 8 µg/mL. Interpretation of MICs for comparators applied CLSI/EUCAST/FDA criteria. Selected isolates with CLA-SPR741 MICs of ≥8 µg/mL were subjected to whole genome sequencing for screening of acquired MLS_B resistance genes.

Results: CLA-SPR741 ($MIC_{50/90}$, 0.12/1 µg/mL) had the lowest MICs against *Enterobacteriaceae* (25.4% ESBL and 14.6% carbapenem-resistant [CRE]), followed by tigecycline ($MIC_{50/90}$, 0.25/1 µg/mL; 94.4–99.7% susceptible) and colistin ($MIC_{50/90}$, ≤0.5/1 µg/mL; 93.2% susceptible). Other agents, including meropenem had MIC_{90} of ≥8 lower than tigecycline ($MIC_{50/90}$, 0.25/2 µg/mL; 86.4–99.1% susceptible) against *E. cloacae*, whereas CLA-SPR741 ($MIC_{50/90}$, 1/>32 µg/mL) was less active against *A. baumannii*. Isolates displaying elevated CLA-SPR741 MICs had combinations of methylases (Erm) and/or inactivating enzymes (Mph).

Conclusions: CLA-SPR741 demonstrated potent activity against this recent collection of *Enterobacteriaceae*, including CRE and ESBL-producing isolates. This study also expanded the knowledge of MLS_B genes in gram-negative pathogens.

Introduction

- *Enterobacteriaceae* isolates account for 27% of health care-associated infections in the US, and a great proportion of these isolates produce extended-spectrum β-lactamases (ESBLs), which account for approximately 14% of *Enterobacteriaceae*
- ESBL-producing *Enterobacteriaceae* isolates have spread in the nosocomial and community settings, complicating the empiric treatment of infections caused by these organisms
- The increased frequency of ESBL-producing *Enterobacteriaceae* isolates may increase the use of more potent antimicrobial agents, including carbapenems
- Although carbapenem-resistant *Enterobacteriaceae* (CRE) isolates are relatively uncommon in the US, the number of US facilities reporting CRE has risen steadily and includes 4% of acute hospitals and 18% of long-term acute care facilities
- Organisms, such as *Acinetobacter baumannii* and other non-fermentative isolates can also express a diverse array of antimicrobial resistance mechanisms, which can compromise therapy
  - These hard-to-treat infections have been targeted as one of the most pressing challenges in the field of infectious diseases
- SPR741 is a novel polymyxin analog that interacts with the outer membrane of gram-negative bacteria and compromises the integrity of the lipopolysaccharide
- SPR741 will increase cell permeability and enable entry of antimicrobial compounds
- This study investigated the activity of clarithromycin combined with SPR741 against a recent collection of *A. baumannii* and *Enterobacteriaceae* clinical isolates

Materials and Methods

Organism collection

- This study used geographically diverse *Enterobacteriaceae* (342) and *A. baumannii* (134) clinical isolates collected worldwide from patients with documented infections
- Isolates originated from 34 countries, including 7 countries in Asia-Pacific, 21 countries/regions in Europe, 5 countries in Latin America, and the US
- Selected isolates were mostly responsible for urinary tract infections (70.0%), followed by bloodstream infections (10.3%), pneumonia in hospitalized patients (10.1%), skin and skin structure infections (5.5%), and other less common infections (4.2%)

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07-A10 document
- Clarithromycin was tested in combination with SPR741 at a fixed concentration of 8 µg/mL
- Bacterial inoculum density was monitored by colony counts to assure 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-S26) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2016) breakpoint criteria, as available
  - Tigecycline MIC breakpoints were from the US Food and Drug Administration-approved package insert

Characterization of macrolide resistance mechanisms by next-generation sequencing

- A selection of isolates exhibiting clarithromycin-SPR741 (fixed 8 µg/mL) MIC results at ≥8 µg/mL were subjected to next-generation sequencing (NGS) to screen for acquired macrolide, lincosamide, and streptogramin B (MLS_B) resistance genes
- Selected isolates had total genomic DNA extracted by the fully automated Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, OH, USA), which was used as input material for library construction
- DNA libraries were prepared using the Nextera™ library construction protocol (Illumina, San Diego, CA, USA) following the manufacturer's instructions and were sequenced on a MiSeq Sequencer (JMI Laboratories, North Liberty, IA, USA)
- Assembled genomes were subjected to a proprietary software (JMI Laboratories), which paired their genomes against a curated database containing numerous resistance determinants to screen for MLS_B genes

Results

- Clarithromycin-SPR741 had MIC_{50} and MIC_{90} results of 1 and >32 µg/mL, respectively, when tested against *A. baumannii* clinical isolates (Tables 1 and 2)
- Only colistin ($MIC_{50/90}$, 1/2 µg/mL; 92.5% susceptible) showed *in vitro* activity against *A. baumannii* isolates; other comparator agents demonstrated susceptibility rates of 17.7–47.7% (Table 2)
- Clarithromycin-SPR741 had the lowest MIC_{50} and MIC_{90} results ($MIC_{50/90}$, 0.12/1 µg/mL) against the entire collection of *Enterobacteriaceae* clinical isolates, followed by tigecycline ($MIC_{50/90}$, 0.25/1 µg/mL; 94.4–99.7% susceptible) and colistin ($MIC_{50/90}$, ≤0.5/1 µg/mL; 93.2% susceptible; Table 3)
- A total of 36.2% and 7.8% of *E. coli* isolates were categorized as ESBL and CRE, respectively (Table 1)
- Clarithromycin-SPR741 inhibited 96.6% of all *E. coli*, 92.9% of ESBL-producing *E. coli*, and 88.9% of carbapenem-resistant *E. coli* at ≤8 µg/mL
- A total of 41.0% and 19.7% of *Klebsiella pneumoniae* isolates were categorized as ESBL and CRE, respectively (Table 1)
- Clarithromycin-SPR741 ($MIC_{50/90}$, 0.12/4 µg/mL), colistin ($MIC_{50/90}$, ≤0.5/1 µg/mL; 92.1% susceptible), and tigecycline ($MIC_{50/90}$, 0.25/1 µg/mL; 96.5–100.0% susceptible) were active against *K. pneumoniae* (Table 3)
- Clarithromycin-SPR741 inhibited all *Enterobacter cloacae* isolates at ≤8 µg/mL, including carbapenem-resistant isolates (Table 1)
- Clarithromycin-SPR741 ($MIC_{50/90}$, 0.12/1 µg/mL) showed MIC results 2-fold lower than tigecycline ($MIC_{50/90}$, 0.25/2 µg/mL; 86.4–99.1% susceptible) against *E. cloacae*; other comparator agents were not active (Table 3)
- Isolates exhibiting clarithromycin-SPR741 MIC values of ≥8 µg/mL that were selected for screening of resistance mechanisms often carried the efflux-pump-[*msr*(E)] and phosphorylase-[*mph*] encoding genes (Table 4)

Table 1 Antimicrobial activity of investigational clarithromycin tested in combination with SPR741 at fixed concentration of 8 µg/mL against *A. baumannii* and *Enterobacteriaceae* clinical isolates

Organism (no. tested)	Number (cumulative %) of isolates at MIC (µg/mL) of:											MIC (µg/mL)	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32	50%	90%
A. baumannii (139)	12 (8.6%)	15 (19.4%)	21 (34.5%)	19 (48.2%)	9 (54.7%)	2 (56.1%)	1 (56.8%)	5 (60.4%)	3 (62.6%)	9 (69.1%)	43 (100.0%)	1	>32
Enterobacteriaceae (349)	96 (27.5%)	125 (63.3%)	37 (73.9%)	27 (81.7%)	30 (90.3%)	11 (93.4%)	4 (94.6%)	7 (96.6%)	4 (97.7%)	1 (98.0%)	7 (100.0%)	0.12	1
ESBL (90)	10 (11.1%)	15 (27.8%)	8 (36.7%)	13 (51.1%)	18 (71.1%)	6 (77.8%)	3 (81.1%)	6 (87.8%)	3 (91.1%)	1 (92.2%)	7 (100.0%)	0.5	16
CRE (56)	4 (7.1%)	20 (42.9%)	4 (50.0%)	1 (51.8%)	10 (69.6%)	4 (76.8%)	3 (82.1%)	3 (87.5%)	1 (90.0%)	0 (90.0%)	6 (100.0%)	0.25	16
E. coli (116)	51 (43.6%)	26 (66.4%)	4 (69.8%)	13 (81.0%)	9 (88.8%)	6 (94.0%)	1 (94.8%)	2 (96.6%)	2 (98.3%)	0 (98.3%)	2 (100.0%)	0.12	2
ESBL (42)[a]	6 (14.3%)	7 (31.0%)	3 (38.1%)	8 (57.1%)	8 (76.2%)	4 (85.7%)	1 (88.1%)	2 (92.9%)	1 (95.2%)	0 (95.2%)	2 (100.0%)	0.5	8
CRE (9)[a]	1 (11.1%)	3 (44.4%)	0 (44.4%)	0 (44.4%)	3 (77.8%)	1 (88.9%)	0 (88.9%)	0 (88.9%)	0 (88.9%)	0 (88.9%)	1 (100.0%)	1	[b]
K. pneumoniae (117)	15 (12.8%)	45 (51.3%)	16 (65.0%)	12 (75.2%)	12 (85.5%)	3 (88.0%)	2 (90.0%)	4 (93.2%)	2 (94.9%)	1 (95.7%)	5 (100.0%)	0.12	4
ESBL (48)[a]	4 (8.3%)	8 (25.0%)	5 (35.4%)	5 (45.8%)	10 (66.7%)	2 (70.8%)	2 (75.0%)	4 (83.3%)	2 (87.5%)	1 (90.0%)	5 (100.0%)	1	32
CRE (23)[a]	2 (8.7%)	1 (13.0%)	1 (17.4%)	0 (17.4%)	7 (47.8%)	2 (56.5%)	2 (65.2%)	2 (73.9%)	2 (78.3%)	0 (78.3%)	5 (100.0%)	2	>32
E. cloacae (115)	30 (26.1%)	53 (72.2%)	17 (87.0%)	2 (88.7%)	9 (96.5%)	2 (98.3%)	1 (99.1%)	1 (100.0%)				0.12	1
CRE (24)[a]	1 (4.2%)	16 (70.8%)	3 (83.3%)	1 (87.5%)	0 (87.5%)	1 (91.7%)	1 (95.8%)	1 (100.0%)				0.12	2

[a] ESBL phenotype consisted of isolates displaying MIC values of >1 µg/mL for aztreonam, ceftazidime, and/or ceftriaxone. CRE, carbapenem-resistant *Enterobacteriaceae* showing MIC values of >2 µg/mL for imipenem, meropenem, and/or doripenem
[b] MIC_{90} value shown when number of isolates ≥10

Table 2 Activity of investigational clarithromycin tested in combination with SPR741 at fixed concentration of 8 µg/mL and comparator agents against 134 isolates of *Acinetobacter baumannii-calcoaceticus* species complex

Antimicrobial agent	MIC		% S / % I / % R[a]					
	50%	90%	CLSI			EUCAST		
Clarithromycin-SPR741	1	>32	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Ampicillin-sulbactam	16	>32	35.1	16.4	48.5	—[b]	—[b]	—[b]
Ceftazidime	>16	>16	32.1	1.5	66.4	—[b]	—[b]	—[b]
Cefepime	>16	>16	29.1	9.7	61.2	—[b]	—[b]	—[b]
Imipenem	>8	>8	34.3	3.7	61.9	34.3	9.0	56.7
Meropenem	>8	>8	32.8	0.7	66.4	32.8	7.5	59.7
Amikacin	32	>32	47.7	4.5	47.7	44.7	3.0	52.3
Colistin	1	2	92.5	—[b]	7.5	92.5	—[b]	7.5
Levofloxacin	>4	>4	17.7	3.2	79.0	17.7	0.0	82.3
Tigecycline	1	2	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]

[a] %S / %I / %R, % susceptible / % intermediate / % resistant; criteria as published by CLSI (2016) and EUCAST (2016)
[b] "—" represents breakpoints not available

Table 3 Activity of investigational clarithromycin tested in combination with SPR741 at fixed concentration of 8 µg/mL and comparator agents against *Enterobacteriaceae* clinical isolates

Organism (no. tested)/ antimicrobial agent	MIC		% S / % I / % R[a]					
	50%	90%	CLSI			EUCAST		
Enterobacteriaceae (342)								
Clarithromycin-SPR741	0.12	1	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Piperacillin-tazobactam	4	>64	71.2	6.2	22.6	67.4	3.9	28.8
Ceftazidime	0.5	>16	62.1	3.3	34.6	58.6	3.6	37.9
Cefepime	≤0.5	>16	66.9	7.7	25.4	63.9	8.0	28.1
Imipenem	≤0.12	4	84.6	1.2	14.2	85.8	11.3	3.0
Meropenem	≤0.12	8	85.2	1.5	13.3	86.7	7.1	6.2
Amikacin	2	8	94.1	2.4	3.6	90.5	3.6	5.9
Colistin	≤0.5	1	—[b]	—[b]	—[b]	93.2	—[b]	6.8
Levofloxacin	>4	>4	45.8	0.0	54.2	45.8	0.0	54.2
Tigecycline	0.25	1	99.7	0.3	0.0	94.4	5.3	0.3
E. coli (116)								
Clarithromycin-SPR741	0.12	2	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Piperacillin-tazobactam	2	>64	85.1	3.5	11.4	82.5	2.6	14.9
Ceftazidime	0.25	32	71.1	7.0	21.9	64.0	7.0	28.9
Cefepime	≤0.5	>16	72.8	7.0	20.2	71.1	5.3	23.7
Imipenem	≤0.12	0.5	92.1	0.9	7.0	93.0	5.3	1.8
Meropenem	≤0.06	0.12	91.2	1.8	7.0	93.0	5.3	1.8
Amikacin	2	4	98.2	0.0	1.8	95.6	2.7	1.8
Colistin	≤0.5	≤0.5	—[b]	—[b]	—[b]	97.3	—[b]	2.7
Levofloxacin	>4	>4	35.1	0.0	64.9	35.1	0.0	64.9
Tigecycline	0.12	0.25	100.0	0.0	0.0	100.0	0.0	0.0
K. pneumoniae (115)								
Clarithromycin-SPR741	0.12	8	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Piperacillin-tazobactam	4	>64	71.7	6.2	22.1	65.5	6.2	28.3
Ceftazidime	0.25	>16	65.8	1.8	32.5	64.0	1.8	34.2
Cefepime	≤0.5	>16	65.8	7.0	27.2	64.0	7.9	28.1
Imipenem	≤0.12	8	80.5	1.8	17.7	82.3	12.4	5.3
Meropenem	≤0.06	>8	81.6	1.8	16.7	83.3	6.1	10.5
Amikacin	1	32	88.6	6.1	5.3	85.1	3.5	11.4
Colistin	≤0.5	1	—[b]	—[b]	—[b]	92.1	—[b]	7.9
Levofloxacin	>4	>4	44.4	0.0	55.6	44.4	0.0	55.6
Tigecycline	0.25	1	100.0	0.0	0.0	96.5	3.5	0.0
E. cloacae (111)								
Clarithromycin-SPR741	0.12	1	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Piperacillin-tazobactam	8	>64	56.4	9.1	34.5	53.6	2.7	43.6
Amikacin	1	8	95.5	0.9	3.6	90.9	4.5	4.5
Cefepime	≤0.5	>16	61.8	9.1	29.1	56.4	10.9	32.7
Imipenem	0.25	8	80.9	0.9	18.2	81.8	16.4	1.8
Meropenem	≤0.12	8	82.7	0.9	16.4	83.6	10.0	6.4
Ceftazidime	8	>32	49.1	0.9	50.0	47.3	1.8	50.9
Colistin	≤0.5	>4	—[b]	—[b]	—[b]	89.9	—[b]	10.1
Levofloxacin	≤0.5	>4	58.8	0.0	41.2	58.8	0.0	41.2
Tigecycline	0.25	2	99.1	0.9	0.0	86.4	12.7	0.9

[a] %S / %I / %R, % susceptible / % intermediate / % resistant; criteria as published by CLSI (2016) and EUCAST (2016). Interpretation for tigecycline MIC results used breakpoints approved by the US Food and Drug Administration. Cefepime susceptible-dose dependent breakpoint applied and displayed under the intermediate category.
[b] "—" represents breakpoints not available

Table 4 MIC results for clarithromycin-SPR741 obtained and detected resistance genes

Organism (no. tested)	Clarithromycin-SPR741 (8 µg/mL) MIC	Resistance gene
A. baumannii (35)	8->32	*msr*(E), *mphE*
E. coli (1)	16	*erm*(B)
E. coli (1)	8	mphA
E. coli (1)	>32	ND[a]
E. coli (1)	16	ND[a]
K. pneumoniae (1)	8	*msr*(E), *mphE*
K. pneumoniae (1)	16	mphA
K. pneumoniae (1)	8	mphA
K. pneumoniae (1)	16	mphA

[a] ND, no MLS_B gene detected

Conclusions

- Clarithromycin-SPR741 demonstrated potent activity against this recent collection of multidrug-resistant (MDR) *Enterobacteriaceae*, including CRE and ESBL-producing isolates
- Clarithromycin-SPR741 was less active against MDR *A. baumannii* clinical isolates
- Isolates displaying elevated clarithromycin-SPR741 MIC results carried MLS_B resistance genes, and this study expanded the knowledge of such genes in gram-negative pathogens
- Results presented here indicate that such combinations may be clinically relevant and warrant further development to investigate their roles as anti-gram-negative agents

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