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Antimicrobial Synergistic Effect of a New Anti-Gram-Positive Agent Tested In Combination with a Polymyxin Derivative against Gram-Negative Pathogens, Including ESKAPE Group Organisms

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Abstract

Background: Gram-negative pathogens displaying a multidrug-resistance (MDR) phenotype have become common. Combination therapy is often used to obtain greater potency and therapeutic success when treating infections caused by MDR isolates. This study evaluated a new approach combining a novel gram-positive agent (a gyrase inhibitor; SPR719) with a polymyxin-like compound (SPR741) against *Acinetobacter baumannii* and *Enterobacteriaceae*.

Methods: A total of 153 *A. baumannii* and 543 *Enterobacteriaceae* (10 species) isolates were selected and tested for susceptibility by CLSI methods. SPR719 was tested in combination with SPR741 at a fixed concentration of 8 µg/mL. Interpretation of MICs for comparators applied CLSI/EUCAST/FDA criteria.

Results: SPR719-SPR741 ($MIC_{50/90}$, 0.5/2 µg/mL) and colistin ($MIC_{50/90}$, ≤0.5/2 µg/mL) were similarly active against *A. baumannii* and had MICs 2-fold lower than tigecycline ($MIC_{50/90}$, 1/4 µg/mL). Other agents tested against *A. baumannii* had MIC_{90} results of >4 µg/mL. SPR719-SPR741 ($MIC_{50/90}$, ≤0.03/0.12 µg/mL) and meropenem ($MIC_{50/90}$, ≤0.015/0.03 µg/mL) displayed the lowest MICs, followed by colistin ($MIC_{50/90}$, 0.12/0.25 µg/mL) and tigecycline ($MIC_{50/90}$, 0.12/0.25 µg/mL) against *Escherichia coli* (19.0% ESBL). SPR719-SPR741 ($MIC_{50/90}$, 0.06/0.25 µg/mL) inhibited 99.4% of *Klebsiella pneumoniae* (25.6% ESBL and 7.7% carbapenem resistant) at ≤2 µg/mL. Meropenem (92.9% susceptible), colistin (97.4% susceptible), and tigecycline (97.4–99.4% susceptible) were active against *K. pneumoniae*. SPR719-SPR741 ($MIC_{50/90}$, 0.06/0.12 µg/mL) and meropenem ($MIC_{50/90}$, 0.03/0.03 µg/mL) had MICs at least 2-fold lower than colistin ($MIC_{50/90}$, 0.25/0.25 µg/mL) and tigecycline ($MIC_{50/90}$, 0.25/0.5 µg/mL) against *Citrobacter freundii*. *Enterobacter aerogenes* and *E. cloacae* were inhibited by SPR719-SPR741 ($MIC_{50/90}$, 0.12/0.5 µg/mL for both) at ≤1 µg/mL while other *Enterobacteriaceae* species had higher MICs.

Conclusions: SPR719-SPR741 showed potent activity against *A. baumannii* and *Enterobacteriaceae*. This strategy was not synergistic against *Enterobacteriaceae* species intrinsically resistant to polymyxin.

Introduction

- The dissemination of extended-spectrum β-lactamases (ESBLs) and carbapenemase enzymes in *Enterobacteriaceae* has become a serious health care concern
- Approximately 14% of *Enterobacteriaceae* isolates collected in 63 United States (US) hospitals in 2012–2014 displayed an ESBL phenotype, while a similar percentage (14%) of isolates were classified as ESBL in Europe during 2011–2013
- Carbapenem resistance among *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* comprised 2.3% and 1.8% of US isolates in 2014 and in 2012, respectively. A rate of 1.9% was reported in these organisms in Europe during 2011–2013
- The vast majority of ESBL-producing *Enterobacteriaceae* carry bla_{CTX-M}, while bla_{KPC} is common among carbapenem-resistant *Enterobacteriaceae* (CRE) from the US, bla_{KPC}, bla_{NDM} and $bla_{OXA-48-like}$ genes are common in Europe
- The scenario described above, lack of new antimicrobial agents approved in the last decades, clinical challenges of managing multidrug-resistant (MDR)-caused infections, and limited therapeutic options have recently prompted several agencies to promote new antimicrobial agent development
- Combination therapy is often used to obtain greater potency and therapeutic success when treating infections caused by MDR isolates.
- SPR719 is a novel antimicrobial agent that targets bacterial gyrase (Figure 1), while SPR741 is a polymyxin-derived molecule
- This study evaluated combining a novel gram-positive agent (SPR719) with a polymyxin-like compound (SPR741) against *Acinetobacter baumannii* and *Enterobacteriaceae* clinical isolates

Materials and Methods

Organism collection

- This study used geographically diverse *Enterobacteriaceae* (543) and *Acinetobacter baumannii* (153) clinical isolates collected from patients worldwide with documented infections
- Isolates originated from 21 European countries/regions (49.9%; 347) and the US (50.1%, 349) and caused urinary tract infections (57.8%), pneumonia in hospitalized patients (18.0%), bloodstream infections (10.3%), skin and skin structure infections (9.9%), and other less common infections (4.0%)

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07-A10 document
- SPR719 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

Results

- SPR719-SPR741 had MIC_{50} and MIC_{90} results of 0.5 and 2 µg/mL, respectively, when tested against *A. baumannii* clinical isolates (Table 1)
- SPR719-SPR741 ($MIC_{50/90}$, 0.5/2 µg/mL) and colistin ($MIC_{50/90}$, ≤0.5/2 µg/mL) were similarly active against *A. baumannii* and had MIC values 2-fold lower than tigecycline ($MIC_{50/90}$, 1/4 µg/mL). Other agents tested against *A. baumannii* had MIC_{90} results of >4 µg/mL (Table 2)
- Overall, SPR719-SPR741 showed MIC_{50} and MIC_{90} results of 0.06 and 2 µg/mL, respectively, when tested against all *Enterobacteriaceae* clinical isolates (Table 1)
- MIC_{90} results of 0.12 and 0.25 µg/mL were obtained for SPR719-SPR741 when tested against *E. coli* and *K. pneumoniae* clinical isolates, respectively, regardless of ESBL production (Table 1)
- SPR719-SPR741, meropenem, colistin, and tigecycline showed the lowest MIC_{50} and MIC_{90} results against *E. coli* and ESBL-producing clinical isolates (Table 3)
- When tested against *K. pneumoniae*, SPR719-SPR741 ($MIC_{50/90}$, 0.06/0.25 µg/mL) and meropenem ($MIC_{50/90}$, 0.03/0.12 µg/mL) were the most potent agents, and both had MIC_{90} results at least 4-fold lower than tigecycline ($MIC_{50/90}$, 0.25/1 µg/mL; Table 3)
- SPR719-SPR741 ($MIC_{50/90}$, 0.06/0.25 µg/mL), colistin ($MIC_{50/90}$, ≤0.5/≤0.5 µg/mL), and tigecycline ($MIC_{50/90}$, 0.5/1 µg/mL) were active against ESBL-producing *K. pneumoniae*, while SPR719-SPR741 ($MIC_{50/90}$, 0.12/1 µg/mL) and tigecycline ($MIC_{50/90}$, 0.5/1 µg/mL) were active against carbapenem-resistant *K. pneumoniae* (Table 3)
- SPR719-SPR741 ($MIC_{50/90}$, 0.06/0.12 µg/mL) and meropenem ($MIC_{50/90}$, 0.03/0.03 µg/mL) had MIC values at least 2-fold lower than colistin ($MIC_{50/90}$, 0.25/0.25 µg/mL) and tigecycline ($MIC_{50/90}$, 0.25/0.5 µg/mL) against *Citrobacter freundii* (Table 3)
- *Enterobacter* spp. isolates were inhibited by SPR719-SPR741 ($MIC_{50/90}$, 0.12/0.5 µg/mL) at ≤1 µg/mL while other *Enterobacteriaceae* species had higher MIC results ($MIC_{50/90}$, >8/>8 µg/mL; Tables 1 and 3)

Table 1 Antimicrobial activity of investigational SPR719 tested in combination with SPR741 at fixed concentration of 8 µg/mL against *A. baumannii* and *Enterobacteriaceae* clinical isolates

Organism (no. tested)	Number (cumulative [a]) of isolates at MIC (µg/mL) of:[a]									
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
A. baumannii (153)	2 (1.3%)	2 (2.6%)	18 (14.4%)	**42 (41.8%)**	30 (61.4%)	23 (76.5%)	22 (90.8%)	1 (91.5%)	0 (91.5%)	13 (100.0%)
Enterobacteriaceae (543)	**176 (32.4)**	129 (56.2)	95 (73.7)	49 (82.7)	22 (86.7)	15 (89.5)	9 (91.2)	8 (92.6)	2 (93.0)	38 (100.0)
Enterobacteriaceae (486)[b]	**176 (36.2)**	129 (62.8)	95 (82.3)	49 (92.4)	22 (96.9)	11 (99.2)	2 (99.6)	0 (99.6)	2 (100.0)	
E. coli (153)	**90 (58.8%)**	45 (88.2%)	15 (98.0%)	0 (98.0%)	1 (98.7%)	0 (98.7%)	1 (99.3%)	0 (99.3%)	1 (100.0%)	
ESBL (29)[c]	**14 (48.3%)**	11 (86.2%)	4 (100.0%)							
K. pneumoniae (156)	**48 (30.8%)**	44 (59.0%)	32 (79.5%)	20 (92.3%)	3 (94.2%)	7 (98.7%)	1 (99.4%)	0 (99.4%)	1 (100.0%)	
ESBL (40)[c]	**11 (27.5%)**	10 (52.5%)	8 (72.5%)	8 (92.5%)	0 (92.5%)	2 (97.5%)	0 (97.5%)	0 (97.5%)	1 (100.0%)	
CRE (12)[c]	2 (16.7%)	2 (33.3%)	3 (58.3%)	3 (83.3%)	0 (83.3%)	1 (91.7%)	0 (91.7%)	0 (91.7%)	1 (100.0%)	
C. freundii (14)	4 (28.6%)	**8 (85.7%)**	2 (100.0%)							
Enterobacter spp. (163)[d]	34 (20.9)	32 (40.5)	**46 (68.7)**	29 (86.5)	18 (97.5)	4 (100.0)				
Other species (57)[e]						4 (7.0)	7 (19.3)	8 (33.3)	0 (33.3)	**38 (100.0)**

[a] Modal MIC, MIC_{50}, and MIC_{90} results are shown in bold, underline, and double underline, respectively
[b] Excludes those *Enterobacteriaceae* (13 *Proteus mirabilis*, 14 *P. vulgaris*, 8 *Providencia rettgeri*, 8 *P. stuartii*, and 14 *Serratia marcescens*) intrinsically resistant to polymyxin
[c] 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
[d] Includes 14 *E. aerogenes* and 149 *E. cloacae*
[e] Includes 13 *P. mirabilis*, 14 *P. vulgaris*, 8 *P. rettgeri*, 8 *P. stuartii*, and 14 *S. marcescens*

Table 2 Activity of investigational SPR719 tested in combination with SPR741 at fixed concentration of 8 µg/mL and comparator agents against 153 isolates of *Acinetobacter baumannii-calcoaceticus* species complex

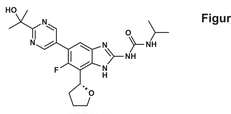
Antimicrobial agent	MIC		% S / % I / % R[a]					
	50%	90%	CLSI			EUCAST		
SPR719-SPR741	0.5	2	—	—	—	—	—	—
Ampicillin-sulbactam	16	>32	37.9	14.4	47.7	—	—	—
Piperacillin-tazobactam	>64	>64	31.6	6.6	61.8	—	—	—
Cefepime	>16	>16	32.0	11.1	56.9	—	—	—
Meropenem	>8	>8	39.9	0.7	59.5	39.9	4.6	55.6
Levofloxacin	>4	>4	25.6	4.9	69.5	24.4	1.2	74.4
Gentamicin	>8	>8	39.0	1.2	59.8	39.0	—	61.0
Colistin	≤0.5	2	94.1	—	5.9	94.1	—	5.9
Tigecycline	1	4	—	—	—	—	—	—

[a] %S / %I / %R, % susceptible / % intermediate / % resistant; criteria as published by CLSI (2016) and EUCAST (2016)

Table 3 Activity of investigational SPR719 tested in combination with SPR741 at fixed concentration of 8 µg/mL and comparator agents against *Enterobacteriaceae* clinical isolates

Organism[a] (no. tested)/ antimicrobial agent	MIC		% S / % I / % R[b]						MIC		% S / % I / % R[b]					
	50%	90%	CLSI			EUCAST			50%	90%	CLSI			EUCAST		
E. coli (153)									*K. pneumoniae* ESBL (40), *continued*							
SPR719-SPR741	≤0.03	0.12	—	—	—	—	—	—	Meropenem	0.03	32	72.5	0.0	27.5	72.5	10.0	17.5
Piperacillin-tazobactam	2	8	95.4	0.7	3.9	94.1	1.3	4.6	Levofloxacin	4	>4	40.0	15.0	45.0	35.0	5.0	60.0
Aztreonam	≤0.12	16	83.7	3.3	13.1	81.0	2.6	16.3	Gentamicin	4	>8	50.0	0.0	50.0	40.0	10.0	50.0
Ceftriaxone	≤0.06	>8	82.4	0.0	17.6	82.4	0.0	17.6	Colistin	≤0.5	≤0.5	—	—	—	95.0	—	5.0
Cefepime	≤0.03	16	85.6	3.9	10.5	84.3	2.6	13.1	Tigecycline	0.5	1	100.0	0.0	0.0	97.5	2.5	0.0
Meropenem	≤0.015	0.03	100.0	0.0	0.0	100.0	0.0	0.0	*K. pneumoniae* CRE (12)								
Levofloxacin	0.06	>4	66.2	3.9	29.9	66.2	0.0	33.8	SPR719-SPR741	0.12	1	—	—	—	—	—	—
Gentamicin	0.5	>8	85.7	0.0	14.3	85.7	0.0	14.3	Piperacillin-tazobactam	>64	>64	0.0	0.0	100.0	0.0	0.0	100.0
Colistin	0.12	0.25	—	—	—	98.7	—	1.3	Aztreonam	>16	>16	8.3	0.0	91.7	8.3	0.0	91.7
Tigecycline	0.12	0.25	100.0	0.0	0.0	100.0	0.0	0.0	Ceftriaxone	>8	>8	8.3	0.0	91.7	8.3	0.0	91.7
E. coli ESBL (29)									Cefepime	>16	>16	8.3	25.0	66.7	8.3	0.0	91.7
SPR719-SPR741	0.06	0.12	—	—	—	—	—	—	Meropenem	16	32	8.3	0.0	91.7	8.3	33.3	58.3
Piperacillin-tazobactam	4	32	89.7	3.4	6.9	86.2	3.4	10.3	Levofloxacin	>4	—	28.6	14.3	57.1	14.3	14.3	71.4
Aztreonam	16	>16	13.8	17.2	69.0	0.0	13.8	86.2	Gentamicin	>8	—	14.3	0.0	85.7	0.0	14.3	85.7
Ceftriaxone	>8	>8	6.9	0.0	93.1	6.9	0.0	93.1	Colistin	≤0.5	>8	—	—	—	83.3	—	16.7
Cefepime	16	>64	24.1	20.7	55.2	24.1	6.9	69.0	Tigecycline	0.5	1	100.0	0.0	0.0	91.7	8.3	0.0
Meropenem	≤0.015	0.03	100.0	0.0	0.0	100.0	0.0	0.0	*C. freundii* (14)								
Levofloxacin	>4	>4	26.7	0.0	73.3	26.7	0.0	73.3	SPR719-SPR741	0.06	0.12	—	—	—	—	—	—
Gentamicin	1	>8	60.0	0.0	40.0	60.0	0.0	40.0	Piperacillin-tazobactam	2	32	85.7	7.1	7.1	85.7	0.0	14.3
Colistin	0.12	0.25	—	—	—	100.0	—	0.0	Aztreonam	≤0.12	16	78.6	0.0	21.4	78.6	0.0	21.4
Tigecycline	0.12	0.25	100.0	0.0	0.0	100.0	0.0	0.0	Ceftriaxone	0.25	>8	78.6	0.0	21.4	78.6	0.0	21.4
K. pneumoniae (156)									Cefepime	≤0.03	1	92.9	7.1	0.0	92.9	0.0	7.1
SPR719-SPR741	0.06	0.25	—	—	—	—	—	—	Meropenem	0.03	0.03	100.0	0.0	0.0	100.0	0.0	0.0
Piperacillin-tazobactam	2	>64	81.3	5.2	13.5	76.1	5.2	18.7	Levofloxacin	0.06	2	92.9	0.0	7.1	85.7	7.1	7.1
Aztreonam	≤0.12	>16	76.3	0.6	23.1	75.6	0.6	23.7	Gentamicin	0.5	>8	78.6	0.0	21.4	78.6	0.0	21.4
Ceftriaxone	≤0.06	>8	74.4	0.0	25.6	74.4	0.0	25.6	Colistin	0.25	0.25	—	—	—	100.0	—	0.0
Cefepime	≤0.5	>16	77.6	5.1	17.3	75.6	3.8	20.5	Tigecycline	0.25	0.5	100.0	0.0	0.0	92.9	7.1	0.0
Meropenem	0.03	0.12	92.9	0.0	7.1	92.9	2.6	4.5	*Enterobacter* spp. (163)[c]								
Levofloxacin	0.06	>4	85.0	3.8	11.2	82.5	2.5	15.0	SPR719-SPR741	0.12	0.5	—	—	—	—	—	—
Gentamicin	0.25	>8	87.5	0.0	12.5	85.0	2.5	12.5	Piperacillin-tazobactam	2	>64	76.1	8.6	15.3	71.8	4.3	23.9
Colistin	≤0.5	≤0.5	—	—	—	97.4	—	2.6	Aztreonam	0.25	>16	68.7	1.8	29.4	65.0	3.7	31.3
Tigecycline	0.25	1	99.4	0.6	0.0	97.4	1.9	0.6	Ceftriaxone	0.5	>8	59.5	0.6	39.9	59.5	0.6	39.9
K. pneumoniae ESBL (40)									Cefepime	≤0.5	16	82.2	6.7	11.0[d]	76.7	11.0	12.3
SPR719-SPR741	0.06	0.25	—	—	—	—	—	—	Meropenem	0.03	0.12	99.4	0.0	0.6	99.4	0.6	0.0
Piperacillin-tazobactam	32	>64	45.0	15.0	40.0	40.0	5.0	55.0	Levofloxacin	≤0.03	>4	89.0	0.0	11.0	83.5	3.3	13.2
Aztreonam	>16	>16	7.5	2.5	90.0	5.0	2.5	92.5	Gentamicin	0.25	1	92.3	0.0	7.7	91.2	1.1	7.7
Ceftriaxone	>8	>8	0.0	0.0	100.0	0.0	0.0	100.0	Colistin	≤0.5	4	—	—	—	89.4		10.6
Cefepime	>16	>16	12.5	20.0	67.5	7.5	12.5	80.0	Tigecycline	0.25	2	100.0	0.0	0.0[e]	89.6	10.4	0.0

[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] %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
[c] Includes 14 *E. aerogenes* and 149 *E. cloacae*



SPR719

Figure 1 Structure of SPR719

Conclusions

- SPR719-SPR741 showed potent activity against *A. baumannii* and *Enterobacteriaceae*
- The potent *in vitro* activity of this combination may be particularly clinically relevant against ESBL-producing *E. coli* and *K. pneumoniae* as a carbapenem-sparing agent
- SPR719-SPR741 also showed potent *in vitro* activity against carbapenem-resistant *K. pneumoniae* where clinically available agents to treat infections caused by these organisms are limited
- The investigational agent SPR719 tested in combination with the polymyxin-like compound SPR741 did not demonstrate synergy against *Enterobacteriaceae* species intrinsically resistant to polymyxins
- The *in vitro* results obtained for this combination strategy warrant further investigations to evaluate safety and *in vivo* efficacy

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