MICROBE 2016

Saturday-490

Boston, MA

June 16 - 20

Synergistic Effect of Gram-positive Agents Tested in Combination with a New Polymyxin Derivative (SPR741) against Multidrug-resistant Gram-negative Pathogens

RE MENDES,[1] PR RHOMBERG,[1] HK BECKER,[1] AP DAVIS,[1] T LISTER,[2] TR PARR JR.[2], M VAARA[3], RK FLAMM,[1]

[1]JMI Laboratories, North Liberty, IA, USA; [2]Spero Therapeutics, Cambridge, MA, USA; [3]Northern Antibiotics, Espoo, Finland

Rodrigo E. Mendes, PhD
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, Iowa 52317
Phone: (319) 665-3370
rodrigo-mendes@jmilabs.com

Abstract

Background: Few options are available for treating multi-drug resistant (MDR) Gram-negative (GN) infections and combination therapy is used to obtain activity greater than each individual component. The activity of clarithromycin (CLA) and rifampin (RIF) combined with SPR741 was assessed against wildtype (WT) and MDR GN isolates.

Methods: WT and MDR *E. coli* (EC), *E. cloacae* (ECL), *K. pneumoniae* (KPN) and *A. baumannii* (ACB) were selected. CLA, RIF and SPR741 were tested by CLSI methods alone and in combination with SPR741 at fixed concentrations of 2 (F2), 4 (F4) and 8 (F8) μg/mL. Isolates with CLA-SPR741 (F8) MICs of ≥8 μg/mL were subjected to whole genome sequencing (WGS) for screening of MLS_B genes.

Results: CLA had MIC_{50} values of 16-128 μg/mL against WT GN isolates, while CLA-SPR741 at F2 (MIC_{50}, 1-16 μg/mL), F4 (MIC_{50}, 0.12-1 μg/mL) and F8 (MIC_{50}, 0.12 μg/mL) showed MIC_{50} 8- to 2,048-fold lower than CLA. SPR741 at F8 had the lowest MICs against MDR ECL ($MIC_{50/90}$, 0.12/2 μg/mL) and EC ($MIC_{50/90}$, 0.5/8 μg/mL), whereas this combination was less active against MDR KPN ($MIC_{50/90}$, 1/>32 μg/mL) and ACB ($MIC_{50/90}$, 32/>32 μg/mL). RIF tested alone had MIC_{50} values of 2-32 μg/mL against WT GN isolates, while RIF-SPR741 tested at F2 (MIC_{50}, 0.5-4 μg/mL), F4 (MIC_{50}, 0.06-0.25 μg/mL) and F8 (MIC_{50}, 0.015-0.12 μg/mL) decreased the RIF MIC_{50} values 4- to 2,048-fold. RIF-SPR741 at F8 had the lowest MICs against MDR EC ($MIC_{50/90}$, 0.015/0.12 μg/mL) and ECL ($MIC_{50/90}$, 0.03/0.12 μg/mL), representing a 128- to 1,024-fold reduction in MICs compared to RIF. RIF had MICs 4- to 16-fold higher than the RIF-SPR741 combinations against MDR ACB. 14 GN were selected for WGS and all showed *mph*(A or E), except for one EC that carried *erm*(B).

Conclusions: CLA, RIF and SPR741 did not show direct *in vitro* activity against GN isolates. However, potent synergistic activity was observed when CLA, and especially RIF, were combined with SPR741, which decreased CLA and RIF MIC up to 2048-fold.

Introduction

Enterobacteriaceae are a common cause of community-acquired and healthcare–acquired infections, with *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. among the most common organisms. During the late 1990s, carbapenem-resistant Enterobacteriaceae (CRE) began to emerge. In 2012, 4.6% of acute-care hospitals reported at least one CRE and the proportion of Enterobacteriaceae that were CRE increased from 1.2% in 2001 to 4.2% in 2011 in both the National Nosocomial Infection Surveillance system (NNIS) and the National Healthcare Safety Network (NHSN), and from 0% in 2001 to 1.4% in 2010 in the Surveillance Network–USA (TSN).

Overall, the majority of CRE isolates in the USA harbor a KPC serine carbapenemase-encoding gene. *bla*$_{KPC}$ genes are mostly detected in *Klebsiella pneumoniae*, but have been observed in numerous Enterobacteriaceae species and have become endemic in several hospitals. However, metallo-β-lactamase production, mainly NDM and VIM enzymes, have been observed among Enterobacteriaceae worldwide, including the detection of NDM-producing isolates in the USA. More recently, a plasmid-borne colistin resistance gene, *mcr*-1, has been documented primarily in *E. coli*, but has also been detected in other Enterobacteriaceae species from human, animal, food and environmental samples on every continent.

With limited remaining options available for treating multidrug-resistant (MDR) Gram-negative infections, combination therapy is used to obtain activity greater than each individual component. SPR741 is a polymyxin B analog that interacts with the outer membrane of Gram-negative bacteria, compromising the integrity of the lipopolysaccharide barrier. When combined with Gram-positive active agents, SPR741 enables the entry of antimicrobial co-drugs, such as clarithromycin and rifampin, which otherwise would lack significant activity against Gram-negative isolates. This study assessed the activity of clarithromycin and rifampin combined with SPR741 against wildtype and MDR Gram-negative isolates.

Methods

Bacterial strain collection. Wildtype *E. coli* (10 isolates), *K. pneumoniae* (11), *Enterobacter cloacae* (11) and *Acinetobacter baumannii* (10) were used as baseline control clinical isolates, which demonstrated a susceptible antimicrobial profile to most agents, including β-lactams. A set of MDR organisms were included and consisted of the same species described above (24 - 28 isolates each, except for *A. baumannii* [51 isolates]) exhibiting intermediate or resistance phenotypes to several drug classes, including polymyxin B/colistin.

Isolates of *K. pneumoniae* (six isolates), *E. cloacae* (three), *E. coli* (one) and *A. baumannii* (eight) with high colistin MIC results were included. In addition, *E. coli*, *K. pneumoniae* and *E. cloacae* MDR isolates included those producing extended-spectrum β-lactamase (ESBL) and carbapenemase enzymes. Carbapenemases included relevant variants of KPC, NDM, VIM and OXA-48-like β-lactamases.

Antimicrobial susceptibility test methods. 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. Clarithromycin, rifampin and SPR741 were tested alone. The clarithromycin and rifampin combinations utilized the SPR741 at fixed concentrations of 4 and 8 μg/mL. Affirmation of the MIC values was performed by concurrent testing of CLSI-recommended quality control reference strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853).

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

Results

***In vitro* activity against *E. coli*.**

- Clarithromycin showed MIC_{50} and MIC_{90} of 32 and 128 μg/mL, respectively, when tested alone against wildtype isolates of *E. coli* (**Table 1**). The clarithromycin-SPR741 at fixed 8 μg/mL ($MIC_{50/90}$, 0.12/0.12 μg/mL) had the lowest MIC results, followed by clarithromycin-SPR741 at fixed 4 μg/mL ($MIC_{50/90}$, 0.12/0.5 μg/mL).
- Clarithromycin was largely inactive against MDR *E. coli* ($MIC_{50/90}$, 256/>256 μg/mL), while the clarithromycin-SPR741 at 8 μg/mL inhibited 92.9% of these isolates at ≤8 μg/mL (**Table 1**).
- Rifampin (MIC_{90}, 16 μg/mL) had elevated MIC results when tested alone against wildtype or MDR *E. coli* (**Table 1**). Rifampin-SPR741 tested at fixed 4 and 8 μg/mL against wildtype *E. coli* had $MIC_{50/90}$ results of 0.06/0.12 and 0.03/0.03 μg/mL, respectively. All MDR *E. coli* were inhibited by the rifampin-SPR741 combinations at ≤8 μg/mL.

***In vitro* activity against *K. pneumoniae*.**

- Clarithromycin and rifampin displayed MIC values of ≥8 μg/mL when tested alone against wildtype and MDR *K. pneumoniae* (**Table 2**). Wildtype *K. pneumoniae* showed low MIC results for clarithromycin-SPR741 tested at fixed 4 μg/mL ($MIC_{50/90}$, 1/1 μg/mL) and 8 μg/mL ($MIC_{50/90}$, 0.12/0.25 μg/mL). These two combinations were less active against MDR isolates (MIC_{90}, >32 μg/mL).
- Rifampin-SPR741 at fixed 4 μg/mL ($MIC_{50/90}$, 0.12/0.12 μg/mL) and 8 μg/mL ($MIC_{50/90}$, 0.06/0.06 μg/mL) were active against wildtype *K. pneumoniae.* Higher MIC values were obtained against MDR isolates (**Table 2**).

***In vitro* activity against *E. cloacae*.**

- Clarithromycin and rifampin exhibited MIC values of ≥32 and ≥8 μg/mL, respectively, when tested alone against wildtype and MDR isolates *E. cloacae* (**Table 3**). Clarithromycin-SPR741 at fixed 4 μg/mL (MIC_{50}, 0.5-1 μg/mL) and 8 μg/mL (MIC_{50}, 0.12 μg/mL) displayed low MIC_{50} values against both groups of isolates.
- The rifampin-SPR741 tested at fixed concentrations of 4 and 8 μg/mL against *E. cloacae* had MIC_{50} results of 0.12 and 0.015-0.03 μg/mL, respectively, against these isolate subsets (**Table 3**).

***In vitro* activity against *A. baumannii*.**

- Clarithromycin showed MIC_{50} and MIC_{90} values of 16 μg/mL (for both) against wildtype *A. baumannii* (**Table 4**). The clarithromycin MIC_{50} values decreased to 0.5 and 0.12 μg/mL when tested in combination with SPR741 at fixed concentrations of 4 and 8 μg/mL, respectively, against wildtype *A. baumannii.* In contrast, the clarithromycin-SPR741 combinations were less active against MDR isolates.
- MIC values of ≥2 μg/mL were obtained for rifampin tested alone against wildtype ($MIC_{50/90}$, 2/8 μg/mL) and MDR ($MIC_{50/90}$, 4/16 μg/mL) *A. baumannii* (**Table 4**). MIC_{90} values of 0.5 - 1 μg/mL and 4 μg/mL were obtained for the rifampin-SPR741 combinations against wildtype and MDR isolates, respectively.

Screening of MLS_B resistance mechanisms.

- All *A. baumannii* isolates tested carried the efflux-pump- [*msr*(E)] and phosphorylase- [*mphE*] encoding genes. The *erm*(B) gene was detected in one *E. coli*, while the other isolate had *mphA* (**Table 5**). All three *K. pneumoniae* harbored *mph* phosphorylase genes (*mphA* or *mphE*) and one isolate also had *msr*(E).

Table 1. Cumulative frequency distribution of MIC results for clarithromycin and rifampin alone and in combinations with SPR741 against *E. coli*.

Phenotype / Antimicrobial Combination	Number and cumulative percentage of isolates inhibited by each MIC (μg/mL): ≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[a]	MIC (μg/mL) MIC_{50}	MIC_{90}
Wildtype (10)																	
Clarithromycin										2 (20.0)	4 (60.0)	1 (70.0)	3 (100.0)			32	128
-SPR741 (4 μg/mL)				5 (50.0)	2 (70.0)	3 (100.0)										0.12	0.5
-SPR741 (8 μg/mL)		1 (10.0)	9 (100.0)													0.12	0.12
Rifampin								1 (10.0)	7 (80.0)	2 (100.0)						8	16
-SPR741 (4 μg/mL)	1 (10.0)	6 (70.0)	3 (100.0)													0.06	0.12
-SPR741 (8 μg/mL)	10 (100.0)															0.03	0.03
MDR (28)																	
Clarithromycin											3 (10.7)	2 (17.9)	6 (39.3)	5 (57.1)	12 (100.0)	256	>256
-SPR741 (4 μg/mL)			3 (10.7)	6 (32.1)	0 (32.1)	2 (39.3)	6 (60.7)	4 (75.0)	3 (85.7)	1 (89.3)	0 (89.3)				3 (100.0)	2	>32
-SPR741 (8 μg/mL)		2 (7.1)	7 (32.1)	2 (39.3)	5 (57.1)	5 (75.0)	3 (85.7)	0 (85.7)	2 (92.9)	1 (96.4)	0 (96.4)				1 (100.0)	0.5	8
Rifampin										11 (39.3)	17 (100.0)					16	16
-SPR741 (4 μg/mL)	16 (57.1)	8 (85.7)	2 (92.9)	0 (92.9)	1 (96.4)	0 (96.4)	0 (96.4)	0 (96.4)	1 (100.0)							0.03	0.12
-SPR741 (8 μg/mL)	24 (85.7)	1 (89.3)	1 (92.9)	0 (92.9)	1 (96.4)	0 (96.4)	0 (96.4)	0 (96.4)	1 (100.0)							0.015	0.12

a. ">" represents greater than the highest dilution tested (i.e. >256 μg/mL for agents tested alone or >32 μg/mL for clarithromycin-SPR741 combinations and >8 μg/mL for rifampin-SPR741 combinations).

Table 2. Cumulative frequency distribution of MIC results for clarithromycin and rifampin alone and in combinations with SPR741 against *K. pneumoniae*.

Phenotype / Antimicrobial Combination	Number and cumulative percentage of isolates inhibited by each MIC (μg/mL): 0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[a]	MIC (μg/mL) MIC_{50}	MIC_{90}
Wildtype (11)																
Clarithromycin										1 (9.1)	0 (9.1)	10 (100.0)			128	128
-SPR741 (4 μg/mL)				2 (18.2)	2 (36.4)	7 (100.0)									1	1
-SPR741 (8 μg/mL)			6 (54.5)	4 (90.9)	1 (100.0)										0.12	0.25
Rifampin										6 (54.5)	4 (90.9)	1 (100.0)			16	32
-SPR741 (4 μg/mL)		3 (27.3)	7 (90.9)	1 (100.0)											0.12	0.12
-SPR741 (8 μg/mL)	2 (18.2)	9 (100.0)													0.06	0.06
MDR (26)																
Clarithromycin										5 (19.2)	0 (19.2)	8 (50.0)	1 (53.8)	12 (100.0)	128	>256
-SPR741 (4 μg/mL)		1 (3.8)	2 (11.5)	1 (15.4)	3 (26.9)	0 (26.9)	3 (38.5)	2 (46.2)	3 (57.7)	4 (73.1)				7 (100.0)	16	>32
-SPR741 (8 μg/mL)	1 (3.8)	3 (15.4)	3 (26.9)	0 (26.9)	6 (50.0)	0 (50.0)	2 (57.7)	4 (73.1)	1 (76.9)	1 (80.8)				5 (100.0)	1	>32
Rifampin								1 (3.8)	10 (42.3)	12 (88.5)	0 (88.5)	0 (88.5)	0 (88.5)	3 (100.0)	32	>256
-SPR741 (4 μg/mL)	3 (11.5)	7 (38.5)	4 (53.8)	0 (53.8)	1 (57.7)	2 (65.4)	0 (65.4)	4 (80.8)						5 (100.0)	0.25	>8
-SPR741 (8 μg/mL)	15 (57.7)	1 (61.5)	0 (61.5)	0 (61.5)	1 (65.4)	0 (65.4)	1 (69.2)	3 (80.8)						5 (100.0)	0.06	>8

a. ">" represents greater than the highest dilution tested (i.e. >256 μg/mL for agents tested alone or >32 μg/mL for clarithromycin-SPR741 combinations and >8 μg/mL for rifampin-SPR741 combinations).

Table 3. Cumulative frequency distribution of MIC results for clarithromycin and rifampin alone and in combinations with SPR741 against *E. cloacae*.

Phenotype / Antimicrobial Combination	Number and cumulative percentage of isolates inhibited by each MIC (μg/mL): ≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[a]	MIC (μg/mL) MIC_{50}	MIC_{90}
Wildtype (11)																	
Clarithromycin												1 (9.1)	8 (81.8)	1 (90.9)	1 (100.0)	128	256
-SPR741 (4 μg/mL)				5 (45.5)	3 (72.7)	2 (90.9)	0 (90.9)	1 (100.0)								0.5	1
-SPR741 (8 μg/mL)	1 (9.1)	0 (9.1)	9 (90.9)	1 (100.0)												0.12	0.12
Rifampin									1 (9.1)	3 (36.4)	7 (100.0)					32	32
-SPR741 (4 μg/mL)	2 (18.2)	1 (27.3)	6 (81.8)	0 (81.8)	0 (81.8)	2 (100.0)										0.12	1
-SPR741 (8 μg/mL)	10 (90.9)	1 (100.0)														0.015	0.03
MDR (24)																	
Clarithromycin												1 (4.2)	0 (4.2)	9 (41.7)	9 (79.2)	5 (100.0)	256	>256
-SPR741 (4 μg/mL)			1 (4.2)	2 (12.5)	4 (29.2)	9 (66.7)	3 (79.2)	1 (83.3)	3 (95.8)	0 (95.8)	1 (100.0)						1	8
-SPR741 (8 μg/mL)			14 (58.3)	3 (70.8)	1 (75.0)	2 (83.3)	2 (91.7)	1 (95.8)	1 (100.0)								0.12	2
Rifampin									1 (4.2)	10 (45.8)	8 (79.2)	1 (83.3)	2 (91.7)	0 (91.7)	2 (100.0)		32	128
-SPR741 (4 μg/mL)	5 (20.8)	5 (41.7)	8 (75.0)	4 (91.7)	0 (91.7)	0 (91.7)	0 (91.7)	1 (95.8)	1 (100.0)								0.12	0.25
-SPR741 (8 μg/mL)	13 (54.2)	4 (70.8)	5 (91.7)	0 (91.7)	0 (91.7)	0 (91.7)	1 (95.8)	1 (100.0)									0.03	0.12

a. ">" represents greater than the highest dilution tested (i.e. >256 μg/mL for agents tested alone or >32 μg/mL for clarithromycin-SPR741 combinations and >8 μg/mL for rifampin-SPR741 combinations).

Table 4. Cumulative frequency distribution of MIC results for clarithromycin and rifampin alone and in combinations with SPR741 against *A. baumannii*.

Phenotype / Antimicrobial Combination	Number and cumulative percentage of isolates inhibited by each MIC (μg/mL): 0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[a]	MIC (μg/mL) MIC_{50}	MIC_{90}
Wildtype (10)																
Clarithromycin								2 (20.0)	7 (90.0)	1 (100.0)					16	16
-SPR741 (4 μg/mL)			3 (30.0)	3 (60.0)	3 (90.0)	1 (100.0)									0.5	1
-SPR741 (8 μg/mL)	1 (10.0)	6 (70.0)	2 (90.0)	1 (100.0)											0.12	0.25
Rifampin						5 (50.0)	2 (70.0)	3 (100.0)							2	8
-SPR741 (4 μg/mL)			8 (80.0)	0 (80.0)	1 (90.0)	1 (100.0)									0.25	1
-SPR741 (8 μg/mL)		8 (80.0)	0 (80.0)	1 (90.0)	0 (90.0)	1 (100.0)									0.12	0.5
MDR (51)																
Clarithromycin									1 (2.0)	7 (15.7)	4 (23.5)	11 (45.1)	0 (45.1)	28 (100.0)	>256	>256
-SPR741 (4 μg/mL)			1 (2.0)	2 (5.9)	4 (13.7)	7 (27.5)	2 (31.4)	3 (37.3)	1 (39.2)	5 (49.0)				26 (100.0)	>32	>32
-SPR741 (8 μg/mL)		1 (2.0)	3 (7.8)	8 (23.5)	4 (31.4)	1 (33.3)	0 (33.3)	3 (39.2)	2 (43.1)	9 (60.8)				20 (100.0)	32	>32
Rifampin					2 (3.9)	32 (66.7)	11 (88.2)	2 (92.2)	0 (92.2)	1 (94.1)	0 (94.1)	1 (96.1)		2 (100.0)	4	16
-SPR741 (4 μg/mL)		2 (3.9)	15 (33.3)	14 (60.8)	10 (80.4)	2 (84.3)	4 (92.2)	0 (92.2)						4 (100.0)	0.5	4
-SPR741 (8 μg/mL)	1 (2.0)	21 (43.1)	8 (58.8)	6 (70.6)	5 (80.4)	4 (88.2)	2 (92.2)	0 (92.2)						4 (100.0)	0.25	4

a. ">" represents greater than the highest dilution tested (i.e. >256 μg/mL for agents tested alone or >32 μg/mL for clarithromycin-SPR741 combinations and >8 μg/mL for rifampin-SPR741 combinations).

Table 5. MIC results for clarithromycin and the clarithromycin-SPR741 combinations when tested against MDR clinical isolates and detected resistance genes.

Org Code	Clarithromycin MIC (μg/mL) alone and in combinations with: Alone	SPR741 (4 μg/mL)	SPR741 (8 μg/mL)	Resistance gene
A. baumannii	64	16	8	*msr*(E), *mphE*
A. baumannii	>256	>32	>32	*msr*(E), *mphE*
A. baumannii	>256	>32	32	*msr*(E), *mphE*
A. baumannii	>256	32	16	*msr*(E), *mphE*
A. baumannii	>256	>32	>32	*msr*(E), *mphE*
A. baumannii	>256	>32	>32	*msr*(E), *mphE*
A. baumannii	>256	>32	32	*msr*(E), *mphE*
A. baumannii	>256	>32	32	*msr*(E), *mphE*
A. baumannii	>256	32	16	*msr*(E), *mphE*
E. coli	>256	>32	16	*erm*(B)
E. coli	>256	16	8	*mphA*
K. pneumoniae	256	32	8	*msr*(E), *mphE*
K. pneumoniae	>256	>32	16	*mphA*
K. pneumoniae	>256	>32	8	*mphA*

Conclusions

- The new SPR741 polymyxin B derivative increased the *in vitro* activity (based on MIC_{50} and MIC_{90} results) of two Gram-positive agents against Enterobacteriaceae and *A. baumannii*, including isolates displaying an MDR phenotype.
- Potentiation was more evident for the rifampin-SPR741 combinations, except against MDR isolates of *K. pneumoniae.* These results warrant further investigations to assess the clinical potential for these synergistic approaches.
- Finally, the higher MIC results obtained for the clarithromycin-SPR741 combinations against certain MDR isolates could be due to the presence of methylases (e.g. Erm) and/or inactivating enzymes (e.g. Mph) observed in the study isolates.

Acknowledgements

The research and publication process was supported by Spero Therapeutics.

References

1. Clinical and Laboratory Standards Institute (2015). *M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard: Tenth edition*. Wayne, PA, USA.
2. Clinical and Laboratory Standards Institute (2016). *M100-S26. Performance standards for antimicrobial susceptibility testing: 26th informational supplement.* Wayne, PA, USA.
3. Location of various MLS_B genes. Available at http://faculty.washington.edu/marilyn/ermweb4.pdf.
4. Mingeot-Leclercq MP, Tulkens PM, Denamur S, Vaara T, Vaara M (2012). Novel polymyxin derivatives are less cytotoxic than polymyxin B to renal proximal tubular cells. *Peptides* 35: 248 - 252.
5. Vaara M, Siikanen O, Apajalahti J, Fox J, Frimodt-Møller N, He H, Poudyal A, Li J, Nation RL, Vaara T (2010). A novel polymyxin derivative that lacks the fatty acid tail and carries only three positive charges has strong synergism with agents excluded by the intact outer membrane. *Antimicrob Agents Chemother*. 54: 3341 - 3346.