F1-1492

Spectrum and Activity of Ceftaroline Combined with NXL104 Tested Against a Challenge Collection of Pathogens with Well Characterized Resistances

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ICAAC 2009
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Abstract

Background: Ceftaroline (CPT) is a broad-spectrum cephalosporin with activity against Gram-negative and -positive (including MRSA), but limited activity against ESBL- and AmpC-producing strains. NXL104 (NXL) is a novel β-lactamase (βL) inhibitor that inhibits AmpC, ESBL and KPC type enzymes.

Methods: CPT, NXL, various CPT/NXL combinations (fixed 2 and 4 μg/ml and 1:1, 2:1, 4:1 and 8:1 ratios) and comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 178 clinical strains, including Enterobacteriaceae (ENT) producing CTX-M (22 strains), CMY (10), FOX (5), KPC (20), SME (5) and metallo-βL (MβL; 5), *P. aeruginosa* (PSA; 15), *A. baumannii* (ACB; 15), MRSA (50; SCC*mec* types I-IV), MSSA (10), *S. pneumoniae* (SPN; 16) and *E. faecalis* (EF; 5).

Results: The greatest NXL effect was obtained at a fixed of 4 μg/mL concentration. 93% of ENT were inhibited at ≤2/4 μg/mL of CPT/NXL while only 45 and 63% were S to cefepime and imipenem, respectively. Among ENT, only MβL-producing strains had a CPT/NXL MIC, >2/4 μg/mL. CPT/NXL was very active against MRSA (MIC_{90}, 2/4 μg/mL), MSSA (MIC_{90}, 0.25/4 μg/mL), SPN (highest MIC, 0.25/4 μg/mL) and ampicillin-S EF (MIC range, 1/4-4/4 μg/mL). CPT showed only marginal anti-PSA activity, but significant enhanced effect with NXL against wildtype (WT) isolates. CPT/NXL exhibited good activity against WT ACB (MIC range, 0.5/4-4/4 μg/mL), but limited activity against OXA- producing ACB.

Organism (no. tested)	No. of strains (cumulative %) inhibited at MIC (μg/mL of):								
	≤0.12	0.25	0.5	1	2	4	8	16	>16
Enterobacteriaceae (67)									
CPT/NXL[a]	24 (36)	13 (55)	18 (82)	5 (90)	2 (93)	-	-	-	5 (100)
Cefepime	5 (8)	4 (13)	5 (21)	2 (24)	6 (33)	6 (42)	2 (45)	5 (55)	32 (100)
Imipenem	8 (12)	12 (31)	15 (52)	2 (55)	2 (58)	3 (63)	8 (75)	-	17 (100)
MRSA (50)									
CPT/NXL[a]	-	-	3 (6)	27 (60)	18 (96)	2 (100)	-	-	
Cefepime	-	-	-	-	-	-	4 (8)	11 (30)	35 (100)
Imipenem	4 (8)	9 (26)	1 (28)	3 (34)	2 (38)	2 (42)	2 (46)	-	27 (100)
S. pneumoniae (16)									
CPT/NXL[a]	14 (88)	2 (100)	-	-	-	-	-	-	-
Cefepime	8 (50)	2 (63)	1 (69)	4 (94)	1 (100)	-	-	-	-
Imipenem	11 (69)	0 (69)	2 (81)	3 (100)	-	-	-	-	-

a. NXL at fixed 4 μg/mL.

Conclusions: CPT alone was very active against Gram-positives. CPT/NXL combinations showed significant enhanced potencies compared with CPT alone against ENT strains producing CTX-M, plasmidic AmpC, and KPC βLs.

Introduction

The objective of this study was to evaluate the spectrum of activity and potency of ceftaroline combined with NXL104 when tested against a challenge set of pathogens with well-characterized resistance phenotypes and genotypes, often geographically specific.

Ceftaroline, a broad-spectrum cephalosporin currently in clinical development, demonstrates bactericidal activity against gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP), as well as common gram-negative pathogens. Positive results have been reported from phase 2 and phase 3 clinical trials on the efficacy and safety of ceftaroline for the treatment of complicated skin and skin structure infections (cSSSI) and for community-acquired bacterial pneumonia (CABP).

As with other cephalosporins, ceftaroline is less active against extended-spectrum β-lactamase (ESBL)-producing organisms. NXL 104 is a new non-β-lactam inhibitor of β-lactamases currently in clinical development (Figure 1). NXL104 displays a broad-spectrum inhibition profile against both class A and class C enzymes. To enhance the activity of ceftaroline against ESBLs, its utility when combined with NXL104 (Figure 1) was investigated.

Materials and Methods

Susceptibility Testing

MIC values were determined using the reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (CLSI, 2009a). Frozen-form assay panels were produced by JMI Laboratories (North Liberty, Iowa, USA) and consisted of cation-adjusted Mueller-Hinton broth (supplemented with 3-5% lysed horse blood for testing of streptococci). CLSI quality control (QC) ranges and interpretive criteria for comparator compounds were used (CLSI, 2009b); tested QC strains included *Escherichia coli* ATCC 25922 and ATCC 35218 (inhibitor effect only), *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *S. pneumoniae* ATCC 49619.

Antimicrobial Agents

Ceftaroline was tested alone (128 – 0.06 μg/mL) and with various NXL104 combinations (fixed NXL104 concentrations of 2 and 4 μg/mL and ceftaroline:NXL104 ratios of 8:1, 4:1, 2:1, and 1:1). To evaluate the antimicrobial activity of NXL104, this compound was also tested alone (32 – 1 μg/mL). The comparator compounds included ceftriaxone, ceftazidime, cefotaxime, cefepime, piperacillin/tazobactam, penicillin, imipenem, meropenem, and ciprofloxacin.

Organism Collection (178 strains)

A. β-Lactamase-producing challenge sets of prevalent types (87 strains):
   1. *Enterobacteriaceae* producing CTX-M-series (CTX-M-15, -14, -3, and -2; 22 strains, various species)
   2. Plasmidic AmpC in *Enterobacteriaceae* (15 strains), including CMY-series (CMY-2 and others; 10 strains) and FOX-series (FOX-5 and others; 5 strains)
   3. Serine carbapenemases in *Enterobacteriaceae* (25 strains), including KPC-series (KPC-2, -3; 20 strains, various species) and SME-series (SME-2; 5 strains)
   4. Metallo-β-lactamases (MBL) in *Enterobacteriaceae* (5 strains): VIM-1 (1), VIM-2 (1), IMP-1 (1), IMP-11 (1), and IMP-21 (1)
   5. Other carbapenem resistances expressed in non-*Enterobacteriaceae* (20 strains)
      a. *P. aeruginosa* (SPM-, VIM-, and IMP-series; 5 strains)
      b. Efflux, overexpression of AmpC and outer membrane protein (OMP) alterations in *P. aeruginosa* (5 strains)
      c. *Acinetobacter baumannii* (OXA-23, -24, and -58; 10 strains)

B. Wild-type *P. aeruginosa* and *A. baumannii* strains (10 strains)

C. Gram-positive cocci (81 strains)
   1. MRSA strains carrying different SCC*mec* types from various geographic regions each representing predominant national clones (50 total), including: SCC*mec* type I, II, and III (10 strains each) and SCC*mec* type IV (20 strains, including a special set [10 strains] of dominant USA clonal types and variants, and different subtypes of SCC*mec* IV)
   2. Methicillin-susceptible *S. aureus* (MSSA, 10 strains)
   3. *S. pneumoniae* (16 strains), including penicillin-resistant (MIC, ≥2 μg/mL; 5 strains), -intermediate (MIC, 0.12 – 1 μg/mL; 5 strains), and -susceptible (MIC, ≤0.06 μg/mL; 6 strains)
   4. *Enterococcus faecalis* (5 strains), including 1 ampicillin-resistant strain.

Results

- Against *Enterobacteriaceae*, the optimal enhanced effect of NXL104 was obtained at a fixed concentration of 4 μg/mL (MIC_{50}, 0.25/4 μg/mL and MIC_{90}, 2/4 μg/mL). Among the tests using ratio combinations, the 2:1 and 1:1 ratios showed the greatest activity, with MIC_{50}s of 1/0.5 and 1/1 μg/mL, respectively (Table 1).
- Ceftaroline plus NXL104 at a fixed concentration of 4 μg/mL [CPT/NXL4] was highly active against *Enterobacteriaceae* producing CTX-M (MIC_{50}, 0.12/4 μg/mL and MIC_{90}, 0.25/4 μg/mL), plasmidic AmpC (MIC_{50}, 0.12/4 μg/mL and MIC_{90}, 0.5/4 μg/mL), KPC (MIC_{50}, 0.5/4 μg/mL and MIC_{90}, 1/4 μg/mL) and SME (MIC_{50} and MIC_{90}, 0.5/4 μg/mL) β-lactamases, but showed limited activity against MBL-producing strains (Tables 2 and 3).
- In general, CPT/NXL4 was more active than the carbapenems against the β-lactamase-producing *Enterobacteriaceae* (Table 4). Against KPC-producing strains, CPT/NXL4 (MIC_{50}, ≤0.5/4 μg/mL) was >32-fold more potent than imipenem (MIC_{50}, >8 μg/mL) and meropenem (MIC_{50}, >8 μg/mL).
- CPT/NXL4 exhibited good in vitro activity against wild-type strains of *Acinetobacter* spp. (MIC range, 1/4 - 4/4 μg/mL); however, OXA-producing strains showed elevated MIC results (16/4 - >32/4 μg/mL; Tables 2 and 3).
- Ceftaroline alone showed limited activity against wild-type *P. aeruginosa* (MIC, 8 - 32 μg/mL). A 4- to 8-fold reduction in the ceftaroline MIC was observed when combined with NXL104. The highest enhanced effect was obtained for CPT/NXL4 (MIC_{50}, 2/4 μg/mL). CPT/NXL4 was only 2-fold less active than cefepime against wild-type strains of *P. aeruginosa.*
- Ceftaroline alone exhibited limited activity (MIC range, 16 - >128 μg/mL) against *P. aeruginosa* strains with efflux, overexpression of AmpC and/or OMP alterations (5 strains), but significant enhanced effects were observed when combined with NXL104 (2- to ≥16-fold). The greatest reductions of ceftaroline MIC results were obtained with 1:1 ratio test (MIC_{50}, 8/8 μg/mL).
- Ceftaroline alone and all ceftaroline/NXL104 combinations tested exhibited excellent activity against MRSA and no significant enhanced activity or antagonism were observed with NXL104. Strains with SCC*mec* type IV showed ceftaroline MIC values slightly lower (0.5 – 1 μg/mL) compared strains with SCC*mec* types I, II, and III (1 - 4 μg/mL; Table 3). Ceftaroline and all ceftaroline/NXL104 combinations were also very active against MSSA strains, with all MIC values at 0.25 μg/mL. No enhanced activity was observed with NXL104 (Table 2).
- Ceftaroline was highly active against *S. pneumoniae* alone and no enhanced activity was observed with NXL104. Against PRSP strains (MIC, ≥2 μg/mL), ceftaroline was 8- to 16-fold more potent than ceftriaxone, cefotaxime, cefepime, or imipenem (Tables 3 and 4).
- Ceftaroline and all ceftaroline/NXL104 combinations tested were active against ampicillin-susceptible *E. faecalis* (4 strains; MIC values, 1 - 4 μg/mL) but showed limited activity against the 1 ampicillin-resistant *E. faecalis* strain (MIC value, >128 μg/mL; Table 2). No enhanced activity was observed with the addition of NXL104.

Table 1. MIC Distributions for Ceftaroline, NXL104, and Ceftaroline/NXL104 Combinations.

Compound[a]/organism (no. tested)	No. of isolates (cumulative %) inhibited at CPT/NXL104 (fixed 4 μg/mL) MIC of :													
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>[b]
Enterobacteriaceae[c] (67)														
Ceftaroline	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.0)	3(7.5)	0(7.5)	4(13.4)	4(19.4)	5(26.9)	3(31.3)	4(37.3)	42(100.0)
CPT/NXL104 fixed 4	2(3.0)	9(16.4)	13(35.8)	13(55.2)	18(82.1)	5(89.6)	2(92.5)	0(92.5)	0(92.5)	0(92.5)	0(92.5)	-[d]	-	5(100.0)
CPT/NXL104 fixed 2	1(1.5)	8(13.4)	5(20.9)	14(41.8)	16(65.7)	10(80.6)	7(91.0)	1(92.5)	0(92.5)	0(92.5)	0(92.5)	-	-	5(100.0)
CPT/NXL104 8:1	0(0.0)	0(0.0)	0(0.0)	1(1.5)	6(10.5)	13(29.9)	24(65.7)	14(86.6)	4(92.5)	0(92.5)	0(92.5)	-	-	5(100.0)
CPT/NXL104 4:1	0(0.0)	0(0.0)	0(0.0)	2(3.0)	12(20.9)	14(41.8)	19(70.2)	14(91.0)	1(92.5)	0(92.5)	2(95.5)	-	-	3(100.0)
CPT/NXL104 2:1	0(0.0)	0(0.0)	0(0.0)	4(6.0)	18(32.8)	15(55.2)	17(80.6)	8(92.5)	0(92.5)	1(94.0)	4(100.0)	-	-	0(100.0)
CPT/NXL104 1:1	0(0.0)	0(0.0)	1(1.5)	9(14.9)	17(40.3)	18(67.2)	15(89.6)	2(92.5)	1(94.0)	2(97.0)	2(100.0)	-	-	0(100.0)
NXL104	-	-	-	-	-	0(0.0)	0(0.0)	2(3.0)	24(38.8)	23(73.1)	4(79.1)			14(100.0)
Non-fermenters[e] (30)														
Ceftaroline	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(3.3)	4(16.7)	2(23.3)	2(30.0)	3(40.0)	0(40.0)	2(46.7)	16(100.0)
CPT/NXL104 fixed 4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(3.3)	0(0.0)	4(16.7)	5(33.3)	1(36.7)	4(50.0)	3(60.0)	-	-	12(100.0)
CPT/NXL104 fixed 2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(20.0)	3(30.0)	2(36.7)	2(43.3)	3(53.3)	-	-	14(100.0)
CPT/NXL104 8:1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(6.7)	6(26.7)	3(36.7)	4(50.0)	4(63.3)	-	-	11(100.0)
CPT/NXL104 4:1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(6.7)	7(30.0)	2(36.7)	3(46.7)	9(76.7)	-	-	7(100.0)
CPT/NXL104 2:1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(10.0)	7(33.3)	1(36.7)	7(60.0)	7(83.3)	-	-	5(100.0)
CPT/NXL104 1:1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(13.3)	6(33.3)	6(53.3)	5(70.0)	4(83.3)	-	-	5(100.0)
NXL104	-	-	-	-	-	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	-	-	30(100.0)

a. CPT = ceftaroline; CPT/NXL104 = ceftaroline/NXL104; fixed 4 = NXL104 at fixed concentration of 4 μg/mL; fixed 2 = NXL104 at fixed concentration of 2 μg/mL; 8:1 = ceftaroline/NXL104 at 8:1 ratio; 4:1 = ceftaroline/NXL104 at 4:1 ratio; 2:1 = ceftaroline/NXL104 at 2:1 ratio; 1:1 = ceftaroline/NXL104 at 1:1 ratio.
b. MIC value greater than the highest concentration tested.
c. Includes *Enterobacteriaceae* strains producing CTX-M (22), plasmidic AmpC (15), KPC (20), SME (5), and metallo-β-lactamases (5).
d. - = concentration not tested.
e. Includes *Acinetobacter* spp. (15 strains; 5 wild-type and 10 OXA-producers) and *P. aeruginosa* (15 strains; 5 wild-type, 5 AmpC hyperproducer +/- OMP alteration, and 5 metallo-β-lactamase producers.

Table 2. Antimicrobial Activity of Ceftaroline/NXL104 (Fixed 4 μg/mL) Tested Against Various Groups of Organisms with Well-Characterized Mechanisms of Resistance.

Organism (no. tested)	No. of isolates (cumulative %) inhibited at ceftaroline/NXL104 (fixed 4 μg/mL) MIC of:											
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
Enterobacteriaceae (67)													
CTX-M producers (22)	-[a]	-	8(36.4)	7(68.2)	6(95.5)	0(95.5)	1(100.0)	-	-	-	-	-	-
Plasmidic AmpC producers (15)	-	2(13.3)	1(20.0)	6(60.0)	4(86.7)	2(100.0)	-	-	-	-	-	-	-
KPC-producers (20)	-	-	-	-	2(10.0)	12(70.0)	4(90.0)	2(100.0)	-	-	-	-	-
SME-producers (5)					1(20.0)	4(100.0)							
MBL-producers (5)	-	-	-	-	-	-	-	-	-	-	-	-	5(100.0)
Acinetobater spp. (15)	-	-	-	-	-	1(6.7)	0(6.7)	1(13.3)	3(33.3)	0(33.3)	2(46.7)	2(60.0)	6(100.0)
Wildtype (5)	-	-	-	-	-	1(20.0)	0(20.0)	1(40.0)	3(100.0)	-	-	-	-
OXA-producers (10)	-	-	-	-	-	-	-	-	-	-	2(20.0)	2(40.0)	6(100.0)
P. aeruginosa (15)	-	-	-	-	-	-	-	3(20.0)	2(33.3)	1(40.0)	2(53.3)	1(60.0)	2(100.0)
Wildtype (5)	-	-	-	-	-	-	-	3(60.0)	2(100.0)	-	-	-	-
AmpC/OMP (5)	-	-	-	-	-	-	-	-	-	1(20.0)	2(60.0)	0(60.0)	2(100.0)
MBL (5)	-	-	-	-	-	-	-	-	-	-	-	1(20.0)	4(100.0)
MRSA (50)	-	-	-	-	-	8(16.0)	29(74.0)	12(98.0)	1(100.0)	-	-	-	-
MSSA (10)	-	-	-	-	10(100.0)	-	-	-	-	-	-	-	-
S. pneumoniae (16)	9(56.3)	2(68.8)	0(68.8)	4(93.8)	1(100.0)	-	-	-	-	-	-	-	-
E. faecalis (5)	-	-	-	-	-	-	1(20.0)	2(60.0)	1(80.0)	0(80.0)	0(80.0)	0(80.0)	1(100.0)

a. - = no one isolate with this MIC value.
Abbreviations: MBL = metallo-β-lactamase; OMP = outer membrane protein; MRSA = methicillin(oxacillin)-resistant *Staphylococcus aureus*; MSSA = methicillin(oxacillin)-susceptible *S. aureus*.

Figure 1. NXL104 Chemical Structure.

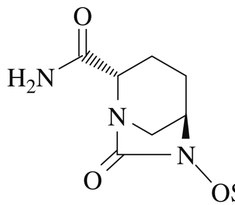

Table 3. Summary of Ceftaroline/NXL104 (Fixed 4 μg/mL) Activity.

Organism (no. tested)	CPT/NXL104 (fixed 4 μg/mL)			Imipenem		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Enterobacteriaceae						
CTX-M-producers (22)	0.12	0.25	0.06-1	≤0.12	0.25	≤0.12-2
Plasmidic Amp C-producers (15)	0.12	0.25	0.03-0.5	0.25	0.5	≤0.12-0.5
KPC-producers (20)	0.5	1	0.25-2	>8	>8	2->8
SME-producers (5)	0.5	NA	0.25-0.5	8	NA	1->8
MBL-producers (5)	>32	NA	>32	>8	NA	≤0.12->8
P. aeruginosa						
Wildtype (5)	16	NA	8->32	>8	NA	1-2
Amp C-producers ± OMP alteration (5)	16	NA	8->32	>8	NA	1->8
MBL-producers (5)	>32	NA	>32	>8	NA	>8
A. baumannii						
Wildtype (5)	4	NA	0.5-4	0.5	NA	≤0.12-0.5
OXA-producers (10)	>32	>32	16->32	>8	>8	>8
Methicillin-resistant *S. aureus*						
SCC*mec* type I (10)	2	2	1-4	>8	>8	>8
SCC*mec* type II (10)	1	2	1-2	>8	>8	0.25->8
SCC*mec* type III (10)	1	2	1-2	>8	>8	>8
SCC*mec* type IV International (10)	1	1	0.5-1	1	4	≤0.12-8
SCC*mec* type IV USA (10)	1	1	0.5-1	0.25	2	≤0.12-4
Methicillin-susceptible *S. aureus* (10)	0.25	0.25	0.25	≤0.12	≤0.12	≤0.12
S. pneumoniae						
Penicillin-resistant (5)	0.12	NA	0.12-0.25	1	NA	0.5-1
Penicillin-intermediate (5)	≤0.015	NA	≤0.015-0.03	≤0.12	NA	≤0.12
Penicillin-susceptible (6)	≤0.015	NA	≤0.015	≤0.12	NA	≤0.12
E. faecalis (5)	2	NA	1->32	2	NA	1->8

Abbreviations: MBL = metallo-β-lactamase; NA = not applicable; OMP = outer membrane protein; SCC*mec* = staphylococcal cassette chromosome.

Table 4. In Vitro Activity of Ceftaroline/NXL104 (Fixed 4 μg/mL) and Selected Antimicrobial Agents Tested Against *Enterobacteriaceae*[a] (67 Strains).

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% Susceptible/ Resistant[b]
CPT/NXL4[c]	0.25	2	0.03->32	-/-
Ceftriaxone	>32	>32	≤8->32	13.4/74.6
Ceftazidime	>32	>32	≤16->32	0.0/74.6
Cefotaxime	>32	>32	0.12->32	13.4/71.6
Cefepime	1613	>16	≤0.012->32	44.8/47.8
Imipenem	≤0.5	>8	≤0.5->8	62.7/25.4
Meropenem	≤0.12	>8	≤0.12->8	64.2/28.4
Piperacillin/tazobactam	64	>64	≤8->64	40.3/46.3
Ciprofloxacin	>2	>2	≤0.25->2	37.3/58.2

a. Includes *Enterobacteriaceae* strains producing CTX-M (22), plasmidic AmpC (15), KPC (20), SME (5) and metallo-β-lactamases (5).
b. Criteria as published by the CLSI (2009).
c. CPT/NXL4 = ceftaroline/NXL104 at fixed concentration of 4 μg/mL.

Conclusions

- The ceftaroline/NXL104 combinations tested showed significant activity against *Enterobacteriaceae* strains producing the prevalent CTX-M, plasmid AmpC, and KPC β-lactamases. The most optimal NXL104 enhanced effect was observed at a fixed concentration of 4 μg/mL.
- Although ceftaroline alone showed only marginal activity against *P. aeruginosa*, significant enhanced effect was observed when ceftaroline was combined with NXL104 against this organism.
- Ceftaroline alone was very active against *S. aureus* (including MRSA), *S. pneumoniae* (including penicillin-resistant strains), and ampicillin-susceptible *E. faecalis*, and as expected, NXL104 did not show enhanced activity when combined with ceftaroline against these organisms. No antagonism was observed in tests against these species.
- The results of this investigation demonstrate that ceftaroline/NXL104 is a potential option for the treatment of infections caused by multidrug-resistant *Enterobacteriaceae*, including KPC-producing strains, as well as gram-positive organisms, including MRSA and PRSP.

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Acknowledgement

Supported by Forest Laboratories, Inc.