

Amended Abstract

Background: Ceftaroline (CPT) is a novel broad-spectrum cephalosporin with activity against resistant gram-positive organisms and common gram-negative pathogens. Like other cephalosporins, its activity is significantly reduced by extended-spectrum β -lactamases (ESBL), AmpC enzymes, or KPCs. NXL-104 (NXL) is a non- β -lactam β -lactamase inhibitor that has been shown to restore *in vitro* activity of many β -lactam antimicrobials against these β -lactam-resistant isolates. We evaluated the MICs of CPT with and without NXL against a collection of molecularly-characterized β -lactamase-producing *Enterobacteriaceae* (ENT).

Methods: Susceptibility of 816 recent clinical ENT isolates previously shown to produce ESBL enzymes (including 6 TEM, 14 SHV, and 20 CTX-M variants), AmpC (10 variants), or KPC (4 variants) to CPT and CPT + NXL (CXL) was determined using broth microdilution panels in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. MICs of CPT were compared to those of CXL and the magnitude of MIC reduction was calculated.

Results: The following table shows CPT and CXL MIC_{50/90} values for isolates with indicated β -lactamase profiles.

Enzyme Profile	N	CPT MIC ₅₀	CPT MIC ₉₀	CXL MIC ₅₀	CXL MIC ₉₀	Reduction in CPT MIC ₅₀
CTX-M	538	>32	>32	≤0.06	0.25	≥256-fold
KPC	118	>32	>32	1	2	≥32-fold
SHV	50	>32	>32	≤0.06	0.5	≥64-fold
AmpC + CTX-M	43	>32	>32	0.25	2	≥32-fold
SHV + CTX-M	28	>32	>32	≤0.06	0.25	≥256-fold
SHV + KPC	18	>32	>32	1	4	≥16-fold
TEM	7*	[4->32]	[4->32]	[≤0.06-0.5]		*
TEM + CTX-M	6*	[>32]	[>32]	[≤0.06-0.5]		*
AmpC + SHV	5*	[4->32]	[4->32]	[≤0.06-0.5]		*
SHV + TEM	1*	[32]	[32]	[0.12]		*
AmpC + TEM	1*	[>32]	[>32]	[0.5]		*
AmpC + SHV + CTX-M	1*	[>32]	[>32]	[0.5]		*

*MIC_{50/90} not calculated if N<10; instead, MIC range is shown in brackets.

Conclusions: NXL lowered CPT MIC₉₀s from ≥16 to ≥256-fold for all variants and combinations of β -lactamases, and lowered MICs by a minimum of 16-fold against KPC-producing isolates. CXL, the combination product of NXL with CPT, promises to greatly expand the spectrum of activity of CPT to include β -lactamase-producing ENT.

Introduction

Ceftaroline (CPT), the active component of the prodrug ceftaroline fosamil, is a novel broad-spectrum cephalosporin with activity against resistant gram-positive organisms and common gram-negative pathogens [1]. Like other cephalosporins, its activity is significantly reduced by extended-spectrum β -lactamases (ESBLs), AmpC enzymes, or KPCs. NXL104 (NXL) is a non- β -lactam, β -lactamase inhibitor that has been shown to restore *in vitro* activity of many β -lactam antimicrobials against these otherwise β -lactam-resistant isolates [2]. In light of the multitude of reports in the literature about growing rates of resistance, NXL may prove a very useful agent in the treatment of infections caused by resistant organisms. We evaluated the MICs of CPT and CXL (the combination of CPT and NXL) against a collection of molecularly characterized β -lactamase-producing *Enterobacteriaceae*.

Materials and Methods

Isolates

• 816 recent clinical isolates of *Enterobacteriaceae* from a global collection previously determined to produce ESBL, AmpC, or KPC β -lactamases (6 TEM, 14 SHV, 20 CTX-M, 10 AmpC, and 4 KPC variants) were selected.

Susceptibility Testing

• Broth microdilution panels were prepared following CLSI guidelines [3]. CPT and CXL were tested in cation-adjusted Mueller Hinton broth at concentrations ranging from 0.06 to 32 μ g/mL of CPT. The NXL concentration was fixed at 4 μ g/mL for all concentrations of CXL.

Quality Control

• *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, and *E. coli* ATCC 35218 were included as quality control strains. Results from clinical isolates were only included if CPT results for the quality control strains were within ranges specified by CLSI [4]

• MIC_{50/90} values were calculated for organisms producing each specific enzyme variant to determine the ability of NXL to restore the antibacterial activity of CPT in the presence of β -lactamases. MIC_{50/90} values were only calculated for groups with ≥10 isolates; otherwise, MIC ranges are shown.

Results

Results are summarized in Figures 1-6 and Table 1.

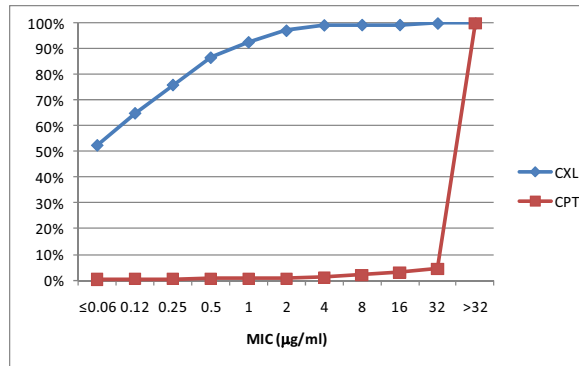
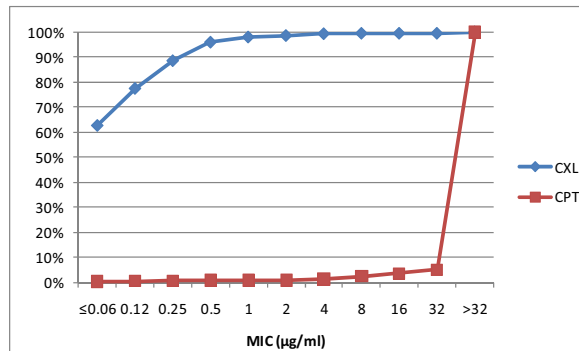
Fig. 1. Cumulative % inhibition of 816 β -lac+ (ESBL, AmpC, KPC) isolates.Fig. 2. Cumulative % inhibition of 680 non-KPC β -lac+ isolates.

Fig. 3. Cumulative % inhibition of 136 KPC and KPC+SHV isolates.

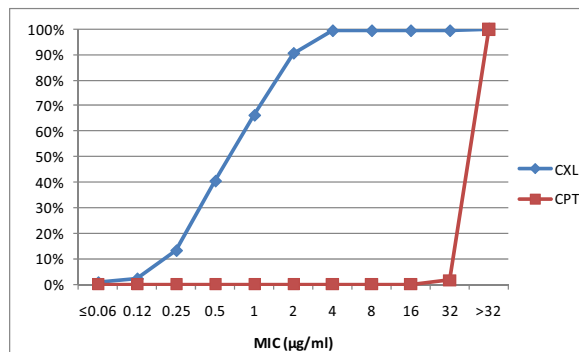


Fig. 4. Cumulative % inhibition of 50 AmpC isolates.

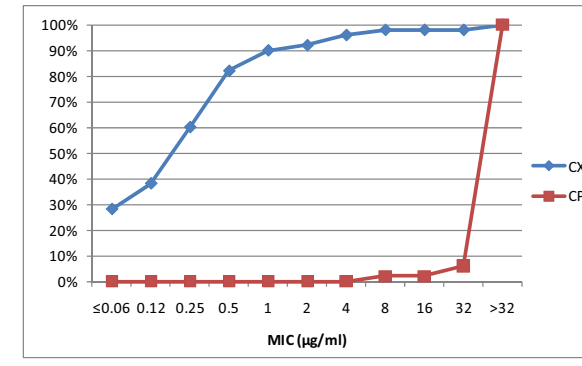


Fig. 5. Cumulative % inhibition of 630 ESBL isolates.

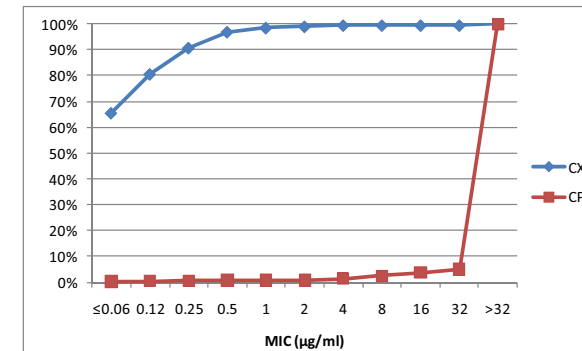
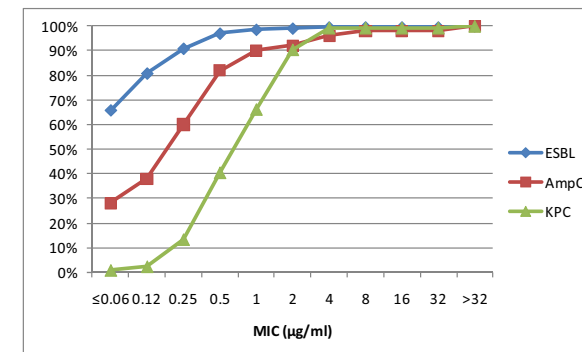


Fig. 6. CXL cumulative % inhibition of beta-lactamase isolates.

Table 1. CPT and CXL MIC_{50/90} values for isolates with indicated β -lactamase profiles

Enzyme Profile	N	CPT MIC ₅₀	CPT MIC ₉₀	CXL MIC ₅₀	CXL MIC ₉₀	Reduction in CPT MIC ₅₀
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SHV + TEM	1*	[32]	[32]	[0.12]		*
AmpC + TEM	1*	[>32]	[>32]	[0.5]		*
AmpC + SHV + CTX-M	1*	[>32]	[>32]	[0.5]		*

*MIC_{50/90} not calculated if N<10; instead, MIC range is shown in brackets.

Conclusions

- NXL lowered CPT MIC₉₀ values 16- to ≥256-fold for all organisms producing the β -lactamase types studied
- The inhibitory effects of NXL104 in combination with CPT were greatest vs. ESBL variants (CXL MIC₉₀ ≤0.25 μ g/mL), followed by AmpC variants (MIC₉₀ ≤1 μ g/mL) and finally, KPC variants (MIC₉₀ ≤2 μ g/mL)
- CXL restores *in vitro* activity to CPT against β -lactamase-producing *Enterobacteriaceae*, including drug-resistant gram-negative pathogens.

References

1. Steed, M.E., Rybak, M.J. Ceftaroline: A New Cephalosporin with Activity against Resistant Gram-Positive Pathogens. *Pharmacotherapy*. 2010; 30(4): 375-389.
2. Livermore, D.M., Mushtaq, S., Warner, M., Miossec, C., Woodford, N. NXL104 combinations versus *Enterobacteriaceae* with CTX-M extended-spectrum β -lactamases and carbapenemases. *J Antimicrob Chemother* 2008; 62(5):1053-1056.
3. Clinical and Laboratory Standards Institute. 2009. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition, M07-A8. CLSI, Villanova, PA, USA.
4. Clinical and Laboratory Standards Institute. 2010. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement, M100-S20. CLSI, Villanova, PA, USA.

Acknowledgement

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