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Activity of Ceftaroline/NXL104 and Comparator Agents Tested Against Strains Producing KPC Serine-Carbapenemases

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Amended Abstract

Background: KPC serine carbapenemases were first described in the USA and have rapidly been discovered worldwide. *bla*$_{KPC}$-carrying strains are resistant (R) to all β-lactams and other antimicrobial classes due to the association of this gene with plasmids harboring additional R determinants. NXL104 is a β-lactamase inhibitor that inhibits serine enzymes, including KPCs, and restores the activity of β-lactam co-drugs.

Methods: 73 KPC-producing Enterobacteriaceae (ENT) strains (7 species) collected in the USA (65 strains), Israel (7), and Argentina (1) were susceptibility (S) tested against ceftaroline (CPT) alone, CPT combined with fixed 4 µg/mL of NXL104 (CXL), and comparator agents using CLSI broth microdilution methods. KPC encoding genes were identified by PCR and sequencing.

Results: Most isolates were *Klebsiella pneumoniae* (KPN; 34) and *Enterobacter cloacae* (ECL; 17), and usually produced KPC-2, -3, and -4 (only 1 strain). CXL showed excellent activity against KPC-producing strains (MIC$_{50}$, 0.5 µg/mL) and 93.2% of KPC-producing strains were inhibited by CXL ≤2 µg/mL. Four strains had CXL MIC of 4 µg/mL (3 KPN and 1 KPC-4-producing ECL) and 1 KPC-3-producing *E. gergoviae* displayed CXL MIC at >16 µg/mL. The majority of KPC-producing strains were R to all comparator agents, including carbapenems. Using recently approved CLSI breakpoints (≤1 µg/mL for S [M100-S20]), 100.0% of KPC producers were non-S to imipenem and meropenem. One KPC-2 KPN was S to ceftriaxone and ceftazidime (MICs, 0.5 and 2 µg/mL, respectively). Among comparators, cefepime and ciprofloxacin showed the highest activity, but inhibited only 19.2 and 20.5% of the strains, respectively, at current S breakpoints (CLSI, 2010).

	Number of strains inhibited at MIC (µg/mL) (cumulative percentage):							
Antimicrobial agent	**≤0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**>16**
Ceftaroline/NXL104	14 (19.2)	25 (53.4)	21 (82.2)	8 (93.2)	4 (98.6)	0 (98.6)	0 (98.6)	1 (100.0)
Ceftaroline				1 (1.4)	0 (1.4)	0 (1.4)	0 (1.4)	72 (100.0)
Ceftriaxone	1 (1.4)	0 (1.4)	0 (1.4)	0 (1.4)	2 (4.1)	1 (5.5)	5 (12.3)	64 (100.0)
Ceftazidime				2 (2.7)	1 (4.1)	4 (9.6)	6 (17.8)	60 (100.0)
Cefepime			1 (1.4)	2 (4.1)	5 (11.0)	6 (19.2)	14 (38.4)	45 (100.0)
Imipenem				3 (4.1)	17 (27.4)	22 (57.5)	31 (100.0)	-[a]
Meropenem				7 (9.6)	19 (35.6)	13 (53.4)	34 (100.0)	-
Piperacillin/tazobactam								73 (100.0)[b]
Ciprofloxacin	8 (11.0)	1 (12.3)	6 (20.5)	7 (30.1)	51 (100.0)[c]	-	-	-

a. - = dilution not tested.
b. All strains had MIC values ≥64 µg/mL.
c. Strains with ciprofloxacin MIC values ≥4 µg/mL.

Conclusions: KPC-producing strains were highly R to all antimicrobials tested except CXL. NXL104 restored CPT activity against the vast majority of KPC-producing ENT from all evaluated species, demonstrating that CXL could be a valuable option for empiric therapy for R ENT strains in countries where serine-carbapenemases and other β-lactamases are prevalent.

Introduction

β-lactamase production is the most significant mechanism of β-lactam resistance. β-lactamase genes have the potential to acquire mutations that can expand their spectrum of hydrolysis against various β-lactams.

KPC enzymes have become the most troublesome group of β-lactamases as a result of their broad spectrum and rapid plasmid-mediated dissemination. KPC serine carbapenemases were first described in the United States (USA) and have subsequently been discovered worldwide. *bla*$_{KPC}$-carrying strains are typically resistant to all β-lactams, and often to other antimicrobial classes, as the *bla*$_{KPC}$ gene is associated with plasmids harboring additional resistance determinants.

Ceftaroline, the active form of the prodrug ceftaroline fosamil, is a novel broad-spectrum cephalosporin exhibiting Gram-positive and -negative activity and extended activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae*. Similar to other cephalosporins, ceftaroline is less active against some β-lactamase-producing Gram-negative organisms. However, when combined with NXL104, a potent non-β-lactam β-lactamase inhibitor, the spectrum of activity of ceftaroline is expanded against strains producing class A β-lactamases, such as TEM, SHV, CTX-M, and KPC enzymes, class C cephalosporinases, and class D oxacillinases with narrow or extended spectrum of activity.

In this study, we evaluated the activity of ceftaroline combined with NXL104 against 73 clinical strains of KPC-producing Enterobacteriaceae.

Methods

Bacterial isolates

A total of 73 KPC-producing Enterobacteriaceae strains (7 species) identified during the 1999-2008 period in 2 surveillance studies (SENTRY Antimicrobial Surveillance Program and MYSTIC Program) were evaluated. Strains were collected in the USA (n = 65), Israel (n = 7), and Argentina (n = 1). Only 1 isolate per patient from documented infections was included in the study. Isolates were collected from bloodstream, respiratory tract, and skin structure infections according to defined protocols. Species identification was confirmed by standard biochemical tests, the Vitek System (bioMerieux; Hazelwood, Missouri), or 16S rRNA sequencing, when necessary.

Antimicrobial susceptibility testing

All strains were tested for antimicrobial susceptibility using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009). Ceftaroline was combined with NXL104 at a fixed concentration of 4 µg/mL. Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI document M100-S20 (2010).

Genotypic detection of β-lactamase genes

Isolates showing reduced susceptibility to imipenem or meropenem (MIC, ≥2 µg/mL) were tested for the presence of carbapenemase genes, including *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$, *bla*$_{SME}$, *bla*$_{GES}$ variants, and for *bla*$_{IMI}$, *bla*$_{NMC-A}$, *bla*$_{OXA-48}$, combined in 2 amplification reactions. PCR amplicons were sequenced on both strands and nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR; Madison, Wisconsin). Sequences were compared with others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Results

- KPC-type enzymes were most frequently found in *Klebsiella pneumoniae* (34 strains; 46.6% of extended-spectrum β-lactamase [ESBL]-producing Enterobacteriaceae), followed by *Enterobacter cloacae* (17; 23.3%), *Citrobacter freundii* (6; 8.2%), *Klebsiella oxytoca* (6; 8.2%), *E. coli* (5; 6.8%), *Serratia marcescens* (3; 4.1%), and *Enterobacter gergoviae* (2; 2.7%; Table 1)

- Ceftaroline/NXL104 showed excellent activity against KPC-producing strains (MIC$_{50}$, 0.5 µg/mL), and 93.2% of KPC-producing strains were inhibited by a ceftaroline/NXL104 MIC of 2 µg/mL or lower (Tables 1 and 2). Four strains had ceftaroline/NXL104 MIC of 4 µg/mL (3 *K. pneumoniae* and 1 KPC-4-producing *E. cloacae*), and 1 KPC-3-producing *E. gergoviae* displayed ceftaroline/NXL104 MIC >16 µg/mL (Table 1)

- The majority of KPC-producing strains were resistant to all comparator agents, including carbapenems (Table 2). Applying recently approved CLSI breakpoints (≤1 µg/mL for susceptibility [M100-S20, mid-2010 Update]), none of the KPC-producing strains were considered susceptible to imipenem or meropenem

- Among comparators, cefepime and ciprofloxacin showed the highest activity, but only inhibited 19.2 and 20.5% of the strains, respectively (Table 2; CLSI, 2010)

Table 1. Ceftaroline/NXL104[a] MIC Distributions Among KPC-producing Strains of Various Enterobacteriaceae Species

	No. of strains (cumulative %) inhibited at ceftaroline/NXL104[a] MIC (µg/mL) of:							
Organism (no. tested)	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**>4**
K. pneumoniae (34)	1 (2.9)	2 (8.8)	4 (20.6)	14 (61.8)	7 (82.4)	3 (91.2)	3 (100.0)	-
E. cloacae (17)	-	-	-	4 (23.5)	8 (70.6)	4 (94.1)	1 (100.0)	-
C. freundii (6)	-	1 (16.7)	2 (50.0)	2 (83.3)	1 (100.0)	-	-	-
K. oxytoca (6)	-	1 (16.7)	2 (50.0)	1 (67.7)	2 (100.0)	-	-	-
E. coli (5)	-	1 (20.0)	-	4 (100.0)	-	-	-	-
S. marcescens (3)	-	-	-	-	3 (100.0)	-	-	-
E. gergoviae (2)	-	-	-	-	-	1 (50.0)	-	1 (100.0)[b]
All (73)	1 (1.4)	5 (8.2)	8 (19.2)	25 (53.4)	21 (82.2)	8 (93.2)	4 (98.6)	1 (100.0)[b]

a. Ceftaroline combined with NXL104 at fixed concentration of 4 µg/mL.
b. One KPC-3-producing *E. gergoviae* with ceftaroline/NXL104 MIC of >16 µg/mL.

Table 2. Antimicrobial Activity of Ceftaroline/NXL104 and Comparator Agents Tested Against 73 Clinical Isolates of KPC-producing Enterobacteriaceae

Antimicrobial agent/ organism (no. tested)	**MIC$_{50}$**	**MIC$_{90}$**	**% Susc.[a]**	**% Res.[a]**
All Enterobacteriaceae (73)				
Ceftaroline/NXL104	0.5	2	-[b]	-
Ceftriaxone	>32	>32	1.4	98.6
Ceftazidime	>32	>32	4.1	90.4
Cefepime	>16	>16	19.2	61.6
Imipenem	8	>8	0.0	95.9
Meropenem	8	>8	0.0	91.4
Piperacillin/tazobactam	>64	>64	0.0	97.3
Ciprofloxacin	4	>4	20.5	69.9
K. pneumoniae (34)				
Ceftaroline/NXL104	0.5	2	-	-
Ceftriaxone	>32	>32	2.9	97.1
Ceftazidime	32	>32	5.9	91.2
Cefepime	>16	>16	11.8	73.5
Imipenem	>8	>8	0.0	100.0
Meropenem	8	>8	0.0	97.1
Piperacillin/tazobactam	>64	>64	0.0	97.1
Ciprofloxacin	4	>4	14.7	82.4
E. cloacae (17)				
Ceftaroline/NXL104	1	2	-	-
Ceftriaxone	>32	>32	0.0	100.0
Ceftazidime	>32	>32	0.0	100.0
Cefepime	>16	>16	11.8	76.5
Imipenem	8	>8	0.0	88.2
Meropenem	8	>8	0.0	76.5
Piperacillin/tazobactam	>64	>64	0.0	100.0
Ciprofloxacin	4	>4	17.7	72.3

a. Percentages susceptible and resistant according to CLSI breakpoint criteria when available (CLSI, 2010).
b. - = No breakpoints have been established by the CLSI (CLSI, 2010).

Conclusions

- Ceftaroline/NXL104 was highly active against clinical isolates of the emerging KPC-producing Enterobacteriaceae
- NXL104 restored ceftaroline activity against the vast majority (93.2% inhibited at ≤2 µg/mL) of KPC-producing Enterobacteriaceae from all evaluated species
- Results from the present study indicate that ceftaroline/NXL104 could be a valuable empiric agent for resistant Enterobacteriaceae strains in countries where serine carbapenemases and other extended-spectrum β-lactamases are prevalent

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