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Antimicrobial Activity of Ceftaroline-Avibactam Tested Against Clinical Enterobacteriaceae Isolates Carrying Multiple β-lactamases from USA Medical Centers

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Amended Abstract

Background: Ceftaroline is a new cephalosporin with broad-spectrum activity against Gram-positive (including MRSA) and Gram-negative organisms. Avibactam is a novel non-β-lactam-β-lactamase inhibitor that inhibits Ambler class A and C enzymes, as well as some class D enzymes. We evaluated the activity of combined ceftaroline-avibactam against Enterobacteriaceae with various types of β-lactamase, with most strains carrying multiple β-lactamases.

Methods: 3,061 blood culture isolates consecutively collected from 26 USA hospitals (20 states) during 2010 were susceptibility tested by CLSI methods. *Klebsiella* spp., *E. coli* and *P. mirabilis* strains were selected according to the CLSI ESBL criteria for further evaluation. Species that hyperexpress chromosomal AmpC were selected using cefepime MIC values of ≥2 μg/mL. Selected isolates were tested by PCR and sequencing for genes encoding TEM, SHV, CTX-M, PER, VEB, GES, PSE, OXA-2, OXA-10, OXA-18/-45, OXA-1/30 and plasmidic AmpCs.

Results: 214 of 3,061 Enterobacteriaceae isolates (7.0% overall) were selected and 186 (86.9%) had at least 1 β-lactamase identified. The most prevalent enzymes were CTX-M-15 (67 strains) and OXA-1/-30 (35 strains); both enzymes were often observed in the same strains (30 of 35). SHV genes were detected in 87 strains (mainly SHV-11, SHV-32 and SHV-1; 10 variants total). KPC-2, KPC-3, SHV-30, and CMY-2 were also prevalent (17, 12, 12 and 12 strains, respectively). Ceftaroline-avibactam exhibited a potent inhibitory effect against all strains producing β-lactamase types encountered in this collection. The highest ceftaroline-avibactam MIC was 4 μg/mL (1 *Klebsiella oxytoca* with bla_{OXY}) and 96.7% of strains were inhibited at ≤1 μg/mL. Ceftaroline-avibactam was active against meropenem-susceptible ($MIC_{50/90}$, 0.06/0.5 μg/mL) and -non-susceptible strains ($MIC_{50/90}$, 0.5/2 μg/mL).

Conclusions: Avibactam, when combined with ceftaroline, effectively lowers the ceftaroline MIC for Enterobacteriaceae that produce the most clinically significant β-lactamases found in USA hospitals. Ceftaroline-avibactam was highly active against Enterobacteriaceae that produce KPC, various ESBL types, and AmpC (chromosomally derepressed or plasmid mediated), as well as those producing more than one of these enzymes. Ceftaroline-avibactam represents a promising therapeutic option for treatment of infections caused by multidrug-resistant Enterobacteriaceae.

Introduction

Infections caused by Gram-negative organisms represent a serious problem in hospital settings. Gram-negative bacilli are currently responsible for 40 - 60% of all pneumonias, approximately 70% of urinary tract infections (UTI) and 23 - 30% of bacteremias in intensive care unit (ICU) patients. These Gram-negative pathogens often exhibit decreased susceptibility or resistance phenotypes to several agents, challenging the antimicrobial therapies currently available. Moreover, the number of new anti-Gram-negative antimicrobial molecules in development and licensed has been limited.

Ceftaroline fosamil is the prodrug of ceftaroline, a new cephalosporin with broad *in vitro* spectrum activity against *Streptococcus pneumoniae* and *Staphylococcus aureus*, including methicillin-resistant strains (MRSA). Ceftaroline is also active against Enterobacteriaceae but has limited activity against strains producing AmpC, extended-spectrum β-lactamases (ESBLs), and carbapenemases, since ceftaroline is recognized as a substrate and hydrolyzed by these enzymes. However, the spectrum of activity of ceftaroline is expanded when combined with avibactam (formerly NXL-104), a potent non-β-lactam β-lactamase inhibitor that inhibits classes A, C and some D β-lactamases. In this study, the activity of ceftaroline-avibactam was evaluated against contemporary Enterobacteriaceae clinical isolates collected in USA hospitals confirmed to produce various types of enzymes, and most strains carried multiple β-lactamase-encoding genes.

Methods

Bacterial Isolates. A total of 3,061 Enterobacteriaceae isolates from bloodstream infections were submitted to JMI Laboratories in 2010 from USA medical centers. Only one isolate per patient from documented bloodstream infections was included in the study. Among those, *Klebsiella* spp., *Escherichia coli* and *Proteus mirabilis* strains that met the Clinical and Laboratory Standards Institute (CLSI) ESBL criteria were selected and included in this study. In addition, *Serratia* spp., *Providencia* spp., *Citrobacter* spp. and *Enterobacter* spp. isolates displaying cefepime MIC values of ≥2 μg/mL were selected. A total of 214 (7.0%) Enterobacteriaceae met the selection criteria. These strains were recovered from patients in 26 hospitals located in 20 USA states (nine Census regions). Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial Susceptibility Testing. Isolates were tested for susceptibility by the broth microdilution method using 96-well frozen-form panels with cation-adjusted Mueller-Hinton broth according to the (CLSI) recommendations. The ceftaroline-avibactam combination was tested in 11 $\log_2$ dilution steps of ceftaroline (32 − 0.03 μg/mL) and a fixed concentration (4 μg/mL) of the β-lactamase inhibitor (avibactam). Validation of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S22) quality control (QC) strains: *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event. Interpretation of meropenem MIC results was in accordance with published CLSI breakpoint criteria (M100-S22).

Genotypic Characterization of β-lactamases. Screening for β-lactamase-encoding genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{GES}, bla_{VEB}, bla_{PER}, bla_{PSE}, bla_{BEL}, bla_{OXA-2}, bla_{OXA-10} and $bla_{OXA-1/30}$, bla_{OXA-18} and bla_{OXA-45}, and bla_{OXY}) was performed by single (bla_{TEM} and bla_{SHV}) or combinations of multiplex reactions. Plasmidic AmpC-encoding genes ($bla_{CMY-1-41}$, $bla_{CMY-43-44}$, bla_{CMY-49}, $bla_{FOX-1-7}$, $bla_{ACC-1-4}$, $bla_{ACT-1-7}$, $bla_{DHA-1-3}$, bla_{LAT-1}, $bla_{MIR-1-5}$, $bla_{MOM-1-7}$) were screened using a multiplex reaction. Selected isolates exhibiting imipenem or meropenem MIC results of ≥2 μg/mL were screened for carbapenemase genes by PCR (bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{KPC}, bla_{SME}, bla_{IMI}, bla_{NMC-A}, bla_{GES} and bla_{OXA-48}). Following PCRs, amplicons were purified and both strands were subjected to sequencing. Nucleotides and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Results

- Ceftaroline-avibactam ($MIC_{50/90}$, 0.12/1 μg/mL) inhibited 96.7% of strains at ≤1 μg/mL (Table 1). The highest ceftaroline-avibactam MIC value observed was 4 μg/mL (one *K. oxytoca* strain with bla_{OXY})
- Ceftaroline-avibactam was active against meropenem-susceptible ($MIC_{50/90}$, 0.06/0.5 μg/mL) and -non-susceptible strains ($MIC_{50/90}$, 0.5/2 μg/mL), including KPC-producing Enterobacteriaceae clinical isolates ($MIC_{50/90}$, 1/2 μg/mL; Table 1)
- Table 2 describes the β-lactamases identified among Enterobacteriaceae isolates that met the screening criteria (214/3,061; 7.0%). Among these strains 186 (86.9%) had at least one β-lactamase detected. The most prevalent enzymes were CTX-M-15 (64 strains) and OXA-1/-30 (34 strains). In addition, both enzymes were often observed in the same strains (31 of 34)
- Narrow- and extended-spectrum SHV-encoding genes were observed among 86 strains (mainly SHV-11 and SHV-32). Other less prevalent enzymes were those associated to OXA-2 group (two occurrences), PSE (five) and PER (one; Table 2)
- Among plasmidic AmpC enzymes (20 occurrences), CMY-2 predominated (13/20; 65.0%) followed by FOX-5 (5/20; 25.0%). All CMY-2 pAmpC-encoding genes were observed among *E. coli*, except for one gene detected in a *Klebsiella pneumoniae* strain, while bla_{FOX-5} were noted in *K. pneumoniae* (three strains), and one strain each of *P. mirabilis* and *Enterobacter cloacae* (data not shown)
- Carbapenemase KPC-2 and KPC-3 were also prevalent (17 and 12 events, respectively) and these strains usually (27/29; 93.1%) exhibited a non-susceptible phenotype to meropenem (i.e. MIC value of ≥2 μg/mL; Table 2).

Table 1. Ceftaroline-avibactam MIC Distribution Obtained against Enterobacteriaceae Strains Producing ESBLs Selected for this Study

Organisms (no. tested)	No. of isolates (cumulative %) inhibited at ceftaroline-avibactam MIC (μg/mL) of:							
	≤0.03	0.06	0.12	0.25	0.5	1	2	4
All organisms (214)	45(21.0)	59(48.6)	38(66.4)	21(76.2)	27(88.8)	17(96.7)	6(99.5)	1(100.0)
E. coli (93)	39(41.9)	43(88.2)	10(98.9)	1(100.0)	-	-	-	-
K. pneumoniae (74)	5(6.8)	9(18.9)	22(48.6)	15(68.9)	11(83.8)	9(95.9)	3(100.0)	-
Others (47)	1(2.1)	7(17.0)	6(29.8)	5(40.4)	16(74.5)	8(91.5)	3(97.9)	1(100.0)
Meropenem-susceptible (179)	44(24.6)	56(55.9)	36(76.0)	17(85.5)	18(95.5)	6(98.9)	1(99.4)	1(100.0)
E. coli (93)	39(41.9)	43(88.2)	10(98.9)	1(100.0)	-	-	-	-
K. pneumoniae (50)	4(8.0)	8(24.0)	21(66.0)	11(88.0)	5(98.0)	1(100.0)	-	-
Others (36)	1(2.8)	5(16.7)	5(30.6)	5(44.4)	13(80.6)	5(94.4)	1(97.2)	1(100.0)
Meropenem-non-susceptible (35)	1(3.0)	3(11.4)	2(17.1)	4(28.6)	9(54.3)	11(85.7)	5(100.0)	-
K. pneumoniae (24)	1(4.2)	1(8.3)	1(12.5)	4(29.2)	6(54.2)	8(87.5)	3(100.0)	-
Others (11)	0(0.0)	2(18.2)	1(27.3)	0(27.3)	3(54.5)	3(81.8)	2(100.0)	-
KPC producers (29)	2(6.9)	0(6.9)	3(10.3)	7(24.1)	14(48.3)	24(82.7)	29(100.0)	-

Table 2. β-lactamase (Enzymes) Arrays Detected among Enterobacteriaceae Strains that Met the Screening Criteria Collected in USA Hospitals (2010)

Enzyme[a]	Number of occurrences	MIC range (μg/mL)		
		Ceftaroline	Ceftaroline-avibactam	Meropenem
ACT-1+SHV-5	1	>32	0.12	≤0.12
CMY-2	5	8->32	≤0.03-0.12	≤0.12
CMY-2+CTX-M-14	1	>32	0.12	≤0.12
CMY-2+SHV-33	1	32	0.12	≤0.12
CMY-2+TEM-1	6	8->32	≤0.03-0.12	≤0.12
CTX-M-14	3	>32	≤0.03	≤0.12-0.25
CTX-M-14+SHV-like+TEM-1	1	>32	0.12	≤0.12
CTX-M-14+SHV-11+TEM-1	2	>32	0.25	≤0.12
CTX-M-14+TEM-1	4	>32	≤0.03-0.25	≤0.12
CTX-M-15	9	>32	≤0.03-0.06	≤0.12
CTX-M-15+OXA-1/30	16	2->32	≤0.03-0.12	≤0.12
CTX-M-15+OXA-1/30+PSE-like	1	>32	0.06	≤0.12
CTX-M-15+OXA-1/30+SHV-1	1	>32	0.25	≤0.12
CTX-M-15+OXA-1/30+SHV-1+TEM-1	2	>32	0.12-0.5	≤0.12-2
CTX-M-15+OXA-1/30+SHV-11+TEM-1	4	>32	≤0.03-1	≤0.12-8
CTX-M-15+OXA-1/30+TEM-1	6	>32	≤0.03-0.06	≤0.12
CTX-M-15+SHV-1	3	>32	0.12-0.25	≤0.12
CTX-M-15+SHV-1+TEM-1	9	>32	0.06-0.5	≤0.12-1
CTX-M-15+SHV-11	2	>32	0.12	≤0.12
CTX-M-15+TEM-1	8	>32	≤0.03-0.06	≤0.12
CTX-M-27	2	>32	≤0.03	≤0.12
CTX-M-27+OXA-1/30	1	>32	≤0.03	≤0.12
CTX-M-27+SHV-11+TEM-1	2	>32	0.06-1	≤0.12-0.5
CTX-M-27+TEM-1	1	>32	0.06	≤0.12
CTX-M-91+TEM-1	1	>32	2	≤0.12
DHA-1+TEM-1	1	>32	0.12	0.25
FOX-5+PSE	4	8->32	0.12-1	≤0.12-0.25
FOX-5+SHV-11	1	32	0.25	≤0.12
KPC-2	1	>32	1	8
KPC-2+OXA-2	1	>32	0.5	4
KPC-2+SHV-1+TEM-1	3	>32	0.25-0.5	8->8
KPC-2+SHV-11	1	>32	1	8.1
KPC-2+SHV-11+TEM-1	5	>32	0.5-2	>8
KPC-2+TEM-1	4	>32	0.25-2	8->8
KPC-3+SHV-12+TEM-1	10	32->32	≤0.03-2	0.5->8
KPC-3+TEM-1	1	>32	1	8
KPC-2+CTX-M-15+ SHV-1+TEM-1	2	>32	1	8.1
KPC-3+CTX-M-15+ OXA-1/30+SHV-11+TEM-1	1	>32	0.5	8.1
OXA-1/30	1	>32	0.12	≤0.12
OXA-1/30+SHV-7+TEM-1	1	8	0.12	≤0.12
OXA-2+SHV-1+TEM-1	1	32	0.25	≤0.12
OXA-9+SHV-7+TEM-1	3	1->32	≤0.03-0.5	≤0.12
PER-1+SHV-12+TEM-1	1	>32	1	≤0.12
SHV-1	2	0.06-0.12	0.06	0.5-8
SHV-1+SHV-12+TEM-1	1	>32	0.12	≤0.12
SHV-1+TEM-1	1	>32	0.12	≤0.12
SHV-11	3	32->32	≤0.03-0.12	≤0.12
SHV-11+SHV-5+TEM-1	1	>32	0.12	≤0.12
SHV-11+TEM-1	1	>32	0.12	≤0.12
SHV-11+TEM-155	1	>32	0.06	≤0.12
SHV-12+TEM-1	5	1->32	≤0.03-0.5	≤0.12
SHV-2	1	32	0.12	0.25
SHV-2+TEM-1	1	32	0.06	≤0.12
SHV-27	1	0.5	≤0.03	≤0.12
SHV-30	4	4->32	0.12-0.5	≤0.12-0.25
SHV-7+TEM-1	1	>32	0.06	0.5
SHV-like	6	1->32	0.06-0.5	≤0.12
TEM-like	14	0.06->32	≤0.03-0.5	≤0.12-0.25

a. Enzymes were named as "-like" when sequencing did not exhibit enough coverage to differentiate among variants or when new enzymes were detected.

Conclusions

- In this study, avibactam, when combined with ceftaroline, effectively restored the *in vitro* activity of ceftaroline ($MIC_{50/90}$, 0.12/1 μg/mL) when tested against Enterobacteriaceae clinical isolates from USA hospitals that met the CLSI screening criteria for ESBL
- Ceftaroline-avibactam was highly active against strains that produced KPC, various ESBL types, and plasmid-mediated AmpC enzymes, as well as those producing combinations of these enzymes
- β-lactamase production constitutes the most relevant β-lactam resistance mechanism among Enterobacteriaceae. Therefore, the β-lactam-β-lactamase inhibitor combination (ceftaroline-avibactam) evaluated in this study represents a promising therapeutic option for further investigation for the treatment of infections caused by multidrug-resistant Enterobacteriaceae.

References

1. Bush K (2010). Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. *Curr Opin Microbiol* 13: 558-564.
2. Bush K (2008). Extended-spectrum β-lactamases in North America, 1987-2006. *Clin Microbiol Infect* 14 Suppl 1: 134-143.
3. Castanheira M, Farrell SE, Mendes RE, Jones RN (2011). Contemporary (2010) diversity of β-lactamases among Enterobacteriaceae collected from bacteremias in USA hospitals: Report from the SENTRY program. Abstr. C2-1250. *51st ICAAC, September 12-15, 2011,* Chicago, IL, USA.
4. Castanheira M, Mendes RE, Woosley LN, Sader H, Fritsche T, Jones RN (2008). Emergence of bla_{CTX-M} among Enterobacteriaceae isolates in USA Hospitals: Report from the SENTRY Antimicrobial Surveillance Program. *ICAAC/IDSA, October 25-28, 2008,* Washington, DC, USA.
5. Castanheira M, Sader HS, Jones R N (2010). Antimicrobial susceptibility patterns of KPC-producing or CTX-M-producing Enterobacteriaceae. *Microb. Drug Resist.* 16: 61-65.
6. Castanheira M, Woosley LN, Brady KM, Jones RN (2009). High Prevalence of CTX-M Producing Isolates and Emergence of KPC-encoding Genes in the Western United States. *ICAAC, September 11-14, 2009,* San Francisco, CA, USA.
7. Clinical and Laboratory Standards Institute (2011). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard: Ninth edition.* Wayne, PA: CLSI.
8. Clinical and Laboratory Standards Institute (2012). *M100-S22. Performance standards for antimicrobial susceptibility testing: 22nd Informational Supplement.* Wayne, PA: CLSI.
9. Livermore DM (1998). β-lactamase-mediated resistance and opportunities for its control. *J. Antimicrob. Chemother.* 41 Suppl D: 25-41.
10. Pitout JD, Laupland KB (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: An emerging public-health concern. *Lancet. Infect. Dis.* 8: 159-166.

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