ICAAC 2013
C2-1634

Ceftaroline-Avibactam Tested against Contemporary (2012) Isolates Collected from United States Hospitals with Well-Characterized β-Lactamases

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Amended Abstract

Background: Ceftaroline (CPT), like other cephalosporins, has limited activity against isolates producing broad-spectrum β-lactamases (BLs); however, CPT activity can be restored when combined with the serine-BL inhibitor avibactam (AVI). We evaluated the activity of CPT-AVI and comparators tested against 701 isolates displaying the CLSI ESBL phenotype and characterized by Check MDR CT-101 (CP).

Methods: 701 clinical isolates collected from 73 hospitals located in all nine USA Census regions (CR) were tested for susceptibility (S) by reference broth microdilution methods and evaluated for the presence of genes encoding ESBLs, KPC and plasmidic AmpC enzymes by CP.

Results: 328 *E. coli* (11.9% ESBL phenotype for this species), 296 *K. pneumoniae* (KPN; 16.0%), 44 *K. oxytoca* (KOX; 10.0%) and 33 *P. mirabilis* (4.8%) were tested. South Atlantic (SAt) and Mid-Atlantic CRs had the highest ESBL rates and West North Central had the lowest rates. KPN had the highest ESBL rates in 3/9 CRs. 303 isolates carried CTX-M Group (G) 1 (which includes CTX-M-15) alone or with 1-4 other BLs. CPT-AVI was active against CTX-M G1 isolates ($MIC_{50/90}$, 0.06/0.25 µg/mL). SHV ESBLs were detected among 176 isolates (all species and CRs). Only 68 isolates carried SHV ESBL alone. CPT-AVI had good coverage against these isolates ($MIC_{50/90}$, 0.12/0.25 µg/mL). 118 isolates carried bla_{KPC}; 112 were KPN. SAt, West South Central and East North Central had 18, 14 and 13 KPC occurrences, respectively. 13 KPC-producers also carried CTX-M G1. KPC-producers were very resistant to all β-lactams, except for CPT-AVI ($MIC_{50/90}$, 0.25/1 µg/mL). CPT-AVI was active against isolates carrying CTX-M G9 (72 isolates; $MIC_{50/90}$, 0.06/0.25 µg/mL) or CMY II (64; $MIC_{50/90}$, 0.06/0.12 µg/mL). Other BLs detected were FOX (10 isolates), TEM ESBL (9), DHA (7), CTX-M G2 (3), NDM-1 (2; Colorado) and CTX-M G8+25 (1). 62.9% of isolates carried ≥2 BLs. 70 strains had negative BL CP results and had borderline ESBL phenotype criteria or were KOX strains with high aztreonam and ceftriaxone and low ceftazidime MICs (OXY-hyperexpression profile). Elevated CPT-AVI MICs (>4 µg/mL) were only detected in 2 NDM-producers.

Conclusions: Regardless of the BL enzymes or combinations produced, CPT-AVI displayed excellent activity against BL-producing isolates from USA hospitals (2012).

Introduction

The β-lactamase scenario among Enterobacteriaceae isolates collected in United States (USA) hospitals differs from other countries due to the late appearance of CTX-M-producing isolates, high prevalence of KPC-producers and low incidence of isolates carrying metallo-β-lactamase genes. Among the USA regions, differences in ESBL rates and prevalence of KPC-producing isolates have been observed.

Avibactam is a non-β-lactam, β-lactamase inhibitor of Ambler structural class A, C, and some class D enzymes. When combined with a cephalosporin, avibactam is able to reduce the MIC values of β-lactamase-producing isolates, including those carrying bla_{KPC}, from the resistant category to susceptible ranges in the vast majority of tested isolates. Ceftaroline (CPT), like other cephalosporins, has limited activity against isolates producing broad-spectrum β-lactamases (BLs); however, CPT activity can be restored when combined with the serine-BL inhibitor avibactam (AVI).

In this study, we evaluated 701 ESBL phenotype-positive isolates of *Escherichia coli*, *Klebsiella* spp. and *Proteus mirabilis* identified using the Clinical and Laboratory Standards Institute (CLSI) screening criteria for the prevalence of common β-lactamase-encoding genes and analyzed the distribution of these genes by USA Census regions. Additionally, we analyzed the activity of ceftaroline-avibactam and comparator agents tested against isolates with characterized β-lactamases.

Methods

Bacterial isolates. A total of 5,739 isolates of *E. coli* (n=2,767), *Klebsiella* spp. (n=2,289; 1,847 *K. pneumoniae* and 442 *K. oxytoca*) and *P. mirabilis* (n=683) consecutively collected from 72 USA hospitals during 2012 were analyzed. Only one isolate per patient was included in the study from the following sites of infection: bloodstream (n=991); respiratory tract from hospitalized patients (n=1,047); intra-abdominal (n=285); skin and skin structure (n=1,565); urinary tract (n=1,558) and other or unknown sources (n=293). Species identification was confirmed when necessary by Matrix-Assisted Laser Desorption Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) by following manufacturer instructions.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using reference broth microdilution methods as described by the CLSI. Ceftaroline was tested alone and in combination with 4 µg/mL of avibactam. Categorical interpretations for all antimicrobials were those found in M100-S23 (2013) and quality control (QC) was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within published ranges as in CLSI (M100-S23, 2013) documents.

Screening for β-lactamases. Isolates displaying the CLSI criteria for ESBL phenotype (MIC >1 µg/mL for aztreonam and/or ceftazidime and/or ceftriaxone; M100-S23) were tested for β-lactamase-encoding genes using the microarray based assay Check-MDR CT101 kit (Check-points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M Groups 1, 2, 8+25 and 9, TEM wildtype (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM-1.

Multilocus sequence type (MLST). NDM-1-producing *K. pneumoniae* were evaluated by MLST according to the instructions on the website http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Results

- An ESBL phenotype was noted among 701 (12.2%) isolates and included 328 *E. coli* (11.9% of the overall samples for this species), 296 *K. pneumoniae* (16.0%), 44 *K. oxytoca* (10.0%) and 33 *P. mirabilis* (4.8%) with varying rates recorded by region and pathogen (Figure 1). The West North Central region had the lowest ESBL rates and the Mid-Atlantic region (Figure 1) had the highest rates
- CTX-M Group 1 (CTX-M-15-like) was detected in 303 isolates (Figure 2a) from all species evaluated and across all USA Census regions (Figure 2b), being more prevalent in East North Central (50 strains; 30.5%), West North Central (9; 29.0%), West South Central (67; 27.0%) and New England (19; 25.3%; Figure 2b) regions
- SHV ESBLs were detected among 176 isolates from all bacterial species examined (Figure 2a) and Census regions (Figure 2b), with these enzymes detected alone in only 68 isolates
- bla_{KPC} was detected among 118 isolates (Figure 2a; 112 *K. pneumoniae*) and were noted in all Census regions, being more prevalent in the Mid-Atlantic region (58 isolates; Figure 2b). Thirteen isolates (all *K. pneumoniae*) carrying CTX-M-15-like-encoding genes were observed
- CTX-M Group 9 (CTX-M-14-like) was found among 72 isolates, mostly *E. coli* (57 isolates) and were obtained from all Census regions (Figure 2b)
- CMY-2-like (CMYII probe; 64 isolates in all regions), FOX (10 isolates, four regions), TEM ESBL (9 isolates, five regions), DHA (7 isolates, four regions), CTX-M Group 2 (3 *K. pneumoniae*, Mid-Atlantic only [one hospital]) and CTX-M Group 8+25 (1 *P. mirabilis*; Mid-Atlantic) were also detected
- Two NDM-1-producing *K. pneumoniae* isolates were detected from one medical center in Colorado. These isolates were clonally related (MLST type 147), also carried CTX-M-15-like
- KPC-producers were very resistant to all β-lactams tested, with the exception of ceftaroline-avibactam ($MIC_{50/90}$, 0.25/1 µg/mL). Among other antimicrobial classes, tigecycline was the only agent that had acceptable activity against these strains ($MIC_{50/90}$, 0.5/1 µg/mL)
- Piperacillin/tazobactam (67.2% susceptible), tigecycline (98.3% susceptible) and meropenem (99.7% susceptible) were active against CTX-M-15-like-producers. All CTX-M-15-like-producing isolates had ceftaroline-avibactam ($MIC_{50/90}$, 0.06/0.25 µg/mL) MIC values at ≤2 µg/mL
- Ceftaroline-avibactam ($MIC_{50/90}$, 0.06/0.25 µg/mL), meropenem ($MIC_{50/90}$, ≤0.06/≤0.06 µg/mL), and tigecycline ($MIC_{50/90}$, 0.12/1 µg/mL) were the most active agents tested against CTX-M-14-like-producing strains
- Meropenem ($MIC_{50/90}$, ≤0.06/0.12 µg/mL), ceftaroline-avibactam ($MIC_{50/90}$, 0.12/0.25 µg/mL) and tigecycline ($MIC_{50/90}$, 0.5/1 µg/mL) were the most active agents tested against isolates carrying SHV ESBL without the presence of carbapenemases
- NDM-1 producers (two strains) were highly resistant to all agents tested, including ceftaroline-avibactam (MIC, >32 µg/mL), but were susceptible to tigecycline displaying MIC values of 0.5 µg/mL for both strains.

Figure 1. ESBL phenotype rates among 701 Enterobacteriaceae isolates collected in 72 hospitals located in the nine USA Census regions.

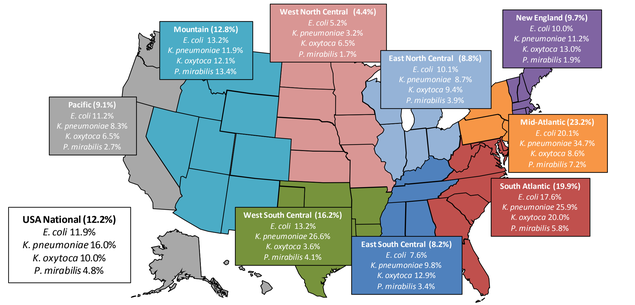

Figure 2. Distribution of most common (>5 occurrences) β-lactamases detected among 701 ESBL phenotype-positive strains collected in USA hospitals (a) overall and (b) by census regions.

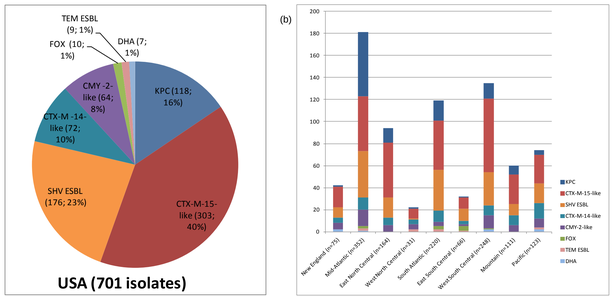

Table 1. Activity of ceftaroline-avibactam and comparator antimicrobial agents when tested against 701 ESBL phenotype-positive Enterobacteriaceae isolates collected in 72 hospitals located in the nine USA Census regions.

Isolate group (no. tested)/ Antimicrobial agent	MIC (µg/mL) 50%	90%	Range	CLSI[a] %S / %I / %R	EUCAST[b] %S / %I / %R
KPC-producers (118)					
Ceftaroline-avibactam	0.25	1	≤0.015 – 4	- / - / -	- / - / -
Ceftazidime	>32	>32	16 – >32	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Ceftriaxone	>8	>8	8 – >8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	>8	>8	2 – >8	0.0 / 4.2 / 95.8	4.2 / 24.6 / 71.2
Piperacillin/tazobactam	>64	>64	>64	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Levofloxacin	>4	>4	≤0.12 – >4	10.2 / 1.7 / 88.1	5.9 / 4.3 / 89.8
Gentamicin	8	>8	≤1 – >8	47.5 / 17.8 / 34.7	32.2 / 15.3 / 52.5
Tetracycline	4	>32	1 – >32	63.6 / 15.2 / 21.2	- / - / -
Tigecycline[b]	0.5	1	0.06 – 8	98.3 / 0.8 / 0.9	93.2 / 5.1 / 1.7
CTX-M-15-like-producers (288)[c]					
Ceftaroline-avibactam	0.06	0.25	≤0.015 – 2	- / - / -	- / - / -
Ceftazidime	16	>32	0.5 – >32	14.6 / 14.2 / 71.2	2.8 / 11.8 / 85.4
Ceftriaxone	>8	>8	8 – >8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	≤0.06	≤0.06	≤0.06 – 2	99.7 / 0.3 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	16	>64	≤0.5 – >64	67.2 / 17.3 / 15.5	48.3 / 18.9 / 32.8
Levofloxacin	>4	>4	≤0.12 – >4	12.5 / 4.2 / 83.3	11.5 / 1.0 / 87.5
Gentamicin	2	>8	≤1 – >8	53.3 / 2.1 / 44.6	51.6 / 1.7 / 46.7
Tetracycline	>32	>32	0.5 – >32	30.2 / 0.7 / 69.1	- / - / -
Tigecycline[b]	0.12	1	0.06 – 4	98.3 / 1.7 / 0.0	94.8 / 3.5 / 1.7
CTX-M-14-like-producers (70)[d]					
Ceftaroline-avibactam	0.06	0.25	≤0.015 – 0.5	- / - / -	- / - / -
Ceftazidime	2	16	0.12 – >32	74.3 / 11.4 / 14.3	35.7 / 38.5 / 25.7
Ceftriaxone	>8	>8	>8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	≤0.06	≤0.06	≤0.06 – 0.25	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	2	8	≤0.5 – >64	92.9 / 2.8 / 4.3	91.4 / 1.5 / 7.1
Levofloxacin	>4	>4	≤0.12 – >4	17.1 / 2.9 / 80.0	17.1 / 0.0 / 82.9
Gentamicin	2	>8	≤1 – >8	55.7 / 0.0 / 44.3	54.3 / 1.4 / 44.3
Tetracycline	>32	>32	0.5 – >32	22.9 / 0.0 / 77.1	- / - / -
Tigecycline[b]	0.12	1	0.06 – 4	94.3 / 5.7 / 0.0	94.3 / 0.0 / 5.7
SHV ESBL-producers (83)[e]					
Ceftaroline-avibactam	0.12	0.25	≤0.015 – 0.5	- / - / -	- / - / -
Ceftazidime	>32	>32	1 – >32	12.0 / 7.3 / 80.7	2.4 / 9.6 / 88.0
Ceftriaxone	>8	>8	0.12 – >8	9.6 / 7.3 / 83.1	9.6 / 7.3 / 83.1
Meropenem	≤0.06	0.12	≤0.06 – 2	98.8 / 1.2 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	>64	>64	1 – >64	45.8 / 3.6 / 50.6	36.1 / 9.7 / 54.2
Levofloxacin	>4	>4	≤0.12 – >4	39.8 / 7.2 / 53.0	37.3 / 2.5 / 60.2
Gentamicin	2	>8	≤1 – >8	61.4 / 10.9 / 27.7	51.8 / 9.6 / 38.6
Tetracycline	4	>32	0.5 – >32	55.4 / 9.7 / 34.9	- / - / -
Tigecycline[b]	0.5	1	0.06 – 2	100.0 / 0.0 / 0.0	94.0 / 6.0 / 0.0
CMY-2-like-producers (54)[f]					
Ceftaroline-avibactam	0.06	0.12	≤0.015 – 1	- / - / -	- / - / -
Ceftazidime	16	>32	2 – >32	13.0 / 24.0 / 63.0	0.0 / 13.0 / 87.0
Ceftriaxone	>8	>8	0.5 – >8	1.9 / 14.8 / 83.3	1.9 / 14.8 / 83.3
Meropenem	≤0.06	0.12	≤0.06 – 0.25	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	4	>64	≤0.5 – >64	81.5 / 7.4 / 11.1	72.2 / 9.3 / 18.5
Levofloxacin	≤0.12	>4	≤0.12 – >4	70.4 / 1.8 / 27.8	64.8 / 5.6 / 29.6
Gentamicin	≤1	>8	≤1 – >8	79.6 / 1.9 / 18.5	77.8 / 1.8 / 20.4
Tetracycline	>32	>32	0.5 – >32	33.3 / 1.9 / 64.8	- / - / -
Tigecycline[b]	0.12	2	0.06 – 4	96.3 / 3.7 / 0.0	85.2 / 11.1 / 3.7

a. Criteria as published by the CLSI [2013] and EUCAST [2013].
b. US-FDA breakpoints were applied [Tygacil Product Insert, 2012].
c. CTX-M-15-like do not include KPC- or NDM-1-producing isolates.
d. CTX-M-14-like do not include CTX-M-15-like-producing isolates.
e. SHV ESBL do not include CTX-M-15-like-, CTX-M-14-like-, KPC- or NDM-1-producing isolates.
f. CMY-2-like do not include CTX-M-15-like-, CTX-M-14-like-, KPC- or NDM-1-producing isolates.

Conclusions

- Significant variations were observed in the prevalence of ESBL phenotype-positive strains and β-lactamase production among isolates collected from different USA Census regions; however, genes encoding KPC and CTX-M enzymes are widely disseminated throughout the USA
- Ceftaroline-avibactam was very active against the vast majority of the isolates tested (exception of NDM-1-producers). This cephalosporin/β-lactamase inhibitor combination was potent against KPC-producers (MIC_{90}, 1 µg/mL) that have been increasingly reported as resistant to all clinically available antimicrobials, including some resistances to colistin and/or tigecycline
- The knowledge of the regional epidemiology of β-lactamases in the USA will be valuable guiding hospital and public health policies to minimize further dissemination of these resistance genes, and also to locally establish antimicrobial stewardship/treatment guidelines.

References

1. Bush K (2008). Extended-spectrum β-lactamases in North America, 1987-2006. *Clin Microbiol Infect* 14 Suppl 1: 134-143.
2. Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN (2013). Prevalence of β-lactamase encoding genes among Enterobacteriaceae bacteremia isolates collected in 26 USA hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2010). *Antimicrob Agents Chemother* 57: 3012-3020.
3. Castanheira M, Mendes RE, Rhomberg PR, Jones RN (2008). Rapid emergence of bla_{CTX-M} among Enterobacteriaceae in U.S. Medical Centers: molecular evaluation from the MYSTIC Program (2007). *Microb Drug Resist* 14: 211-216.
4. Castanheira M, Sader HS, Farrell DJ, Mendes RE, Jones RN (2012). Activity of ceftaroline-avibactam tested against gram-negative organism populations, including strains expressing one or more β-lactamases and methicillin-resistant *Staphylococcus aureus* carrying various SCCmec types. *Antimicrob Agents Chemother* 56: 4779-4785.
5. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition*. Wayne, PA: CLSI.
6. European Committee on Antimicrobial Susceptibility Testing (2013). Breakpoint tables for interpretation of MICs and zone diameters. Version 3.0, January 2013. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed January 1, 2013.
7. Flamm RK, Sader HS, Farrell DJ, Jones RN (2012). Ceftaroline potency among 9 US census regions: Report from the 2010 AWARE program. *Clin Infect Dis* 55 Suppl 3: S194-S205.
8. Pisney L, Barron M, Janelle S, Bamberg W, MacCannell D, Kallen A, Gould C, Limbago B, Epson E, Wendt J (2013). Hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing New Delhi metallo-β-lactamase-Denver, Colorado, 2012. *MMWR Mor Mortal Wkly Rep* 62: 108.

Acknowledgments

This study was supported by Forest Laboratories, Inc. Forest Laboratories, Inc., was involved in the study design and in the decision to present these results. Forest Laboratories, Inc., had no involvement in the analysis, collection, and interpretation of data. Scientific Therapeutics Information, Inc., provided editorial coordination, which was funded by Forest Research Institute, Inc.