Ceftibuten-Avibactam Activity against β-Lactam-Resistant *Enterobacteriaceae* Clinical Isolates

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Introduction

- Avibactam is a potent diazabicyclooctane inhibitor of class A, C, and some D β-lactamases that is approved for IV dosing in combination with ceftazidime, but the oral bioavailability of avibactam is negligible
- To extend the utility of avibactam, Arixa Pharmaceuticals is developing a novel oral avibactam prodrug (ARX-1796/AV-006; Figure 1) to be combined with oral β-lactams like ceftibuten to rescue their activity against resistant *Enterobacteriaceae* that produce β-lactamase enzymes
  - The synthesis and bioavailability of avibactam prodrugs is described in the accompanying poster SUNDAY – AAR-716
- To support upcoming clinical studies, the ability of avibactam to potentiate the activity of oral β-lactams (ceftibuten, cefixime, and amoxicillin) was evaluated against specific subsets of resistant *Enterobacteriaceae* isolates that contained 1 or more characterized β-lactamase genes
- Several oral and IV gram-negative antimicrobial agents with various modes of action were included as comparators

Materials and Methods

Bacterial isolates

- A total of 158 antimicrobial-resistant *Enterobacteriaceae* clinical isolates with specific molecularly characterized β-lactamase genes were selected from the SENTRY Antimicrobial Surveillance Program (Figure 2)
  - A total of 155 isolates were collected in 2016, and 3 isolates were collected in 2015
  - Species identities were confirmed by standard microbiological methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry, when necessary
  - The isolates were collected from 79 medical centers in the United States (51%), Europe (40%), the Asia-Pacific region (5%), and Latin America (4%)
  - The isolates were from the following infection types: bloodstream infections (35%), urinary tract infections (25%), pneumonia in hospitalized patients (20%), skin and skin structure infections (15%), intra-abdominal infections (4%), and other infection types (2%)
- The genomes of 154 isolates were sequenced using a MiSeq (Illumina, San Diego, CA) and subjected to *in silico* analysis to identify β-lactamase genes
  - β-lactamase genes were characterized in 4 isolates using PCR-based methods with β-lactamase gene-specific primers and standard DNA sequencing methods
- Figure 2 displays the distribution of species within each β-lactamase isolate group
  - Many isolates carried more than 1 β-lactamase gene

Susceptibility testing

- Broth microdilution MIC testing was carried out using Clinical and Laboratory Standards Institute (CLSI) susceptibility methods and frozen-form panels containing cation-adjusted Mueller-Hinton broth
- CLSI (2019) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2019) interpretive criteria were applied, where applicable
  - The *Enterobacteriaceae* susceptibility cutoff value for ceftibuten was ≤8 mg/L (CLSI) and ≤1 mg/L (EUCAST)
- MIC values were validated by concurrently testing CLSI-recommended ATCC quality control (QC) reference strains; QC ranges were published by CLSI (2019)
- The concentrations of β-lactamase inhibitor compounds used during MIC testing are shown in Tables 1–6
  - Where indicated, avibactam was present at 4 mg/L

Antimicrobials

- Tables 2–6 list the tested β-lactams and β-lactam/β-lactamase inhibitor combinations
- Fosfomycin, levofloxacin, and trimethoprim-sulfamethoxazole were included as non-β-lactam comparators

Results

- Avibactam restored the *in vitro* activity of ceftibuten against subsets of *Enterobacteriaceae* containing various class A, C, and/or D β-lactamases, including non-class B carbapenemases (Table 1)
  - As expected, ceftibuten-avibactam combinations were inactive against all isolates containing class B (NDM) enzymes (MIC_{50}, >32 mg/L; Table 1 and data not shown)
  - Although they contained only 1 isolate each, avibactam also restored the activity of ceftibuten against additional isolate subsets that contained various combinations of β-lactamase enzymes (Table 1)
- Ceftibuten-avibactam was the most potent β-lactam/β-lactamase inhibitor combination tested against *Enterobacteriaceae* isolates containing genes for extended-spectrum β-lactamases (ESBLs; Table 2; $MIC_{50/90}$, ≤0.03/0.06 mg/L; meropenem was equipotent), ESBL + OXA-48-like (Table 3; $MIC_{50/90}$, 0.06/0.25 mg/L), KPC (Table 4; $MIC_{50/90}$, 0.06/0.5 mg/L), or KPC + ESBL (Table 5; $MIC_{50/90}$, 0.06/0.12 mg/L) enzymes
  - Meropenem was the most potent β-lactam tested ($MIC_{50/90}$, 0.03/0.06 mg/L) against the subset of isolates containing genes for plasmidic AmpC enzymes (pAmpC; Table 6), although ceftibuten-avibactam also displayed potent activity ($MIC_{50/90}$, 0.12/1 mg/L)
- If the *in vitro* CLSI (2019) ceftibuten breakpoint (≤8 mg/L) were applied to the ceftibuten-avibactam combination, all isolates tested here would be categorized as susceptible to the combination, except for isolates containing metallo-β-lactamases
- Many isolate subsets had poor susceptibility to fosfomycin, levofloxacin, and trimethoprim-sulfamethoxazole (Tables 2–6)

Conclusions

- Avibactam restored the *in vitro* activity of oral cephalosporins against *Enterobacteriaceae* isolates that produced specific class A, class C, and class D β-lactamases, including ESBL, plasmidic AmpC, KPC, and/or OXA-48 enzymes
  - As expected, avibactam did not restore the antimicrobial activity of β-lactams against *Enterobacteriaceae* that carried genes encoding metallo-β-lactamases like NDM
- Of the oral β-lactams tested, ceftibuten displayed the most potent $MIC_{50/90}$ values against the resistant isolate subsets in combination with avibactam
- These results suggest that the combination of an oral avibactam prodrug with a cephalosporin like ceftibuten could lead to a novel oral antimicrobial to treat infections caused by β-lactam-resistant *Enterobacteriaceae* that produce class A, C, or D enzymes, including carbapenemases

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Table 1 *In vitro* antimicrobial activity of ceftibuten and ceftibuten-avibactam against *Enterobacteriaceae* subsets containing genes for specific β-lactamase enzymes

β-lactamase enzyme class isolate subset (no. of isolates) Antimicrobial	MIC values (mg/L) MIC_{50}	 MIC_{90}	 Range
ESBL (50) [a]			
Ceftibuten	4	32	≤0.03–>32
Ceftibuten-avibactam (fixed 4 mg/L)	≤0.03	0.06	≤0.03–0.12
ESBL + OXA-48-like (16)			
Ceftibuten	16	>32	4–>32
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.25	≤0.03–0.25
KPC (28)			
Ceftibuten	8	>32	0.12–>32
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.5	≤0.03–4
KPC + ESBL (13)			
Ceftibuten	16	>32	2–>32
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.12	≤0.03–0.25
OXA-48-like (6)			
Ceftibuten	0.5	-	0.06–4
Ceftibuten-avibactam (fixed 4 mg/L)	≤0.03	-	≤0.03–0.25
Plasmidic AmpC (28) [b]			
Ceftibuten	>32	>32	8–>32
Ceftibuten-avibactam (fixed 4 mg/L)	0.12	1	≤0.03–1
NDM + ESBL (9)			
Ceftibuten	>32	-	32–>32
Ceftibuten-avibactam (fixed 4 mg/L)	>32	-	16–>32
KPC + ESBL + pAmpC (1)			
Ceftibuten	32 [c]	-	-
Ceftibuten-avibactam (fixed 4 mg/L)	0.25 [c]	-	-
KPC + OXA-48-like (1)			
Ceftibuten	32 [c]	-	-
Ceftibuten-avibactam (fixed 4 mg/L)	0.5 [c]	-	-
KPC + OXA-48-like + ESBL (1)			
Ceftibuten	32 [c]	-	-
Ceftibuten-avibactam (fixed 4 mg/L)	0.25 [c]	-	-
pAmpC + ESBL + OXA-48-like (1)			
Ceftibuten	>32 [c]	-	-
Ceftibuten-avibactam (fixed 4 mg/L)	4 [c]	-	-
pAmpC + OXA-48-like (1)			
Ceftibuten	>32 [c]	-	-
Ceftibuten-avibactam (fixed 4 mg/L)	0.12 [c]	-	-

ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase
[a]The majority of isolates contained a CTX enzyme gene, but SHV and TEM enzymes were also represented.
[b]Isolates contained genes for CMY, FOX, or DHA enzymes.
[c]Single MIC values.

Table 2 Activity of ceftibuten, ceftibuten-avibactam, and comparator antimicrobial agents against 50 *Enterobacteriaceae* isolates with bla_{ESBL} genes

Antimicrobial agent	MIC (mg/L) MIC_{50}	 MIC_{90}	 Range	CLSI[a] %S	 %I	 %R	EUCAST[a] %S	 %I	 %R
Ceftibuten [b]	4	32	≤0.03–>32	78.0	10.0	12.0 [c]	24.0		76.0 [c]
Ceftibuten-avibactam (fixed 4 mg/L)	≤0.03	0.06	≤0.03–0.12						
Amoxicillin-clavulanate (2:1)	16	32	4–32	38.0	48.0	14.0	38.0		62.0 [d]
							100.0		0.0 [e]
Amoxicillin-avibactam (fixed 4 mg/L)	2	16	0.25–32						
Carbenicillin	>256	>256	>256–>256						
Carbenicillin-avibactam (fixed 4 mg/L)	16	64	≤0.25–128						
Cefixime	>32	>32	0.12–>32	6.0	4.0	90.0	6.0		94.0 [e]
Cefixime-avibactam (fixed 4 mg/L)	0.06	0.12	≤0.03–0.25						
Ceftazidime	16	>32	0.12–>32	20.0	16.0	64.0	10.0	10.0	80.0
Ceftazidime-avibactam (fixed 4 mg/L)	0.12	0.5	≤0.03–0.5	100.0		0.0	100.0		0.0
Fosfomycin	1	64	0.25–>256				84.0		16.0
Levofloxacin	>8	>8	0.03–>8	22.0	0.0	78.0	22.0	0.0	78.0
Meropenem	0.03	0.06	0.015–0.25	100.0	0.0	0.0	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole	>8	>8	≤0.12–>8	30.0		70.0	30.0	4.0	66.0

S, susceptible; I, intermediate; R, resistant
[a] Criteria as published by CLSI (2019) and EUCAST (2019).
[b] The current S/I/R breakpoints for ceftibuten are ≤8/16/≥32 mg/L (CLSI) and ≤1/–/≥2 mg/L (EUCAST).
[c] Using urinary tract infection-only breakpoints.
[d] Using other than uncomplicated urinary tract infection breakpoints.
[e] Using uncomplicated urinary tract infection-only breakpoints.
Organisms include: *Escherichia coli* (26), *Klebsiella pneumoniae* (19), *Proteus mirabilis* (5)

Figure 1 Structures of avibactam and the investigational oral prodrug of avibactam (ARX-1796/AV-006)

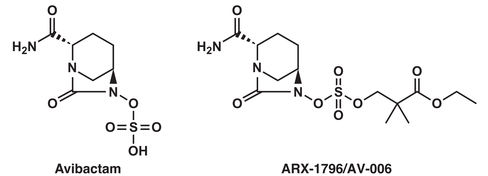

Figure 2. Number and species of molecularly characterized isolates by β-lactamase subgroup

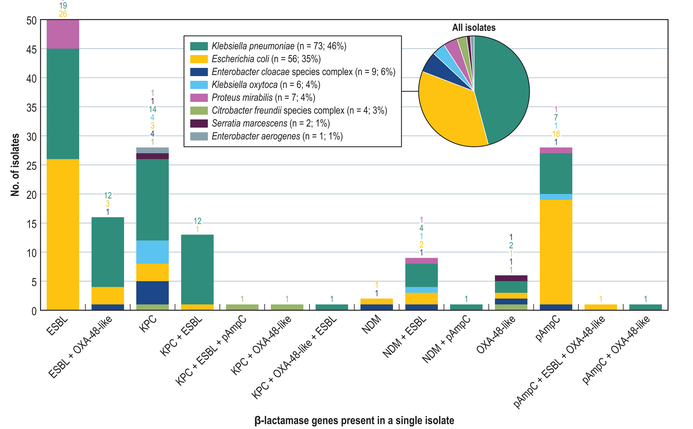

Table 3 Activity of ceftibuten, ceftibuten-avibactam, and comparator antimicrobial agents against 16 *Enterobacteriaceae* isolates with bla_{ESBL} + bla_{OXA-48}-like genes

Antimicrobial agent	MIC (mg/L) MIC_{50}	 MIC_{90}	 Range	CLSI[a] %S	 %I	 %R	EUCAST[a] %S	 %I	 %R
Ceftibuten [b]	16	>32	4–>32	31.2	18.8	50.0 [c]	0.0		100.0 [c]
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.25	≤0.03–0.25						
Amoxicillin-clavulanate (2:1)	>32	>32	>32–>32	0.0	0.0	100.0	0.0		100.0 [d]
							0.0		100.0 [e]
Amoxicillin-avibactam (fixed 4 mg/L)	>32	>32	16–>32						
Carbenicillin	>256	>256	>256–>256						
Carbenicillin-avibactam (fixed 4 mg/L)	>256	>256	128–>256						
Cefixime	>32	>32	32–>32	0.0	0.0	100.0	0.0		100.0 [e]
Cefixime-avibactam (fixed 4 mg/L)	0.25	0.5	0.06–1						
Ceftazidime	>32	>32	1–>32	12.5	6.2	81.2	6.2	6.2	87.5
Ceftazidime-avibactam (fixed 4 mg/L)	0.5	2	0.12–2	100.0		0.0	100.0		0.0
Fosfomycin	16	64	0.5–>256				81.2		18.8
Levofloxacin	>8	>8	0.25–>8	6.2	0.0	93.8	6.2	0.0	93.8
Meropenem	2	>16	0.12–>16	25.0	31.2	43.8	56.2	6.2	37.5
Trimethoprim-sulfamethoxazole	>8	>8	4–>8	0.0		100.0	0.0	12.5	87.5

S, susceptible; I, intermediate; R, resistant
[a] Criteria as published by CLSI (2019) and EUCAST (2019).
[b] The current S/I/R breakpoints for ceftibuten are ≤8/16/≥32 mg/L (CLSI) and ≤1/–/≥2 mg/L (EUCAST).
[c] Using urinary tract infection-only breakpoints.
[d] Using other than uncomplicated urinary tract infection breakpoints.
[e] Using uncomplicated urinary tract infection-only breakpoints.
Organisms include: *Enterobacter cloacae* species complex (1), *Escherichia coli* (3), *Klebsiella pneumoniae* (12)

Table 4 Activity of ceftibuten, ceftibuten-avibactam, and comparator antimicrobial agents against 28 *Enterobacteriaceae* isolates with bla_{KPC} genes

Antimicrobial agent	MIC (mg/L) MIC_{50}	 MIC_{90}	 Range	CLSI[a] %S	 %I	 %R	EUCAST[a] %S	 %I	 %R
Ceftibuten [b]	8	>32	0.12–>32	67.9	10.7	21.4 [c]	21.4		78.6 [c]
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.5	≤0.03–4						
Amoxicillin-clavulanate (2:1)	>32	>32	32–>32	0.0	0.0	100.0	0.0		100.0 [d]
							17.9		82.1 [e]
Amoxicillin-avibactam (fixed 4 mg/L)	32	>32	≤0.015–>32						
Carbenicillin	>256	>256	>256–>256						
Carbenicillin-avibactam (fixed 4 mg/L)	128	>256	≤0.25–>256						
Cefixime	32	>32	0.5–>32	7.1	0.0	92.9	7.1		92.9 [e]
Cefixime-avibactam (fixed 4 mg/L)	0.12	2	≤0.03–16						
Ceftazidime	>32	>32	8–>32	0.0	7.1	92.9	0.0	0.0	100.0
Ceftazidime-avibactam (fixed 4 mg/L)	0.5	2	≤0.03–16	96.4		3.6	96.4		3.6
Fosfomycin	8	32	0.25–256				96.4		3.6
Levofloxacin	>8	>8	0.03–>8	14.3	7.1	78.6	14.3	7.1	78.6
Meropenem	8	>16	1–>16	3.6	14.3	82.1	17.9	42.9	39.3
Trimethoprim-sulfamethoxazole	2	>8	≤0.12–>8	50.0		50.0	50.0	3.6	46.4

S, susceptible; I, intermediate; R, resistant
[a] Criteria as published by CLSI (2019) and EUCAST (2019).
[b] The current S/I/R breakpoints for ceftibuten are ≤8/16/≥32 mg/L (CLSI) and ≤1/–/≥2 mg/L (EUCAST).
[c] Using urinary tract infection-only breakpoints.
[d] Using other than uncomplicated urinary tract infection breakpoints.
[e] Using uncomplicated urinary tract infection-only breakpoints.
Organisms include: *Citrobacter freundii* species complex (1), *Enterobacter aerogenes* (1), *E. cloacae* species complex (4), *Escherichia coli* (3), *Klebsiella oxytoca* (4), *K. pneumoniae* (14), *Serratia marcescens* (1)

Table 5 Activity of ceftibuten, ceftibuten-avibactam, and comparator antimicrobial agents against 13 *Enterobacteriaceae* isolates with bla_{KPC} + bla_{ESBL} genes

Antimicrobial agent	MIC (mg/L) MIC_{50}	 MIC_{90}	 Range	CLSI[a] %S	 %I	 %R	EUCAST[a] %S	 %I	 %R
Ceftibuten [b]	16	>32	2–>32	23.1	38.5	38.5 [c]	0.0		100.0 [c]
Ceftibuten-avibactam (fixed 4 mg/L)	0.06	0.12	≤0.03–0.25						
Amoxicillin-clavulanate (2:1)	>32	>32	32–>32	0.0	0.0	100.0	0.0		100.0 [d]
							15.4		84.6 [e]
Amoxicillin-avibactam (fixed 4 mg/L)	32	>32	8–>32						
Carbenicillin	>256	>256	>256–>256						
Carbenicillin-avibactam (fixed 4 mg/L)	256	>256	32–>256						
Cefixime	>32	>32	32–>32	0.0	0.0	100.0	0.0		100.0 [e]
Cefixime-avibactam (fixed 4 mg/L)	0.25	0.25	0.06–0.5						
Ceftazidime	>32	>32	16–>32	0.0	0.0	100.0	0.0	0.0	100.0
Ceftazidime-avibactam (fixed 4 mg/L)	1	1	0.12–1	100.0		0.0	100.0		0.0
Fosfomycin	16	64	0.5–64				84.6		15.4
Levofloxacin	>8	>8	1–>8	0.0	15.4	84.6	0.0	15.4	84.6
Meropenem	16	>16	1–>16	7.7	7.7	84.6	15.4	30.8	53.8
Trimethoprim-sulfamethoxazole	>8	>8	0.5–>8	15.4		84.6	15.4	15.4	69.2

S, susceptible; I, intermediate; R, resistant
[a] Criteria as published by CLSI (2019) and EUCAST (2019).
[b] The current S/I/R breakpoints for ceftibuten are ≤8/16/≥32 mg/L (CLSI) and ≤1/–/≥2 mg/L (EUCAST).
[c] Using urinary tract infection-only breakpoints.
[d] Using other than uncomplicated urinary tract infection breakpoints.
[e] Using uncomplicated urinary tract infection-only breakpoints.
Organisms include: *Escherichia coli* (1), *Klebsiella pneumoniae* (12)

Table 6 Activity of ceftibuten, ceftibuten-avibactam, and comparator antimicrobial agents against 28 *Enterobacteriaceae* isolates with bla_{pAmpC}

Antimicrobial agent	MIC (mg/L) MIC_{50}	 MIC_{90}	 Range	CLSI[a] %S	 %I	 %R	EUCAST[a] %S	 %I	 %R
Ceftibuten [b]	>32	>32	8–>32	7.1	3.6	89.3 [c]	0.0		100.0 [c]
Ceftibuten-avibactam (fixed 4 mg/L)	0.12	1	≤0.03–1						
Amoxicillin-clavulanate (2:1)	32	>32	32–>32	0.0	0.0	100.0	0.0		100.0 [d]
							50.0		50.0 [e]
Amoxicillin-avibactam (fixed 4 mg/L)	2	16	0.12–>32						
Carbenicillin	256	>256	8–>256						
Carbenicillin-avibactam (fixed 4 mg/L)	4	32	≤0.25–>256						
Cefixime	>32	>32	16–>32	0.0	0.0	100.0	0.0		100.0 [e]
Cefixime-avibactam (fixed 4 mg/L)	0.5	1	≤0.03–16						
Ceftazidime	32	>32	8–>32	0.0	7.1	92.9	0.0	0.0	100.0
Ceftazidime-avibactam (fixed 4 mg/L)	0.12	0.5	≤0.03–16	96.4		3.6	96.4		3.6
Fosfomycin	0.5	8	0.25–>256				96.4		3.6
Levofloxacin	0.5	>8	0.03–>8	53.6	7.1	39.3	53.6	7.1	39.3
Meropenem	0.03	0.06	0.015–0.12	100.0	0.0	0.0	100.0	0.0	0.0
Trimethoprim-sulfamethoxazole	>8	>8	≤0.12–>8	32.1		67.9	32.1	0.0	67.9

S, susceptible; I, intermediate; R, resistant
[a] Criteria as published by CLSI (2019) and EUCAST (2019).
[b] The current S/I/R breakpoints for ceftibuten are ≤8/16/≥32 mg/L (CLSI) and ≤1/–/≥2 mg/L (EUCAST).
[c] Using urinary tract infection-only breakpoints.
[d] Using other than uncomplicated urinary tract infection breakpoints.
[e] Using uncomplicated urinary tract infection-only breakpoints.
Organisms include: *Enterobacter cloacae* species complex (1), *Escherichia coli* (18), *Klebsiella oxytoca* (1), *K. pneumoniae* (7), *Proteus mirabilis* (1)