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ß-lactamase Characterization of *Enterobacteriaceae* Baseline Pathogens from Two Phase III Trials of Ceftazidime-Avibactam

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Abstract

Background: Ceftazidime-avibactam (CAZ-AVI) is a novel β-lactam/non-β-lactam β-lactamase inhibitor combination for the treatment of Gram-negative bacterial infections. This study characterized the β-lactamase content of *Enterobacteriaceae* from patients with complicated intra-abdominal infections (cIAI) in Phase III trials of CAZ-AVI (NCT01499290; NCT01500239).

Methods: Susceptibility testing of clinical *Enterobacteriaceae* isolates was centrally performed based on CLSI (M07-A10/M100-S25). MIC criteria were pre-established for selecting *Enterobacteriaceae* for screening of extended-spectrum β-lactamase (ESBL) and/or carbapenemase genes. Selected isolates underwent microarray-based assay, complemented by PCR/sequencing. Relative *ampC* transcription levels were assessed.

Results: 15.9% (131/823) of patients (mMITT population) had baseline pathogens that met the MIC screening criteria. Seven patients had two pathogens that met the criteria. Isolates consisted of 138 *Enterobacteriaceae* (mostly *E. coli* [81] and *K. pneumoniae* [29]). CAZ-AVI (MIC_{50}/MIC_{90}, 0.12/2 µg/mL) inhibited all *Enterobacteriaceae* at ≤4 µg/mL, except for seven NDM-1 or -4 producers. All but two (79/81; 97.5%) *E. coli* had ≥1 ESBL gene. The other two isolates showed high expression of the intrinsic AmpC. 87.7% (71/81) *E. coli* harbored bla_{CTX-M}-like, most commonly $bla_{CTX-M-15}$ (47/71; 66.2%), followed by $bla_{CTX-M-27}$ (10/71; 14.1%). CTX-M producers often carried $bla_{OXA-1/30}$ (37/71; 52.1%). 14.8% (12/81) *E. coli* carried CMY-encoding genes. 28/29 (96.6%) of *K. pneumoniae* had bla_{CTX-M}-like (one $bla_{CTX-M-3}$- and 27 $bla_{CTX-M-15}$). Among these, three carbapenemase-producing *K. pneumoniae* were detected (NDM-1 [Romania], -4 [India] and OXA-48 [Romania]). 16/28 (57.1%) of bla_{CTX-M}-carrying *K. pneumoniae* also had $bla_{OXA-1/30}$. Among other *Enterobacteriaceae*, *P. mirabilis* and *S. marcescens* had plasmid AmpC and $_{CTX-M}$-like enzymes, respectively; *C. freundii* carried bla_{CTX-M} or had elevated expression of AmpC. Most *E. cloacae* (11/15; 73.3%) carried $bla_{CTX-M-15}$; five were NDM-1 producers (all from India).

Conclusions: CAZ-AVI showed potent *in vitro* activity against these *Enterobacteriaceae* responsible for cIAI, excluding seven NDM-producing isolates (all from India/Romania). $bla_{CTX-M-15}$ alone or in combination with $bla_{OXA-1/30}$ has spread and become the dominant ESBL gene in *Enterobacteriaceae* other than *E. coli*.

Introduction

- *Enterobacteriaceae* organisms are a common cause of community-acquired and healthcare-acquired infections, with *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. among the most common organisms. β-lactam resistance among *Enterobacteriaceae* mostly reflects the worldwide emergence and dissemination in the late 1980s of extended-spectrum β-lactamases (ESBLs), such as bla_{TEM} and bla_{SHV} allelic variants. However, the epidemiology of these isolates changed during the early 2000s, and TEM and SHV ESBL-encoding genes have slowly been replaced by bla_{CTX-M} genes, which have been detected in community- and hospital-acquired *Enterobacteriaceae*.
- During the late 1990s, carbapenem-resistant *Enterobacteriaceae* (CRE) began to emerge. In 2012, 4.6% of acute care hospitals reported at least one CRE. The proportion of isolates that were CRE increased from 1.2% in 2001 to 4.2% in 2011 in the National Nosocomial Infection Surveillance system (NNIS) and the National Healthcare Safety Network (NHSN), and from 0% in 2001 to 1.4% in 2010 in the Surveillance Network–USA (TSN). Overall, the majority of CRE isolates in the US harbor the KPC serine carbapenemase-encoding gene. However, isolates producing OXA-48- and NDM-like enzymes, which are increasingly common in Europe and Asia-Pacific countries, are now also being detected in the US.
- Ceftazidime-avibactam is a novel β-lactam/non-β-lactam β-lactamase inhibitor combination approved by the Food and Drug Administration (FDA) early in 2015 (February) for the treatment of complicated intra-abdominal infections (cIAI) (used in combination with metronidazole) and complicated urinary tract infections (cUTI), including pyelonephritis, in patients with limited or no treatment options. Clinical approval was mostly based on data from Phase II trials, but several Phase III trials have now completed. This present study characterized the β-lactamase content of *Enterobacteriaceae* from patients with cIAI enrolled in two Phase III trials of ceftazidime-avibactam.

Materials and Methods

Patients and clinical isolates

- Isolates were recovered from patients enrolled in two Phase III, randomized, multi-center, double-blind, double-dummy, parallel-group, comparative studies combined to determine the efficacy, safety, and tolerability of ceftazidime-avibactam (2 g/0.5 g, every 8 hours) plus metronidazole (0.5 g, every 8 hours) versus meropenem (1 g, every 8 hours) in the treatment of cIAIs in hospitalized adults (NCT01499290; NCT01500239).
- The microbiological modified intent-to-treat (mMITT) population was comprised of 1,043 patients, and 823 had ≥1 baseline pathogen identified at study entry. A total of 138 baseline *Enterobacteriaceae* causing cIAI in the mMITT met the MIC criteria for ESBL and/or carbapenemase screening, and included 81 *E. coli*, 29 *Klebsiella pneumoniae*, 14 *Enterobacter cloacae*, five *Citrobacter freundii*, five *Proteus mirabilis*, three *Serratia marcescens*, and one *Enterobacter aerogenes*. These isolates were recovered from 131 patients (age 18–86) hospitalized in the Americas (US, one subject; Peru, two), Europe (Belgium, one; Bulgaria, nine; Czech Republic, 10; Greece, one; Hungary, two; Romania, 36; Ukraine, 23), Russia (three), Turkey (one), Israel (one), and Asia-Pacific (India, 37; Thailand, four).

Antimicrobial susceptibility testing and MIC screening criteria

- Susceptibility testing of clinical *Enterobacteriaceae* isolates was centrally performed using broth microdilution method following the Clinical Laboratory Standard Institute (CLSI) guidelines. Ceftazidime was tested in combination with avibactam at a fixed concentration of 4 µg/mL. Ceftazidime-avibactam breakpoints approved by the FDA (≤8 µg/mL for susceptible and ≥16 µg/mL for resistance) were applied for all *Enterobacteriaceae* species. *Enterobacteriaceae* displaying ceftriaxone and/or ceftazidime MIC results of ≥2 µg/mL were selected for further characterization of β-lactamase-encoding genes. Enterobacteriaceae exhibiting imipenem and/or meropenem MIC results ≥2 µg/mL were tested for the presence of carbapenemase-encoding genes.

Screening of β-lactamase and AmpC expression

- Isolates that met the MIC screening criteria were subjected to a microarray-based assay Check-MDR CT101 kit according to the manufacturer's instructions (Check-points, Wageningen, Netherlands). This kit has the capability to detect CTX-M groups 1, 2, 8+25 and 9, TEM, SHV, ACC, ACT/MIR, CMY, DHA, FOX, KPC and NDM encoding genes. Supplemental multiplex PCR assays were utilized to detect additional ESBL-(bla_{GES}, bla_{VEB}, bla_{PER}, and oxacillinase enzymes [bla_{OXA-2}-, bla_{OXA-10}- and bla_{OXA-13}- groups, bla_{OXA-18}, and bla_{OXA-45}]) and carbapenemase-encoding genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{GES}, bla_{NMC-A}, bla_{SME}, bla_{IMI}).
- All amplicons generated were sequenced on both strands (Sanger method); nucleotide and amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Amino acid sequences were compared with those available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/). The transcription levels of the chromosomal *ampC* gene were determined using quantitative Real-Time–PCR assays (qRT-PCR). Quantification of the target mRNA gene applied a normalized expression analysis method and relative comparison to susceptible control strains. A given strain was considered to overexpress the *amp*C gene when at least a 5-fold greater difference of *amp*C transcripts was detected as compared with a species-specific wildtype reference control strain.

Table 1. MIC results for ceftazidime, ceftazidime-avibactam, and meropenem obtained against baseline *Enterobacteriaceae* pathogens recovered from Phase III trials of ceftazidime-avibactam

		Number of isolates at each MIC (µg/mL)												
Organism	**Agent***	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**
Enterobacteriaceae (138)†	CAZ				1	2	4	9	9	13	18	32	10	40
	CAZ-AVI	3	17	49	26	12	11	9	4	0	0	0	0	7‡
	MER	101	13	8	1	4	3	1	0	2	5§			
E. coli (81)	CAZ					2	3	7	8	10	12	23	5	11
	CAZ-AVI	2	17	37	13	2	1	5	4					
	MER	76	3	1	0	1								
K. pneumoniae (29)	CAZ									1	3	7	2	16
	CAZ-AVI			8	5	5	7	2						2‡
	MER	15	3	2	1	2	3	0	0	1	2§			
Other (28)¶	CAZ				1	0	1	2	1	2	3	2	3	13
	CAZ-AVI	1	0	4	8	5	3	2						5‡
	MER	10	7	5	0	1	0	1	0	1	3§			

*CAZ = ceftazidime; CAZ-AVI = ceftazidime-avibactam; MER = meropenem.

CAZ-AVI MICs measured in the presence of a constant concentration of 4 µg/mL for avibactam.

†Includes 81 *E. coli*, 29 *K. pneumoniae*, 14 *E. cloacae*, five *C. freundii*, five *P. mirabilis*, three *S. marcescens* and one *E. aerogenes*.

‡The highest concentration tested for ceftazidime-avibactam was 256 µg/mL; therefore, ‡represents ceftazidime-avibactam MIC >256/4 µg/mL for two NDM-producing *K. pneumoniae* and five NDM-producing *E. cloacae* (all from India, except for one *K. pneumoniae* from Romania).

§The highest concentration tested for meropenem was 8 µg/mL; therefore, §represents meropenem MIC >8 µg/mL.

¶Includes 14 *E. cloacae*, five *C. freundii*, five *P. mirabilis*, three *S. marcescens* and one *E. aerogenes*.

Results

- 15.9% (131/823) of patients (mMITT population) had one *Enterobacteriaceae* baseline pathogen that met the pre-defined MIC screening criteria. Seven patients had two baseline pathogens that were selected for further molecular testing.
- Ceftazidime alone tested against *Enterobacteriaceae* had MIC_{50} and MIC_{90} results of 32 and >64 µg/mL, respectively, while ceftazidime-avibactam (MIC_{50}/MIC_{90}, 0.12/2 µg/mL) inhibited all *Enterobacteriaceae* at ≤4 µg/mL (below the FDA breakpoint for susceptibility), except for seven NDM-1 or -4 producers (Table 1).
- Meropenem was also active against *Enterobacteriaceae* that met the MIC screening criteria with MIC_{50} and MIC_{90} values of ≤0.03 and 0.5 µg/mL, respectively, inhibiting all strains at ≤2 µg/mL. Exceptions were observed for seven NDM-producing isolates (MIC ≥8 µg/mL; Table 1).
- CTX-M alone (31%) and in combination with other enzymes (52.9%) represented the most common β-lactamase observed among selected Enterobacteriaceae (Figure 1 and Table 2). Moreover, CTX-M and OXA-1/30 (36%) comprised the most frequent combination noted among studied isolates.
- Among screen-positive *E. coli*, two isolates showed high expression of the intrinsic AmpC enzyme (Table 2). The remaining *E. coli* (79/81; 97.5%) had ≥1 ESBL gene, and 87.7% (71/81) harbored bla_{CTX-M}-like, most commonly $bla_{CTX-M-15}$ (47/71; 66.2%), followed by $bla_{CTX-M-27}$ (10/71; 14.1%).
- CTX-M-producing *E. coli* clinical trial isolates often carried $bla_{OXA-1/30}$ (37/71; 52.1%). In contrast, plasmid-mediated AmpC genes (i.e. CMY) and bla_{SHV-12} were only detected in 14.8% (12/81) and 4.9% (4/81) of *E. coli,* respectively (Table 2).
- A total of 28/29 (96.6%) *K. pneumoniae* isolates had bla_{CTX-M}-like (one $bla_{CTX-M-3}$- and 27 $bla_{CTX-M-15}$). Moreover, the majority of CTX-M-producing *K. pneumoniae* (16/28; 57.1%) also carried the β-lactamase-encoding $bla_{OXA-1/30}$ (Table 2).
- Among *K. pneumoniae* that met the MIC β-lactamase screening criteria, three carbapenemase-producing isolates were detected as follows: one NDM-1 from Romania (ceftazidime-avibactam MIC >256/4 µg/mL); one NDM-4 from India (ceftazidime-avibactam MIC >256/4 µg/mL); and one OXA-48 from Romania (ceftazidime-avibactam MIC, 1/4 µg/mL).
- CTX-M enzymes (73.3%; 11/15) also prevailed among *Enterobacter* spp. (14 *E. cloacae* and one *E. aerogenes*), and were most frequently (72.7%; 8/11) associated with OXA-1/30 (Table 2). In addition, five *E. cloacae* from India carried bla_{NDM-1} in combination with several β-lactamase-encoding genes.
- All *P. mirabilis* that met the MIC screening criteria carried plasmid-encoded AmpC enzymes (ACC-4 and CMY-16; Table 2), while *S. marcescens* and *C. freundii* produced CTX-M-like enzymes. Two *C. freundii* also had elevated expression of AmpC.

Figure 1. Distribution of ESBL and carbapenemase enzymes detected among *Enterobacteriaceae* collected from patients enrolled in the Phase III cIAI trials of ceftazidime-avibactam. Narrow-spectrum β-lactamase enzymes are not depicted here, but included are TEM-1, TEM-79, TEM-209, SHV-1, SHV-11 and SHV-76 (inhibitor-resistant β-lactamase; ceftazidime-avibactam MIC 0.12 - 0.25/4 µg/mL); cAmpC = over-expression of chromosomal AmpC; pAmpC = plasmid AmpC.

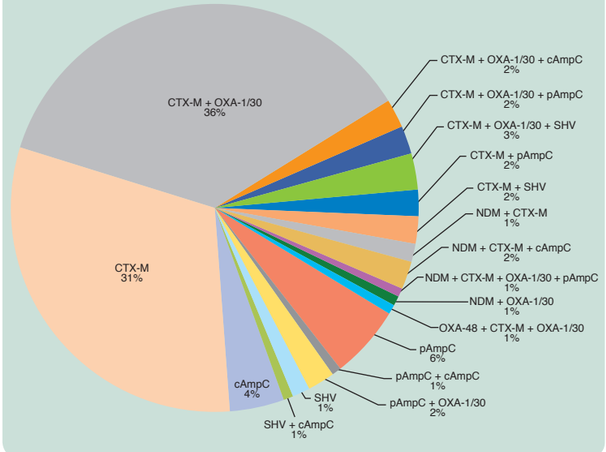

Table 2. Summary of β-lactamase enzymes detected among baseline *Enterobacteriaceae* pathogens recovered from patients enrolled in the cIAI Phase III trials for ceftazidime-avibactam

Pathogen (No.; % of total)	**Results* (No. of isolates; % within each species)**
E. coli (81; 58.7)	CTX-M-15 (10; 12.3) CTX-M-27 (9; 11.1) CTX-M-14 (3; 3.7) CTX-M-3 (3; 3.7) CTX-M-1 (2; 2.5) CTX-M-55 (2; 2.5) CTX-M-2 (1; 1.2) CTX-M-1/-3 (1; 1.2) CTX-M-15 + CMY (2; 2.5) CTX-M-27 + CMY (1; 1.2) CTX-M-15 + OXA-1/30 (31; 38.3) CTX-M-142 + OXA-1/30 (1; 1.2) CTX-M-15 + OXA-1/30 + CMY (3; 3.7) CTX-M-15 + OXA-1/30 + SHV-12 (2; 2.5) SVH-12 (2; 2.5) CMY + OXA-1/30 (2; 2.5) CMY (4; 4.9) cAmpC (2; 2.5)
K. pneumoniae (29; 21.0)	NDM-1/-4 + CTX-M-15 (2; 6.9) OXA-48 + CTX-M + OXA-1/30 (1; 3.4) CTX-M-15 (8; 27.6) CTX-M-3 (1; 3.4) CTX-M-15 + OXA-1/30 (14; 48.3) CTX-M-15 + SHV-12 (1; 3.4) CTX-M-15 + OXA-1/30 + SHV-27 (1; 3.4) DHA-1 + OXA-1/30 (1; 3.4)
Enterobacter spp. (15; 10.9)	NDM-1 + CTX-M-15 + cAmpC (3; 20.0) NDM-1 + CTX-M + OXA-1/30 + CMY-6 (1; 6.7) NDM-1 + OXA-1/30 (1; 6.7) CTX-M-15 + OXA-1/30 (2; 13.3) CTX-M-15 + OXA-1/30 + cAmpC (3; 20.0) CTX-M-15 + OXA-1/30 + SHV-12 (1; 6.7) CTX-M-3 (1; 6.7) SVH-12 + cAmpC (1; 6.7) cAmpC (2; 13.3)
P. mirabilis (5; 3.6)	ACC-4 (4; 80.0) CMY-16 (1; 20.0)
C. freundii (5; 3.6)	cAmpC (2; 40.0) CTX-M-15 + OXA-1/30 (1; 20.0) CTX-M-3 (2; 40.0)
S. marcescens (3; 2.2)	CTX-M-15 + OXA-1/30 (1; 33.3) CTX-M-3 (2; 66.7)

*Narrow-spectrum β-lactamase enzymes are not depicted here, but included TEM-1, TEM-79, TEM-209, SHV-1, SHV-11 and SHV-76 (inhibitor-resistant β-lactamase; ceftazidime-avibactam MIC, 0.12 - 0.25/4 µg/mL); cAmpC = over-expression of chromosomal AmpC.

Conclusions

- Ceftazidime-avibactam showed potent *in vitro* activity against baseline *Enterobacteriaceae* pathogens recovered from patients enrolled in Phase III trials for cIAI, with the exception of seven NDM-producing isolates (one and six isolates from Romania and India, respectively).
- CTX-M variants alone or in combination (e.g. OXA-1/30) with other enzymes represented the most common β-lactam resistance mechanisms among isolates included in this study. The only exception was observed among *P. mirabilis*, where plasmid-encoded AmpC enzymes prevailed.
- This study clearly shows the spread of CTX-M and CTX-M + OXA-1/30-carrying plasmids among clinical isolates of *Enterobacteriaceae*. This enzyme profile has been established among ESBL-producing *E. coli* in the last decade, but has now also become common in other *Enterobacteriaceae* species, such as *K. pneumoniae*, where SHV previously prevailed.
- Furthermore, NDM variants predominated among CRE clinical trial isolates. These pathogens were mostly recovered from patients hospitalized in the Indian subcontinent, where such isolates have supposedly originated and become prevalent.

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References

AVYCAZ Prescribing Information. Available from http://www.avycaz.com. [Accessed March 23, 2015].
Castanheira M et al. *J Antimicrob Chemother* 2011;66:1409–1411.
Castanheira M et al. *Antimicrob Agents Chemother* 2013;57:3012–3020.
Castanheira M et al. *Antimicrob Agents Chemother* 2014;58:7358–7366.
Castanheira M et al. *J Antimicrob Chemother* 2014;69:1804–1814.
Castanheira M et al. *Antimicrob Agents Chemother* 2014;58:833–838.
Chen L et al. *Trends Microbiol* 2014;22:686–696.
Clinical and Laboratory Standards Institute. 10th ed. 2015; Wayne, PA.
Clinical and Laboratory Standards Institute. 25th informational supplement. 2015; Wayne, PA.
Jacob JT et al. *MMWR Morb Mortal Wkly Rep* 2013;62:162–170.

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