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β-lactamase characterization of baseline Enterobacteriaceae from Phase 3 trials of ceftazidime-avibactam for the treatment of complicated urinary tract infections

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Abstract

Background: Ceftazidime-avibactam (CAZ-AVI) received U.S. Food and Drug Administration approval for treatment of complicated urinary tract infections (cUTI), including pyelonephritis, and complicated intra-abdominal infections (in combination with metronidazole) in adult patients with limited or no alternative treatment options. This study characterized the β-lactamase content of Enterobacteriaceae recovered from patients with cUTI in two identical, randomized, multicenter RECAPTURE 1 and 2 Phase 3 trials of CAZ-AVI (NCT01595438; NCT01599806).

Methods: A total of 1,033 patients were randomized for treatment in both trials combined. Susceptibility testing was centrally performed by standard CLSI methods. MIC criteria were pre-established for selecting Enterobacteriaceae for screening of extended-spectrum β-lactamase (ESBL), class C β-lactamase (plasmid AmpC; pAmpC), and/or carbapenemase genes. Isolates underwent microarray-based assay, complemented by PCR/sequencing. Relative transcription of chromosomal AmpC (cAmpC) levels were assessed.

Results: A total of 91 Enterobacteriaceae isolates recovered from 90 subjects at the baseline visit met the MIC screening criteria. One patient had a polymicrobial cUTI caused by *E. coli* and *P. mirabilis*. *E. coli* isolates were most prevalent (42/91; 46.2%), followed by *Enterobacter* spp. (15/91; 16.5%), *K. pneumoniae* (13/91; 14.3%) and *P. mirabilis* (12/91; 13.2%). A total of 17 (18.7%) and 36 (39.6%) isolates were susceptible to CAZ using EUCAST and CLSI breakpoints, respectively. CAZ-AVI inhibited growth of all but two strains at ≤4 mg/L (89/91; 97.8% at the breakpoint for susceptibility [≤8 mg/L]). Isolates with higher CAZ-AVI MICs were one NDM-1-producing *K. pneumoniae* and one *P. rettgeri* with none of the screened β-lactamases (MIC, 32/4 and >64/4 mg/L, respectively). Doripenem inhibited 97.9% (89/91) of isolates at the breakpoint for susceptibility (i.e. ≤1 mg/L). A total of 35 (83.3%) *E. coli* harbored *bla*$_{\text{CTX-M}}$. The other isolates had SHV-2 (1 isolate) SHV-12 (1), SHV-12/CMY-2 (1), KLUC-2 (1), DHA-1 (1) and screened β-lactamases were not detected in two *E. coli*. Among *Klebsiella* spp., 9 (60.0%) and 2 (13.3%) isolates produced CTX-M and SHV-2, respectively. Also, three isolates produced NDM-1 (Ukraine), OXA-48 (Romania) or OXA-9 (Romania), whereas none of the β-lactamase genes screened were detected in one *Klebsiella* spp. The majority of *Enterobacter spp.* (12/15; 80.0%) showed overexpression of cAmpC alone or in combination with CTX-M and/or SHV-12 and/or OXA-1, whereas the remaining isolates produced CTX-M (3/15; 20.0%) alone or in combination with DHA-1 or SHV-12. *P. mirabilis* produced pAmpC (7/12; 58.3%), CTX-M (4/12; 33.3%) or TEM-93 (1/12; 8.3%). *Citrobacter* spp., *Providencia* spp. and *S. marcescens* had overexpression of cAmpC (3/7; 42.9%), CTX-M (2/7; 28.6%), PER-1 (1/7; 14.3%) and one isolate had CTX-M as well as overexpression of cAmpC (1/7; 14.3%).

Conclusions: CAZ-AVI demonstrated potent *in vitro* activity against these β-lactamase-producing organisms causing cUTI in phase 3 clinical trials. CTX-M enzymes prevailed among *E. coli* and *Klebsiella* spp. organisms, whereas pAmpC was most common among *P. mirabilis*. Other organisms often overexpressed cAmpC.

Introduction

- Recently, two identical, prospective, randomized, multicentre, double-blind, double-dummy, parallel-group, comparative Phase 3 trials (RECAPTURE 1, NCT01595438 and RECAPTURE 2, NCT01599806) were conducted to determine the efficacy, safety and tolerability of ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections (cUTI). Hospitalized adult patients with pyelonephritis were also included.
- The study presented here reports the characterization of the β-lactamase content of baseline Enterobacteriaceae isolates that met the predefined MIC criteria for extended-spectrum β-lactamase (ESBL) and/or carbapenemase production. The transcriptional levels of intrinsic AmpC were also measured in selected isolates.

Materials and methods

Patients and clinical isolates

- Isolates were recovered from patients enrolled in two Phase 3 clinical trials comparing ceftazidime-avibactam (2 g/0.5 g, every 8 h) versus doripenem (0.5 g, every 8 h) for the treatment of cUTIs in hospitalized adults (RECAPTURE 1 and RECAPTURE 2). The microbiological modified intent-to-treat (mMITT) population was composed of 810 patients, and a total of 91 Enterobacteriaceae isolates recovered from 90 subjects at the baseline visit met the MIC screening criteria. Baseline isolates included in this analysis and countries of origin are described in Table 1.

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.[1] Ceftazidime was tested in combination with avibactam at a fixed concentration of 4 mg/L. Ceftazidime-avibactam breakpoints approved by the FDA (≤8 mg/L for susceptible and ≥16 mg/L for resistance) were applied for all Enterobacteriaceae species.[2] Enterobacteriaceae displaying ceftriaxone and/or ceftazidime MIC results of ≥2 mg/L were selected for further characterization of β-lactamase-encoding genes.[3] Enterobacteriaceae exhibiting imipenem and/or meropenem MIC results ≥2 mg/L were tested for the presence of carbapenemase-encoding genes.[3]

Table 1. Baseline isolates meeting MIC criteria and included in this analysis

Species	Number
Escherichia coli	42
Enterobacter cloacae	14
Klebsiella pneumoniae	13
Proteus mirabilis	12
Citrobacter freundii	3
Klebsiella oxytoca	2
Providencia rettgeri	2
Citrobacter koseri	1
Enterobacter aerogenes	1
Serratia marcescens	1

Country of origin	Number	Country of origin	Number
Americas		Europe	
United States	1	Bulgaria	7
Argentina	1	Croatia	5
Mexico	2	Poland	6
Peru	10	Romania	18
Israel	1	Russia	16
Asia-Western Pacific		Serbia	2
Japan	2	Slovakia	2
Korea	1	Spain	2
Taiwan	2	Ukraine	13

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 capabilities to detect CTX-M groups 1, 2, 8+25 and 9, TEM, SHV, ACC, ACT/MIR, CMY, DHA, FOX, KPC and NDM encoding genes.[4] Supplemental multiplex PCR assays were utilized to detect additional ESBL- (*bla*$_{\text{GES}}$, *bla*$_{\text{VEB}}$, *bla*$_{\text{PER}}$, and oxacillinase enzymes [*bla*$_{\text{OXA-2}}$-, *bla*$_{\text{OXA-10}}$- and *bla*$_{\text{OXA-13}}$-groups, *bla*$_{\text{OXA-18}}$ and *bla*$_{\text{OXA-45}}$]) and carbapenemase-encoding genes (*bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{NMC-A}}$, *bla*$_{\text{SME}}$, *bla*$_{\text{IMI}}$). 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 *ampC* gene when at least a 5-fold greater difference of *ampC* transcripts was detected as compared with a species-specific wildtype reference control strain.[4]

Table 2. MIC results for ceftazidime, ceftazidime-avibactam and doripenem obtained against baseline Enterobacteriaceae pathogens recovered from cUTI Phase 3 trials of ceftazidime-avibactam

Organism	Agent[a]	Number of isolate at each MIC (mg/L)													
		≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Enterobacteriaceae (91)[b]	CAZ			1 (1.1)	3 (4.4)	1 (5.5)	6 (12.1)	6 (18.7)	9 (28.6)	10 (39.6)	13 (53.8)	11 (65.9)	7 (73.6)	7 (81.3)	17 (100.0)
	CAZ-AVI	2 (2.2)	7 (9.9)	11 (22.0)	20 (44.0)	24 (70.3)	11 (82.4)	9 (92.3)	3 (95.6)	2 (97.8)	0 (97.8)	0 (97.8)	1 (98.9)[c]	0 (98.9)	1 (100.0)[c]
	DOR	10 (11.0)	39 (53.8)	12 (67.0)	15 (83.5)	5 (89.0)	7 (96.7)	1 (97.9)	1 (98.9)	0 (98.9)	1 (100.0)				
E. coli (42)	CAZ	0 (0.0)	0 (0.0)	1 (2.4)	1 (4.8)	0 (4.8)	1 (7.1)	4 (16.7)	8 (35.7)	5 (47.6)	9 (69.0)	5 (81.0)	4 (90.5)	2 (95.2)	2 (100.0)
	CAZ-AVI	2 (4.8)	0 (4.8)	5 (16.7)	19 (61.9)	16 (100.0)									
	DOR	10 (23.8)	25 (83.3)	7 (100.0)											
Enterobacter spp. (15)	CAZ			0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (6.7)	1 (13.3)	1 (20.0)	0 (20.0)	1 (26.7)	4 (53.3)	7 (100.0)
	CAZ-AVI	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (26.7)	3 (46.7)	5 (80.0)	1 (86.7)	2 (100.0)					
	DOR	0 (0.0)	3 (20.0)	2 (33.3)	3 (53.3)	1 (60.0)	5 (93.3)	1 (100.0)							
K. pneumoniae (13)	CAZ			0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)	1 (23.1)	0 (23.1)	1 (30.8)	2 (46.2)	2 (61.5)	1 (69.2)	0 (69.2)	4 (100.0)
	CAZ-AVI	0 (0.0)	0 (0.0)	2 (15.4)	0 (15.4)	3 (38.5)	5 (76.9)	1 (84.6)	1 (92.3)	0 (92.3)	0 (92.3)	0 (92.3)	0 (92.3)	0 (92.3)	1 (100.0)[c]
	DOR	0 (0.0)	6 (46.2)	2 (61.5)	3 (84.6)	1 (92.3)	0 (92.3)	0 (92.3)	0 (92.3)	0 (92.3)	1 (100.0)				
Other (21)[d]	CAZ			0 (0.0)	2 (9.5)	1 (14.3)	3 (28.6)	0 (28.6)	1 (33.3)	3 (47.6)	1 (52.4)	4 (71.4)	1 (76.2)	1 (81.0)	4 (100.0)
	CAZ-AVI	0 (0.0)	7 (33.3)	4 (52.4)	1 (57.1)	1 (61.9)	3 (76.2)	3 (90.5)	1 (95.2)	0 (95.2)	0 (95.2)	0 (95.2)	1 (100.0)[c]		
	DOR	0 (0.0)	5 (23.8)	1 (28.6)	9 (71.4)	3 (85.7)	2 (95.2)	0 (95.2)	1 (100.0)						
Enterobacteriaceae (55)[e] with CAZ MIC >4 mg/L	CAZ-AVI	1 (1.8)	1 (3.6)	6 (14.5)	11 (34.5)	12 (56.4)	9 (72.7)	8 (87.3)	3 (92.7)	2 (96.4)	0 (96.4)	0 (96.4)	1 (98.2)	0 (98.2)	1 (100.0)
	DOR	4 (7.3)	21 (45.5)	8 (60.0)	12 (81.8)	3 (87.3)	5 (96.4)	1 (98.2)	0 (98.2)	0 (98.2)	1 (100.0)				

[a]CAZ = ceftazidime; CAZ-AVI = ceftazidime-avibactam; DOR = doripenem. [b]Includes 42 *E. coli*, 15 *Enterobacter spp.*, 13 *K. pneumoniae*, 12 *P. mirabilis*, four *Citrobacter spp.*, two *Providencia spp.*, two *K. oxytoca* and one *S. marcescens*. [c]AmpC hyper-producing *P. rettgeri* (screened acquired β-lactamases were not detected) and one NDM-producing *K. pneumoniae* from Ukraine. [d]Includes 12 *P. mirabilis*, four *Citrobacter spp.*, two *Providencia* spp., two *K. oxytoca* and one *S. marcescens*. [e]Enterobacteriaceae isolates displaying ceftazidime MIC values >4 mg/L, which would indicate non-susceptible and resistant isolates according to CLSI[3] and EUCAST[5] breakpoints, respectively.

Results

- A total of 91 Enterobacteriaceae isolates recovered from 90 subjects (11.2% of the mMITT population) at the baseline visit met the MIC screening criteria (Tables 1 and 2). One patient had a polymicrobial cUTI caused by *Escherichia coli* and *Proteus mirabilis*.
- Among isolates that met the screening criteria, *E. coli* was the most prevalent (42/91; 46.2%), followed by *Enterobacter* spp. (15/91; 16.5%), *Klebsiella pneumoniae* (13/91; 14.3%) and *P. mirabilis* (12/91; 13.2%; Table 1).
- Ceftazidime-avibactam ($MIC_{50/90}$, 0.25/1 mg/L) inhibited growth of all but two strains at ≤4 mg/L (89/91; 97.8% at the breakpoint for susceptibility [≤8 mg/L]), whereas 96.4% of ceftazidime-resistant Enterobacteriaceae were inhibited by ceftazidime-avibactam ($MIC_{50/90}$, 0.25/2 mg/L) at ≤8 mg/L (Table 2).
- Isolates with higher ceftazidime-avibactam MIC values were one NDM-1-producing *K. pneumoniae* and one *Providencia rettgeri* (MIC, >64/4 and 32/4, respectively; Tables 2 and 3).
- The *P. rettgeri* with an elevated ceftazidime-avibactam MIC value had none of the acquired screened β-lactamase-encoding genes, but demonstrated hyper-expression of AmpC (Tables 2 and 3).
- Doripenem ($MIC_{50/90}$, 0.03/0.5 mg/L) inhibited 97.9% (89/91) of all Enterobacteriaceae isolates at the breakpoint for susceptibility (i.e. ≤1 mg/L).
- A total of 35 (83.3%) *E. coli* harboured *bla*$_{\text{CTX-M}}$. The other isolates had SHV-2 (one isolate), SHV-12 (one), SHV-12/CMY-2 (one), KLUC-2 (one), DHA-1 (one). Two *E. coli* isolates did not have any of the screened acquired β-lactamase genes nor demonstrated hyper-expression of chromosomal AmpC (cAmpC; Table 3).
- Among *Klebsiella* spp., nine (60.0%) and two (13.3%) isolates produced CTX-M and SHV-2, respectively (Table 3). Also, three isolates produced NDM-1 (*K. pneumoniae*; Ukraine), OXA-48 (*Klebsiella oxytoca*; Romania) or OXA-9 (*K. pneumoniae*; Romania), whereas none of the screened β-lactamase genes were detected in one *K. oxytoca*, which exhibited a phenotype resembling hyper-production of K1 (data not shown).
- The majority of *Enterobacter* spp. (12/15; 80.0%) showed hyper-expression of cAmpC alone or in combination with CTX-M and SHV-12, whereas the remaining isolates produced CTX-M (3/15; 20.0%) alone or in combination with DHA-1 and/or SHV-12 (Table 3).
- The majority of *P. mirabilis* produced plasmid AmpC (pAmpC; 7/12; 58.3%; CMY-16 [three], CMY-2 [two], CMY-22 [one] and AAC-4 [one]) (Table 3). Other isolates had CTX-M (4/12; 33.3%) or TEM-93 (1/12; 8.3%).
- *Citrobacter* spp., *Providencia* spp. and *S. marcescens* had overexpression of cAmpC (3/7; 42.9%), CTX-M (2/7; 28.6%), PER-1 (1/7; 14.3%) and one isolate had CTX-M, as well as overexpression of cAmpC (1/7; 14.3%; Table 3).

Table 3. Summary of β-lactamase enzymes detected among baseline Enterobacteriaceae pathogens recovered from patients enrolled in the cUTI Phase 3 trials for ceftazidime-avibactam

Pathogen (No; % of total)	Results[a] (No of isolates; % within each Genus/Species)	Pathogen (No; % of total)	Results[a] (No of isolates; % within each Genus/Species)
E. coli (42; 46.2)	CTX-M-14 (9; 21.4)	*Enterobacter* spp. cont' (15; 16.5)	CTX-M-3 + SHV-12 + cAmpC (2; 13.3)
	CTX-M-1 (7; 16.7)		OXA-1 + cAmpC (1; 6.7)
	CTX-M-27 (6; 14.3)	*K. pneumoniae* (13; 14.3)	CTX-M-14 (4; 30.8)
	CMY-2 (3; 7.1)		CTX-M-3 (4; 30.8)
	CTX-M-3 (2; 4.8)		CTX-M-3 + OXA-1 (1; 7.7)
	CTX-M-55 + OXA-1 (2; 4.8)		NDM-1 (1; 7.7)
	Unknown (2; 4.8)		OXA-9 (1; 7.7)
	CTX-M-65 (1; 2.4)		SHV-12 (2; 15.4)
	CTX-M-55 + OXA-2 (1; 2.4)	*K. oxytoca* (2; 2.2)	OXA-48 (1; 50.0)
	CTX-M-55 (1; 2.4)		K1 (1; 50.0)
	CTX-M-3 + OXA-1 (1; 2.4)	*P. mirabilis* (12; 13.2)	CMY (6; 50.0)
	CTX-M-138 (1; 2.4)		CTX-M-65 (2; 16.6)
	CTX-M-132 (1; 2.4)		CTX-M-3 (1; 8.3)
	SHV-2 (1; 2.4)		CTX-M-116 + OXA-2 (1; 8.3)
	SHV-12 (1; 2.4)		AAC-4 (1; 8.3)
	SHV-12 + CMY-2 (1; 2.4)		TEM-93 (1; 8.3)
	DHA-1 (1; 2.4)	*Citrobacter* spp. (4; 4.4)	cAmpC (2; 50.0)
	KLUC-2 (1; 2.4)		CTX-M-3 + cAmpC (1; 25.0)
Enterobacter spp. (15; 16.5)	cAmpC (9; 60.0)		CTX-M-1 (1; 25.0)
	CTX-M-3 (1; 6.7)	*P. rettgeri* (2; 2.2)	PER-1 (1; 50.0)
	CTX-M-3 + DHA-1 (1; 6.7)		cAmpC (1; 50.0)
	CTX-M-3 + SHV-12 (1; 6.7)	*S. marcescens* (1; 1.1)	CTX-M-3 (1; 100.0)

[a]Narrow-spectrum β-lactamase enzymes are not depicted here; cAmpC = over-expression of chromosomal AmpC; Unknown = screened β-lactamases were not detected; K1 = screened β-lactamases were not detected, but isolate demonstrated a phenotype compatible with hyper-production of the intrinsic K1 enzyme.

Conclusions

- Ceftazidime-avibactam ($MIC_{50/90}$, 0.25/1 mg/L) and doripenem ($MIC_{50/90}$, 0.03/0.5 mg/L) demonstrated similar *in vitro* potencies against these β-lactamase-producing organisms causing cUTI in Phase 3 clinical trials.
- The ceftazidime-avibactam and doripenem *in vitro* potencies were irrespective of β-lactamases present, except when tested against one *P. rettgeri* and one NDM-1-producing *K. pneumoniae*.
- CTX-M enzymes prevailed among *E. coli* and *Klebsiella spp.*, whereas pAmpC was most common among *P. mirabilis*. Other organisms often showed hyper-expression of cAmpC.

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Acknowledgements

The clinical trial studies and the microbiology and molecular characterization of isolates were funded by AstraZeneca Pharmaceuticals (Waltham, MA, USA). JMI Laboratories also received compensation fees for services with regards to abstract and poster preparation, which was also funded by AstraZeneca.

Disclosures

PAB and GGS are employees and shareholders of AstraZeneca.

This study was funded by AstraZeneca. Poster typesetting support was provided by Prime Medica Ltd, Knutsford, Cheshire, funded by AstraZeneca.
Presented at the 26th European Congress of Clinical Microbiology and Infectious Diseases, 9–12 April 2016, Amsterdam, the Netherlands.

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