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Antimicrobial Resistance Patterns and Activity of Ceftazidime-Avibactam and Comparators in Gram-Negative Pathogens Isolated from Intra-Abdominal Infections in the European Union: International Network For Optimal Resistance Monitoring (INFORM) Global Surveillance Study 2013

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Abstract

Background: Avibactam (AVI), a novel non-β-lactam β-lactamase inhibitor, can inhibit a variety of β-lactamases, such as extended-spectrum β-lactamases (ESBL), class C β-lactamases, serine carbapenemases, and some class D β-lactamases. These enzymes are an increasing problem in Gram-negative pathogens, including those causing intra-abdominal infections (IAI). AVI in combination with ceftazidime (CAZ) may therefore represent a new treatment option for IAI. This report presents the *in vitro* activity of CAZ-AVI and comparators against Gram-negative IAI pathogens collected in selected countries of the European Union in 2013.
Methods: 63 sites in 17 countries (Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Romania, Spain, Sweden, United Kingdom) collected 1,085 clinically relevant Gram-negative isolates from patients with IAI in 2013. Only one strain per patient infection episode was included. Susceptibility was determined using the current CLSI broth microdilution method and EUCAST breakpoints. Isolates phenotypically positive for ESBL or non-susceptible to a carbapenem were analysed for the presence of β-lactamases via multiplex PCR, followed by sequencing.
Results: The MIC_{90} values (mg/L) of CAZ-AVI and comparators against selected Gram-negative species (including molecularly characterized ESBL+ subsets and CAZ-non-susceptible phenotypes) are shown below.

Organisms (n) /	CAZ-AVI	CAZ	FEP	ATM	TZP	MEM	LVX	TGC
S|R Breakpoints†	na	≤1/>4	≤1/>4	≤1/>4	≤8/>16	≤2/>8	≤1/>2	≤1/>2
Escherichia coli (504)	0.25	8	2	8	16	0.03	>4	0.5
ESBL+ (53)	0.5	128	>16	128	64	0.03	>4	0.5
Klebsiella spp. (245)	0.25	32	16	64	32	0.06	>4	1
ESBL+ (31)	1	>128	>16	>128	>128	8	>4	2
Enterobacter spp. (106)	1	128	4	64	128	0.12	1	1
CAZ-NS (36)	8	>128	>16	128	>128	8	>4	2
Citrobacter spp. (59)	0.5	128	4	64	128	0.12	1	0.5
CAZ-NS (23)	1	>128	4	64	>128	0.12	4	1
S|R Breakpoints†	na	≤8/>8	≤8/>8	≤1/>16	≤16/>16	≤2/>8	≤1/>2	na
Pseudomonas aeruginosa (66)	8	32	16	32	128	>8	>4	>8
CAZ-NS (14)	32	64	16	64	>128	>8	>4	>8

CAZ-AVI, ceftazidime-avibactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; MEM, meropenem; LVX, levofloxacin; TGC, tigecycline; na, not available; NS, non-susceptible.
† EUCAST v3.1 breakpoints.

79% of CAZ-non-susceptible *P. aeruginosa* had CAZ-AVI MICs of ≤8 mg/L (using the CAZ PK/PD cut-off as reference value in the absence of a CAZ-AVI susceptibility breakpoint), compared to 50% susceptible to meropenem and 43% to levofloxacin. The MIC_{90} values for 11 *A. baumannii* isolates were 64 and 128 mg/L for CAZ-AVI and CAZ, respectively. The most common ESBLs found among the molecularly characterized CAZ-non-susceptible *Enterobacteriaceae* isolates included in this report were CTX-M-15 (n=60), SHV-12 (n=9), and CTX-M-27 (n=6).

Conclusions:
- With the exception of *A. baumannii*, CAZ-AVI shows very promising activity against Gram-negative pathogens isolated from IAI.
- MIC_{90} values of CAZ-AVI were reduced at least 32-fold for *Enterobacteriaceae* and 4-fold for *P. aeruginosa* compared to CAZ alone.
- A sizable proportion of *E. coli* (11%) and *Klebsiella* spp. (13%) isolated from patients with IAI were ESBL-positive (mostly CTX-M-15); the *in vitro* activity of CAZ-AVI was excellent against these ESBL-positive strains (MIC_{90} ≤1 mg/L).
- The addition of avibactam resulted in a vastly improved *in vitro* activity of CAZ against CAZ-non-susceptible *Enterobacteriaceae*. CAZ-AVI could be considered as a potential treatment option against this subset of strains that have MIC_{90} values in the resistant range for most other agents, and for which treatment options are limited.

Introduction

Avibactam (AVI), a novel non-β-lactam β-lactamase inhibitor, can inhibit a variety of β-lactamases, such as extended-spectrum β-lactamases (ESBL), class C β-lactamases, serine carbapenemases, and some class D β-lactamases. These enzymes are an increasing problem in Gram-negative pathogens, including those causing intra-abdominal infections (IAI). AVI in combination with ceftazidime (CAZ) may therefore represent a new treatment option for IAI. This report presents the *in vitro* activity of CAZ-AVI and comparators against Gram-negative IAI pathogens collected in selected countries of the European Union in 2013.

Materials & Methods

63 sites in 17 countries in the European Union collected 1,085 clinically relevant Gram-negative isolates from patients with IAI in 2013 (Table 1). One strain per patient infection episode was included. MICs were determined and phenotypic ESBL screening and confirmation were done using the CLSI broth microdilution method [1, 2]. Susceptibility was determined using EUCAST breakpoints where available [3]. In the absence of EUCAST breakpoints, the CAZ-AVI PK/PD breakpoint was used for comparative purposes. *E. coli, K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* isolates phenotypically positive for ESBL activity or having MICs ≥16 mg/L for ceftazidime or cefotaxime, and all *Enterobacteriaceae* isolates non-susceptible to carbapenems were screened for β-lactamase (*bla*) genes using a combination of multiplex PCR and microarray (Check-Points, Wageningen, The Netherlands) [4]. Isolates were screened for *bla* genes encoding ESBLs (CTX-M, TEM, SHV, VEB, PER, GES), serine carbapenemases (KPC, OXA), and MBLs (NDM, IMP, VIM, SPM), and AmpC β-lactamases (ACC, ACT, CMY, DHA, FOX, MIR, MOX). Detected genes were sequenced and enzyme variants were identified by comparison to the NCBI database (www.ncbi.nlm.nih.gov) and the Lahey Clinic website (www.lahey.org/studies).

Results

Table 1. Number of Gram-Negative IAI Isolates and Hospital Sites from Each Country.

Country	AUT	BEL	CZE	DNK	FRA	DEU	GRC	HUN	IRL	ITA	NLD	POL	PRT	ROM	ESP	SWE	UK	EU
Sites	2	4	3	2	6	7	3	3	1	7	2	3	4	2	6	3	5	63
Gram-negative	45	52	24	39	104	101	80	68	15	92	42	40	87	69	126	50	51	1085
Enterobacteriaceae																		
All	43	52	23	39	100	88	75	60	15	82	41	37	83	57	120	47	46	1008
CAZ-NS	3	12	3	4	17	14	17	10		24	3	9	15	13	19	4	5	172
ESBL+	2	3	3		9	7	9	7		16	3	5	7	8	7	3		89
Non-fermenters*	2		1		4	13	5	8		10	1	3	4	12	6	3	5	77

* *A. baumannii* and *P. aeruginosa*.

Table 2. ESBL Enzyme Types and Variants Found in Molecularly Characterized, Genotypically ESBL-Positive *Enterobacteriaceae*.

		CTX-M								SHV				VEB
	n*	-15	-27	-1	-14	-9	-3	-55	-8	-12	-5	-2	-2A	-2
C. freundii	1					1				1				
E. aerogenes	1	1												
E. cloacae	2					1				1				
E. coli	53	34	6	4	4			1	1	2	1	1		
K. oxytoca	2	1								1				
K. pneumoniae	29	24					1			4	2		1	
P. stuartii	1										1			1
Enterobacteriaceae	89	60	6	4	4	2	1	1	1	9	4	1	1	1

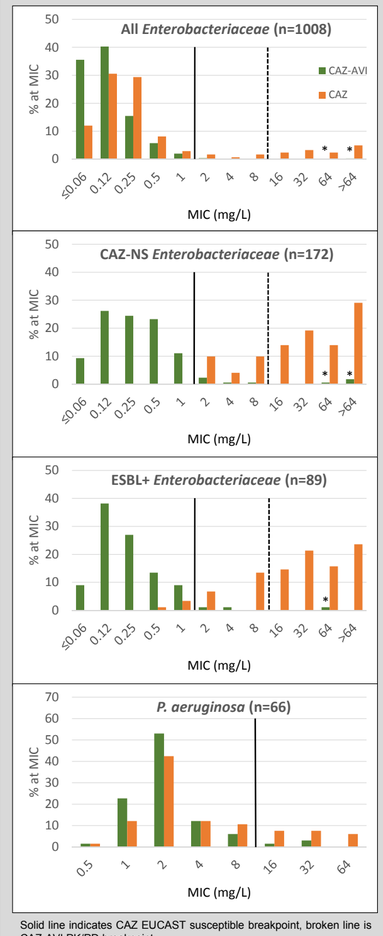
* Number of molecularly characterized isolates in which bla_{ESBL} were detected by PCR.
Some isolates carried more than one ESBL. No TEM-ESBL, ESBL-like GES, or PER enzymes were found. Ten isolates also carried AmpC β-lactamases or carbapenemases (including one *P. stuartii* isolate with a VIM-1 metallo-β-lactamase).

Table 3. Activity of Ceftazidime-Avibactam and Comparators against Gram-Negative IAI Pathogens.

Organisms (n)	CAZ-AVI*		CAZ		FEP		ATM		TZP		MEM		LVX		TGC		AMK		CST	
	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}
Escherichia coli (504)	100	0.25	87.7	8	89.1	2	87.1	8	88.5	16	100	0.03	76.0	>4	99.4	0.5	95.0	8	98.8	≤0.12
CAZ-NS (62)	100	0.5	0	128	21.0	>16	6.5	128	61.3	64	100	0.03	25.8	>4	100	0.5	75.8	16	98.4	≤0.12
ESBL+ (53)	100	0.5	7.6	128	5.7	>16	1.9	128	66.0	64	100	0.03	15.1	>4	100	0.5	75.5	16	100	≤0.12
Klebsiella spp. (245)	100	0.25	83.3	32	85.7	16	82.5	64	81.6	32	97.6	0.06	83.7	>4	90.2	1	95.5	4	98.4	0.25
CAZ-NS (41)	100	1	0	>128	19.5	>16	9.8	>128	17.1	>128	85.4	>8	39.0	>4	70.7	2	75.6	32	97.6	0.25
ESBL+ (31)	100	1	0	>128	6.5	>16	3.2	>128	22.6	>128	87.1	8	35.5	>4	71.0	2	77.4	32	96.8	≤0.12
Enterobacter spp. (106)	97.2	1	66.0	128	87.7	4	68.9	64	70.8	128	96.2	0.12	92.5	1	92.5	1	96.2	4	96.2	0.25
CAZ-NS (36)	91.7	8	0	>128	63.9	>16	8.3	128	16.7	>128	88.9	8	77.8	>4	86.1	2	88.9	16	97.2	≤0.12
Citrobacter spp. (59)	100	0.5	61.0	128	84.8	4	61.0	64	64.4	128	100	0.12	93.2	1	98.3	0.5	98.3	4	100	0.25
CAZ-NS (23)	100	1	0	>128	65.2	4	4.4	64	21.7	>128	100	0.12	87.0	4	95.7	1	95.7	4	100	0.25
P. aeruginosa (66)	95.5	8	78.8	32	84.9	16	0	32	72.7	128	74.2	>8	62.1	>4	na	>8	87.9	32	100	0.5
CAZ-NS (14)	78.6	32	0	64	42.9	16	0	64	14.3	>128	50.0	>8	42.9	>4	na	>8	64.3	>32	100	0.5
A. baumannii (11)	27.3	64	na	128	na	>16	na	128	na	>128	45.5	>8	27.3	>4	na	2	36.4	>32	100	0.12

* In the absence of EUCAST breakpoints, the CAZ-AVI PK/PD breakpoint of ≤8 mg/L was used for comparative purposes. For all other agents EUCAST v3.1 breakpoints were used.
% susceptible values ≥90% are shaded green.
CAZ-AVI, ceftazidime-avibactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; MEM, meropenem; LVX, levofloxacin; TGC, tigecycline; AMK, amikacin; CST, colistin; NS, non-susceptible; na, not available.

Figure 1. MIC Distribution of Ceftazidime-Avibactam and Ceftazidime.



Solid line indicates CAZ EUCAST susceptible breakpoint, broken line is CAZ-AVI PK/PD breakpoint.
* *Enterobacteriaceae* isolates (3 *E. cloacae* and 1 *P. stuartii*) with CAZ-AVI MICs above the CAZ-AVI PK/PD breakpoint with VIM-1 metallo-β-lactamases.

Results Summary

- A wide range of ESBL enzymes were identified in *Enterobacteriaceae* from intra-abdominal infections in the European Union, with CTX-M-15 by far the predominant variant (Table 2).
- Using the CAZ-AVI PK/PD breakpoint for comparative purposes, CAZ-AVI showed susceptibility >90% for the most common *Enterobacteriaceae* species from IAI, including CAZ-non-susceptible and ESBL-positive subsets. 79% of CAZ-non-susceptible *P. aeruginosa* were susceptible to CAZ-AVI, compared to 50% susceptible to meropenem and 43% to levofloxacin (Table 3).
- MIC_{90} values of CAZ-AVI were reduced at least 32-fold for *Enterobacteriaceae* and 4-fold for *P. aeruginosa* compared to CAZ alone (Table 3).
- A striking downward shift in MIC distribution by 8 doubling dilutions for CAZ-AVI compared to CAZ was seen for CAZ-non-susceptible and ESBL-positive *Enterobacteriaceae* (Figure 1). *Enterobacteriaceae* isolates (3 *E. cloacae* and 1 *P. stuartii*) with CAZ-AVI MICs above the CAZ-AVI PK/PD breakpoint carried VIM-1 metallo-β-lactamases.

Conclusions

- CAZ-AVI showed very promising activity against *Enterobacteriaceae* and *P. aeruginosa* isolated from IAI.
- The addition of avibactam resulted in vastly improved *in vitro* activity of CAZ against CAZ-non-susceptible and ESBL-positive *Enterobacteriaceae* from IAI.
- Based on the data presented, it is likely that CAZ-AVI will become recognised as a credible treatment option for IAI as existing standards of care demonstrate varied susceptibility rates across common strains.

References and Acknowledgments:
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