P1291

Comparative *in vitro* Activity of Ceftazidime-Avibactam against Gram-Negative Pathogens from Hospital-Acquired Lower Respiratory Tract Infections in the European Union: 2013 INFORM Surveillance Program

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Abstract

Objectives: Treatment options are limited in hospital-acquired lower respiratory tract infections (HA-LRTI) because of high resistance rates due to extended-spectrum β-lactamases (ESBL) and other resistance mechanisms. As a non-β-lactam β-lactamase inhibitor capable of inhibiting Ambler class A, C, and some class D β-lactamases, avibactam combined with ceftazidime may represent an additional therapeutic option. Therefore, the *in vitro* activity of ceftazidime-avibactam was tested against Gram-negative clinical isolates from HA-LRTI collected in 2013 in the European Union as part of the International Network For Optimal Resistance Monitoring (INFORM) global surveillance program.
Methods: 65 sites in 17 countries in the European Union (Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Portugal, Romania, Spain, Sweden, United Kingdom) collected 1,314 clinically relevant Gram-negative isolates from patients with hospital-acquired (isolates collected ≥48 hours post-admission) LRTI in 2013. One strain per patient infection episode was included. Susceptibility was determined using the CLSI broth microdilution method and EUCAST breakpoints. Isolates phenotypically positive for ESBL or non-susceptible to a carbapenem were analysed for β-lactamases via multiplex PCR, followed by sequencing.
Results: MIC_{90} values (mg/L) of ceftazidime-avibactam and comparators against selected Gram-negative species (including molecularly characterized ESBL+ subsets and ceftazidime-non-susceptible phenotypes) are shown below.

Organisms (n) /	CAZ-AVI	CAZ	FEP	ATM	TZP	MEM	LVX	AMK
S|R Breakpoints†	na	≤1/>4	≤1/>4	≤1/>4	≤8/>16	≤2/>8	≤1/>2	≤8/>16
Klebsiella spp. (373)	1	128	>16	128	>128	0.25	>4	8
ESBL+ (95)	2	>128	>16	>128	>128	>8	>4	32
Escherichia coli (238)	0.25	16	16	16	32	0.03	>4	8
ESBL+ (37)	0.25	64	>16	128	>128	0.06	>4	>32
Enterobacter spp. (179)	1	128	4	64	128	0.12	1	4
CAZ-NS (88)	1	>128	8	64	>128	0.25	>4	8
Citrobacter spp. (66)	0.5	128	1	32	64	0.06	1	4
CAZ-NS (17)	1	>128	>16	128	128	0.12	>4	16
S|R Breakpoints†	na	≤8/>8	≤8/>8	≤1/>16	≤16/>16	≤2/>8	≤1/>2	≤8/>16
Pseudomonas aeruginosa (250)	8	64	16	64	>128	>8	>4	16
CAZ-NS (60)	32	128	>16	128	>128	>8	>4	>32

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

68% of CAZ-non-susceptible *P. aeruginosa* had CAZ-AVI MICs ≤8 mg/L (using the CAZ PK/PD cut-off as reference value in the absence of a CAZ-AVI breakpoint), compared to 73% susceptible to amikacin and 25% to meropenem. The MIC_{90} values for 90 *A. baumannii* isolates were 128 and >128 mg/L for ceftazidime-avibactam and ceftazidime, respectively. The most commonly found ESBL enzymes in *Klebsiella* spp. were CTX-M-15 (70 of 95) and SHV-12 (18 of 95), whereas in *E. coli* they were CTX-M-15 (26 of 37) and CTX-M-14 (4 of 37).

Conclusions:
- MIC_{90} values of ceftazidime-avibactam were reduced at least 64-fold for *Enterobacteriaceae* and 8-fold for *P. aeruginosa* compared to ceftazidime alone. The *in vitro* activity of ceftazidime-avibactam was excellent against ESBL+ and ceftazidime-non-susceptible *Enterobacteriaceae* (MIC_{90} ≤2), subsets for which the MIC_{90} values of most other tested agents were in the resistant range.
- Not surprisingly, ESBL+ rates were high in these hospital-acquired LRTI isolates from the European Union (16% in *E. coli* and 25% in *Klebsiella* spp.), and will have to be considered when selecting therapeutic agents.
- Even in this collection of pathogens with high resistance levels to several classes of antimicrobials, ceftazidime-avibactam showed very promising activity against Gram-negative pathogens, except *A. baumannii*.

Introduction

Treatment options for Gram-negative pathogens are limited in hospital-acquired lower respiratory tract infections (HA-LRTI) because of high resistance rates due to extended-spectrum β-lactamases (ESBL) and other resistance mechanisms. As a non-β-lactam β-lactamase inhibitor capable of inhibiting Ambler class A, C, and some class D β-lactamases, avibactam combined with ceftazidime may represent an additional therapeutic option. Therefore, the *in vitro* activity of ceftazidime-avibactam (CAZ-AVI) was tested against Gram-negative clinical isolates from HA-LRTI collected in 2013 in the European Union as part of the International Network For Optimal Resistance Monitoring (INFORM) global surveillance program.

Materials & Methods

65 sites in 17 countries in the European Union collected 1,314 clinically relevant Gram-negative isolates from patients with hospital-acquired (isolates collected ≥48 hours post-admission) LRTI 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 HA-LRTI Isolates and Hospital Sites from Each 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	2	1	7	2	3	4	3	7	4	5	65
Gram-negative	54	135	110	9	123	150	60	13	30	150	58	63	97	15	115	38	94	1314
Enterobacteriaceae																		
All	47	108	82	6	83	121	40	9	22	112	40	50	64	9	79	27	75	974
CAZ-NS	9	31	23	1	23	32	14	0	7	45	5	19	32	7	15	4	12	279
ESBL+	5	9	14	0	8	9	12	0	2	24	1	16	18	2	12	0	6	138
Non-fermenters*	7	27	28	3	40	29	20	4	8	38	18	13	33	6	36	11	19	340

* *A. baumannii* and *P. aeruginosa*
AUT, Austria; BEL, Belgium; CZE, Czech Republic; DNK, Denmark; FRA, France; DEU, Germany; GRC, Greece; HUN, Hungary; IRL, Ireland; ITA, Italy; NLD, Netherlands; POL, Poland; PRT, Portugal; ROM, Romania; ESP, Spain; SWE, Sweden; UK, United Kingdom; EU, European Union.

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

		CTX-M								SHV				TEM				VEB	GES
	n*	-15	-14	-3	-1	-9	-27	-32	-55	-12	-2A	-5	-31	-4	-24	-26	-52	-2	-6
E. aerogenes	1														1				
E. cloacae	3	2				1				1									1
E. coli	37	26	4	1	2		1	1	1	1							1		
K. oxytoca	4	2		1								1							
K. pneumoniae	91	68	3	1						18	2		1	1				1	
P. mirabilis	1															1			
S. marcescens	1			1															
Entero-bacteriaceae	138	98	8	3	2	1	1	1	1	20	2	1	1	1	1	1	1	1	1

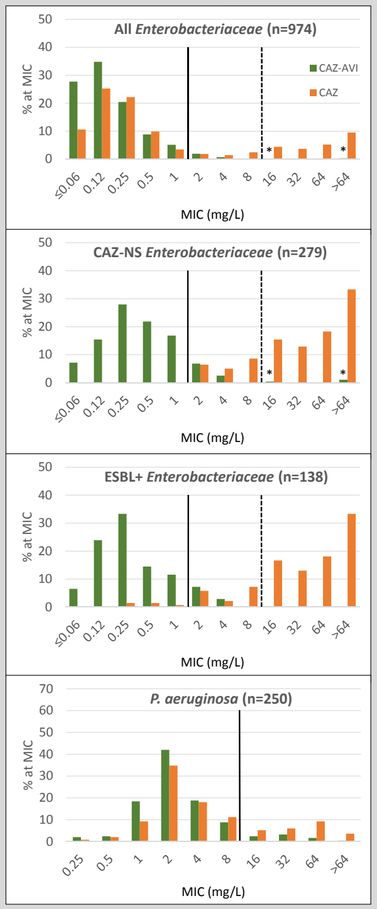
* Number of molecularly characterized isolates in which bla_{ESBL} were detected by PCR.
Some isolates carried more than one ESBL. No PER enzymes were found. 24 isolates also carried AmpC β-lactamases or carbapenemases.

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

Organisms (n)	CAZ-AVI*		CAZ		FEP		ATM		TZP		MEM		LVX		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}
Klebsiella spp. (373)	99.5	1	68.1	128	69.7	16	66.5	128	64.6	128	91.2	0.25	77.2	4	90.9	8	94.9	0.25
CAZ-NS (119)	98.3	2	0	128	10.1	16	5.9	128	15.1	128	72.3	8	37.0	4	73.1	32	86.6	4
ESBL+ (95)	100	2	2.1	128	5.3	16	1.1	128	16.8	128	82.1	8	37.9	4	79.0	32	86.3	4
E. coli (238)	100	0.25	82.8	16	85.3	16	83.2	16	83.2	32	99.6	0.03	73.1	4	92.0	8	99.2	0.12
CAZ-NS (41)	97.6	0.5	0	64	22.0	16	9.8	128	51.2	128	97.6	0.06	19.5	4	65.9	32	97.6	0.12
ESBL+ (37)	100	0.25	5.4	64	16.2	16	2.7	128	54.1	128	100	0.06	13.5	4	64.9	32	97.3	0.12
Enterobacter spp. (179)	100	1	50.8	128	82.1	4	55.3	64	54.8	128	97.8	0.12	91.6	1	97.2	4	97.2	0.25
CAZ-NS (79)	100	1	0	128	63.6	8	9.1	64	13.6	128	95.5	0.25	83.0	4	94.3	8	98.9	0.25
Citrobacter spp. (66)	100	0.5	74.2	128	93.9	1	75.8	32	75.8	64	100	0.06	90.9	1	97.0	4	100	0.12
CAZ-NS (17)	100	1	0	128	76.5	16	5.9	128	29.4	128	100	0.12	64.7	4	88.2	16	100	0.25
P. aeruginosa (250)	92.4	8	76.0	64	82.0	16	5.6	64	69.2	128	67.6	8	64.0	4	86.4	16	100	0.5
CAZ-NS (60)	68.3	32	0	128	36.7	16	0	128	5.0	128	25.0	8	30.0	4	73.3	32	100	0.5
A. baumannii (90)	42.2	128	na	128	na	16	na	128	na	128	46.7	8	36.7	4	51.1	32	98.9	0.25

* 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, ceftazidime; AVI, avibactam; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; MEM, meropenem; LVX, levofloxacin; AMK, amikacin; CST, colistin; NS, non-susceptible; na, not available.

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



Solid line indicates CAZ EUCAST breakpoint, broken line is CAZ-AVI PK/PD breakpoint.
* Four *Enterobacteriaceae* isolates had CAZ-AVI MIC values above the CAZ-AVI PK/PD breakpoint: 1 *E. coli*, 1 *K. oxytoca*, and 1 *K. pneumoniae* carried a VIM-1 or NDM-1 metallo-β-lactamase; the fourth isolate (*M. morganii*, CAZ-AVI MIC of 16 mg/L) was not molecularly characterized.

Results Summary

- In these HA-LRTI *Enterobacteriaceae* from the European Union, the CAZ-non-susceptible rate (29%) was high, as were ESBL-positive rates (14% overall, 16% of *E. coli,* and 25% of *Klebsiella* spp.), with CTX-M-15 by far the predominant ESBL variant (Tables 1 and 2).
- Using the CAZ-AVI PK/PD breakpoint for comparative purposes, CAZ-AVI showed susceptibility >90% for the most common *Enterobacteriaceae* species from HA-LRTI, including CAZ-non-susceptible and ESBL-positive subsets. Using the same cut-off of MIC ≤8 mg/L, 68% of CAZ-non-susceptible *P. aeruginosa* were susceptible to CAZ-AVI, compared to 73% susceptible to amikacin and 25% to meropenem (Table 3).
- MIC_{90} values of CAZ-AVI were reduced at least 64-fold for *Enterobacteriaceae* and 8-fold for *P. aeruginosa* compared to CAZ alone (Table 3).
- The addition of avibactam had an especially striking impact on MICs of CAZ-non-susceptible and ESBL-positive *Enterobacteriaceae* with a downward shift of the MIC distribution by about 8 doubling dilutions (Figure 1). Three of the four *Enterobacteriaceae* isolates with CAZ-AVI MIC >8 mg/L were molecularly characterized and carried MBL enzymes.

Conclusions

- Even in this collection of HA-LRTI pathogens with high resistance levels to several classes of antimicrobials, target Gram-negative pathogens showed high susceptibility rates to CAZ-AVI.
- The *in vitro* activity of CAZ-AVI against CAZ-non-susceptible *Enterobacteriaceae* from HA-LRTI was excellent, including those producing ESBLs (MIC_{90} ≤2) whereas the MIC_{90} values for most other agents tested with this subset were in the resistant range.

References and Acknowledgments:

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