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Avibactam Reverts the Ceftazidime MIC_{90} of a Recent Set of European Clinical Isolates of Gram-negative Bacteria Back to the Epidemiological Cut-off Value

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Abstract

Background: Ceftazidime-avibactam (CAZ-AVI) is a combination of ceftazidime (CAZ) and the novel non-β-lactam β-lactamase inhibitor (AVI) targeting Gram-negative (GN) pathogens and is effective against ESBL- and AmpC-producing strains. We examined the effect of AVI on the CAZ MIC distribution profile of Gram-negative European Clinical Isolates and compared the results to EUCAST wild-type distributions and Epidemiological Cut-Off (ECOFFs) values listed for CAZ.

Methods: CAZ and CAZ-AVI MICs were generated for *P. aeruginosa* (PSA, n=439), *E. coli* (EC, n=1175), *Klebsiella* spp. (KSP, n=442), *Serratia* spp. (SSP, n=106) *Enterobacter* spp. (ESP, n=242), and *P. mirabilis* (PM, n=108) isolates collected from medical centers in Europe during 2009. MIC values were determined using CLSI reference broth microdilution methods. QC organisms were those recommended by CLSI. The CAZ and CAZ-AVI MIC distribution profiles were compared to those of CAZ from the EUCAST website for PSA, EC, KSP, SSP, ESP, and PM.

Results: The MIC_{90} for CAZ and CAZ-AVI for the 2009 European PSA isolates was >32 and 8 µg/mL, respectively. The presence of AVI reverted the MIC_{90} for PSA back to the EUCAST MIC distribution ECOFF. The MIC_{90} for CAZ for EC, KSP, SSP, and PM was also reduced to the ECOFF by the presence of AVI. AVI reduced the MIC_{90} for ESP >32-fold down to 1 µg/mL, no ECOFF has been defined yet for these organisms although it is 1 µg/mL for *E. aerogenes* and *E. cloacae*.

Conclusions: The ECOFF which has been used by EUCAST to identify the upper-end of the wild-type MIC distribution for organisms provides a useful tool to understand the MIC range where a normal population of organisms resides. The elevated CAZ MIC_{90} for GN bacteria from the European surveillance data was due to the presence of resistant bacteria. CAZ-AVI was able to revert the CAZ MIC_{90} for GN back to the ECOFF; a marker for the upper-end of the wild-type population.

	Organism (MIC_{90} in µg/mL)					
Drug	**PSA**	**EC**	**KSP**	**SSP**	**ESP**	**PM**
CAZ	>32	2	32	1	>32	0.5
CAZ-AVI	8	0.25	0.5	0.5	1	0.06
CAZ ECOFF	≤8	≤0.5	≤0.5	≤0.5	NA	≤0.125

Introduction

Ceftazidime-avibactam is a β-lactam antibiotic in combination with a non-β-lactam β-lactamase inhibitor showing promising activity against Ambler class A, C and some D β-lactamases. Avibactam inactivates β-lactamase enzymes very efficiently, with low IC_{50} (concentration resulting in 50% inhibition) values and generates a hydrolytically-stable enzyme-avibactam product. The role of avibactam in this combination is to protect ceftazidime from hydrolysis by a variety of serine β-lactamases.

Ceftazidime-avibactam is currently undergoing clinical development for treatment of complicated urinary tract (cUTI) and intra-abdominal infections (cIAI), including hospital infections and serious infections; in each case, including those caused by antimicrobial-resistant Gram-negative bacteria. The Phase II results for cUTI and cIAI were considered promising as the observed efficacy of ceftazidime-avibactam was similar to that of the comparator agent in each case.

The objective of this study was to compare the *in vitro* activity of ceftazidime and ceftazidime-avibactam when tested against a collection of contemporary (2009) clinical isolates from European medical centers. The MIC distribution profiles of these data were compared to the profiles for ceftazidime as viewed on the EUCAST website (http://www.eucast.org/mic_distributions/).

Materials and Methods

Consecutively collected, non-duplicate isolates from patients with clinically significant infections were collected from January through December in 2009 from a total of 26 medical centers in Belgium, France, Germany, Ireland, Italy, Poland, Portugal, Spain, Sweden, Switzerland, Turkey, and the United Kingdom.

Susceptibility testing was performed by broth microdilution (Clinical Laboratory Standard Institute [CLSI], 2012). Gram-negative bacteria were tested in cation-adjusted Mueller-Hinton broth (CA-MHB). The concentration of avibactam was maintained at 4 µg/mL for all test concentrations of ceftazidime. Susceptibility testing results were interpreted according to CLSI (2012) or EUCAST (2012) criteria. The ESBL-phenotype for *Klebsiella* spp. and *Escherichia coli* was defined as a MIC ≥2 µg/mL for ceftazidime, or ceftriaxone or aztreonam (M100-S22). Because there are currently no established susceptibility criteria for ceftazidime-avibactam, no 'susceptibility' rates were reported for this combination.

Quality control (QC) was performed concurrently with *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853; all QC values were within published ranges. Ceftazidime and ceftazidime-avibactam MIC values were generated for *P. aeruginosa* (n=439), *E. coli* (n=1,175), *Klebsiella* spp. (n=442; includes: *Klebsiella oxytoca* [105 strains], *K. ozaenae* [1 strain], and *K. pneumoniae* [336 strains]), *Serratia* spp. (n=106; includes: *Serratia liquefaciens* [5 strains] and *S. marcescens* [101 strains]), *Enterobacter* spp. (n=242), and *Proteus mirabilis* (n=108) isolates. The ceftazidime and ceftazidime-avibactam MIC distribution profiles from the 2009 European surveillance program were compared to those of ceftazidime obtained from the EUCAST website (http://www.eucast.org/mic_distributions/) accessed 25 April 2012.

Results

- Table 1 shows the activity of ceftazidime, ceftazidime-avibactam and comparator agents for the collection of 2,512 European isolates.
- *P. aeruginosa*
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for *P. aeruginosa* was >32 and 8 µg/mL, respectively (Table 1). Ceftazidime susceptibility was at 72.7% whereas the MIC values for 90.2% of isolates were ≤8 µg/mL for ceftazidime-avibactam (Figure 1a).
  - Thus, the presence of avibactam decreased the MIC_{90} value to the upper limit of the EUCAST MIC distribution ECOFF value (see Figure 1a).
- *E. coli*
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for *E. coli* was 2 and 0.25 µg/mL, respectively (Table 1). Ceftazidime susceptibility was at 93.4/87.5% (CLSI/EUCAST interpretive criteria, respectively) whereas the MIC values were ≤1 µg/mL for ceftazidime-avibactam for 100% of isolates (Figure 1b).
  - For strains with an ESBL-phenotype, 99.4% of ceftazidime-avibactam MIC values were at or below the ECOFF (≤0.5 µg/mL; Figure 1b).
  - Interestingly, in the case of *E. coli*, combination with avibactam shifted the ceftazidime MIC frequency distribution to a set of values lower than the ceftazidime MIC values of the EUCAST wild-type distribution (Figure 1b).
- *Klebsiella* spp.
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for all *Klebsiella* spp. was 32 and 0.5 µg/mL, respectively (Table 1). Note that the ECOFF for *Klebsiella* spp. is ≤0.5 µg/mL (Figure 1c). Ceftazidime susceptibility was at 80.3/76.2% (CLSI/EUCAST interpretive criteria, respectively) while the MIC values for 97.5% of isolates were ≤1 µg/mL for ceftazidime-avibactam.
  - In the presence of avibactam, the MIC_{90} for ceftazidime against strains with an ESBL-phenotype was markedly reduced from >32 µg/mL to 2 µg/mL. 75.9% of the isolate MIC values were at ≤0.5 µg/mL and 89.8% at ≤1 µg/mL.

Figure 1. Comparison of the cumulative MIC frequency distributions for ceftazidime and ceftazidime-avibactam for a) *P. aeruginosa* b) *E. coli* c) *Klebsiella* spp. d) *Enterobacter* spp. e) *P. mirabilis* and f) *Serratia* spp. from a 2009 European surveillance program when compared to the MIC distribution on the EUCAST website

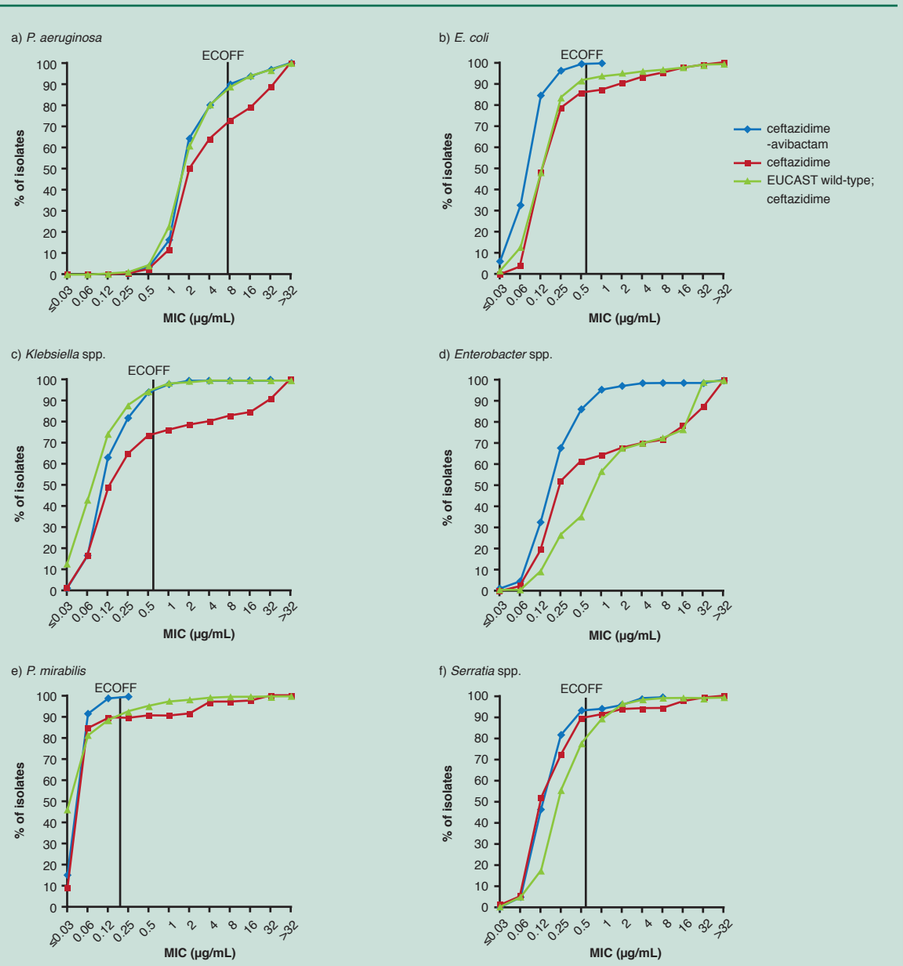

Table 1. Activity of ceftazidime, ceftazidime-avibactam and comparator antimicrobial agents when tested against Gram-negative bacteria (2,512 strains) from Europe (2009 Surveillance)

Organisms/antimicrobial agent (no. tested)	MIC_{50}/ MIC_{90} (µg/mL)	CLSI[a] %S / %R	EUCAST[a] %S / %R
P. aeruginosa (439)			
Ceftazidime-avibactam	2 / 8	- / -	- / -
Ceftazidime	2 / >32	72.7 / 21.0	72.7 / 27.3
Cefepime	4 / >16	72.6 / 11.9	72.6 / 27.4
Imipenem	2 / >8	63.6 / 32.3	67.7 / 24.8
Meropenem	1/ >8	67.0 / 27.3	67.0 / 17.1
Piperacillin-tazobactam	8 / >64	66.3 / 18.0	66.3 / 33.7
Levofloxacin	1 / >4	63.6 / 29.2	57.2 / 36.4
Amikacin	4 / 32	89.5 / 7.1	82.0 / 10.5
Colistin	1 / 1	99.5 / 0.5	99.5 / 0.5
E. coli (1,175)			
Ceftazidime-avibactam	0.12 / 0.25	- / -	- / -
Ceftazidime	0.25 / 2	93.4 / 4.3	87.5 / 6.6
Cefepime	≤0.12 / 2	94.1 / 3.5	89.0 / 7.6
Imipenem	0.25 / 0.25	99.7 / 0.1	99.9 / 0.0
Meropenem	≤0.12 / ≤0.12	99.8 / 0.1	99.9 / 0.1
Piperacillin-tazobactam	2 / 16	91.1 / 4.3	87.9 / 8.9
Levofloxacin	≤0.5 / >4	75.7 / 22.6	75.2 / 24.3
Amikacin	2 / 8	98.9 / 0.3	97.4 / 1.1
ESBL-phenotype (154)			
Ceftazidime-avibactam	0.12 / 0.5	- / -	- / -
Ceftazidime	4 / 32	50.0 / 33.1	8.4 / 50.0
Cefepime	8 / >16	55.2 / 26.6	21.4 / 57.1
Imipenem	0.25 / 0.5	97.4 / 0.6	99.4 / 0.0
Meropenem	≤0.12 / ≤0.12	99.4 / 0.0	100.0 / 0.0
Piperacillin-tazobactam	16 / >64	64.9 / 13.6	49.4 / 35.1
Levofloxacin	>4 / >4	35.7 / 63.0	34.4 / 64.3
Amikacin	4 / 16	93.5 / 1.3	85.1 / 6.5
Klebsiella spp.[b] (442)			
Ceftazidime-avibactam	0.12 / 0.5	- / -	- / -
Ceftazidime	0.25 / 32	80.3 / 17.4	76.2 / 19.7
Cefepime	≤0.12 / >16	85.7 / 12.2	81.4 / 14.5
Imipenem	0.25 / 0.5	95.7 / 1.8	98.2 / 0.2
Meropenem	≤0.12 / ≤0.12	98.4 / 0.5	99.5 / 0.5
Piperacillin-tazobactam	4 / >64	78.1 / 14.3	74.0 / 21.9
Levofloxacin	≤0.5 / >4	83.7 / 12.0	81.4 / 16.3
Amikacin	1 / 4	98.0 / 1.6	95.9 / 2.0
ESBL-phenotype (108)			
Ceftazidime-avibactam	0.5 / 2	- / -	- / -
Ceftazidime	32 / >32	19.4 / 71.3	10.2 / 80.6
Cefepime	16 / >16	41.7 / 50.0	24.1 / 59.3
Imipenem	0.25 / 2	86.1 / 6.5	93.5 / 0.9
Meropenem	≤0.12 / 1	93.5 / 1.9	98.1 / 1.9
Piperacillin-tazobactam	64 / >64	27.8 / 44.4	20.4 / 72.2
Levofloxacin	4 / >4	40.7 / 45.4	38.0 / 59.3
Amikacin	4 / 16	91.7 / 6.5	83.3 / 8.3
Enterobacter spp.[c] (242)			
Ceftazidime-avibactam	0.25 / 1	- / -	- / -
Ceftazidime	0.25 / >32	69.7 / 28.2	64.3 / 30.3
Cefepime	≤0.12 / 2	96.7 / 2.5	87.2 / 3.7
Imipenem	0.5 / 1	91.7 / 2.5	97.5 / 0.4
Meropenem	≤0.12 / ≤0.12	98.3 / 1.7	98.3 / 0.4
Piperacillin-tazobactam	4 / >64	71.9 / 10.3	67.4 / 28.1
Levofloxacin	≤0.5 / 4	88.8 / 7.4	87.2 / 11.2
Amikacin	1 / 2	98.8 / 1.2	97.1 / 1.2
P. mirabilis (108)			
Ceftazidime-avibactam	0.06 / 0.06	- / -	- / -
Ceftazidime	0.06 / 0.5	97.2 / 2.8	90.7 / 2.8
Cefepime	≤0.12 / 0.25	98.1 / 0.9	95.4 / 3.7
Imipenem	2 / 4	39.8 / 13.9	86.1 / 0.0
Meropenem	≤0.12 / ≤0.12	100.0 / 0.0	100.0 / 0.0
Piperacillin-tazobactam	≤0.5 / 1	100.0 / 0.0	100.0 / 0.0
Levofloxacin	≤0.5 / 2	91.7 / 7.4	75.9 / 8.3
Amikacin	4 / 8	100.0 / 0.0	99.1 / 0.0
Serratia spp.[d] (106)			
Ceftazidime-avibactam	0.25 / 0.5	- / -	- / -
Ceftazidime	0.12 / 1	94.3 / 5.7	91.5 / 5.7
Cefepime	≤0.12 / 0.5	100.0 / 0.0	98.1 / 0.0
Imipenem	1 / 2	83.0 / 1.9	98.1 / 0.0
Meropenem	≤0.12 / ≤0.12	100.0 / 0.0	100.0 / 0.0
Piperacillin-tazobactam	2 / 32	89.6 / 0.9	84.0 / 10.4
Levofloxacin	≤0.5 / 2	90.6 / 5.7	84.0 / 9.4
Amikacin	2 / 4	100.0 / 0.0	98.1 / 0.0

[a]Criteria as published by the CLSI [2012] and EUCAST [2012]. [b]Includes: *K. oxytoca* (105 strains), *K. ozaenae* (1 strain), and *K. pneumoniae* (336 strains). [c]Includes: *E. aerogenes* (54 strains), *E. amnigenus* (2 strains), *E. cloacae* (185 strains), and *E. sakazakii* (1 strain). [d]Includes: *S. liquefaciens* (5 strains) and *S. marcescens* (101 strains).

- *Enterobacter* spp.
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for *Enterobacter* spp. was >32 and 1 µg/mL, respectively (Table 1). Ceftazidime susceptibility was at 69.7/64.3% (CLSI/EUCAST interpretive criteria, respectively) whereas the MIC values for 95.5% of isolates were ≤1 µg/mL for ceftazidime-avibactam (Figure 1d).
  - There is no EUCAST ECOFF available for *Enterobacter* spp.; however, the ECOFF for *E. aerogenes* and *E. cloacae* was ≤1 µg/mL (Figure 1d).
  - In the case of *Enterobacter* spp., the ceftazidime MIC wild-type frequency distribution was similar to the distribution of contemporary (2009) clinical isolates. Avibactam reduced the MICs of ceftazidime to a unimodal distribution of lower MICs than the wild-type distribution (Figure 1d).
- *P. mirabilis*
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for *P. mirabilis* spp. was very low at 0.5 and 0.06 µg/mL, respectively (Table 1). Ceftazidime susceptibility was at 97.2/90.7% (CLSI/EUCAST interpretive criteria, respectively) whereas the MIC values for 100.0% of isolates were ≤0.25 µg/mL for ceftazidime-avibactam.
  - The MIC_{90} of ceftazidime-avibactam was 0.06 µg/mL, consistent with the ECOFF of ≤0.125 µg/mL (Figure 1e).
- *Serratia* spp.
  - The MIC_{90} for ceftazidime and ceftazidime-avibactam for *Serratia* spp. was 1 and 0.5 µg/mL, respectively (Table 1). Ceftazidime susceptibility was at 94.3/91.5% (CLSI/EUCAST interpretive criteria, respectively) while the MIC values for 94.3% of isolates were ≤1 µg/mL for ceftazidime-avibactam (Figure 1f).
  - The MIC_{90} of ceftazidime-avibactam (0.5 µg/mL) was consistent with the ceftazidime ECOFF of ≤0.5 µg/mL (Figure 1f).

Conclusions

- The ECOFF, which has been used effectively by EUCAST to identify the upper-end of the wild-type MIC distribution for organisms, provides a useful tool to understand the MIC range where a normal potentially treatable population of organisms resides.
- For *P. aeruginosa* and *Klebsiella* spp., the addition of avibactam converted the ceftazidime MIC distributions of contemporary clinical isolates to close to the corresponding EUCAST wild-type distributions.
- For *E. coli*, *Enterobacter* spp. and *P. mirabilis*, the addition of avibactam converted the ceftazidime MIC distributions of contemporary clinical isolates to distributions of MICs that were lower than the corresponding EUCAST wild-type distributions.
- MIC values of ceftazidime against *Serratia* spp. were somewhat lower than the corresponding EUCAST wild-type distribution except at higher MICs, where a less susceptible sub-population could be discerned. This sub-population was not apparent in the distribution of ceftazidime-avibactam MICs, which overlapped the EUCAST wild-type distribution (Figure 1f).
- The elevated ceftazidime MIC_{90} for Gram-negative bacteria from the 2009 European surveillance data was due to the presence of numerous resistant bacteria, many of which had MIC values for ceftazidime that could be decreased in the presence of the β-lactamase inhibitor avibactam.
- Ceftazidime-avibactam was able to restore the ceftazidime MIC_{90} for Gram-negative bacteria to within/below the ECOFF, a marker for the upper extent of the wild-type population.

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