

# Ceftazidime-Avibactam Disk Mass Studies for EUCAST Disk Diffusion Method Against *Enterobacteriaceae* and *Pseudomonas aeruginosa*

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## Abstract

**Objectives:** Ceftazidime-avibactam is a combination of the extended-spectrum cephalosporin, ceftazidime, and a novel non-β-lactam β-lactamase inhibitor, avibactam. Three studies were performed to compare zone diameters of various ceftazidime-avibactam (CAZ-AVI) disks to MIC results for determination of the optimal disk mass for testing of CAZ-AVI by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method. **Methods:** All studies used the EUCAST disk method and ISO 20776-1 MIC method (based on a fixed avibactam concentration of 4 μg/mL). In Study 1, 5 CAZ-AVI disks (ceftazidime at 10 μg and avibactam at 4, 6, 10, 15 and 20 μg) were tested against a challenge set of 73 *Enterobacteriaceae*, and 32 *Pseudomonas aeruginosa* (most of which were selected based on single or multiple β-lactamase genotypes, including 51 *Enterobacteriaceae* with multiple β-lactamase genotypes) and compared to CAZ-AVI MIC results. CAZ-AVI 10-4 and 10-6 μg disks were then selected for further study by 2 laboratories (Study 2). Each site tested a challenge set of 94 *Enterobacteriaceae* (29 non-ESBL, 46 ESBL, 6 AmpC, 8 carbapenem resistant, 4 NDM and 1 colistin resistant) and 45 *P. aeruginosa* (11 ceftazidime susceptible and 34 ceftazidime resistant) by disk and MIC methods. Study 3 was performed to assess the performance of CAZ-AVI 10-4 and 10-6 μg disks against wild type strains collected from patients (10 *E. coli*, 10 *K. pneumoniae* and 10 *P. aeruginosa*). The first 2 studies included the testing of piperacillin-tazobactam (disk content of 30-6 μg), and all studies included testing of 10 μg ceftazidime disk and quality control organisms *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853. **Results:** CAZ-AVI disk diameters (mm) by CAZ-AVI MIC (categorized by EUCAST ceftazidime MIC breakpoints) for Studies 1 and 2 were:

<i>Enterobacteriaceae</i>	MIC >4 mg/L		MIC = 2-4 mg/L		MIC ≤1 mg/L	
	Study 1 (n=16)	Study 2 (n=16*)	Study 1 (n=9)	Study 2 (n=18*)	Study 1 (n=48)	Study 2 (n=154*)
CAZ-AVI 10-4 μg	6-11	6-13	13-21	13-20	16-34	15-30
CAZ-AVI 10-6 μg	6-14	6-14	14-22	15-21	17-34	19-31
<i>P. aeruginosa</i>	MIC >8 mg/L		MIC ≤8 mg/L			
	Study 1 (n=6)	Study 2 (n=44*)			Study 1 (n=26)	Study 2 (n=46*)
CAZ-AVI 10-4 μg	6-7	6-12			9-24	11-25
CAZ-AVI 10-6 μg	6-7	6-14			10-24	10-28

\*duplicate disk results/strain (2 media manufacturers)

Among wild type isolates tested in Study 3, the range of CAZ-AVI 10-4 and 10-6 μg zones diameters were similar for 20 *Enterobacteriaceae* (21-27 mm) and 10 *P. aeruginosa* (16-26 mm).

**Conclusion:** Separation between susceptible and non-susceptible isolates was similar for 10-4 and 10-6 μg CAZ-AVI disks. The 10-4 μg disk was chosen by EUCAST because the lower avibactam concentration was preferred in order to avoid any possible effects of avibactam alone.

## Methods

Disk Diffusion Method: EUCAST (1) MIC Method: ISO 20776-1 (2)

Antimicrobial Agents:	Disks Tested (μg):
Ceftazidime (CAZ)	10 (Study 1: Mast, Study 2: LSI)
Ceftazidime-avibactam (CAZ-AVI)	10/4, 10/6, 10/10*, 10/15*, 10/20* (Study 1: AZ, Studies 2 & 3: LSI)
Piperacillin-Tazobactam	30/6 (Study 1: AZ, Study 2: BD)
*Tested in Study 1 Only	
CAZ-AVI tested by MIC with AVI at a fixed concentration of 4 mcg/mL	

Media: Study 1 and 3: BD MHA Study 2: BD and Remel MHA

Bacterial Isolates:

Study #	<i>Enterobacteriaceae</i>		<i>Pseudomonas aeruginosa</i>	
	No.	Description	No.	Description
1	73	70 with single or multiple β-lactamases, 3 β-lactamase neg.	32	31 with single or multiple β-lactamases, 1 β-lactamase neg.
2	94	29 non-ESBL, 46 ESBL, 6 AmpC, 8 carbapenem resistant, 4 NDM, 1 colistin resistant	45	11 ceftazidime susceptible, 34 ceftazidime resistant
3	20	10 <i>E. coli</i> , 10 <i>K. pneumoniae</i> , wild type (Sweden)	10	wild type (Sweden)

QC Strains: *E. coli* ATCC 25922 and ATCC 35218, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853

## Results

- 10-10, 10-15 and 10-20 CAZ-AVI disks were not considered for further study because zones for ceftazidime resistant *Enterobacteriaceae* were close to zones for susceptible isolates (up to 15-18 mm)
- Enterobacteriaceae*: CAZ-AVI 10-4 and 10-6 zones were 6-14 mm for all isolates with TAZ-AVI MICs >4 μg/mL, which included isolates with NDM, VEB-1 CMY-2 and AmpC β-lactamase genotypes (Figure 1a-d)
- P. aeruginosa*: CAZ-AVI 10-4 and 10-6 zones were 6-14 for all isolates with CAZ-AVI MICs >8 μg/mL, which included isolates with IMP, VIM, VEB, VEB-1a and PER-1 β-lactamase genotypes (Figure 1e-h)
- CAZ-AVI zones were similar for both media manufacturers (Figure 2)
- Among wild type strains CAZ-AVI 10-4 and 10-6 zones were 21-27 mm for *E. coli* and *K. pneumoniae* and 16-27 mm for *P. aeruginosa* (Figure 3)
- MIC and disk QC results for comparator agents were within expected ranges for all studies (Table 1).

## Results

Figure 1: Distribution of Ceftazidime-Avibactam Disk Diameter Zones by MIC\* (mg/L) for 10-4 and 10-6 μg disks

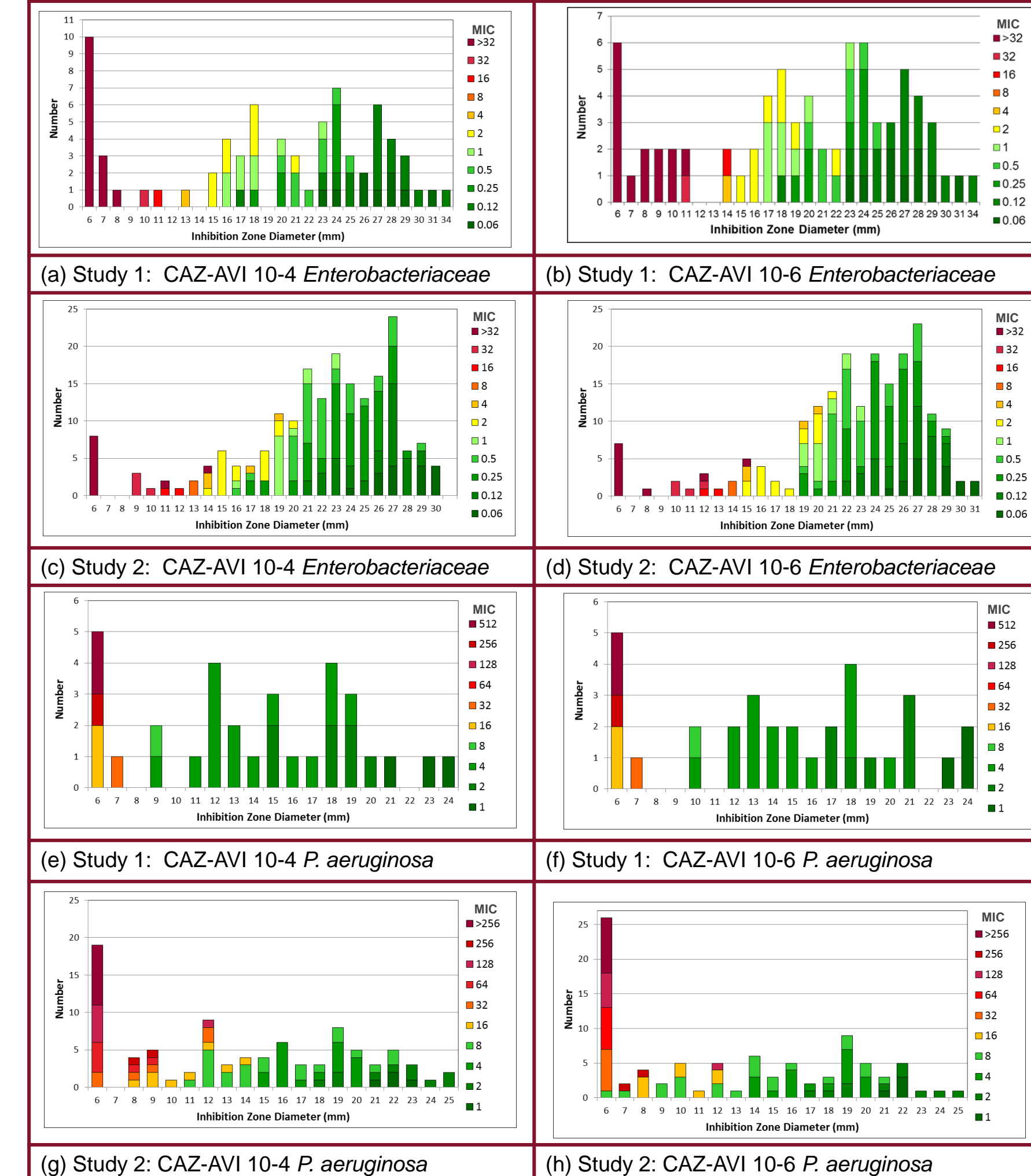


Figure 2: Distribution of CAZ-AVI 10-4 μg Disk Zones by Source of MHA (Study 2)

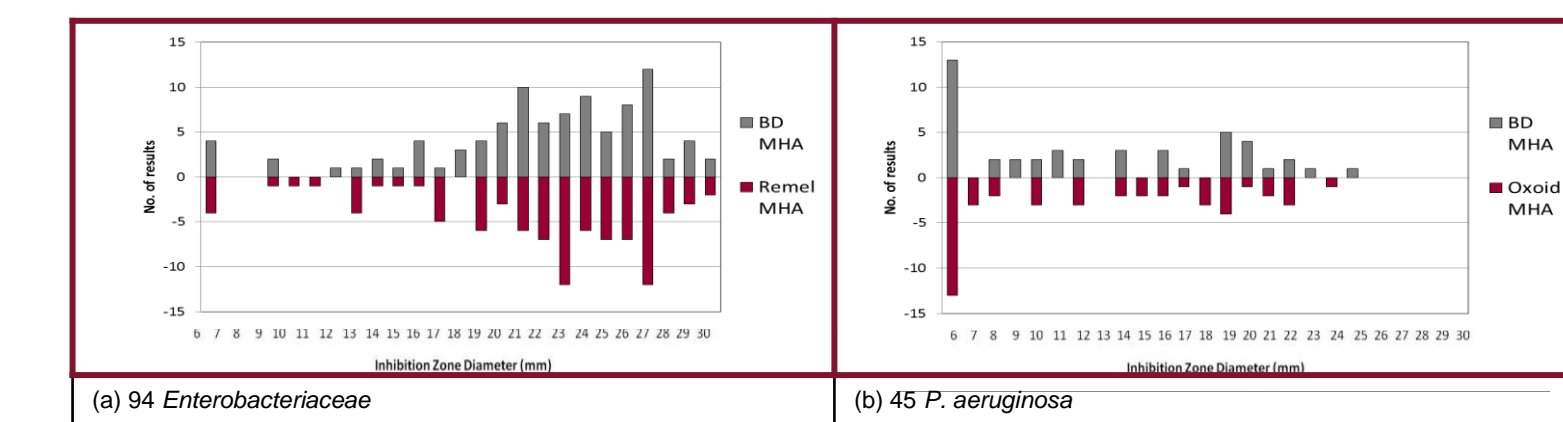


Figure 3: Distribution of CAZ-AVI disk zones for wild-type isolates (Study 3)

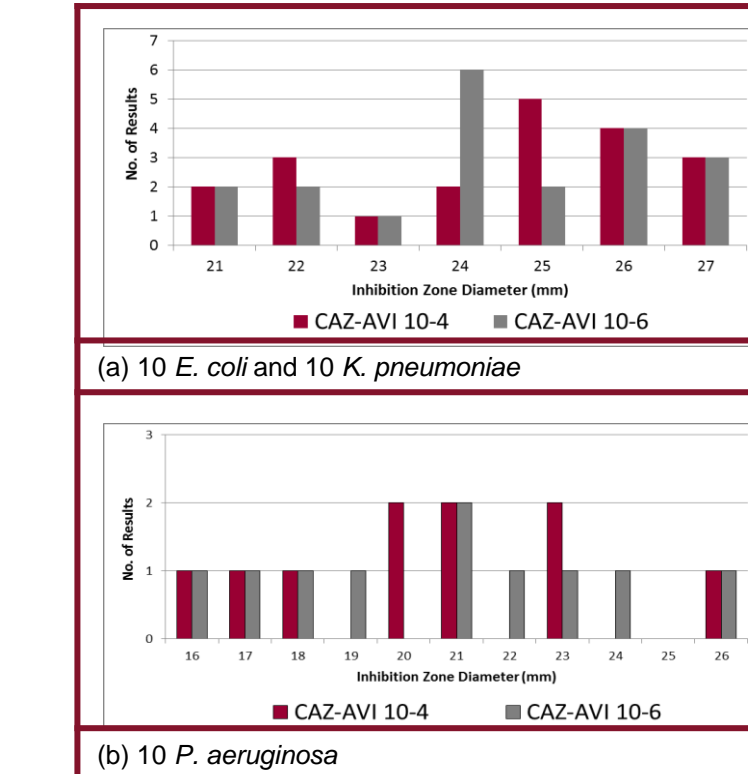


Table 1: Disk QC results (Studies 1-3)

	CAZ-AVI 10-6	CAZ-AVI 10-4	CAZ 10	
<i>E. coli</i> ATCC 25922	n	4	8	18
	Mean	26.75	26.50	26.67
	Range	26-27	25-27	25-29
Expected	NA	NA	23-29	
<i>E. coli</i> ATCC 35218	n	4	4	10
	Mean	27.75	28.25	27.50
	Range	26-29	27-29	27-28
Expected	NA	NA	NA	
<i>K. pneumoniae</i> ATCC 700603	n	4	8	18
	Mean	20.25	19.25	8.00
	Range	20-21	18-20	6-10
Expected	NA	NA	NA	
<i>P. aeruginosa</i> ATCC 27853	n	4	8	18
	Mean	23.25	22.13	23.22
	Range	22-25	20-26	21-26
Expected	NA	NA	21-27	

## Conclusions

- The 10-4 μg disk was chosen because there was no clear distinction between the 10-4 and 10-6 μg disks in these studies and EUCAST preferred the lower avibactam concentration to avoid any possible effects of avibactam alone.
- Subsequent studies were performed to determine EUCAST tentative QC ranges for the CAZ-AVI 10-4 μg disk.
- Subsequent studies will be performed to determine EUCAST disk breakpoints for the CAZ-AVI 10-4 μg disk.

## References

- EUCAST: [http://www.euca.org/antimicrobial\\_susceptibility\\_testing](http://www.euca.org/antimicrobial_susceptibility_testing)
- ISO: SO 20776-1 (2006) Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices ; <http://www.iso.org>.

## Disclosures

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