

## Abstract

**Background:** Ceftazidime-avibactam is a combination of the extended-spectrum cephalosporin, ceftazidime, and a non-β-lactam β-lactamase inhibitor, avibactam, which is being developed for the treatment of complicated urinary tract infection (cUTI), complicated intra-abdominal infection (cIAI) and nosocomial pneumonia. The objective of this study was to compare ceftazidime-avibactam MIC results obtained from three commercial methods (two gradient strip methods and a commercial lyophilized MIC broth method) and ceftazidime-avibactam 10-4 mcg disk diffusion results to reference broth microdilution (BMD) MIC results for a challenge set of *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates.

**Materials/methods:** Two ceftazidime-avibactam gradient strips (MIC Test Strip [MTS], Liofilchem) and Etest (bioMérieux), Sensititre lyophilized MIC panels (Trek, ThermoFisher) and reference broth microdilution panels (ISO) were used to determine MIC results for 82 *Enterobacteriaceae* and 29 *P. aeruginosa* at one testing site. Each isolate was also tested by disk diffusion with ceftazidime-avibactam 10-4 mcg disks (Becton Dickinson & Oxoid) on the same Mueller Hinton agar plates (Becton Dickinson) used for gradient strip testing. Quality control (QC) organisms, *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603, were also tested on each test day.

**Results:** Essential agreements (within +/- one doubling dilution) for the commercial MIC results compared to reference BMD results were 100%, 100% and 95.4% (Trek, Etest and MTS, respectively) for *Enterobacteriaceae* and 97.1% for all three methods for *P. aeruginosa*. Overall, there was good correlation of ceftazidime-avibactam 10-4 disk results to MIC (final category agreement analysis pending breakpoint determination) and between the two disk manufacturers (96% within +/- 2mm for all study isolates). BD and Oxoid 10-4 mean disk zones for *Enterobacteriaceae* were 17.2 and 16.6 mm and for *P. aeruginosa* were 14.2 and 14.1 mm, respectively. QC results for all MIC and disk testing were within established ranges.

**Conclusion:** The ceftazidime-avibactam MTS, Etest and Trek MIC panel performed comparable to reference BMD in this one site study. Ceftazidime-avibactam disk results were similar between BD and Oxoid 10-4 disks and overall demonstrate correlation to MIC results.

## Methods

**Isolates:** A challenge set of *Enterobacteriaceae* and *P. aeruginosa*, some with known resistance mechanisms and many with CAZ-AVI MIC results above or near the US CAZ-AVI FDA susceptible breakpoint of 8/4 µg/mL (1).

Species	No.	Species	No.	Species	No.
<i>Citrobacter freundii</i>	3	<i>Escherichia coli</i>	27	<i>Morganella morganii</i>	4
<i>Enterobacter aerogenes</i>	3	<i>Klebsiella oxytoca</i>	4	<i>Proteus mirabilis</i>	2
<i>Enterobacter cloacae</i>	6	<i>Klebsiella pneumoniae</i>	28	<i>Serratia marcescens</i>	6
		<i>P. aeruginosa</i>	29		
<b>Quality Control Strains:</b>					
<i>E. coli</i> ATCC 25922		<i>P. aeruginosa</i> ATCC 27853		<i>K. pneumoniae</i> ATCC 700603	

**Testing Site:** Laboratory Specialists, Inc., Westlake, OH.

**MIC Method:** Each isolate was tested once by each method (shown below) using the same initial inoculum. 150 mm prepared MHA plates from BD were used for disk and gradient strip testing.

Product Name	Supplier
Reference MIC Panel, CAZ-AVI 0.016/4-256/4 µg/mL (2, 3)	LSI, Westlake OH
Trek Custom MIC Panel, CAZ-AVI 0.03/4-64/4 µg/mL	Thermo-Fisher, E. Grinstead UK
MIC Testing Strip (MTS), CAZ-AVI 0.016/4-256/4 µg/mL	Liofilchem, Roseto degli Abruzzi Italy
Etest, CAZ-AVI 0.016/4-256/4 µg/mL	bioMérieux, Marcy l'Etoile France
CAZ-AVI 10/4 µg disk	Becton Dickinson, Sparks MD
CAZ-AVI 10/4 µg disk	Oxoid, Basingstoke UK

## Results

Table 1 (A-B): CAZ-AVI MIC Dilution Difference

Table 1A: <i>Enterobacteriaceae</i>											Table 1B: <i>P. aeruginosa</i>															
MIC Method	-2	-1	0	1	2	>1*	OS >	Total Eval	Total ALL	EA (Eval)	EA (Eval.+OS>)	MIC Method	-2	-1	0	1	2	>1*	>2*	>3*	OS >	Total Eval	Total ALL	EA (Eval)	EA (Eval.+OS>)	
Trek	2	33	35	2				13	72	85	97.2%	100.0%	Trek		12	11	7					1	30	31	100.0%	100.0%
Etest	1	7	38	25	1	4	10	72	86	97.2%	93.0%	Etest		4	15	5						1	24	31	100.0%	80.6%
MTS	1	12	34	24	1	3	11	72	86	97.2%	96.5%	MTS	1	6	11	6						1	24	31	95.8%	77.4%

\*Result for one method was >highest concentration tested  
OS >: Off-Scale; results for both methods > highest concentration tested

Eval: on scale results (not greater or less than concentrations tested)  
EA: Essential Agreement (within +/- one dilution compared to reference BMD)

Figure 1 (A-F): CAZ-AVI MIC Scatterplots

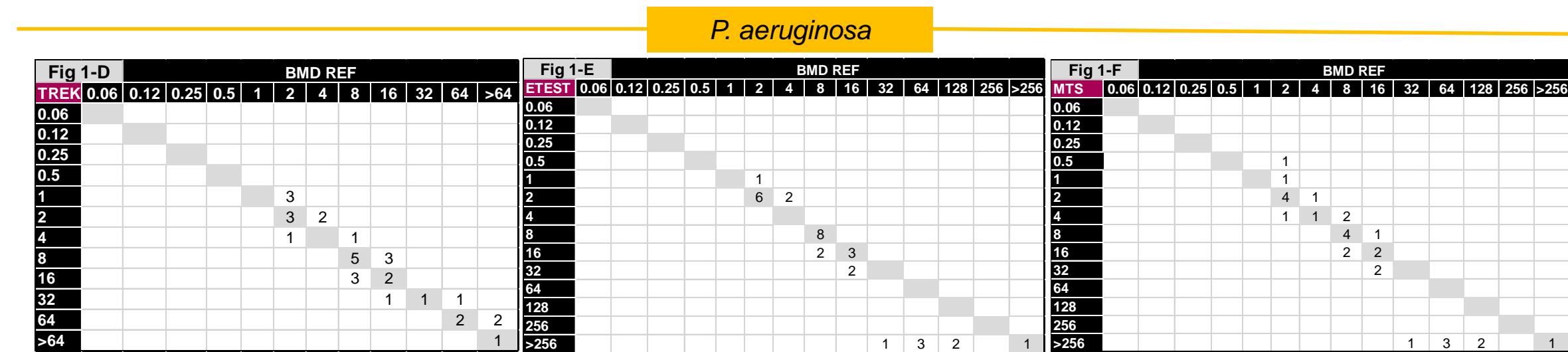
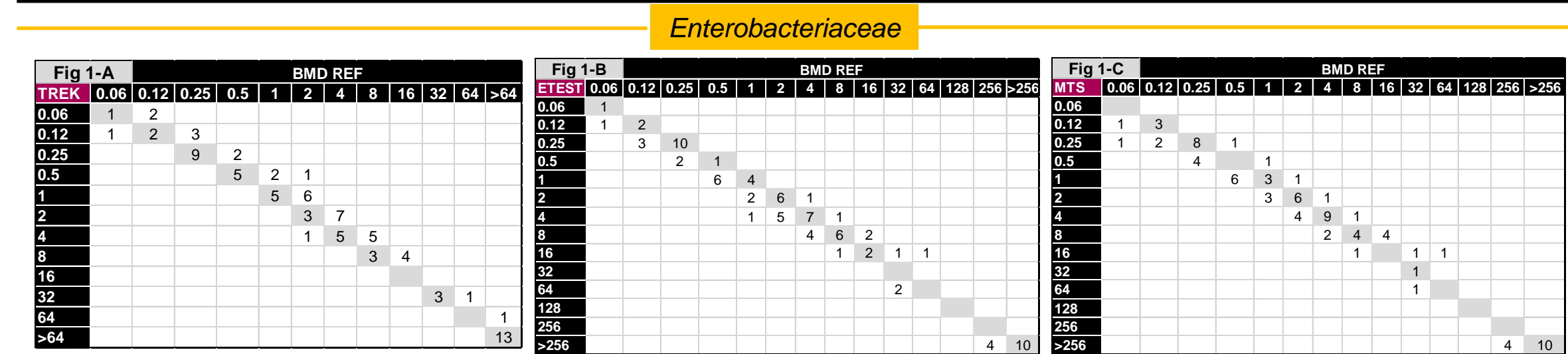


Table 2: Comparison of CAZ-AVI Disks (BD-OX)

Organism	-3	-2	-1	0	1	2	3	4	Total	% ±2 mm	% ±1 mm
<i>Enterobacteriaceae</i>		1	5	31	35	8	3		83	96.4%	85.5%
<i>P. aeruginosa</i>		2	7	11	6	2		1	29	96.6%	82.8%

Table 3: CAZ-AVI Quality Control Results

QC Strain	Expected MIC Range	REF BMD	TREK	ETEST	MTS	10-4 Disk Expected Range	10-4 BD	10-4 OX
<i>E. coli</i> ATCC 25922	0.06-0.5	0.12	0.12	0.25	0.25	24-30	27	27
<i>K. pneumoniae</i> ATCC 700603	0.25-2	0.5	0.5	1	1	18-24	21	20
<i>P. aeruginosa</i> ATCC 27853	0.5-4	2	2	2	2	21-27	22	22

## MIC

- Overall there was good correlation of all CAZ-AVI MIC methods to the reference BMD method (Table 1A-B, Figures 1A-F).
- There was a slight trend to lower Trek CAZ-AVI MIC results compared to reference BMD MICs among *Enterobacteriaceae* (Table 1A, Figure 1A).
- There was a slight trend to higher CAZ-AVI Etest and MTS MIC results compared to reference BMD MIC results among *Enterobacteriaceae* (Table 1A, Figures 1B-C).
- The CAZ-AVI BMD MIC results for 5 *P. aeruginosa* (3 VEB-1a positive and 2 carbapenem resistant) were between 32/4-128/4 µg/mL and Etest and MTS MIC results were >256/4 µg/mL (Figures 1E, 1F).

## Disk

- There was good correlation of CAZ-AVI BD and Oxoid 10-4 disk zones for both *Enterobacteriaceae* and *P. aeruginosa* (Table 2, 96% within +/- 2mm).
- There was a trend to slightly higher CAZ-AVI BD disk zones compared to Oxoid disk zones among *Enterobacteriaceae* (Table 2; 43% of BD zones were 1-2 mm larger than Oxoid zones.).
- CAZ-AVI MIC and 10/4 disk zones demonstrated good correlation (Figure 2).

## Conclusions

The ceftazidime-avibactam Trek MIC panel and two gradient strips (MTS, Etest) performed comparably to reference BMD against a challenge set of *Enterobacteriaceae* and *P. aeruginosa* in this one site study. Further evaluation with additional isolates, Mueller Hinton agar (for gradient diffusion methods) and testing sites is warranted.

Ceftazidime-avibactam 10-4 disk results were similar between BD and Oxoid disks and overall the zone diameters correlate to MIC results, with assessment of categorical agreement awaiting determination of EUCAST ceftazidime-avibactam MIC and disk breakpoints.

## References

- [http://www.accessdata.fda.gov/drugsatfda\\_docs/label/2015/206494s000lbl.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206494s000lbl.pdf)
- EUCAST: [http://www.eucast.org/antimicrobial\\_susceptibility\\_testing](http://www.eucast.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>.

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