Correlation between Broth Microdilution and Disk Diffusion Results When Testing Ceftazidime-Avibactam against a Challenge Collection of *Enterobacterales*: Results from a Multi-Laboratory Study

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INTRODUCTION

- Clinical and Laboratory Standards Institute (CLSI) ceftazidime-avibactam breakpoints for *Enterobacterales* are ≤8/≥16 mg/L (susceptible [S]/resistant [R]) for MICs and ≥21/≤20 mm for disk diffusion (30/20-μg disk)
- According to CLSI guidelines (M23 document, 2018), disk diffusion breakpoints are established after MIC breakpoints have been determined by plotting a scattergram of zone diameters versus MIC values for isolates tested by both methods; thus, the zone diameter interpretative criteria that provide the lowest inter-method error rates (or discrepancy rates) are determined by the error-rate-bound statistical method
- Current ceftazidime-avibactam disk diffusion breakpoints were established with a very small number of carbapenem-resistant *Enterobacterales* (CRE) isolates, and there are reports of high rates of major errors (ME) when testing CRE isolates
- The objective of this study was to evaluate the accuracy of current CLSI ceftazidime-avibactam disk diffusion breakpoints by testing a large challenge collection of *Enterobacterales* isolates containing a high proportion of CRE and isolates with ceftazidime-avibactam MIC values near (+/- 1 doubling dilution) the breakpoints

MATERIALS AND METHODS

Organisms and susceptibility testing

- 112 *Enterobacterales* isolates were tested by broth microdilution and disk diffusion methods in 3 labs
  - 100 isolates from JMI Laboratories were selected to maximize the number of isolates with ceftazidime-avibactam MIC values near the breakpoints (≤8 mg/L for susceptible and ≥16 mg/L for resistance)
    - 20% of isolates at 8 mg/L or 16 mg/L (at the breakpoints)
    - 50% of isolates at 4–32 mg/L (± 1 doubling dilution of the breakpoints)
  - 12 CRE isolates from the University of Pittsburgh were responsible for major errors (Shields *et al.* 2018)
- Participating labs
  - JMI Laboratories
  - University of California at Los Angeles (UCLA)
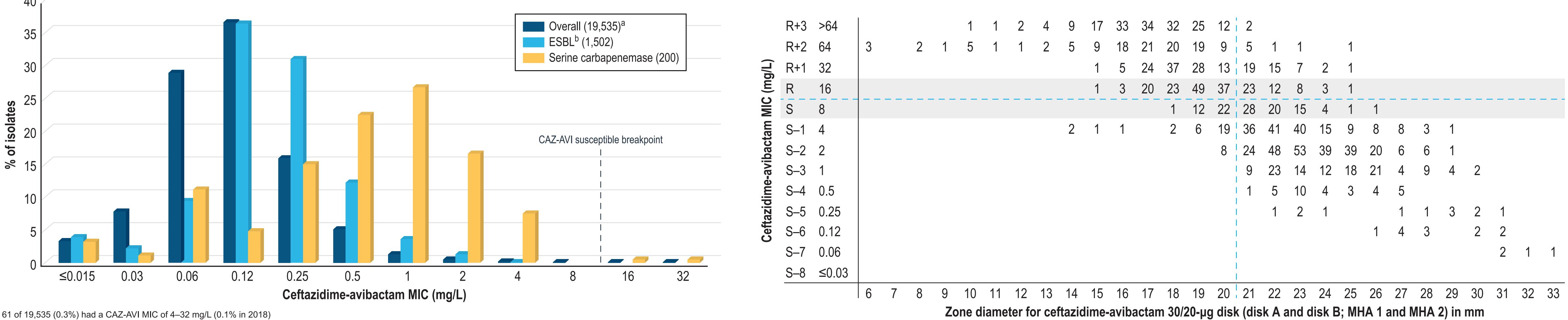 - University of Iowa, Iowa City, Iowa

- 4 disk diffusion results were determined for each MIC result per lab
  - 12 disk results for each isolate – a total of 1,344 results for 112 isolates
  - Ceftazidime-avibactam 30/20-μg disks were obtained from Hardy Diagnostics (Santa Maria, CA) and BD (Franklin Lakes, NJ)
  - Mueller-Hinton agar was obtained from Remel (San Diego, CA) and Hardy Diagnostics

Data analysis

- Discrepancy rates between MIC values and zone diameter test results were calculated according to the CLSI M23 (2018) document
- Discrepancies involving false susceptible disk results were defined as very major (VM) errors, whereas false resistant disk diffusion results were defined as major (Ma) errors
- Optimal disk breakpoints were determined by the error-rate-bound method according to CLSI M23 (2018) using software developed by JMI Laboratories based on dBETS software

Molecular characterization of selected isolates

- A total of 84 isolates were screened for β-lactamases, 64 (76.2%) were submitted to whole genome sequencing, and the remaining 20 isolates were evaluated by microarray, PCR, or WGS as previously described

RESULTS

- Among *Enterobacterales* isolates evaluated in this study, ceftazidime-avibactam MIC results were 8 mg/L or 16 mg/L for 21.1%, and 4–32 mg/L for 46.7% of the isolates; meropenem MIC values were ≥2 mg/L (nonsusceptible) for 76.2% of isolates
- Among 19,535 *Enterobacterales* isolates collected by the ceftazidime-avibactam surveillance program (INFORM Program) in the United States in 2016–2017, only 4 isolates (0.02%) were ceftazidime-avibactam resistant and only 61 (0.3%) had ceftazidime-avibactam MIC values of 4–32 mg/L (Figure 1)
- Current CLSI and US Food and Drug Administration disk diffusion breakpoints for ceftazidime-avibactam (≥21/≤20 mm for S/R) provided the lowest error rates with 12.1% VM (false S) for ≥R+1 and 16.6% for S+R (overall VM error rate of 7.5%), and 12.3% major error rate (false R) for S+R and 6.4% for ≤S-1 (5.5% overall Ma error rate; Table 1 and Figure 2)

- Overall, 46.9% and 89.7% of the errors were observed among MIC values of 8–16 mg/L and 4–32 mg/L, respectively (data not shown)
- Good correlations were noted between MICs and disk diffusion results among the 3 participating labs (data not shown)
- No major differences in error rates were observed between commercial agar lots or disk manufacturers (data not shown)
- Minor error rates were elevated for the comparator compound meropenem, with 9.4% for ≥I+2, 41.8% for I±1, and 4.2% for ≤I-2 (15.5% overall; data not shown)
- Carbapenemases were observed in 67 isolates, including KPC (34), NDM (5), VIM (20), IMP (4), OXA-48 (5), and NMC-A (1)

CONCLUSIONS

- Current ceftazidime-avibactam disk diffusion breakpoints appeared appropriate to minimize discrepancy errors
- The vast majority of discrepancy errors occurred with MIC values ±1 dilution of the breakpoints (4–32 mg/L), which are extremely rare among *Enterobacterales* in the United States

ACKNOWLEDGMENTS

Table 1. Possible scattergram error rates, based on the error-rate-bound method, of ceftazidime-avibactam 30/20-μg disk for isolates from all participating centers combined

Disk breakpoints	Range	Number	Very major (%)	Major (%)	Minor (%)
≥18 mm (S)/≤17 mm (R)	≥R+1	448	249 (55.58)	N/A	0
	S+R	284	156 (54.93)	0	0
	≤S-1	612	N/A	4 (0.65)	0
	Total	1,344	405 (30.13)	4 (0.3)	0
≥19 mm (S)/≤18 mm (R)	≥R+1	448	160 (35.71)	N/A	0
	S+R	284	133 (46.83)	1 (0.35)	0
	≤S-1	612	N/A	6 (0.98)	0
	Total	1,344	293 (21.8)	7 (0.52)	0
≥20 mm (S)/≤19 mm (R)	≥R+1	448	88 (19.64)	N/A	0
	S+R	284	84 (29.58)	13 (4.58)	0
	≤S-1	612	N/A	12 (1.96)	0
	Total	1,344	172 (12.8)	25 (1.86)	0
≥21 mm (S)/≤20 mm (R)	**≥R+1**	**448**	**54 (12.05)**	**N/A**	**0**
	S+R	**284**	**47 (16.55)**	**35 (12.32)**	**0**
	≤S-1	**612**	**N/A**	**39 (6.37)**	**0**
	Total	**1,344**	**101 (7.51)**	**74 (5.51)**	**0**
≥22 mm (S)/≤21 mm (R)	≥R+1	448	28 (6.25)	N/A	0
	S+R	284	24 (8.45)	63 (22.18)	0
	≤S-1	612	N/A	109 (17.81)	0
	Total	1,344	52 (3.87)	172 (12.8)	0
≥23 mm (S)/≤22 mm (R)	≥R+1	448	12 (2.68)	N/A	0
	S+R	284	12 (4.23)	83 (29.23)	0
	≤S-1	612	N/A	227 (37.09)	0
	Total	1,344	24 (1.79)	310 (23.07)	0
≥24 mm (S)/≤23 mm (R)	≥R+1	448	4 (0.89)	N/A	0
	S+R	284	4 (1.41)	98 (34.51)	0
	≤S-1	612	N/A	346 (56.54)	0
	Total	1,344	8 (0.6)	444 (33.04)	0

Current breakpoint in bold.

Figure 1 Ceftazidime-avibactam MIC distributions of *Enterobacterales* isolates from the INFORM Program (2016–2017)

[a] 61 of 19,535 (0.3%) had a CAZ-AVI MIC of 4–32 mg/L (0.1% in 2018)
[b] ESBL, extended-spectrum β-lactamase

Figure 2 Scattergram of disk inhibition zones vs MIC values and table of error rates of ceftazidime-avibactam MIC vs ceftazidime-avibactam 30/20-μg disk for isolates from all participating centers combined when current CLSI breakpoints (broken lines; S at ≥21 mm and R at ≤20 mm) for disk were applied

Scattergram and error rate table for the optimal calculated disk breakpoints of ≥21 mm (S) and ≤20 mm (R).

MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥R+1	448	54 (12.05)	N/A	0
S+R	284	47 (16.55)	35 (12.32)	0
≤S-1	612	N/A	39 (6.37)	0
Total	1,344	101 (7.51)	74 (5.51)	0

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