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Determining Effect of Media and Disk Source on Reproducibility and Relationship between Broth Microdilution and Disk Diffusion Testing of Cefiderocol

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INTRODUCTION

- Cefiderocol (CFDC) is a siderophore cephalosporin with activity against a wide variety of Gram-negative bacteria, including carbapenemase-producing isolates.
- In this study susceptibility testing was performed using the broth microdilution (BMD) and disk diffusion methods with isolates that have been evaluated in the murine thigh infection model using a humanized CFDC PK profile [3] to assess reproducibility across different media and disks.

MATERIALS AND METHODS

• Bacterial strains

- 12 Enterobacterales (5 *E. coli* and 7 *K. pneumoniae*), 12 *P. aeruginosa*, and 12 *A. baumannii* which had been evaluated in the murine thigh infection model using a humanized CFDC PK profile [1] were used.

• MIC determination

- MIC values of CFDC were determined by BMD using iron-depleted cation-adjusted Mueller Hinton broth (ID-CAMHB) as recommended by CLSI over three days with10 replicates per media per isolate per day. ID-CAMHB was prepared from Mueller Hinton broth sourced by Becton Dickinson© (BD) BBL, BD Difco, Oxoid and Merck and iron content of all ID-CAMHBs was confirmed to be ≤0.03 mg/L.
- Susceptibility was interpreted according to 2023 CLSI breakpoints.

• Disk diffusion

- The zone of inhibition was determined with CFDC disks from Hardy and Liofilchem using Mueller Hinton Agar from BD BBL and bioMerieux over three days with 3 replicates per media/disk per isolate per day. The inoculum that was used for BMD was used in the disk diffusion studies.
- Susceptibility was interpreted according to 2023 CLSI breakpoints.

REFERENCES

1. Monogue ML et al., Antimicrob Agents Chemother 61: e01022-17, 2017.

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RESULTS

• MIC determination

- Good reproducibility of MIC was observed for the ID-CAMHB from each manufacturer for all isolates, except for two *A. baumannii* which showed heavy trailing and skipped wells (**Fig. 1**).
- MIC variation was observed between ID-CAMHB from each manufacturer (**Fig. 2**)
  - ➢ Compared with the MIC determined in BD BBL medium, the MIC determined in BD Difco medium were within ±1-dilution for 30 of 36 isolates.
  - ➢ On the other hand, the MIC determined in Oxoid and Merck medium showed larger variations with MIC determined in BD BBL, with only 22 and 19 of 36 isolates showing MIC within ±1-dilution, respectively.
- MIC values obtained with ID-CAMHB sourced from BD BBL and BD Difco showed best categorical agreement with *in vivo* efficacy (**Table 1**)

• Disk diffusion

- Good reproducibility of disk inhibition zones was obtained for Enterobacterales and *P. aeruginosa* irrespective of the manufacturer of disks and agar medium. The relationship between MIC and disk zone was also good, although 4 of 24 isolates showed minor categorical errors (**Fig. 3**)
- *A. baumannii* isolates with elevated MIC values frequently showed colonies within the inhibition zones, with more colonies appearing on BBL agar (**Fig. 4**). This phenomenon was not reproducible, so larger variation of inner inhibition zones were observed with these isolates (**Fig. 5**)

Figure 1. MIC reproducibility in ID-CAMHB from different manufacturers

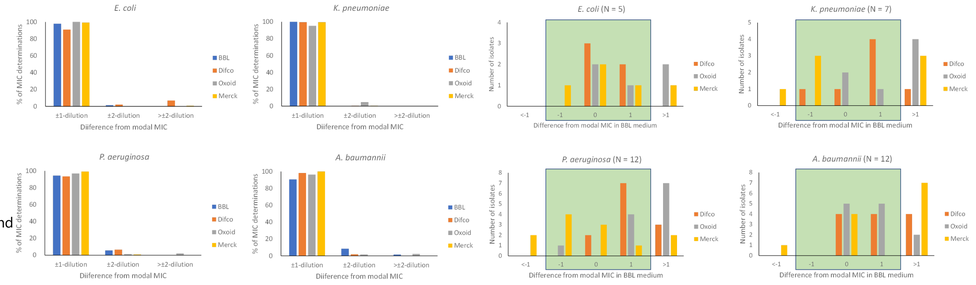


Figure 2. Modal MIC variation in ID-CAMHB between different manufacturers

Table 1. Relationship between modal MIC from each manufacturer and *in vivo* efficacy in murine thigh infection models under humanized PK

Media	log CFU reduction *in vivo* ("S")		log CFU increase *in vivo* ("R")*		Agreement of *in vivo* CFU response with *in vitro* S/I/R
	MIC ≤4 μg/mL (S)	MIC ≥8 μg/mL (I/R)	MIC ≤4 μg/mL (S)	MIC ≥8 μg/mL (I/R)	
Historical (BBL)	25/25 (100%)	0/25 (0%)	0/10 (0%)	10/10 (100%)	35/35 (100%)
BBL	24/25 (96%)	1/25 (4%)	3/10 (30%)	7/10 (70%)	31/35 (89%)
DIFCO	22/25 (88%)	3/25 (12%)	2/10 (20%)	8/10 (80%)	30/35 (86%)
MERCK	19/25 (76%)	6/25 (24%)	3/10 (30%)	7/10 (70%)	26/35 (74%)
OXOID	19/25 (76%)	6/25 (24%)	2/10 (20%)	8/10 (80%)	27/35 (77%)

*A. baumannii AB97 omitted from analysis due to difficulties determining modal MIC in several media

Figure 3. Zone of inhibition observed with CFDC disks for each isolate of Enterobacterales and *P. aeruginosa*

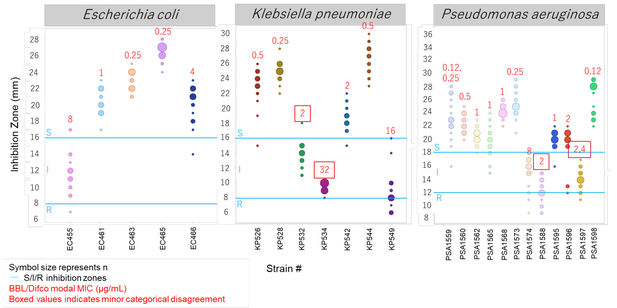


Figure 4. Micro-colony appearance within the zone of inhibition for CFDC-resistant *A. baumannii* isolates

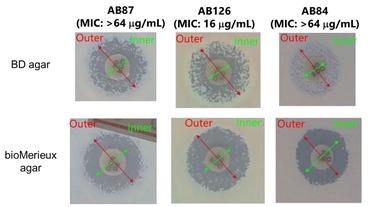
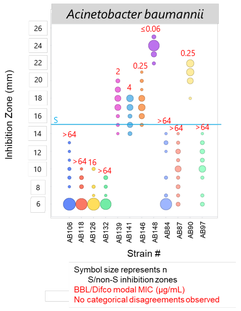


Figure 5. Zone of inhibition observed with CFDC for each isolate of *A. baumannii*



CONCLUSION

- MIC determinations were highly reproducible for each ID-CAMHB medium, but different ID-CAHMBs produced different MIC values for isolates, despite all ID-CAMHB showing an iron content ≤0.03 mg/L.
- MIC values obtained with BD BBL ID-CAMHB and BD Difco ID-CAMHB are deemed the most relevant, as those showed the best correlation with *in vivo* data. **ID-CAMHB sourced from BD BBL and BD Difco are recommended for measuring CFDC values.**
- Zones of inhibition were reproducible irrespective of the manufacturer of disks and agar medium, and overall good categorical agreement was observed between the MICs determined in BD BBL and BD Difco medium and disk inhibition zone.
- The (irreproducible) appearance of micro colonies within the zone of inhibition, most frequently observed for *A. baumannii* isolates with elevated MIC values, complicates measuring the inhibition zone and its interpretation in the presence of micro-colonies.