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Note

Comparison of available methods to evaluate cefiderocol susceptibility in *Acinetobacter* spp

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ABSTRACT

Recently, considerable uncertainty has arisen concerning the appropriate susceptibility testing for cefiderocol in gram-negative bacilli, particularly in the context of its application to *Acinetobacter* spp. The optimal method for assessing the susceptibility levels of *Acinetobacter* spp. to cefiderocol remains a subject of debate due to substantial disparities observed in the values obtained through various testing procedures. This study employed four minimum inhibitory concentration (MIC) methodologies and the disk diffusion to assess the susceptibility of twenty-seven carbapenem resistant (CR)-*Acinetobacter* strains to cefiderocol. The results from our study reveal significant variations in the minimum inhibitory concentration (MIC) values obtained with the different methods and in the level of agreement in interpretation categories between the different MIC methods and the disk diffusion test. Among the MIC methods, there was relatively more consistency in reporting the interpretation categories. For European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, the categorical agreement (CA) for MIC methods ranged between 66.7 and 81.5%. On the other hand, the essential agreement (EA) values were as low as 18.5–29.6%. The CA between MIC methods and disk diffusion was 81.5%. These results emphasize the need for a reliable, accurate, and clinically validated methodology to effectively assess the susceptibility of *Acinetobacter* spp. to cefiderocol. The wide variability observed in our study highlights the importance of standardizing the susceptibility testing process for cefiderocol to ensure consistent and reliable results for clinical decision-making.

1. Introduction

Acinetobacter baumannii is a Gram-negative bacillus that often causes infections in critically ill patients, particularly those with compromised immune systems (Piperaki et al., 2019). The emergence of carbapenem-resistant *A. baumannii* (CRAB) is a significant global health concern. The lack of effective treatments has elevated this bacterium to the status of a "critical priority pathogen" (Centers for Diseases Control and Prevention, 2019). Furthermore, the rapid dissemination of difficult-to-treat (DTR) *A. baumannii* strains emphasizes the urgent need for new antimicrobial therapies (Ramirez et al., 2013; Castanheira et al., 2023). However, efforts by researchers and pharmaceutical companies have generally met with less-than-ideal results (He et al., 2015; Theuretzbacher et al., 2020; Paterson et al., 2020; Watkins and Bonomo,

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2023). One exception is represented by the recently the Food and Drug Administration (FDA)-approved cefiderocol, a drug with demonstrated activity against CRAB (He et al., 2015; Theuretzbacher et al., 2020). A limitation in managing the use of this antimicrobial is the discrepancies and difficulties observed when determining susceptibility levels (Bonnin et al., 2022; Brauncajs et al., 2024; Liu et al., 2023).

The minimum inhibitory concentration (MIC) determination using broth microdilution (BMD) with cation-adjusted iron-depleted Mueller-Hinton medium (ID-CAMHB) is considered the "gold standard" method for evaluating cefiderocol susceptibility (Clinical and Laboratory Standards Institute (CLIS), 2023). However, preparing the ID-CAMHB for routine use in clinical microbiology laboratories is challenging and time-consuming. Significant differences in values are usually reported in tests carried out by different laboratories or in repeated determinations within the same laboratory (Clinical and Laboratory Standards Institute (CLIS), 2023). There are currently three commercial kits to determine susceptibility, two of them are based on BMD (ComASP® and UMIC® panel) and the one on gradient diffusion *E*-strips (Liofilchem S.r.l., Roseto degli Abruzzi, Italy). EUCAST recommends laboratories to test cefiderocol resistance levels with disk diffusion (DD). According to this standard, when correctly performed and calibrated using quality material and recommended quality control guidelines, DD predicts susceptibility and resistance: zone diameters $\geq$ 17 mm correspond to MIC values below the pharmacokinetics (PK)/ pharmacodynamics (PD) breakpoint of susceptible $\leq$ 2 mg/L. (https://www.eucast.org/eucast_news/news_singleview?tx_ttnews%5Btt_news%5D=493&cHash=22779384b74c8cf2c55aa3f7fd69d173).

The assessment of susceptibility levels of *Acinetobacter* spp. to cefiderocol remains controversial due to significant discrepancies observed in values derived from different testing procedures. This study seeks to identify the most suitable method and the discrepancies among four MIC methodologies and DD by testing the susceptibility of twenty-seven carbapenem-resistant (CR) *Acinetobacter* strains to cefiderocol to reduce potential biases inherent in the analysis.

2. Material and methods

2.1. Bacterial strains

A total of 27 CR-*Acinetobacter* clinical, including 19 *A. baumannii* (12 NDM-1 + PER-7, 3 NDM-1, 1 OXA-23, 1 OXA-23 + PER-7, 1 OXA-58 and 1 dual carbapenemase producer of NDM-1 + OXA-23) and 8 *A. non-baumannii* (6 NDM-1, 1 IMP-1, and 1 OXA-23) strains were used to test cefiderocol susceptibility by four different methods (Table S1). PCR and whole genome sequencing were used to profile the genomes of the isolates.

2.2. Antibiotic susceptibility testing (AST)

To determine the cefiderocol susceptibility and compare the obtained results, five different methods, commercial MTS™ (MIC Test Strip) (Liofilchem S.r.l., Roseto degli Abruzzi, Italy), iron-depleted cation adjusted Mueller-Hinton broth (BMD), agar-dilution (BD-difco, Becton Dickson and company, Heidelberg, Germany) (ADIL), ComASP® ((Liofilchem S.r.l.), and the commercial DD method using cefiderocol (FDC) 30 μg disk (Liofilchem S.r.l.) and cation adjusted Mueller-Hinton agar (BBL Mueller Hinton II agar) (Becton Dickson and company), were evaluated. The iron-depleted cation adjusted Mueller-Hinton broth was prepared following the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLIS), 2023). The methods were performed according to the manufacturer's instructions and EUCAST PK-PD breakpoints (https://www.eucast.org/clinical_breakpoints). "Trailing" in the BMD test (multiple wells of tiny or faint growth relative to the growth control) was ignored. Zone diameters were determined using the colony-free inner zone.

Escherichia coli ATCC 25922 was used for quality control purposes. In addition, *A. baumannii* ATCC 17978 (cefiderocol susceptible) and two cefiderocol resistant strains (AMA16 and AMA33) were also used as internal quality control and to assess inter-assay reproducibility of the different methods by quintuplicates.

Values whose interpretation within the categories "susceptibility" or "resistance" agree with those established by EUCAST PK-PD standards were defined as "categorical agreement (CA)." Essential agreement (EA) was defined as MIC variation up to 1-fold. As recommended in the ISO 20776-2:2021 document, essential agreement (EA) and bias were calculated to evaluate the performance of the tested methods. Congruent expected performances were: EA $\geq$ 90%, $-30\% \leq$ bias $\leq +30\%$. Rates of categorical agreement (CA), major errors (ME), and very major errors (VME) were also calculated following the definitions from ISO 20776-2:2007.

3. Results and discussion

Firstly, all methodologies underwent validation for repeatability, with the quality control strains consistently yielding identical categorical results for each respective method. Using the reference method (BMD), we found that 9 out of 27 isolates (33.3%) had MIC values of cefiderocol $\leq$2 mg/L, susceptible according to the EUCAST breakpoints (Fig. 1 and Table S1). Fig. 1A illustrates the disparities in MIC values for cefiderocol susceptibility among different methods. Compared to BMD, alternative MIC methods yielded EA values between 18.5 and 29.6%. A lower impact was observed on interpretation categories, with CA values ranging from 66.7 to 74.1%, reaching the lowest for agar-based MIC methods (gradient strips and agar dilution). DD demonstrated a CA of 81.5%, with only major errors detected (22.2%). Four out of 5 MEs were associated with genomospecies other than *A. baumannii*, including 2 *A. pittii* and 2 *A. nosocomialis* NDM-producing isolates. The distribution of BMD cefiderocol MIC values relative to zone diameters is shown in Fig. 1B. (See Table 1.)

Cefiderocol susceptibility testing poses a significant challenge for clinical microbiologists, as the reference BMD requires ID-CAMHB. There is substantial variability in reports of the performance of cefiderocol AST for *A. baumannii*. An extensive study of OXA-producing CRABs recently reported CA (84–88%) and EA (44–75%) values for ComASP® and gradient strips higher than those observed in our study (Kolesnik-Goldmann et al., 2023). These differences might be explained, at least partially, by the heteroresistance to cefiderocol associated with *A. baumannii* NDM producers (Le et al., 2022), a characteristic of the subpopulation used in our study. In addition, numerous reports indicate that the lack of susceptibility to cefiderocol in NDM-producing *Acinetobacter* isolates (44.7%) is significantly higher than in those harboring other β-lactamase genes (13.2%) (Karakonstantis et al., 2023). Nevertheless, our study focused on scrutinizing a panel encompassing all MIC ranges for cefiderocol. Our work aimed to counteract potential biases inherent in the analysis.

Considering the constraints, EUCAST recommends starting cefiderocol testing using DD, which has demonstrated reliable predictive accuracy for susceptibility and resistance (Matuschek et al., 2022). DD has shown robustness for CRAB in different series, with CA ranging between 64% for Hardy, 86–96.2% for Mast, and 85–87% for Liofilchem (Bonnin et al., 2022; Liu et al., 2023; Kolesnik-Goldmann et al., 2023; Nayak et al., 2022; Morris et al., 2020). A recent report proposes that employing a combination of methods, including DD and ComASP®, could present a practical solution for addressing the challenge of cefiderocol susceptibility testing in routine microbiology laboratories (Bianco et al., 2023). As evidenced in the current study, it is apparent that DD may serve as an appropriate method for assessing cefiderocol susceptibility, with a recommendation to reserve this approach for *A. baumannii* exclusively. Routine laboratories should exercise caution with other *Acinetobacter* species, particularly in the case of metallo-β-lactamases (MBL) isolates. In these instances, confirming susceptible zones through BMD should be recommended for greater accuracy.

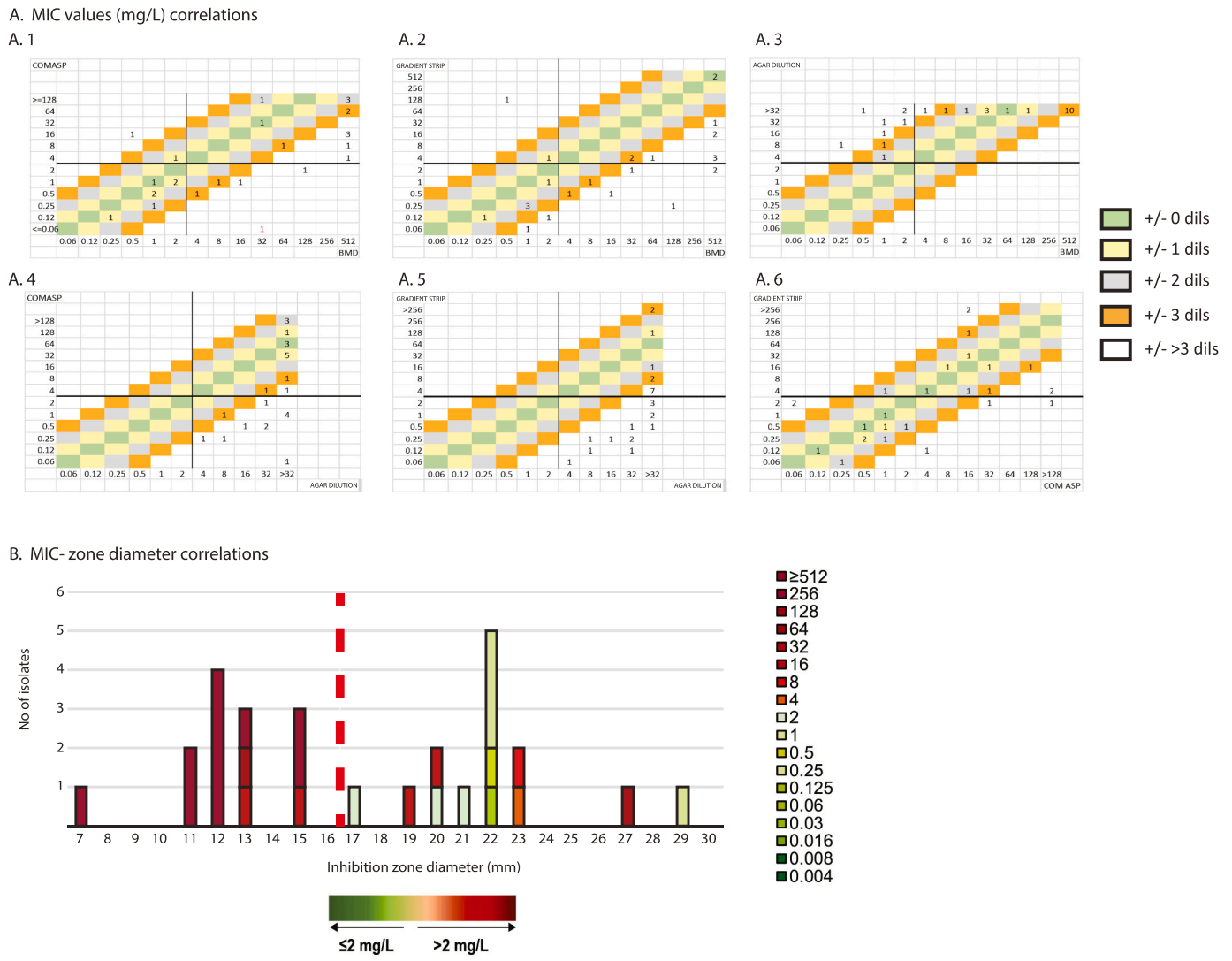

Fig. 1. Comparison between AST methods applying EUCAST clinical breakpoints. A. MIC-based methods. The green boxes indicate coincidence in the MIC values (in mg/L) by the methods under evaluation. Yellow boxes indicate discrepancies of +/− 1 dilution between MICs; gray boxes indicate discrepancies of +/− 2 dilutions between MICs and orange boxes indicate discrepancies of +/− 3 dilutions. BMD: broth microdilution using ID-CAMHB. (A.1) Comparison between BMD and ComASP® MIC values; (A.2) Comparison between BMD and strip test MIC values; (A.3) Comparison between BMD and agar dilution MIC values. (A.4) Comparison between ComASP® and agar dilution MIC values. (A.5) Comparison between strip test and agar dilution MIC values. (A.6) Comparison between strip test and ComASP® MIC values. B. MIC- zone diameter correlations for cefiderocol for *Acinetobacter* spp. Each isolate was tested with cefiderocol discs from one manufacturer on Mueller–Hinton media. Green: below PK/PD MIC breakpoints; orange/red: above PK/PD MIC breakpoints (EUCAST); Red dotted line indicates the proposed zone cut-off values for *A. baumannii* (EUCAST). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Performance characteristics of several routine susceptibility methods compared to cefiderocol broth microdilution (BMD).

Antimicrobial susceptibility method	% with agreement or error				
	EA	CA	VME	ME	BIAS+30
Comparison against reference BMD					
BMD vs GRADIENT STRIP	18.5	66.7	38.9	22.2	40.7
BMD vs ComASP®	29.6	74.1	22.2	7.4	47.4
BMD vs AGAR DILUTION	18.5	66.7	0	100	28.4
BMD vs DISK DIFFUSION	ND	81.5	0	22.2	ND
Comparison between non-reference methods					
GRADIENT STRIP vs ComASP®	37.0	85.2	16.7	11.1	38.1
GRADIENT STRIP vs AGAR DILUTION	3.7	48.1	77.8	0	5.8
ComASP® vs AGAR	29.6	55.6	66.7	0	31.2
GRADIENT STRIP vs DISK DIFFUSION	ND	77.8	23.1	21.4	ND
ComASP® vs DISK DIFFUSION	ND	88.9	13.3	7.1	ND

Since the study described in this article included a relatively small number of isolates, it is important to recognize that the findings may have significant limitations. Furthermore, the study was carried out using CAMHB and cefiderocol disks from a single manufacturer; thus, validating these results in various epidemiological settings for broader generalization will be essential. The findings confirmed the need for a dependable, accurate, and clinically validated approach to evaluate the susceptibility of *Acinetobacter* spp. to cefiderocol. The significant variability noted in our investigation underscored the need to standardize a susceptibility testing procedure for cefiderocol to ensure consistent and reliable results that can effectively guide clinical decision-making.

BMD. broth microdilution using ID-CAMHB; ComASP®: commercial BMD; EA: essential agreement; CA: categorical agreement; VME: very major errors; ME: major errors. NA: not determined.

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CRediT authorship contribution statement

Fernando Pasteran: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Olivia Wong:** Methodology. **Vyanka Mezcord:** Methodology. **Christina Lopez:** Methodology. **Nardin Georgeos:** Methodology. **Venjaminne Fua:** Methodology. **Alonzo Ozuna:** Methodology. **Dema Ramlaoui:** Methodology. **Cristian Sánchez:** Methodology. **Paulina Marchetti:** Methodology. **Alejandra Corso:** Writing – review & editing. **Marcelo E. Tolmasky:** Writing – review & editing, Writing – original draft, Resources. **Robert A. Bonomo:** Writing – review & editing, Writing – original draft, Visualization. **María Soledad Ramirez:** Conceptualization, Methodology, Formal analysis, Validation, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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References

Bianco, G., Boattini, M., Comini, S., Banche, G., Cavallo, R., Costa, C., 2023. Disc diffusion and ComASP((R)) Cefiderocol microdilution panel to overcome the challenge of Cefiderocol susceptibility testing in clinical laboratory routine. Antibiotics (Basel) 12 (3).

Bonnin, R.A., Emeraud, C., Jousset, A.B., Naas, T., Dortet, L., 2022. Comparison of disk diffusion, MIC test strip and broth microdilution methods for cefiderocol susceptibility testing on Carbapenem-resistant Enterobacterales. Clin. Microbiol. Infect. 28 (8), 1156 e1–1156 e5.

Brauncajs, M., Bielec, F., Macieja, A., Pastuszak-Lewandoska, D., 2024. Cefiderocol - an effective antimicrobial for MDR infections but a challenge for routine antimicrobial susceptibility testing. Adv. Med. Sci. 69 (2), 256–263.

Castanheira, M., Mendes, R.E., Gales, A.C., 2023. Global pidemiology and mechanisms of resistance of *Acinetobacter baumannii-calcoaceticus* complex. Clin. Infect. Dis. 76 (Suppl. 2), S166–S178.

Centers for Diseases Control and Prevention, 2019. Antibiotic Resistance Threats in the United States, Atlanta, GA: U.S. Department of Health and Human Services, 2019. CDC.

Clinical and Laboratory Standards Institute (CLIS), 2023. Performance Standards for Antimicrobial Susceptibility Testing Thirty-Three Informational Supplement M100-S33. CLSI, Wayne, PA.

He, S., He, H., Chen, Y., Chen, Y., Wang, W., Yu, D., 2015. In vitro and in vivo analysis of antimicrobial agents alone and in combination against multi-drug resistant *Acinetobacter baumannii*. Front. Microbiol. 6, 507.

Karakonstantis, S., Rousaki, M., Vassilopoulou, L., Kritsotakis, E.I., 2023. Global prevalence of cefiderocol non-susceptibility in Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*: a systematic review and meta-analysis. Clin. Microbiol. Infect. 30 (2), 178–188.

Kolesnik-Goldmann, N., Seth-Smith, H.M.B., Haldimann, K., Imkamp, F., Roloff, T., Zbinden, R., Hobbie, S.N., Egli, A., Mancini, S., 2023. Comparison of disk diffusion, E-test, and broth microdilution methods for testing in vitro activity of Cefiderocol in *Acinetobacter baumannii*. Antibiotics (Basel) 12 (7).

Le, C., Pimentel, C., Pasteran, F., Tuttobene, M.R., Subils, T., Escalante, J., Nishimura, B., Arriaga, S., Carranza, A., Mezcord, V., Vila, A.J., Corso, A., Actis, L.A., Tolmasky, M. E., Bonomo, R.A., Ramirez, M.S., 2022. Human serum proteins and susceptibility of *Acinetobacter baumannii* to Cefiderocol: role of Iron transport. Biomedicines 10 (3).

Liu, Y., Ding, L., Han, R., Zeng, L., Li, J., Guo, Y., Hu, F., 2023. Assessment of cefiderocol disk diffusion versus broth microdilution results when tested against *Acinetobacter baumannii* complex clinical isolates. Microbiol. Spectr. e0535522.

Matuschek, E., Longshaw, C., Takemura, M., Yamano, Y., Kahlmeter, G., 2022. Cefiderocol: EUCAST criteria for disc diffusion and broth microdilution for antimicrobial susceptibility testing. J. Antimicrob. Chemother. 77 (6), 1662–1669.

Morris, C.P., Bergman, Y., Tekle, T., Fissel, J.A., Tamma, P.D., Simner, P.J., 2020. Cefiderocol antimicrobial susceptibility testing against multidrug-resistant gram-negative Bacilli: a comparison of disk diffusion to broth microdilution. J. Clin. Microbiol. 59 (1).

Nayak, G., Behera, B., Mohanty, S., Kar, P., Jena, J., 2022. Analysis of in vitro activity of Cefiderocol against Carbapenem-resistant gram-negative Bacilli by broth microdilution and disk diffusion method: a single-center study in Odisha, India. Infect. Drug Resist. 15, 5887–5897.

Paterson, D.L., Isler, B., Stewart, A., 2020. New treatment options for multiresistant gram negatives. Curr. Opin. Infect. Dis. 33 (2), 214–223.

Piperaki, E.T., Tzouvelekis, L.S., Miriagou, V., Daikos, G.L., 2019. Carbapenem-resistant *Acinetobacter baumannii*: in pursuit of an effective treatment. Clin. Microbiol. Infect. 25 (8), 951–957.

Ramirez, M.S., Nikolaidis, N., Tolmasky, M.E., 2013. Rise and dissemination of aminoglycoside resistance: the *aac(6′)-Ib* paradigm. Front. Microbiol. 4, 121.

Theuretzbacher, U., Bush, K., Harbarth, S., Paul, M., Rex, J.H., Tacconelli, E., Thwaites, G.E., 2020. Critical analysis of antibacterial agents in clinical development. Nat. Rev. Microbiol. 18 (5), 286–298.

Watkins, R.R., Bonomo, R.A., 2023. Sulbactam-durlobactam: a step forward in treating Carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections. Clin. Infect. Dis. 76 (Suppl. 2), S163–S165.