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Cefiderocol Antimicrobial Susceptibility Testing against Multidrug-Resistant Gram-Negative Bacilli: a Comparison of Disk Diffusion to Broth Microdilution

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ABSTRACT Antimicrobial susceptibility testing (AST) of cefiderocol poses challenges because of its unique mechanism of action (i.e., requiring an iron-depleted state) and due to differences in interpretative criteria established by the Clinical and Laboratory Standards Institute (CLSI), U.S. Food and Drug Administration (FDA), and European Committee on Antimicrobial Susceptibility Testing (EUCAST). Our objective was to compare cefiderocol disk diffusion methods (DD) to broth microdilution (BMD) for AST of Gram-negative bacilli (GNB). Cefiderocol AST was performed on consecutive carbapenem-resistant *Enterobacterales* (CRE; 58 isolates) and non-glucose-fermenting GNB (50 isolates) by BMD (lyophilized panels; Sensititre; Thermo Fisher) and DD (30 μg; research-use-only [RUO] MASTDISCS and FDA-cleared HardyDisks). Results were interpreted using FDA (prior to 28 September 2020 update), EUCAST, and investigational CLSI breakpoints (BPs). Categorical agreement (CA), minor errors (mE), major errors (ME), and very major errors (VME) were calculated for DD methods. The susceptibilities of all isolates by BMD were 72% (FDA), 75% (EUCAST) and 90% (CLSI). For DD methods, EUCAST BPs demonstrated lower susceptibility at 65% and 66%, compared to 74% and 72% (FDA) and 87% and 89% (CLSI) by HardyDisks and MASTDISCS, respectively. CA ranged from 75% to 90%, with 8 to 25% mE, 0 to 19% ME, and 0 to 20% VME and varied based on disk, GNB, and BPs evaluated. Both DD methods performed poorly for *Acinetobacter baumannii* complex. There is considerable variability when cefiderocol ASTs are interpreted using CLSI, FDA, and EUCAST breakpoints. DD offers a convenient alternative approach to BMD methods for cefiderocol AST, with the exception of *A. baumannii* complex isolates.

KEYWORDS cefiderocol, antimicrobial susceptibility testing, broth microdilution, disk diffusion

Antimicrobial resistance is a pressing concern in the United States and globally, with an estimated 157,000 deaths from multidrug-resistant Gram-negative bacilli (MDR GNB) in the United States annually (1). Arguably, the greatest antimicrobial resistance threat is that of carbapenem-resistant organisms (2). Carbapenem resistance among Gram-negative bacilli can be mediated by non-carbapenemase-mediated mechanisms (e.g., cell wall permeability defects combined with extended-spectrum β-lactamase or AmpC β-lactamase production) or carbapenemase mediated. Carbapenemases are enzymes that can hydrolyze all or most β-lactam agents, including carbapenems, the broadest class of antimicrobials currently available. Commonly encountered carbapenemases in the United States include *Klebsiella pneumoniae* carbapenemases (KPCs), New Delhi metallo-β-lactamases (NDMs), and OXA-48-like enzymes. Although commonly found in *Enterobacterales*, these carbapenemases are occasionally identified in *Pseu-*

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domonas aeruginosa and *Acinetobacter baumannii* (3). For *P. aeruginosa*, most carbapenem resistance is non-carbapenemase mediated (OprD porin mutations combined with hyperexpression of AmpC or upregulation of efflux pumps), and for the small proportion of isolates that are carbapenemase producers (~2% of carbapenem-resistant isolates), Verona integron-encoded metallo-β-lactamases (VIM) are the most common in the United States (4, 5). On the other hand, carbapenem resistance in *Acinetobacter* spp. is mediated mostly via acquisition of Ambler class D enzymes, in particular, OXA-23 and OXA-24 enzymes (6). Novel β-lactam–β-lactamase inhibitor combinations (e.g., ceftazidime-avibactam, imipenem-cilastatin-relebactam, and meropenem-vaborbactam) may provide protection against non-carbapenemase-mediated carbapenem resistance mechanisms, KPCs, and OXA-48-like carbapenemases but not against NDM and other metallo-β-lactamases (MBL) (7).

Cefiderocol, a novel siderophore-conjugated cephalosporin, has activity against a broad array of MDR GNB, including both carbapenem-resistant *Enterobacterales* and non-glucose-fermenting organisms. The cephalosporin portion of cefiderocol is structurally similar to ceftazidime and cefepime. The novelty lies in the presence of a catechol moiety on the C-3 side chain, which mimics naturally occurring siderophores (8). Cefiderocol is able to chelate ferric iron and thus can be actively transported into the periplasmic space via bacterial iron transport systems (9). Within the periplasmic space, the cephalosporin component binds to penicillin-binding protein 3 (PBP3), prevents side chain cross-linking in peptidoglycan synthesis, and ultimately leads to bacterial demise.

As cefiderocol utilizes active iron transport for bacterial entry and iron transporters are upregulated under iron-depleted conditions, as occurs *in vivo*, special consideration for iron concentrations of media is required when cefiderocol antimicrobial susceptibility testing (AST) is performed (10). Accurate cefiderocol MICs determined through broth microdilution (BMD) require the use of iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB), as standard CAMHB does not provide reproducible MICs that accurately reflect expected *in vivo* activity (10). This can pose challenges for microbiology laboratories, because both preparation of ID-CAMHB and performance of BMD are cumbersome. Alternatively, AST approaches have been developed that improve the ease of obtaining cefiderocol results, including the Sensititre lyophilized BMD panel (Thermo Fisher Scientific, Waltham, MA) and disk diffusion (DD) methods. The Sensititre BMD panel includes cefiderocol with an iron chelator embedded in wells, allowing reconstitution of the entire panel, including cefiderocol wells, with standard CAMHB. Similarly, *in vitro* testing by DD on Mueller-Hinton agar does not require iron depletion, as the iron is sufficiently bound within the agar (11).

Cefiderocol AST interpretation also presents challenges. Three different sets of AST interpretive criteria currently exist, from the Clinical and Laboratory Standards Institute (CLSI) (12), U.S. Food and Drug Administration (FDA; https://www.fda.gov/drugs/development-resources/cefiderocol-injection), and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (13), each with different nuances regarding specific organisms to which breakpoints can be applied. Our objective was to compare cefiderocol DD to Sensititre BMD lyophilized panels for AST of clinically relevant MDR GNB and to investigate differences in their performance characteristics by applying CLSI, FDA (prior to 28 September 2020 update), and EUCAST interpretive criteria.

MATERIALS AND METHODS

Isolate selection. One hundred eight consecutive carbapenem-resistant *Enterobacterales* (CRE; 58 isolates), carbapenem-resistant *Pseudomonas aeruginosa* (14 isolates), carbapenem-resistant *Acinetobacter baumannii* complex (14 isolates), *Achromobacter xylosoxidans* (8 isolates), *Burkholderia cepacia* complex (3 isolates), and *Stenotrophomonas maltophilia* (11 isolates) clinical isolates from unique patients in 2017 were included. Carbapenem resistance was defined as testing resistant to ertapenem for CRE and meropenem for *P. aeruginosa* and *A. baumannii* complex. Specific CRE included *Citrobacter freundii* complex (2 isolates), *Enterobacter cloacae* complex (15 isolates), *Escherichia coli* (15 isolates), *Klebsiella aerogenes* (2 isolates), *Klebsiella oxytoca* (6 isolates), *K. pneumoniae* (15 isolates), and *Serratia marcescens* (3 isolates). Of the 58 CRE, 26 (44%) were carbapenemase-producing CRE (CP-CRE), including those

TABLE 1 Breakpoint interpretations applied to categorize cefiderocol antimicrobial susceptibility testing results[a]

	Investigational CLSI breakpoints						FDA breakpoints[e]						EUCAST breakpoints			
	MIC (μg/ml)			Disk zone diam (mm)			MIC (μg/ml)			Disk zone diam (mm)			MIC (μg/ml)		Disk zone diam (mm)	
Organism	S	I	R	S	I	R	S	I	R	S	I	R	S	R	S	R
Enterobacterales[b]	≤4	8	≥16	≥16	12–15	≤11	≤2	4	≥8	≥18	14–17	≤13	≤2	>2	≥22	<22
Pseudomonas aeruginosa	≤4	8	≥16	≥18	13–17	≤12	≤1	2	≥4	≥25	19–24	≤18	≤2	>2	≥22	<22
Acinetobacter baumannii	≤4	8	≥16	≥15	11–14	≤10							≤2[c]	>2[c]	≥17[d]	
Stenotrophomonas maltophilia	≤4	8	≥16	≥17	13–16	≤12							≤2[c]	>2[c]	≥20[d]	
Achromobacter xylosoxidans													≤2[c]	>2[c]		
Burkholderia cepacia complex													≤2[c]	>2[c]		

[a]CLSI, Clinical and Laboratory Standards Institute; FDA, U.S. Food and Drug Administration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; I, intermediate; R, resistant.
[b]FDA breakpoints for the *Enterobacterales* (listed as *Enterobacteriaceae* on the FDA website) are specific for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex. These breakpoints were used to interpret results for all *Enterobacterales* in this study.
[c]Non-species-specific pharmacokinetic-pharmacodynamic (PK-PD) breakpoint.
[d]EUCAST provided disk correlates associated with the susceptible PK-PD breakpoint for *A. baumannii* and *S. maltophilia*. Although the PK-PD breakpoint for S is set at ≤2 μg/ml, there were no *S. maltophilia* isolates with MICs of >0.5 μg/ml. Thus, the disk correlate for *S. maltophilia* is for isolates with MICs of ≤0.5 μg/ml.
[e]The FDA breakpoints applied in this study were the published breakpoints prior to the 28 September 2020 update.

producing KPC (21 isolates), NDM and OXA-181 (3 isolates), OXA-181 (1 isolate), and *Serratia marcescens* enzymes (SME) (1 isolate).

Laboratory methods. Isolates were identified using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (Bruker Daltonics Inc., Billerica, MA). AST results were determined using the BD Phoenix automated system (BD Diagnostics, Sparks, MD) or DD for mucoid isolates following CLSI guidelines (12), all per routine Johns Hopkins Hospital Medical Microbiology Laboratory protocol. Isolates were stored at −80°C in glycerol until further testing was performed.

Frozen isolates were subcultured twice to tryptic soy agar with 5% sheep blood. Cefiderocol AST was carried out using DD with research-use-only 30-μg cefiderocol MASTDISCS (Mast Group Ltd., Bootle, United Kingdom) and custom, lyophilized Sensititre BMD panels (Thermo Fisher Scientific, Waltham, MA), using the same standardized inoculum. The BMD panel contained cefiderocol concentrations ranging from 0.03 to 64 μg/ml and a proprietary chelator in the wells, removing the requirement for ID-CAMHB. The cefiderocol lyophilized panel has been shown to be substantially equivalent to reference BMD and has received FDA clearance (14) (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K193538). A 30-μl aliquot of the standardized inoculum was added to 11 ml Sensititre CAMHB with TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] buffer for a final concentration of 1×10^5 CFU/ml. The panels were inoculated with 50 μl in each well and incubated for 18 to 24 h (varying by organism as appropriate) at 35 ± 2°C in an aerobic non-CO_2 incubator. As FDA-cleared, 30-μg cefiderocol HardyDisks (Hardy Diagnostics, Santa Maria, CA) became available at a later date, the HardyDisk results were obtained and interpreted separately. The DD methods were carried out on standard Mueller-Hinton agar incubated for 18 to 24 h at 35 ± 2°C following CLSI guidelines (12). If a difference of ≥5 mm was observed between the HardyDisk result and the previous MASTDISC result, both procedures were repeated from the same inoculum, and the repeat result was used for the analysis. Quality control organisms were prepared each day of testing, including *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

MIC interpretation. The cefiderocol MIC was interpreted as the first well where visible bacterial growth was inhibited. When trailing endpoints occurred, the MIC was read at 80% inhibition. Trailing was defined as multiple wells of tiny or faint growth relative to the growth control. Disk zone diameters were read using the innermost colony-free zone when pinpoint colonies were observed within the zone (see reference 15 for examples of trailing endpoints and pinpoint colonies). Results were interpreted by applying three sets of breakpoints, i.e., (i) investigational CLSI (12), (ii) FDA prior to the 28 September 2020 update (https://www.fda.gov/drugs/development-resources/cefiderocol-injection), and (iii) EUCAST (13) breakpoints, as appropriate (Table 1). The MIC_{50} and MIC_{90} were determined for each species by identifying the MIC that would inhibit growth of at least 50% and 90% of the isolates, respectively. DD zone diameter results were compared to BMD results for each of the three sets of breakpoint interpretations. Zone diameters were graphed using GraphPad Prism 8.1.2. Results were compared using linear regression.

Agreement analysis. Using BMD as the reference method, categorical agreement (CA), minor errors (mE), major errors (ME), and very major errors (VME) were assessed according to standard definitions. Acceptance criteria included ≥90% CA, ≤10% mE, and ≤3% ME and VME, based on CLSI guidelines (16).

RESULTS

Cefiderocol broth microdilution. The cefiderocol MIC range was highly variable depending on the species of bacteria, with an overall MIC range of ≤0.03 to 64 μg/ml. The MIC range, MIC_{50}, MIC_{90}, and percent susceptible, intermediate, and resistant isolates are summarized in Table 2, with various breakpoint interpretations applied. *E. coli*, *K. aerogenes*, *K. oxytoca*, and *S. marcescens* uniformly displayed 100% susceptibility

TABLE 2 Summary of cefiderocol broth microdilution results[a]

Organism(s)	No. of isolates	MIC (μg/ml)			% (no.) with breakpoint interpretation							
					CLSI (investigational)			FDA[b]			EUCAST	
		Range	50%	90%	S	I	R	S	I	R	S	R
Carbapenem-resistant *Enterobacterales*	58	≤0.03–64	0.5	8	88 (51)	3 (2)	9 (5)	76 (44)	12 (7)	12 (7)	76 (44)	24 (14)
Carbapenemase-producing CRE	26	≤0.03–32	0.25	4	92 (24)	0	8 (2)	77 (20)	15 (4)	8 (2)	77 (20)	23 (6)
Non-carbapenemase-producing CRE	32	0.06–64	0.5	16	85 (27)	6 (2)	9 (3)	75 (24)	9 (3)	16 (5)	75 (24)	25 (8)
Citrobacter freundii complex	2	0.06–8	0.06	8	50 (1)	50 (1)	0	50 (1)	0	50 (1)	50 (1)	1 (50)
Enterobacter cloacae complex	15	0.06–64	0.5	16	90 (12)	7 (1)	13 (2)	60 (9)	20 (3)	20 (3)	60 (9)	40 (6)
Escherichia coli	15	0.06–2	0.25	1	100 (15)	0	0	100 (15)	0	0	100 (15)	0
Klebsiella aerogenes	2	0.5–1	0.5	1	100 (2)	0	0	100 (2)	0	0	100 (2)	0
Klebsiella oxytoca	6	≤0.03–1	0.06	1	100 (6)	0	0	100 (6)	0	0	100 (6)	0
Klebsiella pneumoniae	15	0.06–32	2	32	80 (12)	0	20 (3)	53 (8)	27 (4)	20 (3)	53 (8)	47 (7)
Serratia marcescens	3	≤0.03–2	≤0.03	2	100 (3)	0	0	100 (3)	0	0	100 (3)	0
Non-glucose-fermenting Gram-negative bacilli	50	≤0.03–8	0.5	4	92 (36)	5 (2)	3 (1)	57 (8)	14 (2)	29 (4)	74 (37)	26 (13)
Pseudomonas aeruginosa	14	0.5–8	1	8	93 (13)	7 (1)	0	57 (8)	14 (2)	29 (4)	71 (10)	29 (4)
Acinetobacter baumannii complex	14	0.06	4	8	86 (12)	7 (1)	7 (1)				36 (5)[c]	64 (9)[c]
Achromobacter xylosoxidans	8	0.06–1	0.25	1							100 (8)[c]	0
Burkholderia cepacia complex	3	0.12–0.25	0.12	0.25							100 (3)[c]	0
Stenotrophomonas maltophilia	11	≤0.03–0.5	0.12	0.25	100 (11)	0	0				100 (11)[c]	0
All isolates	108	≤0.03–64	0.5	4	90 (87)	4 (4)	6 (6)	72 (52)	13 (9)	15 (11)	75 (81)	25 (27)

[a]CLSI, Clinical and Laboratory Standards Institute; FDA, U.S. Food and Drug Administration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; I, intermediate; R, resistant.

[b]Breakpoints used for this analysis were prior to the 28 September 2020 update. FDA breakpoints for the *Enterobacterales* (listed as *Enterobacteriaceae* on the FDA website) are specific for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex. These breakpoints were used to interpret results for all *Enterobacterales* in this study.

[c]Non-species-specific pharmacokinetic-pharmacodynamic (PK-PD) breakpoints.

to cefiderocol regardless of the breakpoint criteria applied (Table 2). In contrast, both *E. cloacae* and *K. pneumoniae* had much more variable results, with susceptibility ranging from 60% to 90% and 53% to 80%, respectively, depending on the breakpoint criteria used. Similarly, both *P. aeruginosa* and *A. baumannii* had wide ranges in their susceptibility, 57% to 93% and 36% to 86%, respectively. When the EUCAST non-species-specific pharmacokinetic-pharmacodynamic (PK-PD) breakpoints were applied, all *A. xylosoxidans* and *B. cepacia* complex isolates tested susceptible. Similarly, *S. maltophilia* displayed 100% susceptibility to cefiderocol, regardless of the breakpoint criteria applied.

Overall, *Enterobacterales* susceptibilities were 88% when CLSI breakpoints were applied, which was higher than those obtained with either FDA or EUCAST breakpoints, at 76% each. MICs of ≥4 μg/ml were observed in 15 isolates, including 9 non-CP-CRE isolates (7 *E. cloacae* isolates, 1 *C. freundii* complex isolate, and 1 *K. pneumoniae* isolate) and 6 CP-CRE isolates, which were all *K. pneumoniae* (3 KPC-producing and 3 NDM- and OXA-181-producing isolates). Trailing endpoints were observed for 3 CRE, including 1 *S. marcescens* isolate (MIC, 2 μg/ml), 1 *E. coli* isolate (MIC, 0.12 μg/ml), and 1 *K. oxytoca* isolate (MIC, 1 μg/ml).

Similar to the CRE, *P. aeruginosa* and *A. baumannii* complex had higher susceptibilities when CLSI breakpoints were applied than with other available breakpoints. *A. baumannii* complex had the most striking difference in susceptibility, driven by 6 isolates with MICs of 4 μg/ml, which were susceptible by CLSI but resistant by EUCAST criteria. Three (21%) *A. baumannii* complex isolates were the only nonfermenters that demonstrated trailing endpoints, with MICs of 0.5, 2, and 4 μg/ml.

Cefiderocol disk diffusion. Cefiderocol disk diffusion susceptibility results are summarized in Table 3. Correlation between the zone diameter results of the two disks is shown in Fig. 1. A strong correlation was noted between the HardyDisks and MASTDISCS ($R^2 = 0.84$). Testing of three isolates (1 *E. cloacae* complex, 1 *K. oxytoca*, and 1 *P. aeruginosa* isolate) was repeated due to ≥5-mm differences between the disk results, which resolved on repeat testing. Pinpoint colonies were observed for 7

TABLE 3 Summary of cefiderocol disk diffusion results for HardyDisks and MASTDISCS[a]

Organism(s)	No. of isolates	% (no.) with breakpoint interpretation															
		30-μg HardyDisks (FDA cleared)								30-μg MASTDISCS (RUO)							
		CLSI (investigational)			FDA[b]			EUCAST		CLSI (investigational)			FDA[b]			EUCAST	
		S	I	R	S	I	R	S	R	S	I	R	S	I	R	S	R
Carbapenem-resistant *Enterobacterales*	58	88 (51)	9 (5)	3 (2)	84 (49)	7 (4)	9 (5)	64 (37)	36 (21)	90 (52)	9 (5)	2 (1)	79 (46)	16 (9)	5 (3)	66 (38)	34 (20)
Carbapenemase-producing CRE	26	88 (23)	12 (3)	0	88 (23)	4 (1)	8 (2)	65 (17)	35 (9)	88 (23)	12 (3)	0	81 (21)	19 (5)	0	69 (18)	31 (8)
Non-carbapenemase-producing CRE	32	88 (28)	6 (2)	6 (2)	81 (26)	9 (3)	9 (3)	63 (20)	37 (12)	91 (29)	6 (2)	3 (1)	78 (25)	13 (4)	9 (3)	63 (20)	37 (12)
Citrobacter freundii complex	2	100 (2)	0	0	50 (1)	50 (1)	0	0	100 (2)	100 (2)	0	0	50 (1)	50 (1)	0	0	100 (2)
Enterobacter cloacae complex	15	80 (12)	13 (2)	7 (1)	73 (11)	13 (2)	13 (2)	47 (7)	53 (8)	86 (13)	7 (1)	7 (1)	67 (10)	20 (3)	13 (2)	53 (8)	47 (7)
Escherichia coli	15	100 (15)	0	0	100 (15)	0	0	80 (12)	20 (3)	100 (15)	0	0	100 (15)	0	0	87 (13)	13 (2)
Klebsiella aerogenes	2	100 (2)	0	0	100 (2)	0	0	100 (2)	0	100 (2)	0	0	100 (2)	0	0	100 (2)	0
Klebsiella oxytoca	6	83 (5)	17 (1)	0	83 (5)	17 (1)	0	83 (5)	17 (1)	100 (6)	0	0	83 (5)	17 (1)	0	83 (5)	17 (1)
Klebsiella pneumoniae	15	80 (12)	13 (2)	7 (1)	80 (12)	0	20 (3)	53 (8)	47 (7)	73 (11)	27 (4)	0	67 (10)	27 (4)	7 (1)	47 (7)	53 (8)
Serratia marcescens	3	100 (3)	0	0	100 (3)	0	0	100 (3)	0	100 (3)	0	0	100 (3)	0	0	100 (3)	0
Non-glucose-fermenting Gram-negative bacilli	39	85 (33)	3 (1)	13 (5)	29 (4)	64 (9)	7 (1)	67 (26)	33 (13)	87 (34)	10 (4)	3 (1)	43 (6)	43 (6)	14 (2)	67 (26)	33 (13)
Pseudomonas aeruginosa	14	100 (14)	0	0	29 (4)	64 (9)	7 (1)	64 (9)	36 (5)	93 (13)	7 (1)	0	43 (6)	43 (6)	14 (2)	71 (10)	29 (4)
Acinetobacter baumannii complex	14	57 (8)	7 (1)	36 (5)				43[c] (6)	57[c] (8)	71 (10)	21 (3)	7 (1)				36 (5)[c]	64 (9)[c]
Stenotrophomonas maltophilia	11	100 (11)	0	0				100[c] (11)	0[c]	100 (11)	0	0				100 (11)[c]	0
All isolates	97	87 (84)	6 (6)	7 (7)	74 (53)	18 (13)	8 (6)	65 (63)	35 (34)	89 (86)	9 (9)	2 (2)	72 (52)	21 (15)	7 (5)	66 (64)	34 (33)

[a]CLSI, Clinical and Laboratory Standards Institute; FDA, U.S. Food and Drug Administration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, MIC; S, susceptible; I, intermediate; R, resistant; RUO, research use only.

[b]Breakpoints used for this analysis were prior to the 28 September 2020 update. FDA breakpoints for the *Enterobacterales* (listed as *Enterobacteriaceae* on the FDA website) are specific for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex. These breakpoints were used to interpret results for all *Enterobacterales* in this study.

[c]EUCAST provided disk correlates associated with the susceptible PK-PD breakpoint for *A. baumannii* and *S. maltophilia*. Although the PK-PD breakpoint for S is set at ≤2 μg/ml, there were no *S. maltophilia* isolates with MICs of >0.5 μg/ml. Thus, the disk correlate for *S. maltophilia* is for isolates with MICs of ≤0.5 μg/ml.

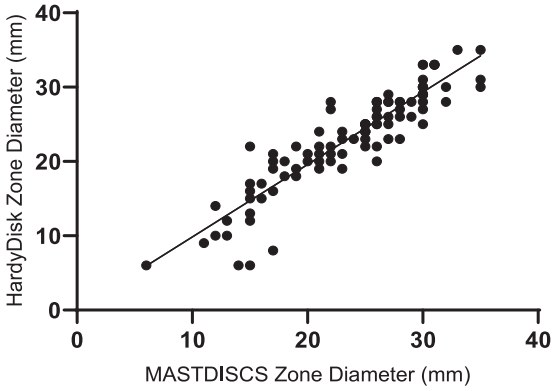


FIG 1 Zone diameter comparison between HardyDisks and MASTDISCS (cefiderocol, 30 μg). $R^2 = 0.84$; slope = 0.97; *y* intercept = 0.09.

organisms and occurred with the same frequency with both disks: 5 *A. baumannii* complex isolates, 1 *K. oxytoca* isolate, and 1 *C. freundii* complex isolate.

EUCAST breakpoints demonstrated lower overall susceptibility at 65% and 66%, compared to 74% and 72% for FDA and 87% and 89% for CLSI breakpoints, by HardyDisks and MASTDISCS, respectively. Similar to BMD, the species with the consistently lowest percent susceptibility to cefiderocol by both disk brands was *A. baumannii* complex. Most species had similar or identical results between BMD and DD. However, there were some notable differences observed between *E. coli*, *K. pneumoniae*, and *E. cloacae* complex between BMD and DD methods.

Correlation of HardyDisks with BMD. The distribution of BMD cefiderocol MICs to HardyDisk zone diameters is shown in Fig. 2. Collectively, CA ranged from 75% to 89%, 8 to 25% mE, 0 to 17% ME, and 0 to 12% VME, with variations by species and breakpoint criteria applied (Table 4). CLSI interpretations provided the highest correlation, with 89% CA, 8% mE, and 3% ME. However, neither the CLSI, FDA, nor EUCAST susceptibility categorization met all acceptance criteria when all isolates were evaluated. When *A. baumannii* complex isolates were excluded from the CLSI results, acceptable results were obtained, with 93% CA, 8% mE, and no ME or VME. Using EUCAST guidance, CA was achieved for 85% of isolates and reached 88% when *A. baumannii* complex was excluded. However, as there is no EUCAST intermediate category, ME were observed for 17% of isolates and VME for 12% of isolates. VME were observed with 3 *A. baumannii* complex isolates and 1 *P. aeruginosa* isolate. FDA disk correlates had the lowest performance, with 75% CA and 25% mE. There were 18 mE, including mE in 9 *Enterobacterales* and 9 *P. aeruginosa* isolates. The majority of *Enterobacterales* tested more susceptible by DD, while *P. aeruginosa* was variable (4/9 isolates tested more susceptible and 5/9 tested more resistant). No ME or VME were observed. When the *Enterobacterales* were limited to the specific species with FDA breakpoints compared to all *Enterobacterales*, similar results were obtained, i.e., CA of 84% and 15% mE.

Correlation of MASTDISCS and BMD. The distribution of cefiderocol MICs to MASTDISCS zone diameters is displayed in Fig. 3. When all isolates were evaluated, CA ranged from 79% to 90%, with 13 to 19% mE, 0 to 13% ME, and 0 to 12% VME, with variations based on species and breakpoint criteria applied (Table 4). CA was slightly higher with the MASTDISCS than the HardyDisks with FDA and EUCAST breakpoints. Similar to the HardyDisk results, neither the CLSI, FDA, nor EUCAST results met all acceptance criteria when all isolates were analyzed. EUCAST interpretations provided the highest CA at 90%, with 13% ME and 12% VME. VME were limited to non-glucose-fermenting organisms. Regarding the *Enterobacterales* only, the CA was 88% with 12% mE when all isolates were evaluated by CLSI breakpoints. The CA was 90% for the *Enterobacterales*, with 14% ME and no VME, when EUCAST breakpoints were applied. MASTDISCS and BMD results were similar with the *Enterobacterales* and the non-

A. *Enterobacterales*

	0	0	0	1	1	0	2	1	0	2	1	1	1	4	4	3	4	6	4	8	3	3	7	2	0	0	0	0	0	0	
64				1																											1
32					1			1																							2
16							2																								2
8										1		1																			2
4											1		1	2	2	1															7
2														1					1		1										3
1										1					2			2	1												6
0.5																1		2		2		1	1								7
0.25																	2	2	1	2			2								9
0.12														1			1		1	2	1		2	1							9
0.06																1	1			1	1		2	1							7
</=0.03																				1		2									3
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	

B. *Pseudomonas aeruginosa*

	0	0	0	0	0	0	0	1	0	2	2	3	1	1	1	1	1	1	0	0	0	0	0	0	0	14
64																										0
32																										0
16																										0
8								1																		1
4											1	1	1													3
2												1						1								2
1											1	1														2
0.5												1	1	1	1	1	1									6
0.25																										0
0.12																										0
0.06																										0
</=0.03																										0
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	

C. *Acinetobacter baumannii* complex

	3	0	1	0	1	0	0	0	1	1	1	2	1	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
64																															0
32																															0
16					1																										1
8	1																														1
4	2								1		1	2	1																		7
2			1							1																					2
1																															0
0.5																	1														1
0.25																1															1
0.12																															0
0.06																	1														1
</=0.03																															0
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	

FIG 2 Distribution of cefiderocol MICs to zone diameters for HardyDisks. Broth microdilution MICs (micrograms per milliliter) are on the *x* axis, and zone diameters (millimeters) are on the *y* axis. The blue lines denote the investigational CLSI breakpoints, the red lines denote EUCAST breakpoints, and the gray highlighted areas denote the intermediate category determined by FDA breakpoints (applying the breakpoints prior to the 28 September 2020 update).

glucose fermenters. FDA breakpoints resulted in a categorical agreement of only 79%, with 19% mE and 20% VME (1 *P. aeruginosa* isolate).

DISCUSSION

Cefiderocol is a welcome addition to the existing antimicrobial armamentarium. For several pathogens and resistance mechanisms, cefiderocol functions as the last active agent before pan-resistance ensues, owing to its broad activity against Gram-negative organisms, which underscores the importance of accurate cefiderocol AST methods. Cefiderocol AST poses challenges both because of its unique mechanism of action (i.e., requiring an

TABLE 4 Disk diffusion performance characteristics compared to broth microdilution[a]

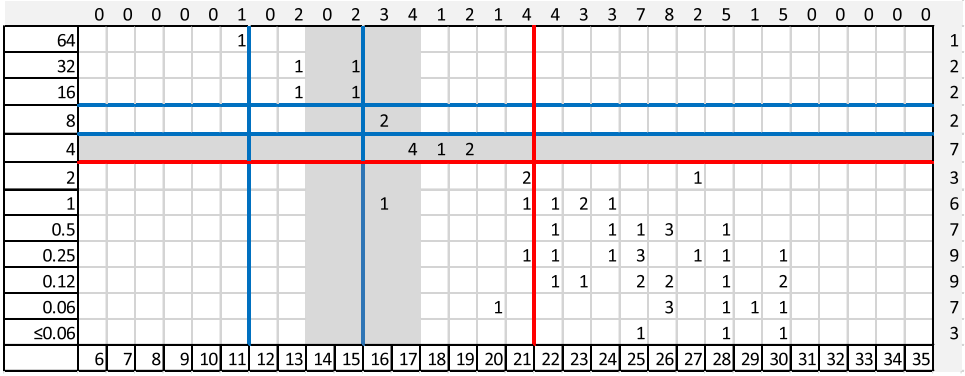
Organism(s)	No. of isolates	% (no.) with agreement or error																				
		30-μg HardyDisks (FDA cleared)											30-μg MASTDISCS (RUO)									
		CLSI				FDA[b] (*n* = 73)				EUCAST			CLSI				FDA[b]				EUCAST		
		CA	mE	ME	VME	CA	mE	ME	VME	CA	ME	VME	CA	mE	ME	VME	CA	mE	ME	VME	CA	ME	VME
Carbapenem-resistant *Enterobacterales*	58	91 (53)	9 (5)	0	0	84 (49)	16 (9)	0	0	88 (51)	17 (7)	0	88 (51)	12 (7)		0	86 (50)	14 (8)	0	0	90 (52)	14 (6)	0
Carbapenemase-producing CRE	26	88 (23)	12 (3)	0	0	81 (21)	19 (5)	0	0	88 (23)	12 (3)	0	88 (23)	12 (3)	0	0	81 (21)	19 (5)	0	0	92 (24)	8 (2)	0
Non-carbapenemase-producing CRE	32	94 (30)	6 (2)	0	0	88 (28)	12 (4)	0	0	88 (28)	12 (4)	0	88 (28)	12 (4)	0	0	91 (29)	9 (3)	0	0	88 (28)	12 (4)	0
Citrobacter freundii complex	2	50 (1)	50 (1)	0	0	50 (1)	50 (1)	0	0	50 (1)	100 (1)	0	50 (1)	50 (1)	0	0	50 (1)	50 (1)	0	0	50 (1)	100 (1)	0
Enterobacter cloacae complex	15	93 (14)	7 (1)	0	0	80 (12)	20 (3)	0	0	87 (13)	25 (2)	0	87 (12)	13 (2)	0	0	87 (13)	13 (2)		0	93 (14)	7 (1)	0
Escherichia coli	15	100 (15)	0	0	0	100 (15)	0	0	0	80 (12)	20 (3)	0	100 (15)	0	0	0	100 (15)	0	0	0	87 (13)	13 (2)	0
Klebsiella aerogenes	2	100 (2)	0	0	0	100 (2)	0	0	0	100 (2)	0	0	100 (2)	0	0	0	100 (2)	0	0	0	100 (2)	0	0
Klebsiella oxytoca	6	83 (5)	17 (1)	0	0	83 (5)	17 (1)	0	0	83 (5)	17 (1)	0	100 (6)	0	0	0	83 (5)	17 (1)	0	0	83 (5)	17 (1)	0
Klebsiella pneumoniae	15	87 (13)	13 (2)	0	0	73 (11)	27 (4)	0	0	100 (15)	0	0	73 (11)	27 (4)	0	0	73 (11)	27 (4)	0	0	83 (14)	7 (1)	0
Serratia marcescens	3	100 (3)	0	0	0	100 (3)	0	0	0	100 (3)	0	0	100 (3)	0	0	0	100 (3)	0	0	0	100 (3)	0	0
Non-glucose-fermenting Gram-negative bacilli	39	85 (33)	8 (3)	12 (3)	0	36 (5)	64 (9)	0	0	79 (31)	15 (4)	31 (4)	85 (33)	15 (6)	0	0	50 (7)	43 (6)	0	25 (1)	90 (35)	8 (2)	15 (2)
Pseudomonas aeruginosa	14	93 (13)	7 (1)	0	0	36 (5)	64 (9)	0	0	79 (11)	14 (2)	25 (1)	86 (12)	14 (2)	0	0	50 (7)	43 (6)	0	25 (1)	86 (12)	10 (1)	25 (1)
Acinetobacter baumannii complex	14	64 (9)	14 (2)	25 (3)	0					64 (9)	20 (2)	33 (3)	71 (10)	29 (4)	0	0					86 (12)[c]	20 (1)[c]	11 (1)[c]
Stenotrophomonas maltophilia	11	100 (11)	0	0	0					100 (11)	0	0	100 (11)	0	0	0					100 (11)[c]	0	0
All isolates	97	89 (86)	8 (8)	3 (3)	0	75 (54)	25 (18)		0	85 (82)	17 (11)	12 (4)	87 (84)	13 (13)	0	0	79 (57)	19 (14)	0	20 (1)	90 (87)	13 (8)	12 (4)

[a]CRE, carbapenem-resistant *Enterobacterales*; FDA, U.S. Food and Drug Administration; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; S, susceptible; I, intermediate; R, resistant; RUO, research use only.

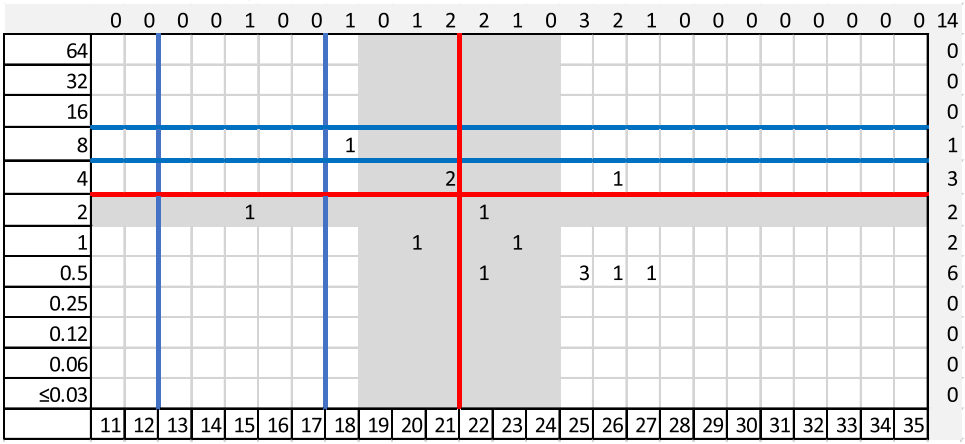
[b]Breakpoints used for this analysis were prior to the 28 September 2020 update. The FDA breakpoints for the *Enterobacterales* (listed as *Enterobacteriaceae* on the FDA website) are specific for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* complex. However, we used the breakpoints to interpret all *Enterobacterales* results in this study.

[c]EUCAST provided disk correlates associated with the susceptible PK-PD breakpoint for *A. baumannii* and *S. maltophilia*. Although the PK-PD breakpoint for S is set at ≤2 μg/ml, there were no *S. maltophilia* isolates tested by EUCAST with MICs of >0.5 μg/ml. Thus, the disk correlate for *S. maltophilia* is for isolates with MICs of ≤0.5 μg/ml.

A. *Enterobacterales*



B. *Pseudomonas aeruginosa*



C. *Acinetobacter baumannii* complex

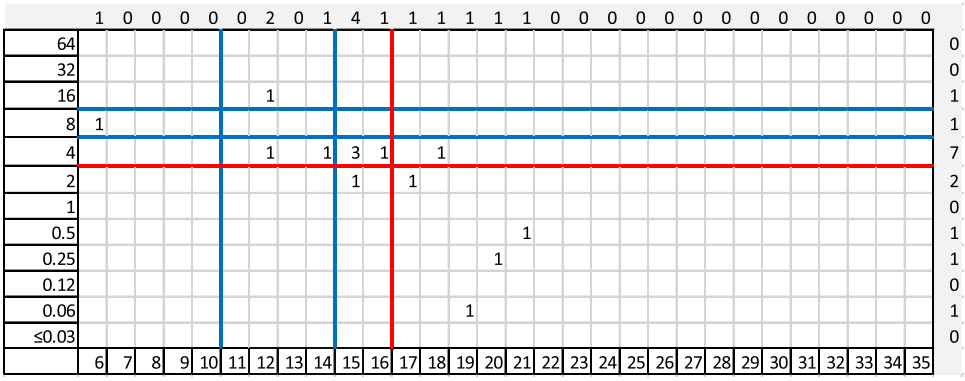


FIG 3 Distribution of cefiderocol MICs to zone diameters for MASTDISCS. Broth microdilution MICs (micrograms per milliliter) are on the *x* axis, and zone diameters (millimeters) are on the *y* axis. The blue lines denote the investigational CLSI breakpoints, the red lines denote EUCAST breakpoints, and the gray highlighted areas denote the intermediate category determined by FDA breakpoints (applying the breakpoints prior to the 28 September 2020 update).

iron-depleted state to replicate *in vivo* efficacy) and because of differences in established susceptibility interpretative criteria established by the CLSI, FDA, and EUCAST (15). We evaluated baseline susceptibility of cefiderocol against 108 MDR GNB clinical isolates and the accuracy of two different cefiderocol disks (i.e., HardyDisks and MASTDISCS) compared to BMD.

Overall, the MICs ranged from ≤0.03 to 64 μg/ml, with a MIC_{50} of 0.5 μg/ml for all GNB and MIC_{90}s of 4 μg/ml for non-glucose fermenters and 8 μg/ml for CRE. These results are similar to those of other studies that limited testing to GNB found to be not

carbapenem susceptible (17, 18). Interestingly, the MIC_{90} was 4-fold higher among non-carbapenemase-producing CRE (16 μg/ml) than CP-CRE (4 μg/ml) in our cohort. Cefiderocol susceptibility of CRE determined by BMD varied from 76% (FDA and EUCAST) to 88% (CLSI). Although susceptibility was lower by all breakpoints for non-CP-CRE than CP-CRE, there were no significant differences. Susceptibility of the non-glucose-fermenting organisms determined by BMD varied from 57% (FDA) to 74% (EUCAST) to 92% (CLSI). As the FDA and EUCAST cefiderocol breakpoints are more stringent than the CLSI investigational breakpoints, it is not surprising that susceptibility to cefiderocol across organisms is higher when CLSI interpretive criteria are applied. The lack of FDA breakpoints for several non-glucose fermenters with a low MIC_{90} of cefiderocol (e.g., *A. xylosoxidans*, *B. cepacia* complex, and *S. maltophilia*) further contributes to the particularly low overall percentages of susceptible isolates when FDA cefiderocol breakpoints are applied, compared to CLSI or EUCAST breakpoints.

Both DD approaches had variable CA for CRE, ranging from 84% to 91%, across the three sets of interpretative criterion recommendations. Categorical agreement was more limited for the non-glucose-fermenting organisms, ranging from 36% to 90%, across the three sets of criteria, with reduced agreement being largely attributable to *A. baumannii* complex isolates. Results of both DD methods had a high degree of correlation with each other; however, variability with *A. baumannii* was observed.

For MASTDISCS, EUCAST breakpoints resulted in the highest CA. However, due to the lack of an intermediate category (or "area of technical uncertainty" category, as defined by EUCAST), higher rates of ME and VME were observed. On the other hand, HardyDisks performed best and met the acceptance criteria when results for the *Enterobacterales* and *P. aeruginosa* were interpreted with the investigational CLSI breakpoints. FDA breakpoints resulted in the lowest CA, which was driven by a high number of minor errors. Although it is recommended that laboratories apply FDA breakpoints to the FDA-cleared HardyDisks, they should proceed with caution, especially when testing MDR GNB. For the *Enterobacterales*, HardyDisks yielded more susceptible results than BMD, and zone diameter results within 19 to 22 mm would benefit from confirmation by BMD. For *P. aeruginosa*, the FDA disk correlates were more variable and did not consistently test one way or another (i.e., more susceptible or resistant) but resulted in a low CA of 36% with 64% mE. These errors occurred with zone diameters between 20 and 24 mm, which would also benefit from confirmation of results by BMD.

CLSI set investigational breakpoints (i.e., research use only) prior to the availability of clinical trial outcome data, whereas data from the complicated urinary tract infection trial (NCT02321800) were available when the FDA breakpoints were set, and this accounts for the difference in breakpoints (15). Both the FDA and CLSI plan to revisit or have revisited the cefiderocol breakpoints as they analyze results from two recent cefiderocol clinical trials (NCT02714595 and NCT03032380). Laboratories should be aware of potential challenges when verifying cefiderocol HardyDisks with FDA breakpoints using the breakpoints published prior to 28 September 2020 (outlined in Table 1). The FDA updated the cefiderocol breakpoints on 28 September 2020; laboratories should be aware of this update as revalidation of AST devices may be required prior to applying the updated breakpoints for patient care.

A consistent finding across both BMD and DD methods is the limited reliability of cefiderocol AST results for *A. baumannii*. Accurate cefiderocol MICs are confounded by the observation of trailing endpoints with BMD. Trailing endpoints are the phenomenon of observing multiple wells with faint growth relative to the growth control. We identified trailing endpoints with 21% of *A. baumannii* isolates. Indeed, multiple assays have demonstrated issues with reliable phenotypic testing for *A. baumannii*, such as the colistin broth disk elution method and the modified carbapenem inactivation method (19–22). DD also posed issues with providing reliable *A. baumannii* complex results. *A. baumannii* isolates frequently had pinpoint colonies within inner zones, making zone diameter measurements difficult. Although, admittedly, both methods have their limitations for *A. baumannii*, as BMD remains the reference standard for MIC testing, we

recommend that cefiderocol AST against *A. baumannii* complex be done by using BMD rather than DD methods.

This study included a relatively small number of isolates, particularly for any individual species. Further, isolates were derived from a single region, which may limit the generalizability of our findings to other regions with differences in the composition of common Gram-negative resistance mechanisms. Our results need to be verified in a larger study that includes geographically diverse isolates. Last, lyophilized BMD panels that have demonstrated equivalency to reference BMD were used as the comparator. These limitations notwithstanding, as cefiderocol is not currently included in any commercial automated AST panel and gradient diffusion methods are also not available, our results indicate that DD offers a convenient, alternative approach to BMD methods for cefiderocol AST. However, caution must be used when results based on the bacterial species and the BP applied are interpreted.

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