Improved Accuracy of Cefepime Susceptibility Testing for Extended-Spectrum-Beta-Lactamase-Producing *Enterobacteriaceae* with an On-Demand Digital Dispensing Method

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ABSTRACT Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* generally cannot be treated with penicillins and cephalosporins. However, some later-generation cephalosporins, including cefepime, are poorly hydrolyzed by specific ESBL enzymes, and certain strains demonstrate *in vitro* susceptibility to these agents, potentially affording additional treatment opportunities. Moreover, the ability to adjust both the dose and dosing interval of beta-lactam agents allows the treatment of strains with elevated MICs that were formerly classified in the intermediate range. The ability to treat strains with elevated cefepime MICs is codified in new susceptible dose-dependent (SDD) breakpoints promulgated by the Clinical and Laboratory Standards Institute. In the interest of validating and implementing new cefepime SDD criteria, we evaluated the performances of Vitek 2, disk diffusion, and a MicroScan panel compared to that of reference broth microdilution (BMD) during the testing of 64 strains enriched for presumptive ESBL phenotype (based on non-susceptibility to ceftriaxone). Surprisingly, categorical agreement with BMD was only 47.6%, 57.1%, and 44.6% for the three methods, respectively. Given these findings, we tested the performance of the HP D300 inkjet-assisted broth microdilution digital dispensing method (DDM), which was previously described by our group as an at-will testing alternative. In contrast to commercial methods, DDM results correlated well with the reference method, with 86% categorical agreement, 91.1% evaluable essential agreement, and no major or very major errors. The reproducibility and accuracy of MIC determinations were statistically equivalent to BMD. Our results provide support for the use of the DDM as a BMD equivalent methodology that will enable hospital-based clinical laboratories to support cefepime MIC-based dosing strategies.

KEYWORDS ESBL, antimicrobial susceptibility testing, broth microdilution, cefepime, digital dispensing, extended-spectrum beta-lactamase producer, inkjet, susceptibility testing, susceptible dose dependent, verification

Few treatment options remain for extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) (1). Therefore, the rapidly increasing worldwide prevalence of these strains is of great concern (2–8). Notably, in China, ESBL-E comprise over half of all *Escherichia coli* bloodstream infections (9), and colonization rates of intensive care unit patients in India have been reported to reach 90% (10). In the United States, prevalence has been rising, especially in certain vulnerable populations, such as patients in adult and pediatric intensive care units, patients with prolonged hospital stays, and patients with exposure to multiple antibiotics (11–15).

ESBL-E harbor serine beta-lactamase enzymes that are, at minimum, capable of hydrolyzing penicillins, at least one of the oxyimino-cephalosporins (commonly referred to as third-generation cephalosporins), and/or aztreonam (16). Of note, ESBL enzymes are both structurally and functionally diverse and potentially expressed at various levels, allowing variable phenotypic resistance, which in some cases can include all cephalosporins (17–20). Previously, Clinical Laboratory and Standards Institute (CLSI) guidance recommended routine ESBL confirmatory testing and classification of positive isolates as resistant (R) to all cephalosporins regardless of MIC data, even if the MIC of the isolate was in the range that would otherwise have been classified as susceptible (S) for a particular agent (21).

Notably, however, cefepime, a fourth-generation cephalosporin, often demonstrates more *in vitro* activity than earlier generation cephalosporins due to greater stability to ESBL hydrolysis (22, 23). This *in vitro* observation correlates with clinical outcome studies, which indicate that cefepime, when administered at higher-than-standard doses, may retain activity against some ESBL-E (24–26). These findings, combined with the observation that certain ESBL enzymes are not active against all third-generation cephalosporins (27), prompted the revisitation of CLSI guidelines, resulting in the lowering of interpretive breakpoints and the elimination of routine ESBL testing in favor of purely MIC-based cephalosporin antimicrobial susceptibility testing (AST) interpretations (26).

The opportunity to treat some presumptive ESBL-E was further codified in the current susceptible dose-dependent (SDD) interpretive category for cefepime (28). Specifically, SDD replaces the "intermediate" category for this drug and, depending on the exact MIC, recommends either an increased dose (MIC = 4 μg ml^{-1}) or an increased dose and frequency (MIC = 8 μg ml^{-1}). Clearly, such specific guidance is predicated on accurate MIC determination.

Unfortunately, these new SDD breakpoints have not yet been cleared by the U.S. Food and Drug Administration (FDA) and adopted by commercial antimicrobial susceptibility testing manufacturers. Therefore, in the interest of validating and implementing the new SDD criteria recommended by the CLSI, we evaluated the performances of several commercial methods (Vitek 2, disk diffusion [DD], and MicroScan panels) that were otherwise approved for testing of cefepime using FDA-cleared breakpoint criteria and a broth microdilution digital dispensing platform recently described by our laboratory (29). Importantly, in order to more informatively gauge accuracy, the strains selected for testing were enriched for presumptive ESBL isolates with MICs often bordering SDD breakpoints, and all methods were compared to reference manual broth microdilution.

RESULTS

The performances of Vitek 2, disk diffusion (DD), MicroScan, and the digital dispensing method (DDM) were compared to the performance of reference broth microdilution (BMD) using a collection of *Enterobacteriaceae* enriched for presumptive ESBL-E on the basis of ceftriaxone resistance by Vitek 2. Notably, we selected for strains that were designated by Vitek 2, our primary clinical platform, as having cefepime MICs in the CLSI susceptible (90.6%; *n* = 58) or SDD (9.4%; *n* = 6) ranges to test whether isolates with presumptive low cefepime MICs were being classified accurately. However, BMD indicated that 49.2%, 25.4%, and 25.4% of strains in this set were susceptible, SDD, and resistant, respectively (Table 1), suggesting a significant skew of Vitek 2 toward a more susceptible categorical interpretation. Notably, 47.6% of strains had a BMD MIC that fell on an interpretive breakpoint.

Cefepime data from Vitek 2, DD, and MicroScan showed categorical agreement of 47.6%, 57.1%, and 44.6%, respectively, with BMD (Table 2); very major errors (VMEs) were 11.1%, 7.1%, and 0%; and major errors (MEs) were 0%, 1.8%, and 23.4%, respectively. The evaluable essential agreement (EA) of Vitek 2 was 21.7% (95% confidence interval [CI] = 9.2% to 40.5%) and overall EA was 52.3% (95% CI = 40.3% to 64.2%). EA for MicroScan was not evaluated because only three results were on scale. Given the

TABLE 1 MIC distribution of the strain set

Genus	No. of strains with a cefepime MIC[a] (μg ml^{-1}) of:					
	≤1	2	4	8	16	>16
Escherichia	11	4	4	11	4	7
Klebsiella	4	2	0	2	1	2
Other[b]	9	1	0	0	1	1
Total no. (%)	24 (37.5)	7 (10.9)	4 (6.3)	13 (20.3)	6 (9.4)	10 (15.6)

[a]The modal consensus value is from at least three BMD measurements.
[b]Other consists of *Enterobacter cloacae* (*n* = 5), *Citrobacter freundii* (3), *Citrobacter amalonaticus* (1),
 Enterobacter aerogenes (1), *Proteus hauseri* (1), and *Proteus vulgaris* (1).

significant discrepancies between clinical methods and BMD, we concluded that SDD CLSI breakpoints could not be reliably implemented with these methods and sought an alternative solution.

Our laboratory previously described the use of an HP D300-based digital dispensing method (DDM) to prepare a BMD equivalent in 384-well plate format with automated spectrophotometric plate reading and susceptibility calls (29). Here, we applied the same technology to generate an antimicrobial doubling dilution series in the standard 96-well plate format recommended for BMD. We first compared the reproducibility of the DDM and reference BMD through repeated measurements of all isolates in our collection. For this analysis, only on-scale measurements were used for calculations, as off-scale results are not log$_2$ transformable. The majority of reference (78.9%) and DDM (80.7%) results were on scale. To compare within-method reproducibility, the log$_2$ difference of each on-scale measurement from the within-method modal MIC was calculated and shown as a distribution in Fig. 1. Each measurement was further classified as "in range" (± 1 twofold dilution from the modal MIC) or "out of range" based on the accepted 2-fold variability of reference BMD. We found that 95.5% (95% CI = 91.1% to 97.8%) of DDM and 92.6% (95% CI = 88.5% to 96.1%) of reference BMD measurements were in range, a nonsignificant difference (Fisher's exact test, P = 0.36).

We next investigated the accuracy of the DDM compared to BMD (Table 3). Evaluable EA, overall EA, and categorical agreement (CA) were 91.1% (95% CI = 85.8% to 94.7%), 88.9% (95% CI = 83.9% to 92.5%), and 86.0% (95% CI = 80.6% to 90.0%), respectively. There were no major or very major errors. There were 14% minor errors (MinEs) (*n* = 29 for the 207 total DDM tests performed), and the MICs for 75.9% (*n* = 22) of the minor errors were in evaluable EA. When SDD was evaluated as two separate categories based on an MIC of 4 or 8 μg ml^{-1}, the DDM showed a CA of 82.6% (95% CI = 76.9% to 87.2%).

Notably, the percentage of in-range results for both the DDM and BMD were ~4% lower than that previously observed in a more diverse set of *Enterobacteriaceae* (i.e., not enriched for ESBL phenotype) (29). This suggested a modestly increased intrinsic biological variability for cefepime response in the current strain set that would lower EA irrespective of intrinsic method accuracy. Moreover, nearly half the strains had an MIC that fell on a breakpoint, leading to an expectation of lower CA.

Therefore, to quantify for comparative purposes the variability of the reference standard, we also determined the within-method agreement of BMD. For each strain,

TABLE 2 Comparison of commercial methods to reference BMD

Method	% agreement (95% CI)			% error category (95% CI)		
	Evaluable essential	Overall essential	Categorical	Minor error	Major error	Very major error
Vitek 2	20.8 (9.2–40.5)	52.3 (40.3–64.2)	47.6 (35.7–59.7)	41.3 (29.9–53.5)	0	11.1 (5.4–21.2)
Disk diffusion	NA[a]	NA[a]	57.2 (44.1–69.2)	33.9 (22.9–47)	1.8 (0.3–9.4)	7.1 (2.8–17.0)
MicroScan	NA[b]	NA[b]	44.7 (31.4–58.8)	31.9 (20.4–46.2)	23.4 (13.6–37.2)	0

[a]NA, not applicable as disk diffusion is not an MIC-based method.
[b]NA due to the limited number (*n* = 3) of on-scale results.
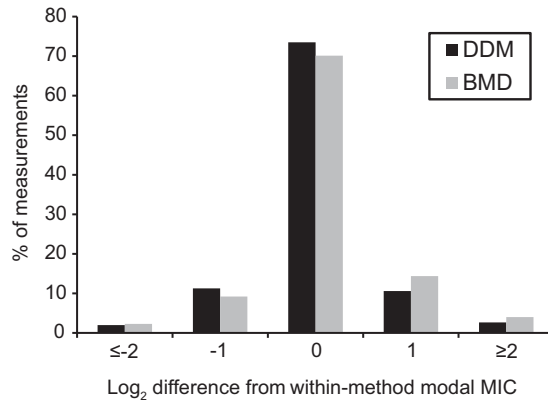
FIG 1 Log$_2$ variance from modal MIC. The log$_2$ differences shown represent the number of 2-fold dilutions away from the modal MIC. A total of 95.5% of DDM ($n = 150$) and 93.2% of BMD measurements ($n = 163$) were within $\pm$ 1 twofold dilution of the modal MIC, respectively.

individual BMD MIC measurements were compared to the modal BMD MIC for a within-method evaluable EA of 93.8% (95% CI = 89.2% to 96.5%), an overall EA of 93.6% (95% CI = 89.5% to 96.1%), and a CA of 90.6% (95% CI = 86% to 93.8%), results not significantly different from the DDM ($P = 0.5$, $P = 0.1$, and $P = 0.2$, respectively). In addition, when SDD was evaluated as two categories based on an MIC of 4 or 8 μg ml^{-1}, within-method BMD showed a CA of 87.4% (95% CI = 82.5% to 91.2%), results not statistically different from the DDM ($P = 0.2$).

DISCUSSION

The new CLSI interpretive criteria for cefepime present a challenge for clinical microbiology laboratories. Updated breakpoint changes must be validated for use with FDA-cleared methods. Importantly, the new SDD category for cefepime recommends distinct dosing regimens predicated on a specific MIC result (i.e., $\leq$2, 4, 8, $\geq$16 μg ml^{-1}). The underlying premise for use of SDD is that available AST methods provide highly accurate MIC values across this range.

Accordingly, prior to implementation of SDD breakpoints, we investigated the clinical performance of the Vitek 2, disk diffusion, and a manual MicroScan panel in interrogation of a strain set enriched for cefepime MICs across the SDD breakpoint range. Strains chosen for analysis were enriched for the presumptive ESBL phenotype (ceftriaxone resistant) and demonstrated by a screening method (Vitek 2) to show a susceptible or SDD phenotype for cefepime. These are the precise types of strains for which SDD is thought to provide utility: strains harboring presumptive enzymes that hydrolyze cefepime relatively poorly and therefore are amenable to treatment with more intense cefepime exposure.

The commercial methods tested performed poorly as assessed by metrics such as EA and CA. These findings were similar to reports prior to SDD introduction and the lowering of cefepime breakpoint susceptibility cutoffs in which Vitek 2 demonstrated a high false-susceptibility rate and a low EA for ESBL-producing organisms (30). Low cefepime CA was also previously observed for ESBL-E strains using other commercial methods, including MicroScan and disk diffusion (31).

We therefore considered an alternative use of the DDM technology that we previously validated in a 384-well plate format with automated interpretation as a BMD equivalent (29). In the present case, we adapted DDM technology to a 96-well plate format with visual reading of MICs in order to replicate exactly classic BMD, with the sole exception that antimicrobial doubling dilutions were created directly through the addition of precise droplet sizes from a stock solution rather than through serial dilution. Importantly, the DDM is technically simple, as it only requires a single pipetting step to create a total of 24 cefepime dilution series in the format described. Therefore,

TABLE 3 Comparison of the DDM to reference BMD

Genus (no.)	No. of measurements with $\log_2$ difference from reference MIC[a]						% agreement (95% CI)			% error category (95% CI)		
	−2	−1	0	1	2	3	Evaluable essential	Overall essential	Categorical	Minor error	Major error	Very major error
Escherichia (41)	0	6	53	40	9	1	90.8 (83.9–94.9)	88.6 (82.1–93.0)	84.1 (76.9–89.4)	15.9 (10.6–23.1)	0	0
Klebsiella (11)	1	4	14	10	3	0	87.5 (71.9–95)	89.1 (75.3–95.7)	94.5 (82.3–95.5)	5.4 (1.5–17.8)	0	0
Other (12)	0	3	5	10	0	0	100 (82.4–100)	89.4 (75.7–95.8)	84.2 (69.6–92.6)	15.7 (7.4–30.0)	0	0
Total no. (%, CI)[b]	1 (0.6)	13 (8.2)	72 (45.3)	60 (37.8)	12 (7.5)	1 (0.6)	145 (91.1, 85.8–94.7)	184 (88.9, 83.9–92.5)	178 (86.0, 80.6–90.1)	29 (14.0, 9.9–19.4)	0	0

[a]Includes only comparisons in which both the DDM result and the reference MIC were on scale (within the range of dilutions tested).

[b]Numerical tabulations include comparison of all individual DDM measurements as applicable, performed in replicate for each strain as described in the Materials and Methods section, to the reference MIC (leading to a total of 207 comparisons for determination of categorical agreement and minor errors).

the DDM can be practically implemented in a clinical laboratory in contrast to laborious manual BMD testing.

Unlike commercial methods, the DDM was highly correlated with the BMD reference method. The modestly lower CA and EA observed compared to our previous DDM study (29) was expected due to the observed biological variability of the strain set for cefepime. Furthermore, the 14% minor error rate, although higher than the general 10% cutoff cited by Cumitech 31A, was completely expected. Specifically, Cumitech guidelines acknowledge the potential for and acceptability of a >10% minor error rate for strain sets containing a significant number of isolates with MICs on an interpretive breakpoint (32). The MICs of 47.6% of our strain set were on an interpretative breakpoint, a reasonable and compelling explanation for a somewhat elevated minor error rate. The accuracy of the DDM was further supported by the observation that DDM EA and CA were statistically indistinguishable from within-method BMD comparisons.

Notably, the ability of at-will dispensing technology to generate customized dilution series allows for more informative quality control (QC). MicroScan panels and other commercial methods, such as Vitek 2, determine or extrapolate MIC values from a narrow range of doubling dilutions surrounding breakpoints. In the MicroScan NM38 panel examined, the lowest panel concentration of cefepime ($2 \mu g \ ml^{-1}$) is 16-fold above the quality control range for the QC strains recommended by the manufacturer and CLSI guidelines. Similarly, in the Vitek 2 GN73 panel, an MIC of $\leq 1 \mu g \ ml^{-1}$ is the lowest value reported. As such, either degradation or unintended misapplication of an antimicrobial by the manufacturer will be undetectable, leaving the end user in total reliance on the manufacturer to ensure adequate performance.

We note for MicroScan that cefepime MICs were consistently high compared to BMD and the DDM, apparent in the large number of observed major errors, and most consistent with low effective cefepime concentrations. Importantly, in this work, we prepared our DDM and BMD quality control dilution series to encompass the QC range of *E. coli* ATCC 25922 MICs, thereby allowing on-scale assessment of QC fidelity, which is of particular importance for relatively labile antibiotics such as cefepime.

Taken together, this study demonstrates that the clinical methods examined do not support the use of SDD breakpoints when tested against ESBL-E strains representative of those for which the SDD category was created. Based on our data, we would recommend that clinical laboratories verify their methodology prior to implementing these or future SDD breakpoints with a strain set whose MIC values span the SDD range and use a highly accurate reference dilution method as a comparator. If satisfactory performance cannot be ensured, SDD criteria should not be implemented. In this case, it is probably prudent for clinical laboratories to mark cefepime as resistant unless ceftriaxone resistance can be otherwise confidently ascribed to derepressed chromosomal *ampC* enzymes, which hydrolyze cefepime inefficiently (33). We also recommend that the CLSI revisit the suitability of disk diffusion for cefepime antimicrobial susceptibility determination in ESBL-E phenotype strains.

In contrast, we found that the DDM, as a true MIC-based methodology, was as accurate and reproducible as reference BMD. Moreover, the DDM has the ability to facilely create any required dilution series of any antimicrobial on demand. This flexibility will enable clinical labs, should they choose to validate this method as a laboratory-developed test, to tailor MIC panels to address this or future SDD implementation. Further, dilution series can be designed to encompass breakpoints for any relevant QC strain, to implement any future breakpoint updates, and to provide subdoubling dilution resolution near breakpoints of interest. Importantly, the DDM is performed with commercially available equipment and reagents. This includes the HP D300 digital dispenser (<$40,000), disposable HP T8+ Dispensehead cassettes (with up to 24 cefepime broth microdilution assays set up per channel at a cost of <$9 per channel), and standard microplates. Disposable and reagent costs per DDM cefepime microdilution assay were <$1. Therefore, we believe that the DDM will provide clinical laboratories with an important and accessible new tool to support strategies, such as SDD, that are designed to help address the emerging antimicrobial resistance threat.

MATERIALS AND METHODS

Bacterial strains and antimicrobials. Sixty-four deidentified *Enterobacteriaceae* clinical isolates were collected at our institution under Institutional Review Board (IRB)-approved protocols between June and September 2016. All strains were colony purified prior to storage at −80°C in tryptic soy broth (BD Diagnostics, Franklin Lakes, NJ) containing 50% glycerol (Sigma-Aldrich, St. Louis, MO). *Escherichia coli* ATCC 25922 was obtained from the American Type Culture Collection (Manassas, VA). Cefepime powder was purchased from Chem-Impex International (Wood Dale, IL). Cefepime stock solution (12.8 mg ml⁻¹) for reference BMD testing was prepared according to CLSI guidelines (34). Cefepime stock solution (30 mg ml⁻¹) for use in digital dispensing was prepared in sterile 0.3% polysorbate 20 (Sigma-Aldrich, St. Louis, MO) as required for liquid handling by the HP D300 (HP, Inc., Palo Alto, CA). Polysorbate 20 was diluted to negligible final levels in a doubling dilution series (ranging from a maximum of 0.0006% to a minimum of 1.3×10^{-6}%). All stock solutions were stored as aliquots at −20°C and discarded after a single use.

Reference broth microdilution testing. BMD panels were prepared in-house according to established guidelines (34). Serial 2-fold dilutions of cefepime at double final concentration were made in sterile, round-bottom, polystyrene 96-well plates (Evergreen Scientific, Los Angeles, CA) using cation-adjusted Mueller-Hinton broth (BD Diagnostics, Franklin Lakes, NJ) in a volume of 50 μl. Panels were stored at −80°C until use (less than 3 weeks).

Susceptibility testing was performed according to CLSI guidelines using the colony suspension method (34). Organisms were grown overnight at 37°C in ambient air on tryptic soy agar containing 5% sheep's blood (Remel, Lenexa, KS). Inoculum was prepared by suspending several colonies in sterile normal saline (0.9% NaCl; Thermo Fisher Scientific, Waltham, MA) and adjusting the suspension to a 0.5 McFarland standard using a DensiChek Plus handheld colorimeter (bioMérieux, Durham, NC). The resulting suspension was diluted 1:150 in sterile, cation-adjusted Mueller-Hinton broth, and 50 μl was added to each well of a microdilution panel. This step brought the bacterial inoculum to a density of approximately 5×10^5 CFU ml⁻¹, as confirmed by CLSI-recommended methods for inoculum validation (34), and cefepime to the final desired concentration, which ranged from 0.125 to 64 μg ml⁻¹. *E. coli* ATCC 25922 was included as a quality control (QC) strain in every experiment. Inoculated panels were incubated at 37°C in ambient air for 16 to 20 h. The MIC was defined as the lowest concentration of antimicrobial resulting in complete inhibition of growth as determined visually (28). Uninoculated wells were included to ensure no contamination occurred during plate preparation.

Digital dispensing method. The HP D300 digital dispensing system (HP, Inc., Palo Alto, CA) is a modified inkjet printer that dispenses droplets varying in size from 11 pl to 10 μl per the manufacturer's specifications (35). It can therefore create doubling dilution series directly from antimicrobial stock solutions by aliquoting the exact volume of antimicrobial desired into specific microwells without the need for serial dilution steps. Using this system, cefepime was dispensed directly from stock solutions into empty, round-bottom, untreated polystyrene 96-well plates (Evergreen Scientific, Los Angeles, CA) to final microwell concentrations ranging from 0.125 to 64 μg ml⁻¹ in a 100-μl final assay volume. Panels were immediately frozen at −80°C until use (less than 3 weeks).

Prior to each experiment, organisms were grown overnight at 37°C in ambient air on tryptic soy agar containing 5% sheep's blood (Remel, Lenexa, KS). Bacterial suspensions with a 0.5 McFarland standard were prepared in normal saline (0.9% NaCl; Thermo Fisher Scientific, Waltham, MA) as for BMD and diluted 1:300 in sterile, cation-adjusted Mueller-Hinton broth (BD Diagnostics, Franklin Lakes, NJ) to yield a bacterial density of approximately 5×10^5 CFU ml⁻¹ as confirmed by CLSI-recommended methods for inoculum validation (34). One-hundred microliters of this suspension was added per well and incubated at 37°C for 16 to 20 h. MIC was defined as for BMD, and *E. coli* ATCC 25922 was again included as a QC strain in every assay plate. Uninoculated wells were included to confirm the absence of contamination during microplate setup.

Determination of reference MIC. To determine the reference MIC, BMD was performed in triplicate for each of the 64 strains in the collection. All replicates were performed on separate days with independent inocula. For each cefepime/organism combination, the modal MIC of triplicate measurements was considered the reference MIC. If a mode was not calculable due to variability among replicates, experiments were repeated in triplicate and the modal MIC was determined from all measurements. In the rare cases (3 out of 430 measurements) where results were bimodal after follow-up testing, the highest mode was recorded.

Clinical validation of Vitek 2, disk diffusion, and MicroScan. Cefepime MIC results from Vitek 2 (GN73 panel), MicroScan (NM38 panel), and disk diffusion (BD BBL Sensi-Disc antimicrobial susceptibility test, 30-μg cefepime discs) zone diameters were interpreted according to current CLSI guidelines (28). Categorical agreement (CA) was evaluated in comparison to the reference BMD MIC, with results considered to be in CA if they fell in the same interpretive category as the BMD result (28). Very major errors (VMEs) were defined as a susceptible result in the test method and a resistant result in the BMD reference method. Major errors (MEs) were defined as a resistant result on the test method and a susceptible BMD result. Minor errors (MinEs) were defined as a susceptible/resistant result in the test method and an SDD result by BMD or the converse. For Vitek 2 only, evaluable essential agreement (EA) was calculated in comparison to BMD. Results were considered to be in evaluable EA if the Vitek 2 result and the BMD result were both on scale and within ±1 twofold dilution. Measurements for which there was growth at the highest concentration of cefepime tested or no growth at the lowest concentration were considered to be off scale. EA was not calculated for MicroScan due to the limited number of on-scale results ($n = 3$).

Verification of DDM. The reproducibility of BMD and the DDM was compared through triplicate measurements of all 64 isolates in our collection. All replicates were performed on separate days with independent inocula. Each on-scale DDM and BMD result was log$_2$ transformed and compared to the within-method modal MIC determined as described above for BMD. The resulting distributions were plotted using Microsoft Excel 2010 (Microsoft, Redmond, WA).

The accuracy of the DDM was determined as previously described (29). All DDM measurements, performed at least in triplicate for each strain, were evaluated in EA and CA determinations. A DDM MIC result was considered to be in evaluable EA if it fell within ±1 doubling dilution of the reference MIC and both results were on scale. A DDM result was considered to be in overall EA if the DDM MIC was either in evaluable EA with the reference MIC, if the DDM MIC and the reference MIC were both off scale in the same direction, or if either the DDM MIC or the reference MIC was at the lowest or highest evaluable MIC tested and the other was off scale in the same direction. Results were considered to be in CA if both methods yielded the same categorical interpretation (S, SDD, or R) based on current CLSI interpretive criteria for cefepime (28). CA analysis was also conducted with an SDD MIC of 4 μg ml^{-1} and an SDD MIC of 8 μg ml^{-1} considered to be two separate categories.

Statistical analysis. For reproducibility studies, measurements that were on-scale were classified as in range (≤1 twofold dilution difference) or out of range (>1 twofold dilution difference) compared to the modal within-method MIC. Proportions of in-range to out-of-range measurements were compared using a two-tailed Fisher's exact test (significance defined as a P value of <0.05). For accuracy studies, EA and CA 95% confidence intervals were calculated as recommended by CLSI (36). JMP 12.0.1 (SAS, Cary, NC) was used for all statistical analyses.

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