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Determination of Disk Diffusion and MIC Quality Control Guidelines for High-Dose Cefepime-Tazobactam (WCK 4282), a Novel Antibacterial Combination Consisting of a β-Lactamase Inhibitor and a Fourth-Generation Cephalosporin

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ABSTRACT High-dose cefepime-tazobactam (1:1; WCK 4282), a novel antibacterial combination consisting of the β-lactamase inhibitor tazobactam and a fourth-generation cephalosporin, is under clinical development for the treatment of serious Gram-negative infections. A quality control (QC) study was performed to establish disk diffusion and MIC ranges for cefepime-tazobactam for multiple QC reference strains. The cefepime-tazobactam QC ranges for a fixed tazobactam MIC of 8 μg/ml and disk diffusion (30/20-μg disk) test methods were approved by the CLSI Subcommittee on Antimicrobial Susceptibility Testing in January 2015 and January 2016. These QC ranges will be important for accurate *in vitro* activity evaluations of cefepime-tazobactam when tested against clinical Gram-negative bacteria during clinical studies and routine patient care.

KEYWORDS cefepime-tazobactam, WCK 4282, quality control

β-Lactam antibacterial agents are among the most commonly used antibiotics worldwide (1). At the same time, the increase in resistance to β-lactam antibacterial agents among Gram-positive and Gram-negative organisms during the past 2 decades is one of the most significant global threats to the efficacy of this class of antimicrobial agents (2, 3). Combining various β-lactam antimicrobial agents with β-lactamase inhibitors has been proven to be an effective therapy for Gram-negative and Gram-positive infections (1). High-dose cefepime-tazobactam (1:1; WCK 4282) is a new antibacterial combination, consisting of 2 g of tazobactam combined with 2 g of cefepime to be administered as an intravenous infusion over 90 min. Cefepime-tazobactam has recently been evaluated in *in vitro* testing and clinical studies (4; A. Bhatia, R. Chugh, M. Gupta, and P. Iwanowski, presented at 26th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Amsterdam, Netherlands, 9 to 12 April, 2016; H. Khande, P. Joshi, S. Palwe, K. Umarkar, S. Takalkar, S. Bhagwat, and M. Patel, presented at 26th ECCMID, Amsterdam, Netherlands, 9 to 12 April, 2016; K. Umarkar, J. Satav, A. Uday, A. Kulkarni, H. Khande, S. Palwe, S. Takalkar, S. Bhagwat, and M. Patel, presented at 26th ECCMID, Amsterdam, Netherlands, 9 to 12 April, 2016). A CLSI M23-styled (tier 2) quality control (QC) study in the style of was performed to establish disk diffusion and broth microdilution QC ranges for several relevant bacterial reference control strains to assist clinical laboratories in monitoring the *in vitro* activity of this combination during clinical trial development and routine antimicrobial susceptibility testing (5–8). Data from these studies were presented at the 2016 ASM Microbe

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TABLE 1 CLSI-approved disk diffusion quality control ranges for cefepime-tazobactam (30/20-μg disks) against reference bacterial strains

Bacterial strain (QC range [mm])	No. of occurrences at zone diameter (mm) of[a]:																	Zone size (mm)	% of zone diameter values in range
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38		
S. aureus ATCC 25923 (24–30)		2	15[a]	78[a]	132[a]	121[a]	83[a]	32[a]	14[a]	3								7	99.0
E. coli ATCC 25922 (32–37)									2	4	22[a]	86[a]	142[a]	113[a]	87[a]	20[a]	4	6	97.9
E. coli NCTC 13353 (27–31)					11	43[a]	156[a]	137[a]	98[a]	30[a]	5							5	96.7
K. pneumoniae ATCC 700603 (25–30)				24[a]	71[a]	170[a]	163[a]	37[a]	12[a]	3								6	99.4
P. aeruginosa ATCC 27853 (27–31)				1	2	58[a]	111[a]	170[a]	99[a]	28[a]	11							5	97.1

[a]Acceptable range for quality control strains according to CLSI document M100-S27 (8).

and ECCMID meetings (M. D. Huband, J. E Ross, R. K. Flamm, R. N. Jones, and H. S. Sader, presented at ASM Microbe, Boston, MA, 16 to 20 June, 2016; J. E Ross, M. D. Huband, R. K. Flamm, R. N. Jones, and H. S. Sader, presented at 26th ECCMID, Amsterdam, Netherlands, 9 to 12 April, 2016).

RESULTS

The CLSI-approved QC ranges of cefepime-tazobactam for bacterial reference strains using disk diffusion and broth microdilution testing are summarized in Tables 1 and 2, respectively. Disk diffusion susceptibility testing was performed for cefepime-tazobactam to establish QC ranges against 5 bacterial reference strains; results are summarized in Table 1. When CLSI M23-A4 analysis criteria were applied (5), 96.7% to 99.4% of the zone of inhibition results for cefepime-tazobactam (30/20-μg disks) against each of the 5 quality control reference strains tested by the 8 participating laboratories were within the proposed QC ranges. *Escherichia coli* strain NCTC 13353, a CTX-M-15 producer, was included to properly evaluate the tazobactam inhibition effect, as cefepime is labile to CTX-M-15 and tazobactam displays potent inhibitory activity against this β-lactamase; the proposed zone diameter QC range of 27 to 31 mm for this strain included 96.7% of reported results. With use of the RangeFinder statistical program (9), there were no laboratories or media lots identified as statistical outliers. Only minor differences ($\leq$1 mm) were observed between the median zone diameter values observed for each QC reference strain among the 3 medium lots tested, regardless of the lot of cefepime-tazobactam disks used. The proposed zone diameter ranges for the 5 QC reference strains tested in this study were subsequently approved at the CLSI meeting of January 2016 (Table 1). Zone of inhibition results for the cefepime (720/720; 100.0%) and piperacillin-tazobactam (720/720; 100.0%) control disks were all within published CLSI QC ranges (8), providing validated internal controls for this study (data not shown).

In addition to disk diffusion testing, broth microdilution susceptibility testing was performed to establish cefepime-tazobactam QC ranges for 7 reference bacterial strains. The cefepime-tazobactam (tazobactam at fixed MIC of 8 μg/ml) broth microdilution MIC results obtained by the 8 laboratories are shown in Table 2 and Fig. S1 to S7

TABLE 2 CLSI-approved broth microdilution quality control ranges for cefepime-tazobactam against QC reference strains

QC strain	Cefepime-tazobactam (fixed MIC, 8 μg/ml)		Figure no.
	CLSI-approved MIC range (μg/ml)	% of MIC values in range	
S. aureus ATCC 29213	1/8–4/8	100.0	S1
E. coli ATCC 25922	0.03/8–0.12/8	100.0	S2
E. coli NCTC 13353[a]	0.06/8–0.25/8	95.8	S3
K. pneumoniae ATCC 700603	0.12/8–0.5/8	99.2	S4
P. aeruginosa ATCC 27853	0.5/8–4/8	100.0	S5
S. pneumoniae ATCC 49619	0.03/8–0.12/8	95.8	S6
H. influenzae ATCC 49247	0.5/8–2/8	100.0	S7

[a]This CTX-M-15-producing strain was needed for proper evaluation of tazobactam enzyme inhibition.

in the supplemental material. A cefepime-tazobactam MIC QC range of 0.03/8 to 0.12/8 μg/ml was recommended for *E. coli* strain ATCC 25922, which included all reported results and an MIC mode at 0.06/8 μg/ml (203/240 MIC values at 0.06 μg/ml; 84.6%). For the *Klebsiella pneumoniae* ATCC 700603 strain, an SHV-18 producer, a QC range of 0.12/8 to 0.5/8 μg/ml was proposed with 99.2% (238/240) of the results included in the range. A 4 doubling dilution range was approved for *Pseudomonas aeruginosa* ATCC 27853 (0.5/8 to 4/8 μg/ml) based on the 87.8% MIC shoulder observed at 2 μg/ml. *E. coli* NCTC 13353 is a CTX-M-15 producer and was included to properly evaluate tazobactam enzyme inhibition effects. The approved MIC QC range of 0.06/8 to 0.25/8 μg/ml for this strain included 95.8% (230/240) of results.

A 3 doubling dilution QC range was approved for both *Haemophilus influenzae* ATCC 49247 (0.5/8 to 2/8 μg/ml) and *Streptococcus pneumoniae* ATCC 49619 (0.03/8 to 0.12/8 μg/ml), which included 100.0% and 95.8% of all MIC results, respectively. Finally, a range of 1/8 to 4/8 μg/ml included 100.0% of cefepime-tazobactam MIC results for *Staphylococcus aureus* ATCC 29213, with 89.2% of the results at the modal MIC (2/8 μg/ml). No significant differences as determined by the RangeFinder statistical program (9) were observed in the performance of the 3 Mueller-Hinton broth media lots used in this study. The MIC results for the cefepime (400/400; 100.0%) and meropenem (318/320; 99.4%) control agents against QC reference strains were within published CLSI QC ranges (≥99.7%, overall), therefore providing validated internal controls for this study (data not shown).

DISCUSSION

Cefepime-tazobactam disk diffusion susceptibility testing demonstrated acceptable interlaboratory and intralaboratory reproducibility for cefepime-tazobactam (30/20-μg) disks with *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853. Broth microdilution susceptibility testing also demonstrated acceptable interlaboratory and intralaboratory reproducibility for cefepime-tazobactam (fixed MIC, 8 μg/ml) with the following CLSI QC reference strains: *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, and *H. influenzae* ATCC 49247. Against *S. pneumoniae* ATCC 49619, a single laboratory was responsible for 10/240 (4.2%) out-of-QC-range (low) cefepime-tazobactam MIC values. The most likely cause is an inoculum at the low end of the acceptable range which was observed for *S. pneumoniae* in this study. Inclusion or exclusion of this laboratory did not alter the calculated cefepime-tazobactam QC range of 0.06/8 to 0.25/8 μg/ml. Furthermore, good interlaboratory and intralaboratory reproducibility was noted for cefepime-tazobactam against *E. coli* NCTC 13353 (CTX-M-15) by both disk diffusion and broth microdilution testing. This reference strain is necessary to effectively QC the tazobactam component of the cefepime-tazobactam combination for β-lactamase inhibition. The CLSI Subcommittee on Antimicrobial Susceptibility Testing approved cefepime-tazobactam (fixed MIC, 8 μg/ml) broth microdilution QC ranges for 7 reference strains in January 2015 and recently published them in Tables 5A and 5B of the CLSI document M100-S26. In January 2016, the subcommittee approved the disk diffusion QC ranges for cefepime-tazobactam against *S. aureus* ATCC 25923, *E. coli* strains ATCC 25922 and NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853. These cefepime-tazobactam disk diffusion QC ranges have recently been published in Table 4A of the CLSI document M100-S27 (8). This study established disk diffusion as well as broth microdilution QC ranges for cefepime-tazobactam against QC reference strains that can be utilized to support accurate antimicrobial susceptibility testing for monitoring the *in vitro* activity of this combination during clinical trial development as well as during routine clinical antimicrobial susceptibility testing.

MATERIALS AND METHODS

Applying CLSI guidelines for the development of *in vitro* susceptibility testing criteria and QC parameters, 8 experienced microbiology laboratories participated in each of the 2 arms of this study to establish QC ranges for broth microdilution (BMD) and disk diffusion (DD) testing (5–8). The following

laboratory sites (principal investigator) participated: JMI Laboratories, North Liberty, IA, USA (R. N. Jones; BMD, DD); TREK Diagnostic Systems/Thermo Fisher Scientific, Cleveland, OH, USA (C. Knapp; BMD, DD); Cleveland Clinic Foundation, Cleveland, OH, USA (G. Procop; BMD, DD); Wheaton Franciscan Laboratory, Wauwatosa, WI, USA (E. Munson; BMD, DD); University of Alberta Hospitals, Edmonton, Alberta, Canada (R. Rennie; BMD, DD); University of Washington Medical Center, Seattle, WA, USA (S. Swanzy; BMD); Johns Hopkins Bayview Medical Center, Baltimore, MD, USA (S. Riedel; BMD); Summa Health Systems, Akron, OH, USA (G. Kallstrom; BMD); University of Rochester Medical Center, Rochester, NY, USA (D. Hardy; DD); Indiana University Health Methodist Hospital, Indianapolis, IN, USA (G. Denys; DD); Henry Ford Hospital, Detroit, MI, USA (M. Zervos; DD).

For disk diffusion testing, 2 different lots of 30/20-μg cefepime-tazobactam disks were manufactured by 2 companies, Mast Group Ltd., Bootle, Merseyside, UK (lot 358874) and Bio-Rad Laboratories, Hercules, CA, USA (lot 5G0010). A single lot each of cefepime (30-μg) and piperacillin-tazobactam (100/10-μg) control disks from Becton, Dickinson (Franklin Lakes, NJ, USA) were used as internal controls (BD lots 5161869 and 5111769, respectively). Three lots of Mueller-Hinton (MH) agar plates produced by Hardy Diagnostics (Santa Maria, CA, USA; lot 15260), Remel (Lenexa, KS, USA; lot 745316), and BD BBL (Franklin Lakes, NJ, USA; lot 5230996) were used for disk diffusion testing. Disk diffusion zones of inhibition testing was performed as described in CLSI document M02-A12 (6). Appropriate starting inoculum concentrations were verified by performing colony counts. The 5 reference QC strains tested included *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853. Ten replicates of each QC strain were tested using 3 different lots of MH agar and 2 lots of cefepime-tazobactam (30/20-μg) disks obtained from 2 separate manufacturers in 8 qualified laboratories generating a total of 480 zone diameters for each QC reference strain. Agar plates were inoculated from a 0.5 McFarland standard suspension of each QC reference strain following standard CLSI procedures for disk diffusion (6), and 2 cefepime-tazobactam 30/20-μg disks as well as 1 cefepime 30-μg disk and 1 piperacillin-tazobactam 100/10-μg disk were applied. Inoculated MH agar plates were incubated for 16 to 18 h at 35°C in an ambient air incubator, after which zone diameters were manually determined.

For broth microdilution testing, 3 different panels (lots CML1WKK, CML2WKK, and CML3WKK) were prepared by a certified GMP source (TREK Diagnostic Systems/Thermo Fisher Scientific) for each panel design, 3 different lots of cation-adjusted MH broth manufactured by 3 different companies were used, Difco Laboratories (Detroit, MI, USA; lot 3120127), Becton Dickinson (Sparks, MD, USA; lot 4044343), and Oxoid (Hampshire, UK; lot 1394228). Cefepime, tazobactam, meropenem, and ceftriaxone powders were provided by TREK Diagnostic Systems (Thermo Fisher Scientific). The 7 QC reference strains tested included *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 for panel 1; *H. influenzae* ATCC 49247 for panel 2; and *S. pneumoniae* ATCC 49619 for panel 3. The 8 participating laboratories performed broth microdilution MIC testing following CLSI guidelines (7); panel 1 was incubated for 16 to 20 h at 35°C in an ambient air incubator, and panels 2 and 3 were incubated for 20 to 24 h at 35°C in an ambient air incubator. All MIC endpoints were read at 100%, i.e., complete inhibition of growth. For inoculum preparation, colonies from a 5% sheep blood agar or chocolate agar plate (overnight growth) were suspended into Mueller-Hinton broth or saline to prepare a solution adjusted to the density of a 0.5 McFarland standard. Experiments were performed over a minimum of 3 days, generating 1 MIC value in 3 different media lots for 10 replicates (30 determinants) for each QC strain per site for cefepime-tazobactam at 8 participating laboratory sites, resulting in a total number of 1,680 MIC values. Internal QC testing was performed using meropenem and ceftriaxone, with test concentration ranges of 0.008 to 8.0 μg/ml for each drug. All meropenem and ceftriaxone MIC QC values obtained were within published CLSI ranges. To verify the accuracy of the prepared inoculums, colony counts were performed by subculturing in a quantitative manner onto antimicrobial-free agar plates and resulted in average counts ranging from 2×10^5 to 5×10^5 CFU/ml; all results were within an acceptable inoculum target range of 2 to 8×10^5 CFU/ml.

For the final data analysis, the RangeFinder statistical program was applied to evaluate the ranges of the MIC and zone diameter results (9), whereas the Gavan statistic (10) was also applied to evaluate the zone diameter results.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00788-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 2, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 3, PDF file, 0.1 MB.
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SUPPLEMENTAL FILE 5, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 6, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 7, PDF file, 0.1 MB.

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