WCK 4282 (Cefepime-Tazobactam) MIC Quality Control Ranges Using a Multi-Laboratory Study Design

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AMENDED ABSTRACT

Background: We conducted a study to establish MIC quality control (QC) ranges for WCK 4282 (high-dose cefepime-tazobactam) with tazobactam at a fixed concentration of 8 mg/L, using the reference broth microdilution (BMD) method. Cefepime-tazobactam is under clinical development for the treatment of serious Gram-negative infections.

Material/methods: An eight laboratory study design followed CLSI M23-A3 guidelines. Seven QC strains were tested (*Escherichia coli* ATCC 25922, *E. coli* NCTC 13353, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Haemophilus influenzae* ATCC 49247, *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213), using three media lots (three manufacturers) of cation-adjusted Mueller-Hinton broth (CA-MHB), Haemophilus Test Medium (HTM) and CA-MHB supplemented with 2.5-5% lysed horse blood. Ten replicate tests were performed for each QC organism generating 240 BMD values/QC strain (1,680 total results). Cefepime and meropenem were used as control agents.

Results: A cefepime-tazobactam MIC QC range of 0.03/8 – 0.12/8 mg/L was proposed for *E. coli* ATCC 25922 (see Table), which included all reported results and a mode at 0.06/8 mg/L (203 of 240 results; 84.6%). *E. coli* NCTC 13353 is a CTX-M-15 producer and was included to properly evaluate tazobactam inhibition effect. The proposed MIC QC range of 0.06/8 – 0.25/8 mg/L for this strain included 96.0% of results. *K. pneumoniae* ATCC 700603, a SHV-18 producer, provided a three doubling dilution QC range of 0.12/8 – 0.5/8 mg/L with 99.2% of the results included. A four doubling dilution range was proposed for *P. aeruginosa* ATCC 27853 (0.5/8 – 4/8 mg/L) due to a bimodal MIC distribution. A three doubling dilution QC range was proposed for both *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619, which included 100.0% of the MIC results. A three doubling dilution range of 1/8 – 4/8 mg/L included all MIC results for *S. aureus* ATCC 29213, with >89% of the results at the modal MIC (2/8 mg/L). No significant differences were noted among media lots. Only two of 1,120 MIC values (0.2%) generated for the control agents were outside the CLSI published QC ranges. The CLSI Subcommittee on Antimicrobial Susceptibility Testing approved these WCK 4282 (cefepime-tazobactam) QC ranges in January 2015.

Conclusions: The recently approved MIC QC ranges for WCK 4282 (cefepime-tazobactam) should accurately guide clinical or reference laboratories participating in the testing of clinical trial isolates, and facilitate the regulatory review process for this investigational antimicrobial combination (see Tables and Figures).

INTRODUCTION

WCK 4282 (high dose cefepime-tazobactam) is a new antibacterial combination consisting of the β-lactamase inhibitor tazobactam (tested at a fixed concentration of 8 mg/L) and the fourth-generation cephalosporin, cefepime. It has demonstrated excellent antibacterial activity against contemporary Gram-negative pathogens including isolates showing resistance to existing drug classes. A Clinical and Laboratory Standards Institute (CLSI) M23-style (tier 2) quality control (QC) study was performed to establish broth microdilution MIC QC ranges for seven reference bacterial strains. These ranges will assist clinical and reference laboratories in monitoring the activity of this combination during clinical trials and in clinical microbiology practice.

MATERIALS AND METHODS

Participating institutions. A total of eight laboratories participated in the QC study and provided WCK 4282 (cefepime-tazobactam) MIC data for the QC reference strains, as follows: JMI Laboratories, North Liberty, Iowa, USA (R.N. Jones, M.D.); Summa Health Systems, Akron, Ohio, USA (G. Kallstrom, Ph.D.); TREK Diagnostic Systems/ThermoFisher Scientific, Cleveland, Ohio, USA (C. Knapp, M.S.); University of Alberta, Edmonton, Canada (R. Rennie, Ph.D.); Wheaton Franciscan Laboratory, Wauwatosa, Wisconsin, USA (E. Munson, Ph.D.); University of Washington Medical Center, Seattle, Washington, USA (S. Swanzy, B.S., M.T. [ASCP]); Cleveland Clinic Foundation, Cleveland, Ohio, USA (G. Procop, M.D.) and Johns Hopkins Bayview Medical Center, Baltimore, Maryland, USA (S. Riedel, M.D., Ph.D.).

Susceptibility testing. Broth microdilution panels were prepared by a certified GMP source (Trek Diagnostic Systems/ThermoFisher Scientific) using three cation-adjusted Mueller-Hinton (MH) broth media lots produced by Difco Laboratories (Detroit, Michigan, USA), Becton Dickinson (BD; Sparks, Maryland, USA), and Oxoid (Hampshire, United Kingdom). Cefepime and tazobactam powders were provided by Wockhardt Limited. Broth microdilution MIC testing was performed as described in CLSI guidelines (M07-A10; 2015) and panels were incubated for 16-20 hours at 35°C in an ambient air incubator. All sites were instructed to read the MIC endpoint at 100% (complete inhibition of growth). Appropriate inoculum concentrations were verified by performing colony counts from broth in the microdilution trays which were subcultured in a quantitative manner onto drug-free agar plates. The QC reference strains tested included: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and NCTC 13353, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Streptococcus pneumoniae* ATCC 49619 and *Haemophilus influenzae* ATCC 49247. Ten replicates of each QC strain were tested in 3 different lots of media producing 1,680 MIC values for WCK 4282 and the control, meropenem.

RESULTS

- Applying CLSI M23 analysis criteria to WCK 4282 (cefepime-tazobactam; tazobactam at fixed 8 mg/L), >95% of MIC results from the eight participating laboratories (seven laboratories for *S. pneumoniae* ATCC 49619) were within the proposed QC ranges recently approved (CLSI) for each of the seven reference strains (**Table 1** and **Figures 1-7**).
- MIC results for the cefepime (560/560; 100.0%) and meropenem (558/560; 99.6%) control agents were within CLSI published QC ranges ≥99.6% overall, providing validated internal controls for this study.
- No significant difference in media performance was observed among the three lots of Mueller-Hinton broth used.
- Colony counts were performed on each of the QC strains tested (count averages ranged from 1.7x10^5 to 4.7x10^5 CFU/mL) and results were within acceptable inoculum targets.

Table 1. Approved quality control ranges for broth microdilution testing of WCK 4282 (cefepime-tazobactam).

QC organism	MIC range (mg/L)	% in proposed range
S. aureus ATCC 29213	1/8 – 4/8	100.0
E. coli ATCC 25922	0.03/8 – 0.12/8	100.0
P. aeruginosa ATCC 27853	0.5/8 – 4/8	100.0
K. pneumoniae ATCC 700603	0.12/8 – 0.5/8	99.2
E. coli NCTC 13353[a]	0.06/8 – 0.25/8	96.0
S. pneumoniae ATCC 49619	0.03/8 – 0.12/8	100.0[b]
H. influenzae ATCC 49247	0.5/8 – 2/8	100.0

a. This CTX-M-15 and OXA-1 producing strain is needed for proper evaluation of tazobactam enzyme inhibition.
b. Excluding data from one laboratory.

Figure 1. WCK 4282 (cefepime-tazobactam) MIC distributions for *S. aureus* ATCC 29213

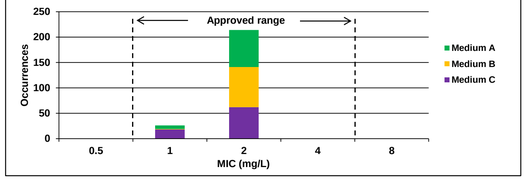


Figure 2. WCK 4282 (cefepime-tazobactam) MIC distributions for *E. coli* ATCC 25922

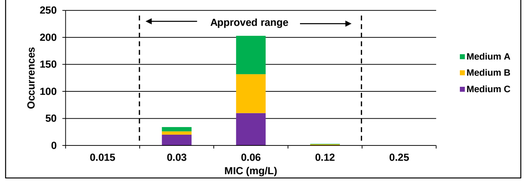


Figure 3. WCK 4282 (cefepime-tazobactam) MIC distributions for *P. aeruginosa* ATCC 27853

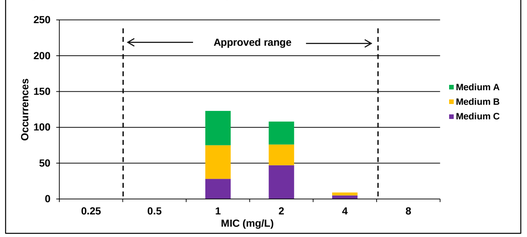


Figure 4. WCK 4282 (cefepime-tazobactam) MIC distributions for *K. pneumoniae* ATCC 700603

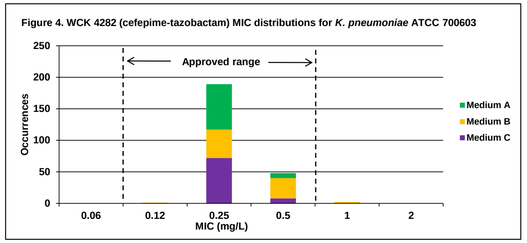


Figure 5. WCK 4282 (cefepime-tazobactam) MIC distributions for *E. coli* NCTC 13353

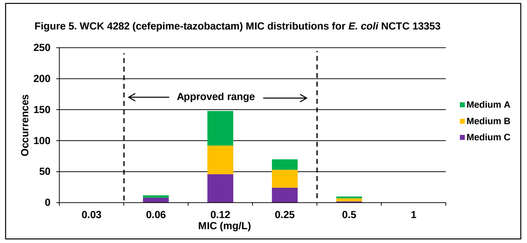


Figure 6. WCK 4282 (cefepime-tazobactam) MIC distributions for *S. pneumoniae* ATCC 49619

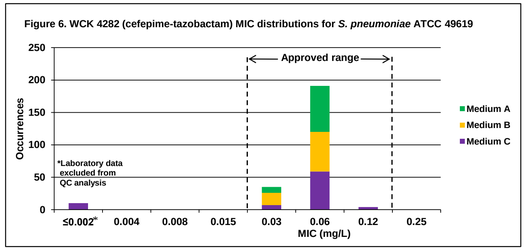
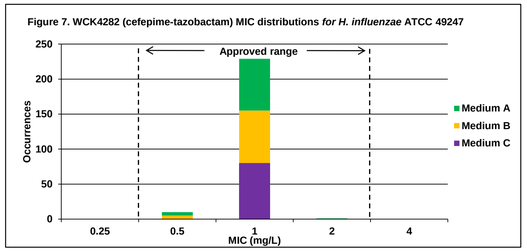


Figure 7. WCK4282 (cefepime-tazobactam) MIC distributions for *H. influenzae* ATCC 49247



CONCLUSIONS

- WCK 4282 (cefepime-tazobactam) broth microdilution susceptibility testing demonstrated acceptable inter- and intra-laboratory reproducibility with the following CLSI QC reference strains: *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247.
- Good inter- and intra-laboratory reproducibility was also noted for WCK 4282 (cefepime-tazobactam) against *E. coli* NCTC 13353. This reference strain would be necessary to QC the tazobactam component of the cefepime-tazobactam combination for β-lactamase inhibition.
- The CLSI subcommittee on Antimicrobial Susceptibility Testing approved WCK 4282 (cefepime-tazobactam) QC ranges for these 7 reference strains in January 2015, and recently published them in Tables 5A and 5B of the M100-S26 document.
- This study established QC ranges for WCK 4282 (cefepime-tazobactam) against seven QC reference strains that can be utilized to support accurate antimicrobial susceptibility testing.

ACKNOWLEDGEMENTS

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