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Cefepime-Enmetazobactam (formerly AAI101; 30/20 µg) and Cefepime (30 µg) Disk Diffusion Quality Control Ranges Using a CLSI M23 (2018) Multi-Laboratory Study Design

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Introduction

- Standard therapies for the treatment of serious Gram-negative infections by third-generation cephalosporin (3GC)-resistant Enterobacteriaceae are losing efficacy. As a result, carbapenems are used more frequently in settings with a high prevalence of 3GC-resistance
- Carbapenem-resistant and 3GC-resistant Enterobacteriaceae have been categorized as "critical priority" pathogens by the World Health Organization
- Enmetazobactam (formerly AAI101) is a novel extended-spectrum β-lactamase inhibitor that targets 3GC-resistant Enterobacteriaceae
- Cefepime-enmetazobactam is intended for the empiric treatment of serious gram-negative infections in settings with an elevated prevalence of ESBL-producing Enterobacteriaceae
- Cefepime-enmetazobactam (2g/0.5g) administered q8h has recently entered phase 3 clinical trials in patients with complicated urinary tract infections and pyelonephritis (NTC03687255)
- In this study, Clinical and Laboratory Standards Institute (CLSI) M23 (2018) Tier-2 Quality Control (QC) studies were conducted to establish disk diffusion QC ranges for cefepime-enmetazobactam (30/20 µg) and cefepime (30 µg) disks against CLSI reference strains

Materials and Methods

- The bacterial QC reference strains tested were *Escherichia coli* ATCC 25922 (β-lactamase-negative), *E. coli* ATCC 35218 (non-ESBL, TEM-1 β-lactamase producing), *E. coli* NCTC 13353 (CTX-M-15, ESBL-producing), *Klebsiella pneumoniae* ATCC 700603 (SHV-18, ESBL-producing), and *Pseudomonas aeruginosa* ATCC 27853
- CLSI M23 Tier-2 disk diffusion QC testing utilized at least 8 participating laboratories (Table 1), 3 lots of Mueller-Hinton agar medium obtained from 3 manufacturers, and ≥10 replicate tests per QC strain
- Testing was performed over a minimum of 3 days with no more than 4 replicates tested per day
- Mueller-Hinton agar for disk diffusion susceptibility testing was obtained from Remel (Thermo Fisher Scientific; Waltham, Massachusetts, medium lot A), Becton Dickinson (BBL; Franklin Lakes, NJ, medium lot B), and Hardy Diagnostics (Santa Maria, California, medium lot C)
- Disk diffusion testing employed 2 lots of cefepime-enmetazobactam (30/20 µg) disks obtained from Oxoid (Thermo Fisher Scientific) and 2 lots of cefepime (30 µg) disks obtained from Becton Dickinson (BBL) and Oxoid (Thermo Fisher Scientific)
- Inoculum densities were monitored by bacterial colony counts
- 2 cefepime-enmetazobactam (30/20 µg) inhibition zone diameter values were generated for 3 media lots at 8 independent laboratories over 10 replicates totaling 480 zone diameter values; a minimum of 420 inhibition zone diameter values are required to fulfil CLSI M23 criteria
- Inhibition zone diameter ranges for each QC reference strain were calculated using the Gavan statistic and RangeFinder statistical program

Results

- 7 mm QC ranges containing 97.1%-100.0% of all cefepime-enmetazobactam (30/20 µg) disk diffusion zone diameter values were approved at the January 2019 CLSI meeting for *E. coli* ATCC 25922 (32-38 mm), *E. coli* ATCC 35218 (32-38 mm), *E. coli* NCTC 13353 (27-33 mm), *K. pneumoniae* ATCC 700603 (26-32 mm), and *P. aeruginosa* ATCC 27853 (26-32 mm) (Table 2 and Figures 1-5)
- A 7 mm QC range (31-37 mm) containing 100.0% of cefepime (30 µg) zone diameter values was approved at the January 2019 CLSI meeting for *E. coli* ATCC 35218 (Table 2 and Figure 6)
- Colony counts were performed on each of the QC reference strains and results were within acceptable inoculum targets of 1-2 x 10^6 CFU/mL for a 0.5 McFarland standard
- Against cefepime (30 µg) tested alone, 97.1% (466/480), 99.5% (418/420), 99.8% (479/480), and 99.2% (476/480) of the inhibition zone diameter values against *E. coli* ATCC 25922, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853, respectively, were within current CLSI-approved QC ranges, providing validated internal controls on each day of susceptibility testing

Table 1 Investigators and laboratories participating in the CLSI M23 disk diffusion quality control study

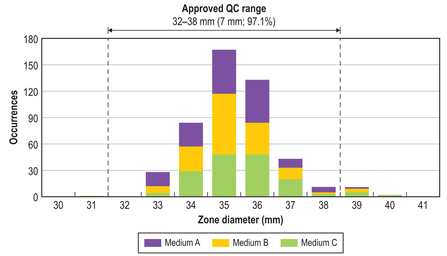
Investigator	Participating Laboratory and Location
R. Flamm, PhD	JMI Laboratories, North Liberty, Iowa, USA
C. Knapp, MS	Thermo Fisher Scientific, Oakwood Village, Ohio, USA
D. Dressel, MS	International Health Management Associates, Schaumberg, Illinois, USA
D. Snydman, MD	Tufts University Medical Center, Boston, Massachusetts, USA
A. Bobenchik, PhD	Rhode Island Miriam Hospital, Providence, Rhode Island, USA
D. Hardy, PhD	University of Rochester Medical Center, Rochester, New York, USA
C. Pillar, PhD	Micromyx Inc., Kalamazoo, Michigan, USA
G. Denys, PhD	Indiana University Health, Methodist Hospital, Indianapolis, Indiana, USA

Table 2 CLSI approved disk diffusion QC ranges for cefepime-enmetazobactam (30/20 µg) and cefepime (30 µg) against reference strains

Reference strain	Approved CLSI disk diffusion QC ranges (mm)	
	FPE[a] (30/20 µg)	Cefepime (30 µg)
Escherichia coli ATCC 25922	**32 – 38** (7 mm)	31 – 37[b] (7 mm)
Escherichia coli ATCC 35218	**32 – 38** (7 mm)	**31 – 37** (7 mm)
Escherichia coli NCTC 13353	**27 – 33** (7 mm)	6 – 15[b] (10 mm)
Klebsiella pneumoniae ATCC 700603	**26 – 32** (7 mm)	23 – 29[b] (7 mm)
Pseudomonas aeruginosa ATCC 27853	**26 – 32** (7 mm)	25 – 31[b] (7 mm)

[a] FPE, cefepime-enmetazobactam (30/20 µg).
[b] Current CLSI QC range.

Figure 1 Cefepime-enmetazobactam (30/20 µg) disk distributions by medium lot for *E. coli* ATCC 25922



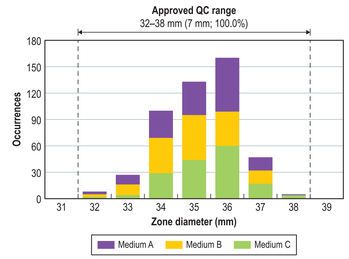


Figure 2 Cefepime-enmetazobactam (30/20 µg) disk distributions by medium lot for *E. coli* ATCC 35218

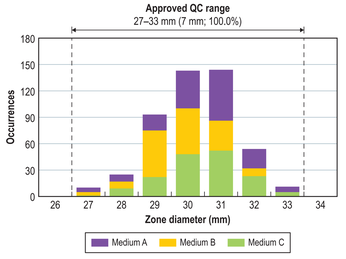


Figure 3 Cefepime-enmetazobactam (30/20 µg) disk distributions by medium lot for *E. coli* NCTC 13353

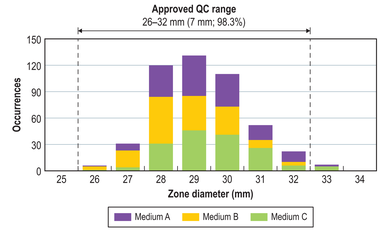


Figure 4 Cefepime-enmetazobactam (30/20 µg) disk distributions by medium lot for *K. pneumoniae* ATCC 700603

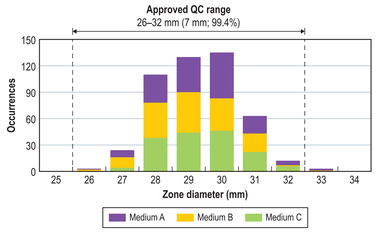


Figure 5 Cefepime-enmetazobactam (30/20 µg) disk distributions by medium lot for *P. aeruginosa* ATCC 27853

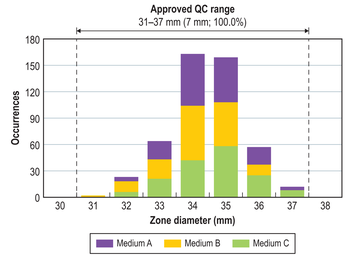


Figure 6 Cefepime (30 µg) disk distributions by medium lot for *E. coli* ATCC 35218

Conclusions

- 7 mm QC ranges containing 97.1-100.0% of all cefepime-enmetazobactam (30/20 µg) disk diffusion zone diameter values were approved for *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 at the January 2019 CLSI meeting
- A 7 mm QC range containing 100.0% of cefepime (30 µg) zone diameter values was approved for *E. coli* ATCC 35218 at the January 2019 CLSI meeting
- *E. coli* NCTC 13353 is recommended for routine QC testing of cefepime-enmetazobactam (30/20 µg) disks as it can effectively control for both β-lactamase activity (hydrolysis of cefepime by CTX-M-15) and inhibition (by enmetazobactam)
- Established disk diffusion QC ranges for cefepime-enmetazobactam (30/20 µg) disks will ensure that appropriate disk diffusion QC standards are implemented by reference and clinical laboratories

Acknowledgements

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