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# Cefepime-AAI101 and Cefepime MIC Quality Control Ranges Using a CLSI M23-A5 Multi-Laboratory Study Design

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## Introduction

- AAI101 is a novel β-lactamase inhibitor highly active against extended-spectrum β-lactamases (ESBLs), the primary mechanism of β-lactam resistance toward oxyiminocephalosporins
- The cefepime-AAI101 combination has completed Phase 2 clinical trials to evaluate the efficacy and safety of the combination cefepime-AAI101 for treatment of complicated urinary tract infections (cUTI)
- Clinical and Laboratory Standards Institute (CLSI) M23-A4 (tier 2) quality control studies were conducted to establish cefepime-AAI101 broth microdilution (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) and cefepime quality control (QC) ranges against CLSI QC reference strains

## Materials and Methods

- Investigators and institutions participating in CLSI M23 (2018) broth microdilution QC studies for cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) and cefepime are listed in Table 1
- Broth microdilution studies utilized a minimum of 7 participating laboratories (Table 1), 3 lots of cation-adjusted Mueller-Hinton medium obtained from at least 2 different manufacturers, and ≥10 replicate tests per QC strain tested over a minimum of 3 days
  - Cation adjusted Mueller-Hinton medium for broth microdilution testing was obtained from Difco (Detroit, Michigan), Becton Dickinson (BD; Sparks, Maryland), and Oxoid (Hampshire, United Kingdom)
- Frozen-form broth microdilution susceptibility panels were prepared in a certified good-manufacturing practice facility (Trek Diagnostic Systems/Thermo Fisher Scientific, Oakwood Village, Ohio) using AAI101 powder provided by Allecra Therapeutics
- QC reference strains tested included *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853
- *Escherichia coli* NCTC 13353 was included as an ESBL-producing (CTX-M-15) QC reference strain specifically to address the activity of the cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) combinations

**Table 1 Investigators and laboratories participating in the cefepime-AAI101 and cefepime M23 broth microdilution quality control studies**

| Investigator | Laboratory and location | M23 study participation[a] |
|---|---|---|
| R. Flamm, PhD | JMI Laboratories, North Liberty, Iowa, USA | F4 and F8 |
| C. Knapp, MS | Thermo Fisher Scientific, Oakwood Village, Ohio, USA | F4 and F8 |
| J. Hindler, MS | UCLA Medical Center, Los Angeles, California, USA | F4 and F8 |
| G. Kallstrom, PhD | Summa Health System, Akron, Ohio, USA | F8 |
| E. Munson, PhD | Wheaton Franciscan Laboratory, Wauwatosa, Wisconsin, USA | F8 |
| S. Riedel, MD, PhD | Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA | F4 and F8 |
| D. Hardy, PhD | University of Rochester Medical Center, Rochester, New York, USA | F4 and F8 |
| T. Fritsche, MD, PhD | Marshfield Laboratories, Marshfield, Wisconsin, USA | F4 and F8 |
| C. Pillar, PhD | Micromyx Inc., Kalamazoo, Michigan, USA | F4 |
| G. Denys, PhD | Indiana University Health, Methodist Hospital, Indianapolis, Indiana, USA | F4 |

[a] F4 = fixed AAI101 concentration of 4 µg/mL, F8 = fixed AAI101 concentration of 8 µg/mL

## Results

- Applying CLSI M23 and RangeFinder statistical analysis criteria to the cefepime-AAI101 combinations, ≥99.2% of cefepime-AAI101 (fixed AAI101 concentration of 4 µg/mL) and ≥99.6% of cefepime-AAI101 (fixed AAI101 concentration of 8 µg/mL) MIC values from the participating laboratories were within the approved QC ranges for each of the reference strains tested (Table 2)
- Colony counts were performed on each of the QC reference strains tested (Table 3) and results were within acceptable inoculum targets
- Three dilution broth microdilution QC ranges were approved by the CLSI for cefepime-AAI101 (fixed AAI101 concentration of 4 µg/mL) against: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, and *K. pneumoniae* ATCC 700603 (Table 2, Table 4, and Figure 1), and a 4-dilution QC range was approved for *P. aeruginosa* ATCC 27853 based on the bimodal (65.7% MIC shoulder at 2/4 µg/mL) distribution of data (Table 2)
- Similarly, 3 dilution broth microdilution QC ranges were approved for cefepime-AAI101 (fixed AAI101 concentration of 8 µg/mL) against *E. coli* ATCC 25922, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 (Table 2, Table 5, and Figure 2), and a 4-dilution QC range was approved for *E. coli* ATCC 35218 based on the bimodal (80.0% MIC shoulder at 0.03/8 µg/mL) distribution of data (Table 2)
- Among the reference bacterial strains tested that have approved cefepime QC ranges, 100.0% of the cefepime MIC values against *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, and *P. aeruginosa* ATCC 27853 and 99.8% of the cefepime MIC results against *K. pneumoniae* ATCC 700603 were within CLSI approved QC ranges, providing validated internal controls for these studies

**Table 2 CLSI approved broth microdilution quality control ranges for cefepime-AAI101 and cefepime against reference strains**

| Quality control reference strain | CLSI approved quality control ranges (µg/mL) (# of dilutions; % of values in range) | | |
|---|---|---|---|
| | Cefepime-AAI101 (fixed 4 µg/mL) | Cefepime-AAI101 (fixed 8 µg/mL) | Cefepime |
| *Escherichia coli* ATCC 25922 | 0.03/4 – 0.12/4 (3; 100.0%) | 0.03/8 – 0.12/8 (3; 100.0%) | 0.016 – 0.12[a] (4; 100.0%) |
| *E. coli* ATCC 35218 | 0.016/4 – 0.06/4 (3; 100.0%) | 0.008/8 – 0.06/8 (4; 100.0%) | 0.008 – 0.06[b] (4; 100.0%) |
| *E. coli* NCTC 13353 | 0.06/4 – 0.25/4 (3; 99.5%) | 0.03/8 – 0.12/8 (3; 100.0%) | ≥64[b] (100.0%) |
| *Klebsiella pneumoniae* ATCC 700603 | 0.12/4 – 0.5/4 (3; 99.2%) | 0.12/8 – 0.5/8 (3; 100.0%) | 0.5 – 2[a] (3; 99.8%) |
| *Pseudomonas aeruginosa* ATCC 27853 | 0.5/4 – 4/4 (4; 100.0%) | 0.5/8 – 2/8 (3; 99.6%) | 0.5 – 4[a] (4; 100.0%) |

[a] Current CLSI quality control range
[b] QC range approved at the June 2017 CLSI meeting (Philadelphia, Pennsylvania)

**Table 3 Colony counts for the quality control reference strains used in broth microdilution susceptibility testing**

| CLSI QC reference strain | Range[a] (CFU/mL) | Average[b] (CFU/mL) |
|---|---|---|
| *Escherichia coli* ATCC 25922 | $8.0 \times 10^4$ – $9.0 \times 10^5$ | $3.1 \times 10^5$ |
| *E. coli* ATCC 35218 | $1.0 \times 10^5$ – $8.0 \times 10^5$ | $3.1 \times 10^5$ |
| *E. coli* NCTC 13353 | $1.0 \times 10^5$ – $7.1 \times 10^5$ | $3.2 \times 10^5$ |
| *Klebsiella pneumoniae* ATCC 700603 | $1.0 \times 10^5$ – $9.0 \times 10^5$ | $3.2 \times 10^5$ |
| *Pseudomonas aeruginosa* ATCC 27853 | $9.0 \times 10^4$ – $9.5 \times 10^5$ | $4.0 \times 10^5$ |

[a] Range of all participating laboratories
[b] Average of all participating laboratories

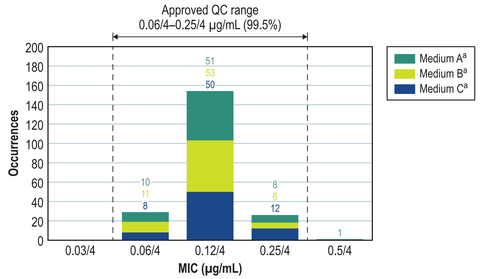
**Table 4 Inter- and intralaboratory comparisons of cefepime-AAI101 (fixed AAI101 concentration of 4 µg/mL) MIC values against *E. coli* NCTC 13353 for an 8-laboratory protocol meeting the study design guidelines found in CLSI M23-A4 (2016)**

| MIC (µg/mL) | Occurrences by media lot[a] | | | Laboratory (occurrences): | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | D | E | F | G | H[b] | |
| 0.03/4 | | | | | | | | | | | | |
| 0.06/4 | 10 | 11 | 8 | 1 | | 2 | 1 | | 20 | 5 | | 29 |
| 0.12/4 | 51 | 53 | 50 | 12 | 25 | 27 | 25 | 30 | 10 | 25 | | 154 |
| 0.25/4 | 8 | 6 | 12 | 16 | 5 | 1 | 4 | | | | | 26 |
| 0.5/4 | 1 | | | 1 | | | | | | | 19 | 1 |
| 1/4 | | | | | | | | | | | 10 | |
| 2/4 | | | | | | | | | | | 1 | |
| **Total** | 70 | 70 | 70 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | — | 210 |
| **Mean** | 0.13/4 | 0.12/4 | 0.14/4 | 0.20/4 | 0.14/4 | 0.12/4 | 0.14/4 | 0.12/4 | 0.08/4 | 0.11/4 | — | 0.13/4 |
| **Median** | 0.12/4 | 0.12/4 | 0.12/4 | 0.25/4 | 0.12/4 | 0.12/4 | 0.12/4 | 0.12/4 | 0.06/4 | 0.12/4 | — | 0.12/4 |
| **Mode** | 0.12/4 | 0.12/4 | 0.12/4 | 0.25/4 | 0.12/4 | 0.12/4 | 0.12/4 | 0.12/4 | 0.06/4 | 0.12/4 | — | 0.12/4 |
| **Geometric mean** | 0.12/4 | 0.11/4 | 0.13/4 | 0.18/4 | 0.14/4 | 0.12/4 | 0.13/4 | 0.12/4 | 0.08/4 | 0.11/4 | | 0.12/4 |
| **Range** | 4 | 3 | 3 | 4 | 2 | 3 | 3 | 1 | 2 | 2 | — | 4 |

[a] A, Difco lot # 5181782; B, BD lot # 5257869; C, Oxoid lot # 1433705
[b] Laboratory H is a statistical outlier for the mean, median, and modal MIC values and was excluded from the data analysis

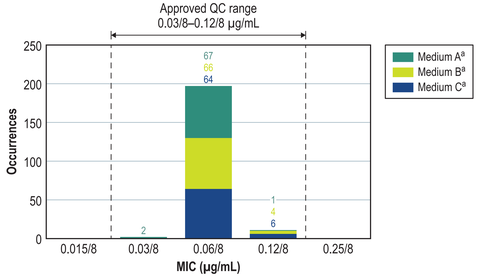
| Approved QC range (µg/mL) | # of dilutions | % in range |
|---|---|---|
| 0.06/4 – 0.25/4 | 3 | 99.5% (209/210) |

**Figure 1 Cefepime-AAI101 (fixed AAI101 concentration of 4 µg/mL) MIC distributions by medium lot for *Escherichia coli* NCTC 13353**




[a] Medium A, Difco (lot 5181782); Medium B, BD (lot 5257869); Medium C, Oxoid (lot 1433705)

**Figure 2 Cefepime-AAI101 (fixed AAI101 concentration of 8 µg/mL) MIC distributions by medium lot for *Escherichia coli* NCTC 13353**




[a] Medium A, Difco (lot 5181782); Medium B, BD (lot 5257869); Medium C, Oxoid (lot 1433705)

**Table 5 Inter- and intralaboratory comparisons of cefepime-AAI101 (fixed AAI101 concentration of 8 µg/mL) MIC values against *E. coli* NCTC 13353 for an 8-laboratory protocol meeting the study design guidelines found in CLSI M23-A4 (2016)**

| MIC (µg/mL) | Occurrences by media lot[a] | | | Laboratory (occurrences): | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | D | E | F[b] | G | H | |
| 0.015/8 | | | | | | | | | | | | |
| 0.03/8 | 2 | | | 1 | | 1 | | | | | | 2 |
| 0.06/8 | 67 | 66 | 64 | 29 | 24 | 29 | 29 | 30 | 1 | 28 | 28 | 197 |
| 0.12/8 | 1 | 4 | 6 | | 6 | | 1 | | | 2 | 2 | 11 |
| 0.25/8 | | | | | | | | | 18 | | | |
| 0.5/8 | | | | | | | | | 11 | | | |
| **Total** | 70 | 70 | 70 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 210 |
| **Mean** | 0.06/8 | 0.06/8 | 0.07/8 | 0.06/8 | 0.07/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.34/8 | 0.07/8 | 0.07/8 | 0.06/8 |
| **Median** | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.25/8 | 0.06/8 | 0.06/8 | 0.06/8 |
| **Mode** | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.25/8 | 0.06/8 | 0.06/8 | 0.06/8 |
| **Geometric mean** | 0.06/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.07/8 | 0.06/8 | 0.06/8 | 0.06/8 | 0.31/8 | 0.06/8 | 0.06/8 | 0.06/8 |
| **Range** | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 4 | 2 | 2 | 3 |

[a] A, Difco lot # 5181782; B, BD lot # 5257869; C = Oxoid lot # 1433705
[b] Laboratory F is an outlier for the mean, median, and modal MIC values and was excluded from the data analysis

| Approved QC range (µg/mL) | # of dilutions | % in range |
|---|---|---|
| 0.03/8 – 0.12/8 | 3 | 100.0% (210/210) |

## Conclusions

- Cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) broth microdilution susceptibility testing demonstrated acceptable intra- and interlaboratory reproducibility with the following CLSI QC reference strains: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853
- At its June 2017 meeting, the CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST) approved cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) broth microdilution QC ranges against the following reference strains: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853
- At its June 2017 meeting, the CLSI Subcommittee on AST also approved the cefepime broth microdilution QC range for *E. coli* ATCC 35218 and updated the QC range for *E. coli* NCTC 13353
- Overall, 99.2%-100.0% of cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) and 99.8%-100.0% of cefepime MIC values against the reference strains tested were within the QC ranges approved by the CLSI
- *E. coli* NCTC 13353 (CTX-M-15; ESBL phenotype), which is resistant to cefepime, provides adequate QC for cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL), and should be tested routinely when evaluating the activity of cefepime-AAI101 combinations
- The approved broth microdilution QC ranges for cefepime and for cefepime-AAI101 (fixed AAI101 concentrations of 4 µg/mL and 8 µg/mL) will assist clinical and reference laboratories in generating reliable, accurate susceptibility testing results during clinical trials and in clinical microbiology practice

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## References

Clinical and Laboratory Standards Institute (2018). *M23-A5. Development of in vitro susceptibility testing criteria and quality control parameters: fifth edition*. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute (2018). *M100Ed28E. Performance standards for antimicrobial susceptibility testing: 28th informational supplement*. Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute (2018). *M07Ed11E. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—eleventh edition*. Wayne, PA: CLSI.

Turnidge J, Bordash G (2007). Statistical methods for establishing quality control ranges for antibacterial agents in Clinical and Laboratory Standards Institute susceptibility testing. *Antimicrob Agents Chemother* 51: 2483–2488.