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# Validation of a TREK Diagnostic Systems Dry-form Broth Microdilution MIC Product for Testing Ceftazidime-Avibactam

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## Abstract

**Background**: Ceftazidime-avibactam (CAZ-AVI) is a broad-spectrum β-lactamase inhibitor combination in late stage clinical development for serious Gram-negative (GN) and -positive (GP) pathogen infections. In preparation for clinical microbiology laboratory use, a validation experiment was initiated to evaluate a commercial (ThermoFisher Scientific) dry-form broth microdilution (BMD) product (Sensititre [ST]) compared to reference CLSI M07 BMD panels against contemporary clinical isolates.

**Methods**: We examined 525 recent GP (285) and GN (240) isolates in 11 pathogen groups. The following organisms were tested: *S. aureus* (110; 53 MRSA), CoNS (20; 10 *S. lugdunensis*, 10 *S. haemolyticus*), enterococci (40; 20 *E. faecalis*, 20 *E. faecium* with 10 VRE), beta streptococci (60; 2 species), *S. pneumoniae* (30), 25 other streptococci (5 species) and 240 GN isolates (see Table). All strains were tested in Mueller-Hinton broth (supplemented as needed) in ST and frozen-form BMD panels. Endpoints were read manually and by automated techniques (ST only; MIC range, 0.015/4-32/4 μg/ml). Quality control used multiple ATCC strains; all results were within CLSI ranges. Reproducibility with 3 replicates across species groups (25 strains) was determined. Target essential agreement (EA) was ± one doubling dilution at ≥95%.

**Results**: Comparisons used all and only on-scale (O-S) analysis for 525 and 416 results, respectively. Among 11 organism groups, all had ST MIC/reference BMD MIC ratios predominantly at 1 (47.5-97.5%) without significant differences between all results and O-S data sets. Automated endpoint results did not differ. Enterobacteriaceae comparisons showed a modest skewing of ST MIC results toward an elevated MIC (33.9%), but the EA was 98.8 and 98.9% for O-S and all data, respectively. Organisms (6; only 1.2%) outside of EA were enterococci, streptococci, enteric bacilli, and *H. influenzae*. Intra-laboratory reproducibility was within ± one doubling dilution (100.0%).

**Conclusions**: ST dry-form BMD panels produced accurate CAZ-AVI MIC results when compared to the reference BMD, read manually (98.8-98.9% EA) and by automated options in this single-site study. This commercial BMD product would allow quality MIC guidance for CAZ-AVI therapy following regulatory approval.

**Abstract Table**

| Organisms or Groups (no. tested) | Sensititre MIC/Reference MIC ratio (occurrences): All comparisons 0.25 | 0.5 | 1 | 2 | 4 | On-scale comparisons[a] 0.25 | 0.5 | 1 | 2 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive species (285) | 0 | 18 | 224 | 40 | 3 | 0 | 18 | 164 | 36 | 2 |
| Gram-negative species (240) | 0 | 37 | 127 | 73 | 3 | 0 | 32 | 112 | 51 | 1 |
| All strains (525) | 0 | 55 | 351 | 113 | 6 | 0 | 50 | 276 | 87 | 3 |

a. MIC results were on the dilution schedule for both compared methods

## Introduction

Ceftazidime-avibactam consists of a broad-spectrum β-lactam antimicrobial agent (ceftazidime) in combination with the non-β-lactam β-lactamase inhibitor avibactam. This combination has activity against bacteria producing Ambler Class A, C, and some Class D β-lactamases. Avibactam is highly potent and inactivates β-lactamase enzymes very efficiently, with low $IC_{50}$ values, thus generating a stable enzyme-avibactam product against common contemporary enzymes (TEM-1, CTX-M-15). The role of avibactam in the combination is to protect ceftazidime from destruction by a variety of serine β-lactamases.

The *in vitro* spectrum of activity of ceftazidime-avibactam includes Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL) and non-metallo-carbapenamases (KPC and some OXA enzymes). Ceftazidime-avibactam has also been shown to be active against *Pseudomonas aeruginosa* strains containing a derepressed AmpC enzyme (MIC, at 4-8 μg/ml), but is not active against *Pseudomonas* strains resistant to ceftazidime due to efflux pump mechanisms.

To become an effective therapeutic agent against emerging multidrug-resistant (MDR) pathogens such as the ESKAPE organisms, laboratories must be able to accurately test the combination to guide treatments. In this report, we describe the results from a commercial method (Sensititre®; ThermoFisher Scientific) developed for ceftazidime-avibactam susceptibility testing when compared to the reference CLSI broth microdilution method.

## Methods

A systematic method development and validation study was designed to compare the Sensititre® dry-form broth microdilution panel results monitoring ceftazidime-avibactam (MIC range, ≤0.015/4 to 32/4 μg/ml) to those results derived from reference CLSI frozen-form panels. Endpoints were read manually and by automated commercially available devices were also compared. All tests were performed in standardized cation-adjusted Mueller-Hinton broth with appropriate supplements (HTM or 2.5-5% lysed horse blood) for testing fastidious species. Study design followed guidelines found in CLSI M23-A3 (2008), FDA guidances, and previously used by these investigators.

The study examined 525 recent Gram-positive (285) and -negative (240) isolates in 11 pathogen groups. The following organisms were tested: *Staphylococcus aureus* (110; 53 MRSA), coagulase-negative staphylococci (CoNS; 20 including 10 *S. lugdunensis,* 10 *S. haemolyticus*), enterococci (40; 20 *E. faecalis,* 20 *E. faecium* with 10 being VRE), β-streptococci (60; two species), *Streptococcus pneumoniae* (30), 25 other streptococci (five species) and 240 Gram-negative isolates (see Table 2). Endpoints were only read manually for *H. influenzae* (85 strains). Quality control used multiple ATCC strains (29212, 29213, 25922, 27853, 49247, 35218, 49619 and 700603); all results were within published CLSI ranges. Reproducibility with three replicates across species groups (25 strains) was also determined. Target essential agreement (EA) between methods was ± one doubling dilution at ≥95% for compared MIC results.

## Results

- Table 1 is reproduced from a recent publication by our laboratories comparing the spectrums for ceftazidime tested alone and combined with avibactam against 5,605 Enterobacteriaceae and 1,259 *P. aeruginosa* (USA strains, 2012)
- Against enteric bacilli the susceptibility rates for ceftazidime at ≤4 μg/ml (85.5-91.8%) were increased significantly to 99.4-100.0% when combined with 4 μg/ml of avibactam. Similarly, *P. aeruginosa* (CLSI breakpoint at ≤8 μg/ml) had ceftazidime susceptibility rates at 79.5-89.7%, but markedly expanded to 95.8-98.7% with avibactam (Table 1)
- To assure an accurate recognition of this enhanced activity by a commercial device, 525 pathogens were tested and compared to the reference CLSI MIC method results (Table 2)
  - Comparisons between methods were analyzed using all data (525 data points) and only those having on-scale MIC results for both methods; results were similar with one overall EA of 98.9%
  - Among the 285 Gram-positive cocci, 78.6% of Sensititre® MIC values were identical to those of the reference test
  - Enterobacteriaceae and *H. influenzae* (manual reads only) MIC comparisons showed a slight skewing of Sensititre® results toward a higher MIC result, but other Gram-negative species showed excellent concordance
  - Automated endpoints did not significantly differ from manually read MIC results (data not shown)
- Organisms (six) outside of EA limits were enterococci, streptococci, enteric bacilli, and *H. influenzae;* only 1.1% (Table 2). Intra-laboratory reproducibility was within ± one doubling dilution for all (100.0%) 25 triplicate comparisons.

**Table 2. Comparisons of the ThermoFisher Scientific (Sensititre®) product ceftazidime-avibactam combination MIC results and those obtained from the reference broth microdilution method (CLSI) when testing 525 organisms**

| Organisms or Groups (no. tested) | Candidate MIC/Reference MIC ratio (occurrences): All comparisons (525) 0.25 | 0.5 | 1 | 2 | 4 | On-scale comparisons (416)[a] 0.25 | 0.5 | 1 | 2 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram-positive species (285) | | | | | | | | | | |
| *S. aureus* (110)[b] | 0 | 7 | 86 | 17 | 0 | 0 | 7 | 68 | 13 | 0 |
| CoNS (20)[c] | 0 | 6 | 14 | 0 | 0 | 0 | 6 | 11 | 0 | 0 |
| Enterococci (40)[d] | 0 | 0 | 39 | 0 | 1[e] | 0 | 0 | 0 | 0 | 0 |
| *S. pneumoniae* (30) | 0 | 4 | 25 | 1 | 0 | 0 | 4 | 25 | 1 | 0 |
| *S. pyogenes* (30) | 0 | 0 | 21 | 9 | 0 | 0 | 0 | 21 | 9 | 0 |
| *S. agalactiae* (30) | 0 | 0 | 25 | 5 | 0 | 0 | 0 | 25 | 5 | 0 |
| Other streptococci (25)[f] | 0 | 1 | 14 | 8 | 2 | 0 | 1 | 14 | 8 | 2 |
| Gram-negative species (240) | | | | | | | | | | |
| Enterobacteriaceae (115)[g] | 0 | 20 | 55 | 39 | 1 | 0 | 18 | 55 | 37 | 1 |
| *P. aeruginosa* (20) | 0 | 6 | 12 | 2 | 0 | 0 | 6 | 12 | 1 | 0 |
| *Acinetobacter* spp. (10) | 0 | 1 | 7 | 2 | 0 | 0 | 1 | 6 | 2 | 0 |
| *H. influenzae* (85) | 0 | 9 | 44 | 30 | 2 | 0 | 7 | 32 | 11 | 0 |
| *M. catarrhalis* (10) | 0 | 1 | 9 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| All strains (525) | 0 | 55 | 351 | 113 | 6 | 0 | 50 | 276 | 87 | 3 |

a. MIC results were on the dilution schedule for both compared methods
b. Includes 53 strains of MRSA
c. Includes: *S. lugdunensis* (10 strains) and *S. haemolyticus* (10 strains)
d. Includes: *E. faecalis* (10 strains; three vancomycin-resistant) and *E. faecium* (10 strains; seven vancomycin-resistant)
e. One strain with a ratio of ≥16
f. Includes five species
g. Includes 13 species

**Table 1. Summary of ceftazidime-avibactam and ceftazidime (alone) activity when tested against selected Gram-negative bacterial isolates from patients in USA medical centers (2012)[a]**

| Organism | Infection type (no.)[b] | Antimicrobial | No. of isolates (cumulative %) inhibited at ceftazidime-avibactam MIC (μg/ml); ≤0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | ≥32 | $MIC_{50}$ | $MIC_{90}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enterobacteriaceae | BSI (1,269) | CAZ-AVI | 121 (11.7) | 464 (48.2) | 450 (83.7) | 137 (94.5) | 53 (98.7) | 12 (99.6) | 3 (99.8) | 0 (99.8) | 1 (99.9) | 1 (100.0) | -- | 0.12 | 0.25 |
| | | Ceftazidime | 26 (2.0) | 201 (17.9) | 428 (51.6) | 295 (74.9) | 111 (83.6) | 31 (86.1) | 15 (87.2) | 9 (87.9) | 22 (89.7) | 36 (92.5) | 34 (100.0) | 0.12 | 16 |
| | Pneumonia (1,738) | CAZ-AVI | 125 (8.9) | 460 (35.3) | 682 (74.6) | 280 (90.7) | 100 (96.4) | 44 (99.0) | 6 (99.3) | 1 (99.4) | 6 (99.7) | 3 (99.9) | 2 (100.0) | 0.12 | 0.25 |
| | | Ceftazidime | 48 (2.8) | 241 (16.6) | 514 (46.2) | 409 (69.7) | 185 (80.4) | 45 (83.0) | 27 (84.5) | 17 (85.5) | 24 (86.9) | 33 (88.8) | 195 (100.0) | 0.25 | 32 |
| | IAI (410) | CAZ-AVI | 36 (11.5) | 122 (41.2) | 157 (79.5) | 43 (90.0) | 28 (96.8) | 10 (99.3) | 3 (100.0) | -- | -- | -- | -- | 0.12 | 0.25 |
| | | Ceftazidime | 12 (2.9) | 46 (14.1) | 143 (49.0) | 91 (71.2) | 38 (80.5) | 17 (84.6) | 4 (85.6) | 2 (86.1) | 6 (87.6) | 7 (89.3) | 44 (100.0) | 0.25 | 32 |
| | UTI (2,188) | CAZ-AVI | 309 (17.0) | 771 (52.3) | 731 (85.7) | 208 (95.2) | 75 (98.6) | 22 (99.6) | 3 (99.8) | 4 (100.0) | 1 (100.0) | -- | -- | 0.06 | 0.25 |
| | | Ceftazidime | 79 (3.6) | 405 (22.1) | 774 (57.5) | 492 (80.0) | 154 (87.0) | 53 (89.4) | 34 (91.0) | 18 (91.8) | 23 (92.9) | 29 (94.2) | 127 (100.0) | 0.12 | 2 |
| *Pseudomonas aeruginosa* | BSI (141) | CAZ-AVI | | | | 1 (0.7) | 4 (3.5) | 56 (43.3) | 46 (75.9) | 18 (88.7) | 11 (96.5) | 1 (97.2) | 4 (100.0) | 2 | 8 |
| | | Ceftazidime | | | | | 2 (1.4) | 13 (10.6) | 70 (60.3) | 25 (78.0) | 7 (83.0) | 2 (84.4) | 22 (100.0) | 2 | >32 |
| | Pneumonia (881) | CAZ-AVI | | 3 (0.3) | 2 (0.6) | 15 (2.3) | 59 (9.0) | 312 (44.4) | 265 (74.5) | 132 (89.4) | 56 (95.8) | 25 (98.6) | 12 (100.0) | 2 | 8 |
| | | Ceftazidime | | 1 (0.1) | 2 (0.3) | 2 (0.6) | 30 (4.0) | 143 (20.2) | 328 (57.4) | 133 (72.5) | 61 (79.5) | 45 (84.6) | 136 (100.0) | 2 | 32 |
| | IAI (82) | CAZ-AVI | | | | | | 35 (42.7) | 30 (79.3) | 11 (92.7) | 3 (96.3) | 2 (98.8) | 1 (100.0) | 2 | 4 |
| | | Ceftazidime | | | | | | 11 (13.4) | 39 (61.0) | 12 (75.6) | 8 (85.4) | 1 (86.6) | 11 (100.0) | 2 | 32 |
| | UTI (155) | CAZ-AVI | | | | | 9 (5.8) | 47 (36.1) | 63 (76.8) | 23 (91.6) | 11 (98.7) | 1 (99.4) | 1 (100.0) | 2 | 4 |
| | | Ceftazidime | | | | | 5 (3.2) | 18 (14.8) | 70 (60.0) | 30 (79.4) | 16 (89.7) | 6 (93.5) | 10 (100.0) | 2 | 16 |

a. From Flamm, Farrell, Sader and Jones (2014)
b. Abbreviations: BSI, bloodstream infections; IAI, intra-abdominal infections; UTI, urinary tract infections; ceftazidime-avibactam,CAZ-AVI

## Conclusions

- Sensititre® ceftazidime-avibactam dry-form broth microdilution MIC panels demonstrated excellent validation agreement with reference frozen-form panel MIC results, regardless of manual or automated endpoint reading or whether the organisms were Gram-positive or -negative pathogens
- These single-center Sensititre® validation study results confirmed in a FDA 510 K-style study, appears to allow accurate determination of ceftazidime-avibactam MIC values by clinical laboratories following regulatory approval. This broad-spectrum agent will be welcomed by physicians to address therapy of infections caused by MDR Gram-negative ESKAPE pathogens.

## References

1. Bonnefoy A, Dupuis-Hamelin C, Steier V, Delachaume C, Seys C, Stachyra T, Fairley M, Guitton M, Lampilas M (2004). In vitro activity of AVE1330A, an innovative broad-spectrum non-β-lactam β-lactamase inhibitor. *J Antimicrob Chemother* 54: 410-417.
2. Boucher HW, Talbot GH, Benjamin DK, Jr., Bradley J, Guidos RJ, Jones RN, Murray BE, Bonomo RA, Gilbert D, for the Infectious Diseases Society of America (2013). 10 x '20 Progress--Development of new drugs active against Gram-negative bacilli: An update from the Infectious Diseases Society of America. *Clin Infect Dis* 56: 1685-1694.
3. Clinical and Laboratory Standards Institute (2008). *M23-A3. Development of in vitro susceptibility testing criteria and quality control parameters: third edition*. Wayne, PA: CLSI.
4. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: ninth edition* Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2014). *M100-S24. Performance standards for antimicrobial susceptibility testing: 24th informational supplement.* Wayne, PA: CLSI.
6. Flamm RK, Farrell DJ, Sader HS, Jones RN (2014). Ceftazidime/avibactam activity tested against Gram-negative bacteria isolated from bloodstream, pneumonia, intra-abdominal, and urinary tract infections in United States medical centers (2012). *J Antmicrob Chemother in press.*
7. Flamm RK, Stone GG, Sader HS, Jones RN, Nichols WW (2014). Avibactam reverts the ceftazidime $MIC_{90}$ of European Gram-negative bacterial clinical isolates to the epidemiological cut-off value. *J Chemother in press*.
8. Fritsche TR, Moet GJ, Jones RN (2004). Commercial broth microdilution panel validation and reproducibility trials for NVP PDF-713 (LBM 415), a novel inhibitor of bacterial peptide deformylase. *Clin Microbiol Infect* 10: 857-860.
9. Jones RN, Streit JM, Fritsche TR (2004). Validation of commercial dry-form broth microdilution panels and test reproducibility for susceptibility testing of dalbavancin, a new very long-acting glycopeptide. *Int J Antimicrob Agents* 23: 197-199.
10. Livermore DM, Mushtaq S, Warner M, Zhang J, Maharjan S, Doumith M, Woodford N (2011). Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* 55: 390-394.
11. Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagace-Wiens PR, Denisuik A, Rubinstein E, Gin AS, Hoban DJ, Lynch JP, 3rd, Karlowsky JA (2013). Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination. *Drugs* 73: 159-177.

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