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# Evaluation of the equivalency of the Thermo Scientific Oxoid Aztreonam/Avibactam (30/20 µg) Antimicrobial Susceptibility Testing Disc to the CLSI M07 broth microdilution frozen reference method

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## ABSTRACT

### Background

Aztreonam/avibactam is a candidate novel injectable combination of a monobactam, aztreonam and a non-β-lactam β-lactamase inhibitor, avibactam to treat infections caused by serious multi-drug resistant (MDR) Gram-negative bacteria including metallo-beta-lactamase (MBL)- producing bacteria. A study was conducted to evaluate the performance and reproducibility of the new Thermo Scientific™ Oxoid™ Aztreonam/Avibactam 30/20 µg (AZA50) Antimicrobial Susceptibility Testing (AST) Disc (Thermo Fisher Scientific, Basingstoke, UK) against minimum inhibitory concentrations (MICs).

### Methods

The Oxoid AZA50 discs were tested against 300 clinical, 75 challenge and 15 reproducibility isolates including Enterobacterales and *Stenotrophomonas maltophilia*. The gold-standard broth-microdilution method (BMD) was performed simultaneously utilising Clinical and Laboratory Standards Institute (CLSI) M07 frozen reference panels to acquire MIC results. Quality control (QC) organisms (recommended by CLSI) were tested on each testing day against 2 lots of Oxoid discs. All isolates were tested in accordance with CLSI M02/M100 using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA supplied by Thermo Fisher). All testing was conducted by Thermo Fisher Scientific (Basingstoke, UK).

### Results

Overall, a categorical agreement of 100% was achieved when the Oxoid AZA50 disc was compared to the reference MIC values obtained using CLSI M07 reference panels. All data showed 99.25% reproducibility within-reader and between-reader calculated as the percent of results which were within 3 mm of the modal result. QC results were within the stated limits 100% of the time for each lot and reader.

### Conclusions

When compared to the reference MICs obtained, the Oxoid AZA50 disc demonstrated an equivalent level of performance. The 100% categorical agreement obtained by the Oxoid AZA50 disc indicates that this is an acceptable method for the antimicrobial susceptibility testing of aztreonam/avibactam.

## INTRODUCTION

Aztreonam (Figure 1) is a monobactam β-lactam active against metallo-β-lactamase (MBL)-producing multidrug-resistant pathogens for which there are limited or no treatment options. However, due to the frequent co-production of class A β-lactamases or AmpC-type determinants within MBL- producing Gram-negative organisms, aztreonam remains active only in one-third of cases. Avibactam (Figure 2) is a novel non-β-lactam β-lactamase inhibitor with a potent activity against class A β-lactamases and AmpC-type determinants[1].

The novel combination of avibactam with aztreonam shows promising results against MBL-producing pathogens, this combination restores the *in vitro* activity and *in vivo* efficacy of aztreonam against these organisms protecting it from inhibition[1].

A single product formulation of aztreonam/avibactam is currently under development in Phase III studies and it has recently received a positive review by European Medicines Agency (EMA) for the treatment of serious infections (i.e., complicated intra-abdominal infections, nosocomial pneumonia including hospital-acquired pneumonia and ventilator-associated pneumonia, complicated urinary tract infections, or bloodstream infections) caused by MBL-producing Gram-negative bacteria[1] such as enterobacterales (*Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis and Serratia marcescens*).

An *in vitro* study was conducted by Thermo Fisher Scientific to evaluate the performance and reproducibility of the new aztreonam/avibactam 30/20 µg (AZA50) Oxoid AST disc against minimum inhibitory concentrations (MICs).

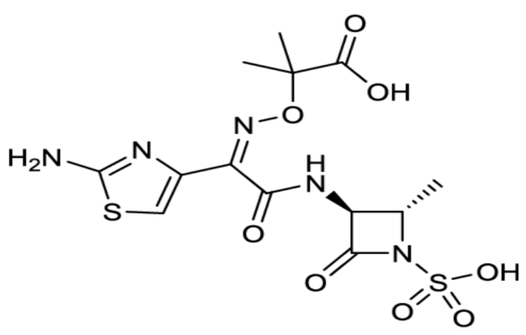

**Figure 1**. The chemical structure of aztreonam.

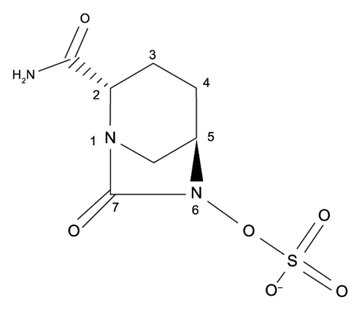

**Figure 2**. The chemical structure of avibactam.

## MATERIALS AND METHODS

All isolates were tested in accordance with CLSI M02[2]/M100[3]/M02QG[4] using FDA-cleared Remel Mueller Hinton Agar (MHA).

Colony counts (CC) were performed as per CLSI M02[2] (13th Ed.) guidelines for at least 10% of clinical and challenge isolates and all QC and reproducibility isolates.

### Quality control

Quality control strains from the American Type Culture Collection (ATCC) were tested daily using two lots of Oxoid discs alongside clinical, challenge and reproducibility isolates to ensure all AST discs were within the QC limits (Table 1). At least 20 replicates of the quality control strains were tested per individual (3 independent individuals) to represent 3 testing sites.

The QC strains must be within specification for at least 95 % of the results during study to meet acceptance criteria[5].

**Table 1.** QC zone size limits for AZA50 AST discs[3].

| QC organism | QC limit (mm) | MIC (µg/L) |
|---|---|---|
| ***Escherichia coli*** (ATCC® 25922™) | 32-38 | 0.03/4.0-0.12/4 |
| ***Pseudomonas aeruginosa*** (ATCC® 27853™) | 24-30 | 2/4-8/4 |
| ***Escherichia coli*** (ATCC® 35218™) | 31-38 | 0.016/4-0.06/4 |
| ***Klebsiella pneumoniae*** (ATCC® 700603™) | 26-32 | 0.06/4-0.5/4 |

### Reproducibility

Two lots of Oxoid AZA50 discs were tested and read by three independent individuals against 15 indicated and on-scale reproducibility isolates over a 3-day testing period to generate a total of 270 data points.

≥ 95 % of the results must be within 3 mm of the modal zone diameter to meet acceptance criteria[5].

### Clinical and challenge isolates

One lot of Oxoid AZA50 discs was tested for a total of 375 clinical and challenge isolates including Enterobacterales and *Stenotrophomonas maltophilia* (Table 2). All isolates were shared between three independent individuals (approximately 125 isolates each) to represent three testing sites which were then analysed using breakpoints set by sponsor (Table 3).

Categorical agreement (CA) must be ≥ 90 % when compared to the predicate device and the very major discrepancy and major discrepancy rate must be ≤ 3 % each to meet acceptance criteria[5].

**Table 2.** Number of isolates tested during the study.

| Isolates | Number Tested |
|---|---|
| **Clinical Isolates** | 300 |
| **Challenge Isolates** | 75 |
| **Reproducibility Isolates** | 15 |
| **ATCC Quality Control Strains** | 4 (x 20 repeats) |
| **TOTAL** | 394 |

**Table 3.** Indicated organisms and breakpoints from Pfizer Inc. (Sponsor).

| Pathogen | Disk Diffusion (zone diameters in mm) | | | MIC (mg/L) | | |
|---|---|---|---|---|---|---|
| | S | I | R | S | I | R |
| **Enterobacterales** | ≥18 | 14-17 | ≤13 | ≤ 8 | N/A | >8 |
| ***Stenotrophomonas maltophilia*** | N/A | N/A | N/A | ≤ 8 | N/A | >8 |

S= Susceptible
R= Resistant.
I= Intermediate

## RESULTS

### Quality control

QC results were within the stated limits for all QC organisms (*Escherichia coli* ATCC® 25922™ and ATCC® 35218™, *Pseudomonas aeruginosa* ATCC® 27853™, and *Klebsiella pneumoniae* ATCC® 700603™) 100% of the time for each lot of Oxoid AZA50 discs.

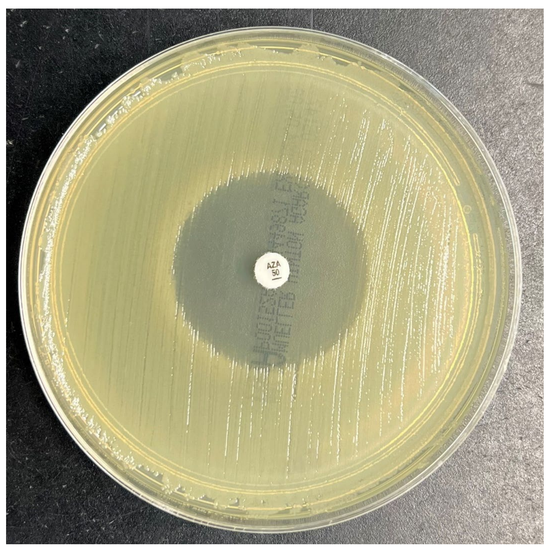

**Figure 3.** Oxoid AZA50 disc zone of inhibition with *Escherichia coli* (ATCC®25922™) on MHA.

### Reproducibility

The reproducibility was calculated as the percent of results which were within 3 mm of the modal value. All results showed reproducibility greater than the acceptance criteria of 95%. The summary is shown in Table 4.

**Table 4.** Summary of the reproducibility of Oxoid AZA50 discs between 2 lots and 3 independent individuals.

| Reproducibility between disc lots | | | Reproducibility between individuals | | | |
|---|---|---|---|---|---|---|
| Lot 1 | Lot 2 | All Lots | Individual 1 | Individual 2 | Individual 3 | All Individuals |
| 97.03% | 97.82% | 99.25% | 100% | 96.69% | 98.90% | 99.25% |

### Clinical and challenge isolates

The categorical agreement of the Oxoid AZA50 disc was analysed using charts such as the example shown in Figure 4 and summarised in Table 5.

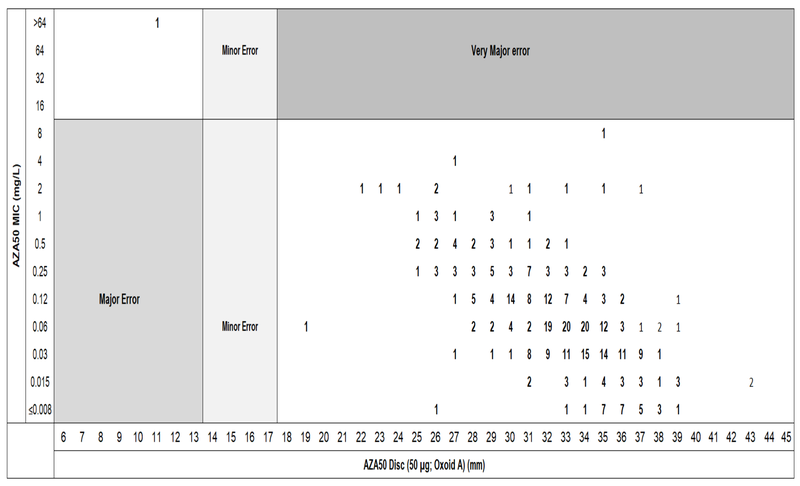


**Figure 4.** Analysis of the AZA50 Oxoid disc against MIC for Enterobacterales using provisional breakpoints.

**Table 5.** Analysis of AZA50 Oxoid disc vs. MIC for indicated species.

| Number of isolates tested | #CA | %CA | #S | #R | MAJ | % MAJ | VMJ | %VMJ |
|---|---|---|---|---|---|---|---|---|
| **Organism** | | | | | | | | |
| **Enterobacterales[a]** | | | | | | | | |
| **Clinical** | 290 | 290 | 100.00% | 289 | 1 | 0 | 0.00% | 0 | 0.00% |
| **Challenge** | 65 | 65 | 100.00% | 65 | 0 | 0 | 0.00% | 0 | 0.00% |
| **Combined** | 355 | 355 | 100.00% | 354 | 1 | 0 | 0.00% | 0 | 0.00% |
| ***Stenotrophomonas maltophilia*[b]** | | | | | | | | |
| **Clinical** | 10 | 10 | 100.00% | 8 | 2 | - | - | - | - |
| **Challenge** | 10 | 10 | 100.00% | 10 | 0 | - | - | - | - |
| **Combined** | 20 | 20 | 100.00% | 18 | 2 | - | - | - | - |

CA= Categorical Agreement, S= Susceptible, R= Resistant, MAJ= Major errors, VMJ= Very Major Errors

[a] includes *E. coli* (80), *E. cloacae* (59), *K. oxytoca* (55), *K. pneumoniae* (65), *S. marcescenes* (25), *C. freundii* (35) and *P. mirabilis* (36).
[b] *S. maltophilia* does not have an intermediate breakpoint and therefore cannot have any minor discrepancies.

The overall categorical agreement achieved for all indicated isolates tested in this study was 100.0%, when the Oxoid AZA50 disc was compared to the MIC values.

No minor discrepancies, no major discrepancies and no very major discrepancies were observed during the study.

All colony counts were in the region of 1-2 x10[8] CFU/mL for all QC and reproducibility isolates and for all the clinical and challenge isolates tested for CC. The average of all the colony counts combined was 1.08 x10[8] CFU/mL.

## CONCLUSION

This study validates that the Oxoid AZA50 AST disc has an equivalent level of performance compared to the CLSI M07 broth microdilution frozen reference method against the provisional breakpoints supplied by the sponsor. The high categorical agreement obtained by the Oxoid AZA50 disc confirms the reliability of the disc diffusion as substantiated alternative, next to the gold standard microbroth dilution, for antimicrobial susceptibility testing of aztreonam/avibactam on MBL-producing Gram-negative bacteria.

## REFERENCES

[1] Mauri C, Maraolo AE, Di Bella S, Luzzaro F, Principe L. The Revival of Aztreonam in Combination with Avibactam against Metallo-β-Lactamase-Producing Gram-Negatives: A Systematic Review of In Vitro Studies and Clinical Cases. Antibiotics (Basel). 2021 Aug 20;10(8):1012. doi: 10.3390/antibiotics10081012. PMID: 34439062; PMCID: PMC8388901.

[2] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

[3] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

[4] CLSI M02QG:2018 1st Ed. QUICK GUIDE — Disk Diffusion Reading Guide

[5] BS EN ISO 20776-2:2007 Clinical laboratory testing and *in vitro* diagnostic test systems. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Evaluation of performance of antimicrobial susceptibility test devices

## TRADEMARKS/LICENSING