

A Multi-Site Study Comparing a Commercially Prepared Dried MIC Susceptibility System to the CLSI Broth Microdilution Method for Aztreonam-avibactam using Non-Fastidious Gram-Negative Organisms

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Abstract

Purpose: Aztreonam-avibactam (AZA) (Pfizer, Inc.) was developed for the treatment of bacteria including infections caused by metallo-β-lactamase producing Enterobacterales. A four-site evaluation was performed to determine the accuracy and reproducibility of AZA susceptibility testing using the Sensititre™ dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI M07/ISO 20776-1/ISO 20776-2 (CLSI/ISO) reference broth microdilution method (BMD). Both auto (Optiread™) and manual read methodologies were employed.

Methods: AZA (0.008/4-64/4 μg/mL) was tested against 518 recent clinical isolates, 209 challenge isolates and 12 reproducibility isolates. These isolates consisted of 122 *Escherichia coli* (including ESBL+/-, AMP C, and, KPC), 117 *Klebsiella pneumoniae* (including ESBL+/-, AmpC and KPC), 27 *Klebsiella oxytoca*, 53 *Proteus mirabilis*, 25 *Proteus vulgaris*, 80 *Enterobacter cloacae*, 29 *Citrobacter koseri*, 30 *Citrobacter freundii*, 86 *Klebsiella aerogenes*, 94 *Morganella morganii*, 27 *Serratia marcescens*, 48 *Providencia spp.*, and 1 *Pseudomonas aeruginosa* reproducibility isolate. The Sensititre dried MIC susceptibility panels were inoculated per manufacturer's instructions. BMD was performed per CLSI/ISO guidelines. Recommended CLSI quality control (QC) organisms were tested daily and all results were within the published QC ranges.

Results: Comparisons of AZA MICs for non-fastidious gram-negative on the Sensititre system for automated and manual reads to the CLSI/ISO BMD MICs resulted in 94.9% and 96.0% essential agreements (EA; +/- 1 log₂ dilution), respectively. Likewise, Categorical agreement was determined to be 98.8% and 99.6% respectively. Overall agreement for the reproducibility (+/- 1 log₂ dilution of the modal MIC) using automated and manual reads was 98.1% and 97.6%, respectively.

Conclusions: The results of this AZA study on the Sensititre susceptibility system (both auto and manual read) demonstrated an equivalent level of performance compared to the CLSI/ISO BMD when tested against gram-negative non-fastidious organisms. This high level of agreement obtained for both clinical and challenge isolates indicates that reliable results are obtained for AZA on the Sensititre system.

Introduction

Aztreonam-avibactam (AZA) (Pfizer, Inc.) was developed for the treatment of bacteria including infections caused by metallo-β-lactamase producing Enterobacterales. It combines aztreonam, an MBL-stable monobactam that causes bacterial death by inactivating penicillin-binding proteins essential for cell wall synthesis with avibactam, a non-β-lactam β-lactamase inhibitor that protects aztreonam from hydrolysis by most serine β-lactamases. Aztreonam-avibactam is indicated for the treatment of Complicated intra-abdominal (cIAI), Hospital-acquired pneumonia including ventilator-associated pneumonia (HAP and VAP) and complicated urinary tract (cUTI) including pyelonephritis. Aztreonam-avibactam is also indicated for the treatment of infections due to aerobic Gram-negative organisms in adult patients with limited treatment options

A four-site evaluation was performed to determine the accuracy and reproducibility of AZA susceptibility testing using the Sensititre™ dried MIC susceptibility system (Thermo Fisher Scientific, Cleveland, OH) compared with the CLSI M07/ISO 20776-1/ISO 20776-2 (CLSI/ISO) reference broth microdilution method (BMD). Both auto (Optiread™) and manual read methodologies were employed.

Materials and methods

- Sensititre dried MIC susceptibility plates (Thermo Fisher Scientific, East Grinstead, UK) (Fig. 1)
- CLSI M07 reference broth microdilution method (BMD) plates (Thermo Fisher Scientific, Cleveland, OH)
- 727 clinical and challenge isolates (Table 1)
- CLSI recommended quality control strains (Table 2)
- 12 reproducibility isolates (Table 1, Table 5)

Materials and methods (cont.)

Table 1. Organisms Tested

Species	Isolates Tested
<i>Enterobacter cloacae</i> complex	80
<i>Escherichia coli</i>	122
<i>Klebsiella pneumoniae</i>	117
<i>Klebsiella oxytoca</i>	27
<i>Proteus mirabilis</i>	53
<i>Proteus vulgaris</i>	25
<i>Citrobacter koseri</i>	29
<i>Citrobacter freundii</i>	30
<i>Klebsiella (Enterobacter) aerogenes</i>	86
<i>Morganella morganii</i>	94
<i>Serratia marcescens</i>	27
<i>Providencia spp.</i>	48
<i>Pseudomonas aeruginosa</i>	1
Total	739

All 727 clinical and challenge isolates were tested against AZA(0.008/4-64/4 μg/mL) on both the Sensititre dried plates and the frozen reference BMD plates. Dried plates were tested according to manufacturer's instructions. Frozen reference plates were tested according to CLSI M07. Dried plates were read manually using the Sensititre™ Optiread and Vizion and frozen plates were read using a manual mirror box.

Figure 1. Sensititre Dried MIC Susceptibility Plates



Table 2. Quality Control Strains

Quality Control Strain	CLSI/EUCAST QC Ranges (μg/ml)	
	Aztreonam/avibactam	
<i>Escherichia coli</i> ATCC 25922	0.03/4 – 0.12/4	
<i>Klebsiella pneumoniae</i> ATCC 700603	0.06/4 – 0.5/4	

Recommended CLSI quality control organisms were tested daily and were within the expected ranges. Colony counts were performed daily for each QC strain to verify the isolates correct inoculum concentration.

Results

Agreement:

Essential agreement for AZA on the Sensititre 18 – 24 hour susceptibility plate compared to the frozen reference microdilution plate was calculated using the ± 1 log₂ dilution standard. Categorical agreement was calculated utilizing the CLSI/EUCAST breakpoints of ≤4, >4.

The essential agreement of AZA after initial testing was 94.9% and 96.0% when testing automated and manual reads respectively. Categorically, agreement was determined to be 98.8 and 99.6% respectively (Not Presented). Total biases were within the ± 30% limits as set in ISO (Table 3 and 4).

Reproducibility:

Reproducibility for AZA on the Sensititre 18 – 24 hour susceptibility plate was determined by comparing individual results to the overall modal MIC determined at 4 sites. Testing was conducted on the test system only in triplicate over 3 days. Overall, both read methods showed acceptable reproducibility as shown in Table 5.

Conclusions

This study validated that the Sensititre susceptibility system demonstrates an equivalent level of performance compared to the CLSI/ISO reference broth microdilution plate when testing AZA against clinically relevant Gram-Negative isolates. This study suggests that the Sensititre system is an acceptable method for susceptibility testing of AZA according to both the CLSI and ISO testing methodology

Table 3. Summary Data and % Essential Agreement of Enterobacterales autoread on the Sensititre 18 – 24 Hour Susceptibility Test System

Organism Group	Total of all Isolates	Total Evaluable Isolates	Aztreonam/avibactam				Total Bias
			Essential Agreement of Total	Essential Agreement of Evaluable	% Essential Agreement of Total	% Essential Agreement of Evaluable	
<i>Enterobacter cloacae</i> complex	79	77	75	73	94.9%	94.8%	-14.6%
<i>Escherichia coli</i>	120	106	112	98	93.3%	92.5%	-43.6%
<i>Klebsiella pneumoniae</i>	115	109	108	102	93.9%	93.6%	13.2%
<i>Klebsiella oxytoca</i>	27	26	26	25	96.3%	96.2%	28.9%
<i>Proteus mirabilis</i>	51	3	51	3	100.0%	100.0%	-64.7%
<i>Proteus vulgaris</i>	25	2	25	2	100.0%	100.0%	-76.0%
<i>Citrobacter koseri</i>	29	28	27	26	93.1%	92.9%	19.1%
<i>Citrobacter freundii</i>	29	29	28	28	96.6%	96.6%	-6.9%
<i>Klebsiella (Enterobacter) aerogenes</i>	83	81	76	74	91.6%	91.4%	-21.7%
<i>Morganella morganii</i>	94	63	90	59	95.7%	93.7%	-2.7%
<i>Serratia marcescens</i>	27	26	26	26	96.3%	96.3%	-6.8%
<i>Providencia spp.</i>	48	15	47	14	97.9%	93.3%	-36.6%
Total	727	566	690	529	94.9%	93.5%	-13.9%

Table 4. Summary Data and % Essential Agreement of Enterobacterales read manually on the Sensititre 18 – 24 Hour Susceptibility Test System

Organism Group	Total of all Isolates	Total Evaluable Isolates	Aztreonam/avibactam				Total Bias
			Essential Agreement of Total	Essential Agreement of Evaluable	% Essential Agreement of Total	% Essential Agreement of Evaluable	
<i>Enterobacter cloacae</i> complex	79	76	76	73	96.2%	96.1%	-15.9%
<i>Escherichia coli</i>	120	106	112	98	93.3%	92.5%	-33.8%
<i>Klebsiella pneumoniae</i>	115	109	109	103	94.8%	94.5%	14.9%
<i>Klebsiella oxytoca</i>	27	26	26	25	96.3%	96.2%	28.9%
<i>Proteus mirabilis</i>	51	3	51	3	100.0%	100.0%	-64.7%
<i>Proteus vulgaris</i>	25	3	25	3	100.0%	100.0%	-36.0%
<i>Citrobacter koseri</i>	29	27	28	26	96.6%	96.3%	12.2%
<i>Citrobacter freundii</i>	29	29	27	27	93.1%	93.1%	3.4%
<i>Klebsiella (Enterobacter) aerogenes</i>	83	81	77	75	88.7%	88.2%	-19.7%
<i>Morganella morganii</i>	94	66	93	65	98.9%	98.5%	5.5%
<i>Serratia marcescens</i>	27	26	27	26	100.0%	100.0%	0.9%
<i>Providencia spp.</i>	48	15	47	14	97.9%	93.3%	-42.9%
Total	727	567	698	538	96.0%	94.9%	-10.2%

Table 5. Reproducibility Data for Automated (Left) and Manual (Right) read methods for Aztreonam-avibactam on the Sensititre 18-24 Susceptibility Plate

AZA	Difference in the number of wells between test result and test mode (Auto Read)						
	OFF-Scale	≤-2	-1	0	+1	≥+2	OFF-Scale
<i>Escherichia coli</i> (ESBL +)				34	2		
<i>Escherichia coli</i> (ESBL +)	2			24	10		
<i>Klebsiella pneumoniae</i> (SHV-11)			2	31	3		
<i>Klebsiella pneumoniae</i> (KPC)			1	18	17		
<i>Enterobacter cloacae</i> complex				26	7	3	
<i>Citrobacter freundii</i>				34	2		
<i>Proteus mirabilis</i>				18	15	3	
<i>Klebsiella (Enterobacter) aerogenes</i> (KPC -)			1	24	11		
<i>Klebsiella (Enterobacter) aerogenes</i> (KPC -)				34	1	1	
<i>Pseudomonas aeruginosa</i>				35	1		
<i>Klebsiella (Enterobacter) aerogenes</i> *				9			
<i>Klebsiella (Enterobacter) aerogenes</i> *				9			
TOTAL	2	0	4	296	69	7	0
Reproducibility Essential Agreement	371/378 = 98.1%						

AZA	Difference in the number of wells between test result and test mode (Manual Read)						
	OFF-Scale	≤-2	-1	0	+1	≥+2	OFF-Scale
<i>Escherichia coli</i> (ESBL +)			1	32	3		
<i>Escherichia coli</i> (ESBL +)				23	13		
<i>Klebsiella pneumoniae</i> (SHV-11)			2	28	6		
<i>Klebsiella pneumoniae</i> (KPC)	1		14	21			
<i>Enterobacter cloacae</i> complex				26	7	3	
<i>Citrobacter freundii</i>				34	2		
<i>Proteus mirabilis</i>				18	15	3	
<i>Klebsiella (Enterobacter) aerogenes</i> (KPC -)				26	9	1	
<i>Klebsiella (Enterobacter) aerogenes</i> (KPC -)				34	1	1	
<i>Pseudomonas aeruginosa</i>			1	32	3		
<i>Klebsiella (Enterobacter) aerogenes</i> *				9			
<i>Klebsiella (Enterobacter) aerogenes</i> *				9			
TOTAL	0	1	18	292	59	8	0
Reproducibility Essential Agreement	369/378 = 97.6%						

*These isolates were run at a single site at the request of the FDA during 510k review

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