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

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ABSTRACT

Background: Plazomicin (PLZ) (Achaogen, South San Francisco, CA) is a next-generation aminoglycoside active against multidrug resistant (MDR) *Enterobacteriaceae* spp., including carbapenem-resistant *Enterobacteriaceae* (CRE). A four site evaluation was performed to determine the accuracy and reproducibility of PLZ susceptibility testing against non-fastidious gram-negative organisms using the Thermo Scientific™ Sensititre™ dried MIC susceptibility system (ThermoFisher 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: PLZ (0.06-128 µg/mL) was tested against 473 recent clinical isolates, 96 challenge isolates and 15 reproducibility isolates. These isolates consisted of 122 *Escherichia coli,* 111 *K. pneumoniae*, 51 *K. oxytoca*, 49 *E. cloacae*., 28 *E. aerogenes*, 22 *C. koseris*, 26 *C. freundii*, 48 *P. mirabilis,* 24 *P. vulgaris,* 28 *M. morganii,* 19 *P. stuartii,* 23 *P. rettgeri* and 29 *S. marcescens*. The Sensititre dried MIC susceptibility plates 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: Comparison of *Enterobacteriaceae* spp. MIC results on the Sensititre system to the CLSI/ISO BMD method for automated and manual reads resulted in 99.5% and 99.5% essential agreements (EA; +/- 1 $\log_2$ dilution) for PLZ, respectively. Overall agreement for the reproducibility (+/- 1 $\log_2$ dilution of the modal MIC) using automated and manual reads was 99.4% and 99.6%.
Conclusions: The Sensititre susceptibility system (both auto and manual read) demonstrated an equivalent level of performance compared to the CLSI/ISO BMD when testing PLZ against *Enterobacteriaceae* spp. This high level of agreement obtained by the Sensititre system and the CLSI/ISO BMD method demonstrates that this is an acceptable method for susceptibility testing of PLZ.

INTRODUCTION

Plazomicin (Figure 1.) is a next generation aminoglycoside active against MDR *Enterobacteriaceae* spp., including CRE. This *in vitro* multi-site comparison study was performed to evaluate the performance of plazomicin on the commercially manufactured Sensititre® 18-24 hour susceptibility system, for both automated and manual reads, compared against the Clinical Laboratory Standards Institute (CLSI) reference broth microdilution (BMD) method (M07/M100). To establish equivalency between the two methods, a 4 lab clinical study was conducted, and the MIC results obtained using the Sensititre dried plate technology were compared to the MIC results obtained from the CLSI M07 frozen reference plate.

MATERIALS AND METHODS

●The Sensititre 18-24 hour MIC or breakpoint susceptibility system (ThermoFisher Scientific, Oakwood Village, OH) is an *in vitro* diagnostic product for clinical susceptibility testing of both fastidious and non-fastidious organisms.
plazomicin was tested against: (Table 1.)

- 473 recent clinical isolates across the four sites
- 96 challenge isolates at a single testing site
- 15 reproducibility isolates at each site (tested in triplicate over a 3 day testing period)
- 2 Quality Control Strains (ATCC)

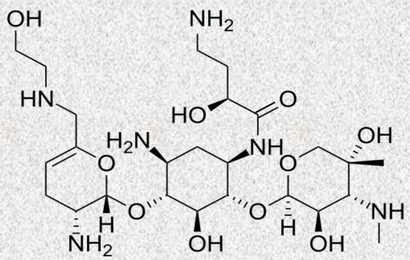

Figure 1. Chemical structure of Plazomicin

MATERIALS AND METHODS Cont.

● Colony Counts and purity plates were performed on the inoculums of the Clinical, Challenge, Reproducibility and QC strains on each day of testing.

●Each isolate was tested using a:

- Dried Sensititre 18–24 susceptibility plate containing plazomicin (0.06-128µg/ml). The dried plates were set up and tested by both automated and manual reading methodologies according to the manufacturer's instructions.
- CLSI reference broth microdilution plate was prepared and tested on each isolate according to the current Clinical Laboratory Standards Institute standard method.

Table 1. Organisms Tested	Number Tested
Clinical Isolates (4 sites)	473
CDC Challenge Isolates (one site)	96
Reproducibility Isolates (4 sites) (3 x day for 3 days)	15 (540)
ATCC Quality Control Strains (20 replicates of each strain at 4 sites)	2 (160)
TOTAL	**1269**

Quality Control

●Recommended CLSI quality control (QC) organisms were tested daily and were within the CLSI expected QC ranges.

●Colony counts were performed and fell within expected ranges
Reference 2-8X10^5, Sensititre 5X10^4-5X10^5

Table 2. Quality Control Strains	CLSI QC Ranges (µg/ml)
Escherichia coli ATCC 25922	0.25-2
Pseudomonas aeruginosa ATCC 27853	1-4

Results

Essential agreement for **plazomicin** on the Sensititre susceptibility plate compared to the reference microdilution plate was calculated for each read method (Auto and Manual) using the +/- one $\log_2$ dilution standard. Essential agreement rates are shown for non-fastidious gram-negative isolates in **Tables 3 and 4**.

RESULTS Cont.

Clinical Isolates and Challenge Organisms

The overall essential agreement for plazomicin within ±1 $\log_2$ dilution was **99.5%** for the manual method and **99.5%** for the auto read method.

Inter-laboratory Reproducibility

Reproducibility testing results for plazomicin within ±1 $\log_2$ dilution from the modal MIC was **99.4%** for the auto read method and **99.6%** for the manual read method.

Table 3. % Essential Agreement of Non-Fastidious gram-negative Clinical and Challenge Isolates Using the Manual Read and Auto Read Method

The overall essential agreement for plazomicin within +/- one $\log_2$ dilution, was 99.5% for both the manual read and auto read methods

Combined Total Isolates

Plazomicin	% Essential Agreement	
Organism Group	Manual Read	Auto Read
Escherichia coli	100%	100%
Klebsiella pneumoniae	100%	100%
Klebsiella oxytoca	100%	100%
Enterobacter cloacae	100%	100%
Enterobacter aerogenes	100%	100%
Citrobacter koseri	100%	100%
Citrobacter freundii	100%	100%
Proteus mirabilis	95.7%	95.7%
Proteus vulgaris	100%	100%
Morganella morganii	96.4%	96.4%
Providencia stuartii	100%	100%
Providencia rettgeri	100%	100%
Serratia marcescens	100%	100%
Total	**99.5%**	**99.5%**

RESULTS Cont.

Table 4. Inter-laboratory Reproducibility % Essential Agreement ±1 $\log_2$ dilution from the Modal Value

Plazomicin	Auto Read	Manual Read
Between-site total isolates tested	539	540
Between-site isolates within +/- 1 well from mode	536	538
Between-site reproducibility ratio	536	538
Between-site reproducibility %	**99.4%**	**99.6%**
Total essential agreement	536/539	538/540
Essential agreement %	**99.4%**	**99.6%**

One isolate did not generate a signal for Auto Read method.

CONCLUSIONS

This study validates that the Sensititre 18–24 hour susceptibility system (both auto read and manual read) demonstrated an equivalent level of performance compared to the CLSI M07/M100 reference broth microdilution plate when testing **plazomicin** against non-fastidious gram-negative clinical and challenge isolates. This study suggests that this is an acceptable method for susceptibility testing of **plazomicin**.

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