Evaluation of Thermo Scientific™ Sensititre™ System for MIC determination of fluoroquinolones with EUCAST breakpoints for Enterobacteriaceae, Acinetobacter and Pseudomonas

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OVERVIEW

Purpose: To evaluate the performance of Thermo Scientific Sensititre susceptibility MIC plates (Thermo Fisher Scientific) for antimicrobial susceptibility testing of *Enterobacteriaceae*, *Acinetobacter* and *Pseudomonas*, using automated (Thermo Scientific™ Sensititre™ OptiRead™ System) and manual (Thermo Scientific™ Sensititre™ Vizion™ System) reading methods, according to ISO 20776[1,2] guidelines for Antimicrobial Susceptibility Testing (AST) as interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[3].

Methods: *Enterobacteriaceae*, *Acinetobacter* and *Pseudomonas* clinical isolates were tested on Sensititre susceptibility MIC plates containing ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin.

Results: Sensititre susceptibility MIC plates showed >95% essential agreement and >96% categorical agreement with the broth microdilution reference method for all fluoroquinolones tested.

INTRODUCTION

The Sensititre System is a leader in antimicrobial susceptibility and identification testing. The Sensititre System consists of 96-well microtitre plates containing dilutions of antimicrobials dried in individual wells (see figure 1) (available in both standard and custom formats), as well as instrumentation to assist inoculation, plate management, reading, and interpretation (see figure 2). The Sensititre System utilizes true MIC results rather than extrapolated (MIC) results and offers flexible, customizable testing options to accommodate formularies and laboratories of all sizes conducting antimicrobial susceptibility and identification (AST/ ID) testing.

FIGURE 1. Thermo Scientific Sensititre MIC susceptibility plate

FIGURE 2. Thermo Scientific Sensititre System

METHODS

Reproducibility
Reproducibility testing of 10 Gram negative isolates in triplicate over three days was performed on the Sensititre susceptibility MIC plates.

Clinical isolate testing
A total of 390 clinical isolates (including 54 Acinetobacter, 271 Enterobacteriaceae and 65 Pseudomonas) were tested in total. The Sensititre susceptibility MIC plate was inoculated and tested according to manufacturer's instructions; plates were read using both the Sensititre Vizion (manual read) and Sensititre OptiRead (automated read); results were interpreted using the Thermo Scientific™ Sensititre™ SWIN™ Software system. The broth microdilution reference method was performed according to ISO 20776-1:2006, and read using the Sensititre Vizion (manual read). Frozen broth microdilution plates were sourced from Thermo Fisher Scientific, Oakwood Village, OH, USA.

Quality control
Recommended quality control (QC) organisms were tested daily on the Sensititre susceptibility MIC, and broth microdilution plates. Purity checks and colony counts were performed daily on the broth microdilution reference method according to ISO 20776-1:2006. Purity checks were conducted daily on the Sensititre susceptibility MIC plates and colony counts performed periodically according to manufacturer's instructions.

TABLE 1. Percentage essential agreement (EA) and categorical agreement (CA) for manual and automatic reads

Antimicrobial	Read type	EA (%)	CA (%)
Ciprofloxacin	Manual	96.4	97.9
	Auto	95.7	97.2
Levofloxacin	Manual	99.4	98.5
	Auto	99.4	97.7
Norfloxacin	Manual	99.1	98.9
	Auto	98.6	98.5
Ofloxacin	Manual	99.1	97.4
	Auto	97.5	96.7

Data Analysis
Using EUCAST breakpoints, essential agreement (EA), categorical agreement (CA) and discrepancy rates were calculated according to ISO 20776-2:2007. The ISO 20776-2:2007 acceptance criteria are ≥90% EA, ≥90% CA and a very major discrepancy (VMD) and major discrepancy (MD) rate of ≤3%. In instances where the testing does not include a significant number of resistant isolates the VMD may be greater than 3%, as the calculation is based on the number of resistant isolates. If the VMD rate is >3% but the EA is ≥97% the device is considered to have acceptable accuracy[1]. Minor discrepancies (mD) were also reported.

RESULTS

Reproducibility
Intra- (daily) and inter- (between days) reproducibility of Gram negative isolates for both manual read and automatic read was within ±1 dilution of the mode for all antimicrobials tested for ≥95% of results from all study sites. Where the mode could not be calculated, the median was used.

Clinical isolate testing
Essential and categorical agreements (EA and CA)
As shown in Table 1, of the 390 clinical isolates tested during the study, EA was >95%, and the CA was >96%, for all fluoroquinolones, for all Gram negative organisms tested, for both manual read and automatic read methods.

Discrepancy rates (VMD and MD)
There were no VMD or MD discrepancies observed for the fluoroquinolone data for all Gram negative organisms tested.

CONCLUSIONS

The performance of the fluoroquinolones; ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin tested on the Sensititre susceptibility system met the reproducibility, essential and categorical agreement acceptance criteria as stipulated in ISO 20776-2. Overall, the Sensititre susceptibility system proved to be an accurate alternative to standard broth microdilution methods MIC determination of ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin for Gram negative organisms using EUCAST breakpoints.

REFERENCES

1. Clinical Laboratory Testing and in vitro Diagnostic Test Systems - Susceptibility Testing of Infectious Agents and Evaluation of Performance of AST Devices – Part 2:Evaluation of Performance of AST devices. ISO 20776-2:2007
2. Clinical Laboratory Testing and in vitro Diagnostic Test Systems - Susceptibility Testing of Infectious Agents and Evaluation of Performance of AST Devices – Part 1: Reference Method for Testing the in vitro Activity of antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases. ISO 20776-1: 2006.
3. European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints v6.0

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