**ABSTRACT**

A multicenter study was performed to evaluate the accuracy of eravacycline on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels. Material Methods: For efficacy, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 414 Enterobacteriaceae clinical isolates were tested using the turbidity and Prompt inoculation methods. MSDGN panels were incubated at 35 ± 2°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 24 and 48 hours. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2°C and read visually. Frozen reference panels were read at 16-20 hours. FDA breakpoints (% susceptibility) used for interpretation of MIC results were: Enterobacteriaceae ≤ 5.

**RESULTS**

**MicroScan Dried Gram Negative MIC Results for Enterobacteriaceae Using MicroScan Dried Gram Negative MIC Panels**

<table>
<thead>
<tr>
<th>Organism Group</th>
<th>Susceptible</th>
<th>Resistant</th>
</tr>
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<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>≤ 0.5</td>
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</table>

- Eravacycline does not have an intermediate or non-susceptible category, therefore potential major and very major errors were calculated using the Non-susceptible (NS) result in place of resistant (R) as well as categorical errors.

- Potential Major Errors: +\[(\text{MIC Reference} \leq 0.5) \land (\text{MIC MSDGN} > 0.5)\]

- Potential Very Major Errors: +\[(\text{MIC Reference} < 0.5) \land (\text{MIC MSDGN} \geq 0.5)\]

**Efficacy**

- **Turbidity** A total of 414 Enterobacteriaceae clinical isolates were tested at three sites. MSDGN panels were inoculated using the turbidity inoculation method (Table 2). The essential Agreement between MicroScan and reference panel was 98.9% (409/414) for manual read method, 97.8% (405/414) for WalkAway System, 95.7% (396/414) for autoSCAN-4 instrument using the turbidity inoculation method.

- **Categorical Agreement** for Enterobacteriaceae between MSDGN panel and reference panel was 98.9% (409/414) for manual read method, 97.8% (405/414) for WalkAway System, 95.7% (396/414) for autoSCAN-4 instrument using the turbidity inoculation method.

**Panel Inoculation, Incubation, and Reading**

- All isolates were subcultured on trypticase soy agar (TSA) with 5% sheep blood and incubated in 18-24 hours at 34-37°C prior to testing. Isolates from frozen stocks were subcultured twice before testing.

- Inoculum suspensions for each strain were prepared with the direct standardization (turbidity standard) method for MSDGN MIC and frozen reference panels. MSDGN MIC panels were also inoculated using the Prompt inoculation method.

- Following inoculation, MSDGN MIC panels were incubated at 35 ± 2°C in the WalkAway system for 18 ± 2 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

**Data Analysis**

- Essential Agreement (EA) = MSDGN panel MIC within ± 1 dilution of the frozen reference result MIC.

- Categorical Agreement (CA) = MSDGN and reference panel categorical results (S) agree using FDA breakpoints for Enterobacteriaceae. (Table 1).

**METHODS**

**Panels**

- Frozen reference and MicroScan Dried Gram Negative MIC panels contained two fold doubling dilutions of eravacycline 0.016 - 32 μg/ml in catalyzed Mueller-Hinton broth.

- Reference panels were prepared and frozen following CLSI/ISO recommendations.

**Quality Control**

- Quality control (QC) testing was performed daily using ATCC 25922 E. coli and ATCC 27853 P. aeruginosa (see https://www.fda.gov/STIC). The essential Agreement between MicroScan and reference panel was 98.9% (409/414) for manual read method, 97.8% (405/414) for WalkAway System, 95.7% (396/414) for autoSCAN-4 instrument using the turbidity inoculation method.

- Categorical Agreement for Enterobacteriaceae between MSDGN panel and reference panel was 98.9% (409/414) for manual read method, 97.8% (405/414) for WalkAway System, 95.7% (396/414) for autoSCAN-4 instrument using the turbidity inoculation method.

**RESULTS**

**Table 1. Eravacycline FDA Interpretable Breakpoints (μg/ml)** (https://www.fda.gov/STIC)

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**Funding Information**

This multicenter study showed that eravacycline MIC results for Enterobacteriaceae obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using FDA interpretive criteria. FDA cleared 18/4/2019. This study was supported by Tetraphase Pharmaceuticals Inc.