Background: A multicenter study was performed to evaluate the accuracy of the EUCAST formulation of amoxicillin/clavulanic acid (2 µg/ml) against fixed clavulanic acid concentration) on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen ISO/CLSI broth microdilution reference methods.

Material/methods: For efficacy, an evaluation was conducted at three sites by comparing MICS using the MSDGN panel to MICs using an ISO/CLSI broth microdilution reference panel. A total of 626 Enterobacteriaceae clinical isolates were tested using the turbidity and Prompt\(^*\) methods of inoculation. Reproducibility, a subset of 11 organisms was tested on MSDGN panels at all three sites. MSDGN panels were incubated at 35-37°C in a WalkAway. The autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2°C and read visually. EUCAST breakpoints (µg/ml) used for interpretation of MIC results were: Enterobacteriaceae ≤ 8 S and >8 R.

Methods: For the purpose of evaluation of the reference panels and MICs using a frozen reference panel.

Quality Control: (QC) testing was performed using ATCC 25922 E. coli and ATCC 35218 E. coli using EUCAST, 9.0 QC references.

Panel inoculation, incubation, and reading:
- All isolates were subcultured onto tryptic soy agar (TSA) with 5% sheep blood and inoculated with the Prompt\(^*\) method prior to testing. Isolates from frozen stocks were subcultured twice before testing.
- Inoculum suspensions for each strain were prepared and directly inoculated onto the standardized turbidity method standard for MICDGN and MICFRCN panels. MICDGN panels were also inoculated using the Prompt Inoculation method.
- Following inoculation, MICDGN panels were incubated at 35°C for 18-20 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 reference MIC obtained using the EUCAST broth microdilution reference method.
- Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, R) agree using EUCAST breakpoints for Enterobacteriaceae (Table 1).

Results and Discussion

Reproducibility among the isolates was greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.

Conclusions: This multicenter study showed that amoxicillin/clavulanic acid MIC results for Enterobacteriaceae obtained using the MSDGN panel with an extended dilution series correlate well with MICs obtained using frozen reference panels.

This multicenter study showed that amoxicillin/clavulanic acid MIC results for Enterobacteriaceae obtained using the MSDGN panel with the EUCAST formulation correlate well with MICs obtained using frozen reference panels. The 2 µg/ml fixed concentration of clavulanic acid follows EUCAST recommendations.

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