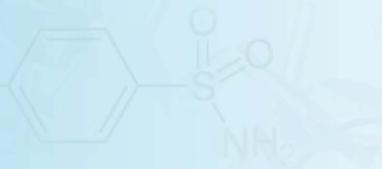


Thermo Scientific M.I.C.Evaluator Strips: An Effective Tool For Streptococcal MIC Testing Using The EUCAST Method.

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Overview

Purpose: To compare the ISO broth dilution method with Thermo Scientific™ M.I.C.Evaluator™ (M.I.C.E.™) strips for *Streptococcus pneumoniae* minimum inhibitory concentration (MIC) testing.

Methods: Organism MICs when following ISO broth dilution and M.I.C.E. strip methods were compared.

Results: All antibiotics showed ≥90% essential agreement (EA) when compared with the ISO* broth dilution method.

Introduction

Thermo Scientific M.I.C.E. strips (Thermo Fisher Scientific), shown in Figure 1, are a quantitative assay which combine the accuracy of traditional broth dilution with the ease of use of antibiotic discs. Each polymer strip contains an antibiotic gradient, which when placed onto the inoculated agar plate, diffuses into the agar. Following appropriate incubation, the MIC of an organism can be visually determined without the need for any measurements or calculations by reading the graduated scale at the point where growth of the organism touches the strip.

This study compared the performance of amikacin (256-0.0015µg/mL), amoxicillin (256-0.0015µg/mL), amoxicillin/clavulanic acid (256-0.0015µg/mL), ampicillin (256-0.0015µg/mL), cefotaxime (32-0.0002µg/mL and 256-0.0015µg/mL), oxacillin (256-0.0015µg/mL), penicillin (32-0.0002µg/mL and 256-0.0015µg/mL) and tigecycline (256-0.0015µg/mL) M.I.C.E. strips to the ISO* broth dilution method for determining the MIC of *S. pneumoniae* isolates.

Methods

Using sterile 0.9% saline, $2-8 \times 10^5$ CFU/mL suspensions of a range of *S. pneumoniae* isolates were prepared from cultures grown on Thermo Scientific Columbia Agar with Horse Blood (PB0122A) (Thermo Fisher Scientific). Suspensions were inoculated into microdilution plates containing the antibiotic serial dilution and Thermo Scientific Mueller-Hinton Broth with 5% lysed horse blood (containing 20mg/L NAD) microdilution plates and incubated at $36 \pm 1^\circ\text{C}$ in ambient air, following ISO guidelines¹. In addition, a 1.0 McFarland suspension of each isolate was used to create a bacterial lawn on Thermo Scientific Mueller-Hinton Agar with 5% horse blood (containing 20mg/L NAD) and, using sterile forceps, M.I.C.E. strips were applied. All plates were incubated at $36 \pm 1^\circ\text{C}$ in 5% CO₂, following EUCAST guidelines², as shown in Figures 2-7.

ISO reference method used in the absence of a EUCAST agar/broth dilution method.

FIGURE 1: Thermo Scientific M.I.C.E. Strips



Results were used to determine the percentage EA between the ISO* reference method and M.I.C.E. strips (EUCAST method).

Results

All strips gave >90% EA with the gold standard ISO method when using the EUCAST agar method. Amoxicillin/clavulanic acid (256-0.0015µg/mL), ampicillin (256-0.0015µg/mL), cefotaxime (256-0.0015µg/mL) and oxacillin (256-0.0015µg/mL) M.I.C.E. strips achieved 100% EA with the ISO broth dilution method, as shown in Table 1. Cefotaxime (32-0.0002µg/mL), penicillin (32-0.0002µg/mL) and tigecycline (256-0.0015µg/mL) achieved 95% EA and amikacin (256-0.0015µg/mL) and amoxicillin (256-0.0015µg/mL) achieved 90% EA with the ISO broth dilution.

Conclusion

Amikacin, amoxicillin, amoxicillin/clavulanic acid, ampicillin, cefotaxime, oxacillin, penicillin and tigecycline M.I.C.E. strips offer an accurate alternative to labour-intensive traditional broth dilution methods for determining the MIC of *S. pneumoniae* when using the EUCAST method.

References

1. ISO guidelines; Clinical laboratory testing and *in vitro* diagnostic test systems – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – ISO 20776-1:2006 (E)
2. EUCAST guidelines: European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0 01/01/2012.

TABLE 1: EA of each of the M.I.C.E. strips tested, shown as a percentage.

M.I.C.E. Strip	EA (%)
Amikacin 256µg/mL	90
Amoxicillin 256µg/mL	90
Amoxicillin/ Clavulanic acid 256µg/mL	100
Ampicillin 256µg/mL	100
Cefotaxime 32µg/mL	95
Cefotaxime 256µg/mL	100
Oxacillin 256µg/mL	100
Penicillin 32µg/mL	95
Penicillin 256µg/mL	100
Tigecycline 256µg/mL	95

FIGURE 2: A 1.0 McFarland bacterial lawn is inoculated into a non-selective plate using a swab.



FIGURE 3: M.I.C.E. Strips are individually packaged for ease of use.



FIGURE 4: M.I.C.E. Strips can be easily removed from packaging using sterile forceps.



FIGURE 5: The M.I.C.E. Strip is placed onto the inoculated agar plate.



FIGURE 6: Upon application the antibiotic begins to diffuse into the agar.



FIGURE 7: Plates are incubated according to EUCAST guidelines.



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