Validation Of M.I.C.Evaluator Strips For Testing Antimicrobial Susceptibilities Of Fastidious Organisms According To EUCAST Guidelines

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Overview

Purpose: The Thermo Scientific™ Oxoid™ M.I.C.Evaluator™ (M.I.C.E.™) strips (Thermo Fisher Scientific) allow the quantitative determination of antimicrobial agent minimum inhibitory concentration (MIC). This study was undertaken to validate the performance of Ceftriaxone 32 M.I.C.E., Clindamycin 256 M.I.C.E., Ciprofloxacin 32 M.I.C.E., Erythromycin 256 M.I.C.E., Imipenem 32 M.I.C.E., Linezolid 256 M.I.C.E., Levofloxacin 32 M.I.C.E., Meropenem 32 M.I.C.E., Teicoplanin 256 M.I.C.E., Tetracycline 256 M.I.C.E. and Vancomycin 256 M.I.C.E. strips for the determination of antimicrobial susceptibility of fastidious organisms against the European Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution method.

Methods: Streptococci and Haemophilus strains isolated from clinical samples were tested during the study. Broth microdilution plates and M.I.C.E. strip plates were inoculated with 0.5 McFarland inoculum suspension of each isolate tested and incubated according to the EUCAST guidelines[1]. Organism MICs were compared and used to determine the essential agreement (EA) between M.I.C.E. strips and EUCAST broth microdilution method.

Results: All M.I.C.E. strips showed an EA greater or equal to 90% for Streptococci and Haemophilus when compared with the EUCAST broth microdilution method.

Introduction

Several methods are available to measure the susceptibility of organisms to antimicrobials, such as broth microdilution, agar dilution, and disk diffusion, which are standard methods recommended by EUCAST. However, broth microdilution and agar dilution methods are time-consuming and labour-intensive and there is a need for simpler and more economical methods for susceptibility testing of Streptococci and Haemophilus.

The M.I.C.Evaluator strip is a patented commercial method for the quantitative determination of antimicrobial agent MICs. It is set up in a manner similar to that of agar disc diffusion test, but the disc is replaced with a calibrated polymer strip impregnated with a continuous concentration gradient of antimicrobial. Following appropriate inoculation and incubation procedures, the MIC of an organism can be visually determined without the need of any measurements or calculations.

Methods

Sample Preparation

All isolates to be tested were retrieved from -80˚C storage and cultures were grown on Thermo Scientific™ Columbia Agar with Horse Blood (Streptococci) or Thermo Scientific™ Columbia Agar with Chocolated Horse Blood (Haemophilus) overnight in the appropriate conditions, according to the EUCAST guidelines.

EUCAST Broth Microdilution Method

Microtitre plates containing cation adjusted Thermo Scientific™ Mueller-Hinton Broth (CAMHB) supplemented with 5% Lysed Horse Blood, 20mg/L β-NAD and appropriate concentrations of each antimicrobial agent (ceftriaxone, clindamycin, ciprofloxacin, erythromycin, imipenem, linezolid, levofloxacin, meropenem, teicoplanin, tetracycline and vancomycin), were inoculated with a 0.5 McFarland inoculum suspension of each Streptococci and Haemophilus isolate (to achieve an inoculum level of $7.5x10^5$CFU/ml) using a multi-channel pipette, as shown in Figure 1. All microtitre plates were incubated in aerobic conditions at 35±1˚C for 16-20 hrs according to EUCAST guidelines.

M.I.C.Evaluator Strip Method

The same inoculum suspension of each Streptococci and Haemophilus isolate was used to create a bacterial lawn on Thermo Scientific™ Mueller-Hinton Agar supplemented with 5% Defibrinated Horse Blood and 20mg/L β-NAD. All tested M.I.C.E. strips (Figure 2) were applied onto each agar plate using sterile forceps. Following the EUCAST guidelines plates were incubated in 5% CO_2 at 35±1˚C for 16-20 hrs.

MIC results were then read and used to determine EA (the percentage of M.I.C.E. strips giving an MIC within +1.0 and -1.5 doubling dilution difference from the EUCAST broth microdilution method results).

FIGURE 1. Microtitre plate for broth microdilution method.

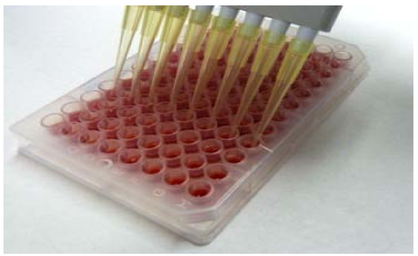
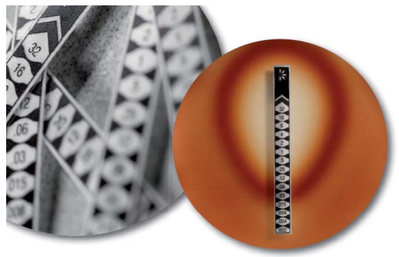


FIGURE 2. Thermo Scientific Oxoid M.I.C.E. strip.



Results

As shown in Table 1, M.I.C.E. strips demonstrated greater than or equal to 90% EA for Streptococci and Haemophilus when compared with the EUCAST broth microdilution method.

Table 1 . Essential agreement (shown as a percentage) between M.I.C.E. strip and EUCAST broth microdilution method.

M.I.C.E. Strip	Streptococci	Haemophilus
Ceftriaxone 32	100 (n=20)	95 (n=19)
Clindamycin 256	100 (n=30)	
Ciprofloxacin 32	100 (n=10)	95 (n=19)
Erythromycin 256	100 (n=20)	100 (n=18)
Imipenem 32	100 (n=20)	90 (n=19)
Linezolid 256	95 (n=20)	
Levofloxacin 32	100 (n=20)	100 (n=18)
Meropenem 32	90 (n=20)	90 (n=20)
Teicoplanin 256	97 (n=30)	
Tetracycline 256	100 (n=20)	100 (n=19)
Vancomycin 256	100 (n=30)	

Conclusion

The presented data confirm that the M.I.C.E. strip method for determination of the antimicrobial susceptibility patterns of fastidious organisms is comparable to the EUCAST broth microdilution method. Our study indicates that the M.I.C.E. strip method constitutes an effective and reliable alternative for susceptibility testing of fastidious bacteria by routine clinical microbiology laboratories.

References

1. www.eucast.org/antimicrobial_susceptibility_testing/

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