Evaluation Of Thermo Scientific Oxoid Ceftaroline 32 M.I.C.Evaluator Strips

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Overview

Purpose: To evaluate the performance of Thermo Scientific™ Oxoid™ Ceftaroline 32 M.I.C.Evaluator™ (M.I.C.E.™) strips (Thermo Fisher Scientific), which are designed to determine the minimum inhibitory concentration (MIC) of the ceftaroline antimicrobial agent against fastidious and non-fastidious organisms isolated from clinical samples.

Methods: A total of two hundred and one organisms, including Streptococci, Staphylococci, Enterobacteriaceae and Haemophilus were used to evaluate the performance of Oxoid Ceftaroline 32 M.I.C.E. strips. All organisms were tested according to M.I.C.E. strips method and the CLSI and EUCAST broth and agar dilution methods. Organism MICs were compared and used to determine essential agreement (EA).

Results: Oxoid Ceftaroline 32 M.I.C.E. strips achieved an essential agreement (EA) of greater than 90% across all tested groups and sub-groups of organisms for both the CLSI and EUCAST broth and agar dilution methods.

Introduction

With the introduction a variety of antimicrobials, it has become necessary to perform antimicrobial susceptibility testing (AST) routinaly. The MIC determination by dilution methods is the most practical and standard method for interpreting and reporting AST results. Dilution AST methods are used to determine the minimum inhibitory concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial compounds in either broth or agar media. The broth dilution method is a simple procedure for testing a small number of isolates, whereas the agar dilution method is used to test several organisms on one plate[1].

Thermo Scientific™ Oxoid™ M.I.C.Evaluator strips are an alternative to the traditional broth and agar dilution methods. Oxoid M.I.C.E. strips are reliable and straightforward tool for determining an exact MIC.

Methods

Two hundred and one Streptococci, Staphylococci (including methicillin-resistant *Staphylococcus aureus*), Enterobacteriaceae and Haemophilus isolated from clinical samples were tested. Organisms were grown on Thermo Scientific™ Columbia Agar with Horse Blood or Thermo Scientific™ Columbia Agar with Chocolate Blood overnight in the appropriate conditions, according to CLSI[2] and EUCAST[3] guidelines.

CLSI and EUCAST Broth dilution method

Microtitre plate wells containing broth media (for EUCAST method listed in Table 1., for CLSI method listed in Table 2.) and appropriate concentrations of ceftaroline antimicrobial agent, were inoculated with 0.5 McFarland inoculum suspension of each isolate (to achieve an inoculum level of $7.5x10^5$ cfu/mL) using a multi-channel pipette. All microtitre plates were incubated in aerobic conditions at 35±2C° according to CLSI and EUCAST guidelines.

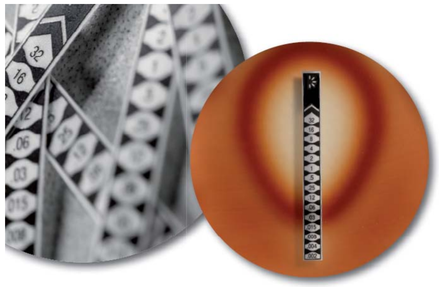
CLSI and EUCAST Agar dilution method

Media for the EUCAST and CLSI agar dilution method as shown in Table 1. and Table 2., with appropriate concentrations of ceftaroline antimicrobial agent, were inoculated with 0.5 McFarland inoculum suspension using a multi-point inoculator. Plates were incubated in aerobic conditions (Staphylococci and Enterobacteriaceae) or 5% CO_2 at 35±2C° (Streptococci and Haemophilus), according to CLSI and EUCAST methodologies.

M.I.C.E. strips method

All agar plates for Oxoid M.I.C.E. strips testing (see Table 1. and Table 2.), were inoculated by swabbing the plate with the same inoculum suspension in 3 different directions using a cotton swab. Oxoid Ceftaroline M.I.C.E. strips (see Figure 1.)

FIGURE 1. Thermo Scientific Oxoid M.I.C.E. strip.



were added to each plate using sterile forceps. M.I.C.E. strip plates, were incubated in appropriate conditions according to the EUCAST and CLSI guidelines, as shown in Table 1. and Table 2.

Organism MIC results were then read and used to determine EA (the percentage of the M.I.C.E. strips giving an MIC within +1.0 and -1.5 doubling dilution difference to the EUCAST and CLSI broth and agar dilution results. EA was calculated for each of the genera and species tested.

Results

Oxoid Ceftaroline 32 M.I.C.Evaluator strips demonstrated greater than 90% EA across all two hundred and one organisms tested, as shown by the results in Table 3.

EUCAST EA results

Oxoid Ceftaroline M.I.C.E. strips achieved an EA of greater than 90% across all tested groups of organisms for the EUCAST broth and agar dilution methods. Streptococci and Staphylococci demonstrated 100% EA for both EUCAST dilution methods. Enterobacteriaceae and Haemophilus achieved 92% EA and 98% EA for both EUCAST dilution methods respectively.

Oxoid Ceftaroline M.I.C.E. strips showed an essential agreement greater or equal to 90% for all tested sub-groups of organisms for both EUCAST broth and agar dilution methods.

CLSI EA results

Streptococci achieved 98% EA with the broth and agar diultion methods. Staphylococci demonstrated 100% EA for both CLSI broth and agar dilution methods. When tested with Enterobacteriaceae, M.I.C.E. strips again performed well, achieving 92% EA for both dilution methods. Withe the CLSI dilution methods, Haemophilus achieved 98% EA.

Additionally, Oxoid Ceftaroline 32 M.I.C.E. Strips achieved at least 90% EA for all sub-groups of organisms tested.

TABLE 1. Media and incubation conditions for each of the EUCAST method.

Organism	Medium for agar dilution method and strips testing	Incubation conditions	Medium for broth dilution method	Incubation conditions
Streptococcus	Thermo Scientific™ Mueller-Hinton Agar with 5% Defibrinated Horse Blood and 20mg/L β-NAD	35±2˚C for 20-24 hrs. in 5%CO_2	Cation-adjusted Thermo Scientific™ Mueller-Hinton Broth with 5% Lysed Horse Blood and 20mg/L β-NAD	35±2˚C for 20-24 hrs.
Staphylococcus	Thermo Scientific Mueller-Hinton Agar	35±2˚C for 16-18 hrs.	Cation-adjusted Thermo Scientific Mueller-Hinton Broth	35±2˚C for 16-20 hrs.
Enterobacteriaceae	Thermo Scientific Mueller-Hinton Agar	35±2˚C for 16-18 hrs.	Cation-adjusted Thermo Scientific Mueller-Hinton Broth	35±2˚C for 16-20 hrs.
Haemophilus	Thermo Scientific Mueller-Hinton Agar with 5% Defibrinated Horse Blood and 20mg/L β-NAD	35±2˚C for 16-18 hrs. in 5%CO_2	Cation-adjusted Thermo Scientific Mueller-Hinton Broth with 5% Lysed Horse Blood and 20mg/L β-NAD	35±2˚C for 20-24 hrs.

TABLE 2. Media and incubation conditions for each of the CLSI method.

Organism	Medium for agar dilution method and strips testing	Incubation conditions	Medium for broth dilution method	Incubation conditions
Streptococcus	Thermo Scientific™ Mueller-Hinton Agar with 5% Sheep Blood	35±2˚C for 20-24 hrs. in 5%CO_2	Cation-adjusted Thermo Scientific™ Mueller-Hinton Broth with 2.5-5% Lysed Horse Blood	35±2˚C for 20-24 hrs.
Staphylococcus	Thermo Scientific Mueller-Hinton Agar with 5% Sheep Blood	35±2˚C for 16-18 hrs.	Cation-adjusted Thermo Scientific Mueller-Hinton Broth	35±2˚C for 16-20 hrs.
Enterobacteriaceae	Thermo Scientific Mueller-Hinton Agar with 5% Sheep Blood	35±2˚C for 16-18 hrs.	Cation-adjusted Thermo Scientific Mueller-Hinton Broth	35±2˚C for 16-20 hrs.
Haemophilus	Thermo Scientific™ Haemophilus Test Medium Agar	35±2˚C for 16-18 hrs. in 5%CO_2	Thermo Scientific™ Haemophilus Test Medium Broth	35±2˚C for 20-24 hrs.

TABLE 3. The EA results for each of organism group and sub-group. Number of organisms tested shown in brackets.

Organism	CLSI EA (%) broth and agar dilution methods	EUCAST EA (%) broth and agar dilution methods
Overall (n=201)	95	95
Streptococcus (n=40)	98	100
S. agalactiae (n=10)	100	100
S. pneumoniae (n=12)	92	100
S. pyogenes (n=9)	100	100
S. viridans (n=9)	100	100
Staphylococcus (n=46)	100	100
MRSA (n=23)	100	100
MSSA (n=13)	100	100
CNS (n=10)	100	100
Enterobacteriaceae (n=75)	92	92
E. coli (n=55)	94	94
Morganella (n=20)	90	90
Haemophilus (n=40)	98	98

Conclusion

M.I.C.E. strips are effective at determining the ceftaroline antimicrobial agent MIC of Streptococci (*Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus viridans*) and Staphylococci (MRSA, methicillin-sensitive *Staphylococcus aureus*, coagulase-negative Staphylococcus), as well as common respiratory gram-negative organisms including Haemophilus, Morganella and *Escherichia coli.* Oxoid Ceftaroline 32 M.I.C.E. strips offer an accurate alternative to traditional agar and broth dilution methods for determining the MIC of many pathogens.

References

1. Manual on Antimicrobial Susceptibility Testing (Under the auspices of indian Association of Medical Microbiologists) Dr. M.K. Lalitha.
2. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard - Eight Edition. CLSI Document M07- A8.
3. EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing – Version 2.1 (February 2012).

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