

# Antimicrobial susceptibility testing of *Listeria monocytogenes* with EUCAST breakpoints: A multi-laboratory study.

Karen Bowker<sup>1</sup>, Jenny Åhman<sup>2</sup>, Olav Natås<sup>3</sup>, Israel Nissan<sup>4</sup>, Pia Littauer<sup>5</sup> and Erika Matuschek<sup>2</sup>

<sup>1</sup> Dept of Microbiology, Southmead Hospital, Bristol, United Kingdom, <sup>2</sup> EUCAST Laboratory for AST, Växjö, Sweden

<sup>3</sup> Dept of Medical Microbiology, Stavanger University Hospital, Norway

<sup>4</sup> Government Central Laboratories, Jerusalem, Israel, <sup>5</sup> Dept of Clinical Microbiology, Hvidovre Hospital, Denmark

## Introduction

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently proposed clinical MIC breakpoints based on epidemiological cut-off (ECOFF) values for *Listeria monocytogenes*.

## Objective

The objective of this study was to establish corresponding zone diameter breakpoints based on the EUCAST Mueller-Hinton Fastidious medium (MH-F) in a multi-laboratory study and to validate the use of a gradient test for MIC determination using broth microdilution (BMD) as reference.

## Methods

A total of 129 clinical isolates of *L. monocytogenes* were collected from five sites in Denmark, Israel, Norway, Sweden and United Kingdom. Antimicrobial susceptibility testing was performed against ampicillin, benzylpenicillin, erythromycin, meropenem and trimethoprim-sulfamethoxazole. Disk diffusion was performed for all isolates at all sites on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) according to EUCAST methodology. Media (commercial and in-house prepared plates) and disks from different manufacturers were used at the various sites. MIC determination was performed with BMD (TREK Diagnostics/Thermo Fischer Scientific) and Etest (bioMérieux) at Southmead Hospital, UK. BMD was performed on custom panels containing MH broth with 5% lysed horse blood (by repeated freezing and thawing) and 20 mg/L β-NAD (MH-F broth) and Etest was performed on MH-F media. All data were analysed by EUCAST.

## Results

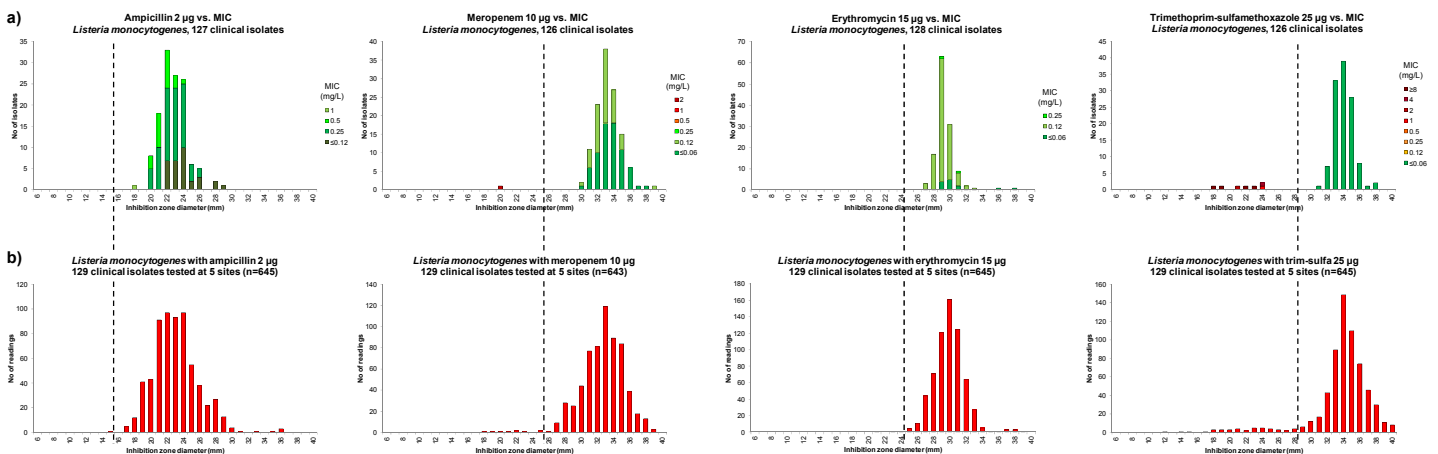
All *L. monocytogenes* isolates grew well within 16 – 20 h on the MH-F medium. The correlation between BMD and Etest MICs was excellent, with 99.4% of Etest values within ± 1 dilution of the BMD values. Zone diameter breakpoints were established (Table 1) in such a way that the MIC ECOFF corresponded to the zone diameter distribution ECOFF (examples in Figure 1a). Reproducibility between the sites was good (Figure 1b) and small variations in inhibition zones were mainly due to variations in media and disks from different manufacturers.

**Table 1**  
EUCAST clinical MIC breakpoints for *Listeria monocytogenes* and corresponding zone diameter breakpoints.  
EUCAST Clinical Breakpoint Table v 2.0

Antibiotic agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Ampicillin	1	1	2	16	16
Benzylpenicillin	1	1	1 unit	13	13
Meropenem	0.25	0.25	10	26	26
Erythromycin	1	1	15	25	25
Trimethoprim-sulfamethoxazole	0.03	0.03	1.25-23.75	29	29

## Conclusions

MH-F is a suitable medium for antimicrobial susceptibility testing of *L. monocytogenes* for both gradient tests and disk diffusion. As a result of a multi-laboratory study, we present zone diameter breakpoints correlated to EUCAST clinical MIC breakpoints.



**Figure 1** a) Inhibition zone distributions from one site (Växjö, SE) for *Listeria monocytogenes* with corresponding MIC values (BMD) as coloured bars. b) Combined inhibition zone distributions from the five test sites. Dotted lines correspond to EUCAST zone diameter breakpoints.

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