Evaluation of the ability of a newly developed Muller Hinton E® agar to detect MRSA carrying the novel *mecA* homologue *mecC*

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Objectives

The objective was to evaluate MH E® (bioMérieux) for AST using a collection of well characterized *mecC* MRSA isolates used in the previous evaluation of phenotypic AST.

Introduction

Accurate phenotypic antimicrobial susceptibility testing (AST) of resistance has proven important to detect MRSA harboring the *mecA* homologue *mecC*, since most genotypic methods fails to detect these isolates due to the low sequence similarity between *mecA* and *mecC*. In a recent evaluation, considerable differences between Muller Hinton (MH) agars from different manufacturers were observed (Skov *et al*,2014). In response to these results, a new MH agar, called MH E®, was developed by bioMérieux.

Methods

The evaluation included 61 MRSA carrying the *mecC* gene and three methicillin susceptible (MSSA) isolates. The *mecC* MRSA strains: 25 from the UK (13 human and 12 bovine), 24 from Denmark (human) and 12 from Scotland (human) wer confirmed by multiplex PCR detecting *mecA, mec*C, *spa* and PVL (*lukF-pv)* genes. Standard inocula (McFarland 0.5) were plated on two lots of MH E® agar plates and incubated overnight in air at 35°C. Inhibition zone diameters were measured for a 30 μg cefoxitin disc (Oxoid) and MICs determined using Etest® (bioMérieux). Interpretation was done according to EUCAST recommendations: zone diameters of ≥ 22 mm and MIC ≤ 4 mg/L were interpreted as susceptible (www.EUCAST.org).

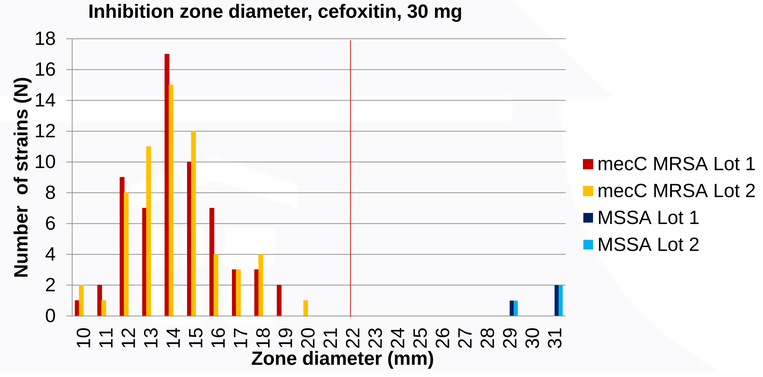

Figure 1. Inhibition zone diameters for cefoxitin 30 mg disc on MH E® agar (two lots). EUCAST breakpoint indicated at S ≥ 22 mm

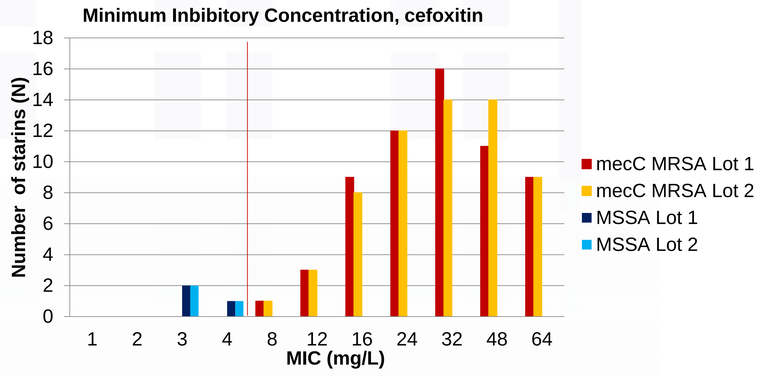

Figure 2 . Cefoxitin minimum inhibitory concentration (MIC) by Etest ®, on MH E® agar (two lots) EUCAST breakpoint indicated at S ≤ 4 mg/L

Results

The strain collection represented 14 *spa* types: t529, t742, t843, t978, t1535, t6220, t6292, t6293, t6386, t7485, t7734, t7945, t7946 and t7947, assigned to CC130, ST425, CC705 and CC1943.
The *mecC* MRSA population had inhibition zone diameters ≤20 mm (median 14 mm) and MIC values in the range 8-64 mg/L (median 32 mg/L). The three susceptible isolates had inhibition zones and MIC values of 29-31 mm and 3-4 mg/L, respectively.
Antimicrobial susceptibility was detected with 100 % specificity and sensitivity. Results are shown in Figure 1 and 2. No significant inter-batch variation was observed
For three isolates double inhibition zones were observed on the MH E® agar, which was not the case when the isolates were tested on other MH agars.

Conclusions

The correct phenotypic identification of *mecC* MRSA on MH E® is an improvement compared to the former MH tested from bioMérieux and proves phenotypic AST using either disc diffusion or Etest to be reliable for detection of *mecC* MRSA.

References

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Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR.Clin Microbiol Infect. 2012 Apr;18(4):395-400

Acknowledgement

Alexandra Medina is thanked for excellent technical assistance
The study was sponsored by bioMérieux