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

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1 Statens Serum Institut, Denmark
2 University of Cambridge, UK
3 Public Health England, UK
4 Scottish MRSA Reference Laboratory, UK
5 Animal Health and Veterinary Laboratories Agency, UK

**Objectives:** Early detection of methicillin-resistant Staphylococcus aureus (MRSA) is important in infection control. Accurate phenotypic antimicrobial susceptibility testing (AST) of resistance has proven important in the detection of MRSA harboring the meca homologue mecC, since most genotypic methods fail to detect these isolates due to the low sequence similarity between meca and mecC and concordant primer mismatches.

In a recent evaluation, cefoxitin proved superior compared to oxacillin in disc diffusion testing for detection of mecA MRSA, as previously observed for mecC MRSA isolates. Furthermore, the study revealed considerable differences between Mueller Hinton (MH) agars from different manufacturers. In response to these results, a new MH agar, called MH E, was developed by bioMérieux. The objective of this study was to evaluate a newly developed MH agar (MH E, bioMérieux) for AST using the same international strain collection of well characterized mecC MRSA isolates as previously used in the evaluation of phenotypic AST.

**Methods:** The evaluation included 62 S. aureus isolates, three methicillin susceptible isolates (MSSA) and 59 carrying the mecC gene as confirmed by multiple PCR detecting mecA, mecC together with the Protein A (pap) and PVL (iok-fv) encoding genes. Spot types were used as previously described. Inhibition zone diameters were measured for a 30 mg cefoxitin disc (Oxoid) using an automated colorimetric and MIC was measured using Etest (bioMérieux). Interpretation was made according to EUCAST recommendations: zone diameters of less than 22 mm and MIC > 4 mg/L were interpreted as resistant.

**Results:** The strain collection represented 13 spa types assigned to CC130, ST425 and ST1943. All 59 mecC isolates and the MSSAs were correctly identified, with median values of 14 mm and 32 mg/L, respectively. Results are shown in Table 1. 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 other disc diffusion or Etest to be a reliable method for detection of mecC MRSA.

**Meropenem-Colistin Synergy Testing of Multi-Drug Resistant Acinetobacter baumannii in Routine Two-Dimensional Etests**

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D. Holm, E. E. Bennett, G. Banos, A. Chekanea, Y. Sauvannet, R. Mantelin, V. Van Belkem, G. Durand, S. Chartier and J. Siffert

1 Ceremnic Research Laboratory, Service of Infectious Diseases Department, Geneva University Hospital (HUG), Geneva, Switzerland
2 bioMérieux SA, Microbiology Unit, La Balme Les Grottes, France

**Objectives:** Confronted with multidrug resistant bacteria, physicians ask clinical microbiologists to supply them with synergy testing data. Whereas conventional checkerboard synergy testing does provide such information, this time-consuming and labor-intensive method cannot be performed on a routine basis. It is especially evident when confronted with Gram-negative bacterial species such as Acinetobacter baumannii. Pseudomonas aeruginosa or other non-fermentative organisms. We focused here on a panel of multidrug-resistant Acinetobacter baumannii and tested them for potential synergy when exposed to meropenem and colistin, one of the preferred therapeutic combinations.

**Methods:** We compared checkerboard testing with the Xact method (bioMérieux), an approach derived from the Etest format. Briefly, an agar plate seeded with a 0.5 McFarland suspension is overlaid with a square strip impregnated by a gradient of antibiotic A and a perpendicular gradient of antimicrobial agent B. After 1 hour of incubation, this test strip is removed and the pattern of bacterial growth is imaged after 16-20h of growth at 35°C. Inhibition zones provide the following information: MIC for antimicrobial A, MIC for antimicrobial B and assessment of synergic, additive or antagonistic effects along various concentration ratios of antimicrobials A and B respectively.

**Results:** This work examined 28 multidrug resistant A. baumannii isolates, most of them isolated from rectal swabs and all collected between 2006 and 2013 in the HUG. All study isolates were resistant to amikacin, ciprofloxacin, cefazidine, and piperacillin-tazobactam. The MIC to imipenem ranged from 0.5-256 mg/L. The MIC to meropenem ranged from 0.5-32 mg/L. The MIC50 and MIC90 values for imipenem were 16 mg/L and 32 mg/L, respectively. For meropenem, the MIC50 and MIC90 values were 64 mg/L and 192 mg/L, respectively. All study isolates were susceptible to colistin. The MIC for colistin ranged from 0.064-0.5 mg/L. The MIC50 and MIC90 values for colistin were 8 mg/L and 192 mg/L, respectively.

According to checkerboard data, Xact revealed a sensitivity of 94% and a specificity of 91%. The Xact method is easier to set-up and analyse, allowing to perform three times more analyses during the working time and offering potential for routine synergy testing.

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