Comparison of Oxacillin-Supplemented AGV Agar and Mueller-Hinton Agar for Detection of Methicillin-Resistance in *Staphylococcus aureus*

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ABSTRACT

Objectives: To evaluate acceptability of AGV agar which is the susceptibility testing medium most widely used in Russia for the detection of resistance to methicillin (oxacillin) in *Staphylococcus aureus* in comparison with Mueller-Hinton (MH) agar.

Methods: A total of 94 double-checked MRSA by screening on MH agar were included in this study. All strains were screened for oxacillin-resistance on AGV agar supplemented with 6 mg/l oxacillin and 4% NaCl. Inoculation, incubation and interpretation of results were done according to NCCLS standards. *S.aureus* ATCC 29213 and ATCC 43300 were used as susceptible and resistant controls, respectively. For 13 strains mecA gene was detected by PCR.

Results: 23 (24.5%) and 15 (16%) MRSA strains failed to grown on AGV agar after 24 h and 48 h of incubation, respectively. *S.aureus* ATCC 43300 and 5 (38.5%) other MRSA with known mecA gene failed to grow on AGV screening plates after 24 h incubation, and one (7.7%) mecA-positive strain did not grow after 48 h incubation.

Conclusions: Screening for MRSA on AGV agar seems to be unsatisfactory method and should not be used as alternative to Mueller-Hinton agar for detection of MRSA.

INTRODUCTION AND PURPOSE

Introduction: Treatment of MRSA infections is one of the greatest problem of antimicrobial therapy. In term of view that MRSA are resistant to practically all beta-lactam antibiotics, it is very important to detect the true resistance to methicillin for choice of appropriate non-beta-lactam antimicrobial agent. The agar screening method is the most popular and reliable method for this purpose. In Russia the most commonly used media for susceptibility testing is AGV agar. And what is more, in some laboratories AGV now is the only available media for susceptibility testing because of economical problems and lack of collaboration with international manufactures.

But agar AGV is not standardised media and different from Mueller-Hinton agar by high Ca, Mg, Zn and thymidine contents. According to some recent studies (O.Stetsiouk) AGV should not be considered as an appropriate media for determination of susceptibility of *S.aureus* to carbapenems and quinolones, *P.aeruginosa* to aminoglicosides, quinolones, carbapenems, *Haemophilus* spp. to any antimicrobials and for testing of any microorganism to antifolates. The above mentioned limitations logically result in a question - “Is AGV agar an reliable media for detection of resistance to methicillin”.

Purpose of the study: To evaluate acceptability of AGV agar for the detection of resistance to methicillin (oxacillin) of *S.aureus* in comparison with Mueller-Hinton agar.

METHODS

A total of 94 strains of *S.aureus* isolated from inpatients hospitalized in Smolensk Regional Hospital in 1998 and double-checked for methicillin-resistance by screening on Mueller-Hinton agar with 6 mg/l oxacillin and 4% NaCl according to NCCLS standards were included in this study.

All strains were screened for oxacillin-resistance on AGV agar supplemented with 6 mg/l oxacillin and 4% NaCl. Bacterial suspension (0.5 McFarland turbidity) was used for inoculation. Inoculated plates were incubated in ambient air at 35°C. Screening plates were examined for presence of growth after 24 and 48 hours. If more than 1 colony was observed result was registered as positive, if one colony or no growth - negative. *S.aureus* ATCC 29213 and ATCC 43300 were used as negative and positive controls, respectively.

For 13 strains presence of mecA gene has been proven by PCR.

RESULTS

Twenty three (24.5%) MRSA strains failed to grow on AGV agar after 24 h and 15 (16%) after 48 h of incubation (see table below). Five (38.5%) of 13 MRSA with known mecA gene shown no growth on AGV screening plates after 24 h incubation, and one (7.7%) mecA-positive strain did not grow after 48 h incubation. There was no growth of referent strain *S.aureus* ATCC 43300 after 24 and 48 hours of incubation that was used as positive control on each AGV screening plate.

Table. Results of screening for methicillin-resistance of MRSA on AGV agar after 24 and 48 hours of incubation

	24 h incubation		48 h incubation	
	all strains	mecA+ve	all strains	mecA+ve
Growth	71 (75.5%)	8 (61.5%)	79 (84%)	12 (92.3%)
No growth	23 (24.5%)	5 (38.5%)	15 (16%)	1 (7.7%)

CONCLUSION

- Screening for methicillin-resistance on AGV agar seems to be an unreliable method even after 48 hours of incubation and should not be used as alternative to Mueller-Hinton agar for detection of MRSA.