**Methicillin susceptibility testing of staphylococci by Etest and comparison with agar dilution and mecA detection**

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The susceptibility to methicillin of 44 *Staphylococcus aureus* and 120 coagulase-negative staphylococci (CNS) was determined by Etest, agar dilution and presence of the *mecA* gene. There was agreement between the results of all methods when testing *S. aureus*. However, discrepancies occurred with CNS when cultural methods were compared with presence of the *mecA* gene. *mecA*-positive isolates tested as resistant more often with agar dilution on Columbia agar plus 5% NaCl than by Etest.

**Introduction**

The results of methicillin susceptibility testing for both Staphylococcus aureus and coagulase-negative staphylococci (CNS) can vary on different media and at different incubation temperatures.\(^1\) Although resistance is detected more often by agar dilution MIC than by disc diffusion,\(^2\) the former is time consuming and difficult to perform on a routine basis. Some workers have therefore promoted the Etest as a possible, simple solution.\(^3,4\) In this investigation the reliability of Etest for detecting methicillin resistance in staphylococci was evaluated and compared with agar dilution MIC and the *mecA* status of the isolates.

**Materials and methods**

A previously described collection of 164 isolates of staphylococci (44 *S. aureus* and 120 CNS)\(^1,2\) was examined. Eight were reference strains, three were quality control strains and the remainder were clinical isolates where difficulty had been encountered determining methicillin susceptibility by disc diffusion.

**Susceptibility testing**

Staphylococci were tested by agar dilution according to the National Committee for Clinical Laboratory Standards (NCCLS).\(^5\) Methicillin was added to Columbia and IsoSensitest agars (Oxoid, Basingstoke, UK) supplemented with 5% NaCl to give final concentrations of 0, 0.5, 1, 2, 4, 8 and 16 mg/L. Plates were inoculated with each isolate at a concentration of 10\(^6\) cfu/spot and incubated for 48 h at 35°C.

The Etest MIC was performed by applying the strip to media inoculated with a suspension of bacteria at a density of MacFarland standard 0.5. Each isolate was tested with methicillin and oxacillin Etest (AB Biodisk, Solna, Sweden) on the same media as above. The plates were incubated at 35°C for 48 h and the MIC was read as recommended by the manufacturer by two separate observers.

**Polymerase chain reaction**

The *mecA* gene was amplified by a set of primers previously described by Tokue et al.\(^6\) MR1 (5’-GTGGAATTGGCAAATACAGG-3’) and MR2 (5’-TGAATTCTGAGTACCAGAT-3’). A third primer, MR3 (5’-ATGATTATGGCTACGTCAACCAAACACCTCCTCCTCCACCCCAATCC-3’) was used for confirmatory semi-nested PCR using methods described by Falla et al.\(^7\) Primers targeting the 16S rRNA gene, RW01 (5’-ACCTGAGGGAAGGTGGG-3’) and DG74 (5’-AGGGTGATCTCAACCCGCA-3’)\(^8\) were used as an internal control for each amplification reaction.

A single colony from overnight growth of each isolate was suspended in 0.5 mL distilled water and the DNA was liberated by boiling for 15 min. Crude DNA extract was diluted 1 in 3 for use as DNA template for PCR.

Each isolate was simultaneously amplified for both the *mecA* and 16S rRNA genes. Each 20 µL reaction mixture contained 2 µL DNA template, 0.5 units Taq polymerase,
and final concentrations of 200 μM dNTPs (each), 1.5 mM MgCl₂, 75 mM Tris-HCl pH 9.0, 20 mM (NH₄)₂SO₄, 0.01% Tween 20, and 250 nM each of primers MR1, MR2, DG74 and RW01. Thirty cycles of amplification consisted of 60 s at 95°C, 60 s at 55°C and 2 min at 72°C. The PCR products were separated by electrophoresis on a 1% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. A further round of semi-nested PCR amplification with 2 μL of a 1 in 10 dilution of the primary mecA product was performed using the primers MR2 and MR3.

β-Lactamase test

β-Lactamase tests were performed on mecA-negative S. aureus that had a methicillin MIC of ≥8 mg/L. Isolates were emulsified in 50 μL nitrocefin. After 30 min those having undergone a colour change from yellow to red were deemed positive for β-lactamase.

Induction of methicillin resistance

mecA-positive isolates classified as susceptible by Etest were cultured in Mueller-Hinton broth containing 0.5 mg/L methicillin. The MIC was then determined by agar dilution on Columbia agar plus 5% NaCl as above.

Results

The 370 bp 16S rRNA gene product was detected in all amplification reactions. The primary mecA gene product of 1339 bp was found in 21 isolates of S. aureus and in 48 CNS, all of which produced the predicted 785 bp product on confirmatory semi-nested PCR. The Table contains the results of agar dilution and Etest susceptibility tests (organisms with an MIC of methicillin or oxacillin ≥8 mg/L were classified as resistant) compared with the mecA genotype of the isolate. The Figure shows the Etest MIC of mecA-positive and mecA-negative isolates on Columbia agar plus 5% NaCl.

There was agreement between the presence of the mecA gene and susceptibility of S. aureus by Etest and by agar dilution on Columbia agar plus 5% NaCl, but not by agar dilution on Isosensitest agar plus 5% NaCl. β-Lactamase production was not detected in either of the two mecA-negative S. aureus that were classified as resistant by agar dilution.

When CNS were tested, greatest agreement with mecA genotype was seen with agar dilution on Columbia agar plus 5% NaCl. However, 20 of the 48 mecA-positive CNS tested as susceptible on one or more combinations of Etest and media. Nine of these 20 isolates were found to have inducible methicillin resistance. A further two isolates exhibited poor growth on the media used and this may have contributed to resistance not being detected.
Methicillin susceptibility testing by Etest

Three mecA-positive CNS remained susceptible when tested on agar containing NaCl, but two of these exhibited resistance when tested on media without NaCl.

Discussion

Determination of methicillin resistance in S. aureus by Etest was equal to that measured by agar dilution and compared well with detection of the mecA gene. This investigation, therefore, confirms the reliability of methicillin MIC testing of S. aureus on Columbia agar plus 5% NaCl observed by Milne et al.\(^1\) Given the relative ease of performing Etest MICs and the reliability of the test demonstrated here and by others\(^3,4\) it is an attractive method of assessing S. aureus isolates for methicillin resistance.

In contrast, neither cultural technique correlated with the presence of the mecA gene in CNS. It is assumed that mecA-positive isolates will be resistant to all \(\beta\)-lactams and all but one in this study was shown to be methicillin-resistant under at least one set of culture conditions. However, mecA genotyping for both CNS and S. aureus is of unknown clinical value. Expression of the mecA gene is influenced by other regulatory genes\(^9\) that affect susceptibility test results in vitro. It is possible, therefore, that some mecA-positive isolates may be susceptible to methicillin and hence be treatable with appropriate \(\beta\)-lactam antibiotics. Differences in the presence of these regulatory genes may account for the poor correlation between mecA status and phenotypic resistance seen with the CNS but not the S. aureus studied here. Whether susceptibility in vivo can be predicted for mecA-negative isolates, particularly those with MICs of 16 or 32 mg/L, is also uncertain.

References


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