DIAGNOSTIC MICROBIOLOGY

A comparison of PCR detection of mecA with two standard methods of oxacillin disk susceptibility testing for coagulase-negative staphylococci

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Coagulase-negative staphylococci (CNS) are common isolates from blood cultures, and an increasing proportion is now methicillin resistant. The National Committee for Clinical Laboratory Standards (NCCLS) recently issued new criteria for zone sizes applicable to oxacillin disk sensitivity testing for CNS and the British Society of Antimicrobial Chemotherapy (BSAC) has also issued guidelines. This study evaluated two standard methods for oxacillin disk sensitivity testing of 67 CNS isolates from blood cultures, and compared these results with detection of the mecA gene by PCR. Over 94% of mecA-positive isolates were detected by conventional disk testing, with no significant differences between the two methods. In this study, the clinical utility of mecA detection in CNS for the determination of methicillin resistance appears to be limited.

Introduction

Coagulase-negative staphylococci (CNS) are common isolates from blood cultures [1]. Although some of these isolates are attributed to contamination of the cultures with skin flora, an increasing proportion [2] is implicated in nosocomial infections. Increasing methicillin resistance in CNS has resulted in the widespread use of glycopeptides as first-line antimicrobial therapy for these micro-organisms. Accurate and quick susceptibility testing will be important if a reduction in the empirical usage of vancomycin is to be achieved. In the UK, oxacillin disk testing is a convenient and affordable method of susceptibility testing. The British Society of Antimicrobial Chemotherapy (BSAC) [3] and the National Committee for Clinical Laboratory Standards (NCCLS) [4] have both published guidelines for oxacillin disk testing. The NCCLS recently revised oxacillin MIC breakpoints and disk diffusion zone sizes for CNS to improve the sensitivity of these tests [5].

Methicillin resistance in CNS is mediated by presence of the mecA gene, although conventional disk sensitivity testing may fail to detect this [6].

This study set out to compare the revised NCCLS criteria with current BSAC guidelines, with conventional oxacillin disk testing and PCR detection of the mecA gene as a standard positive control.

Materials and methods

Micro-organisms

Sixty-seven isolates of CNS from positive blood cultures were examined. They were identified by examination of colonial morphology and a commercial latex agglutination test (Staphaurex, Murex Biotech Ltd). All isolates were also negative by PCR for a Staphylococcus aureus-specific genetic sequence [7]. The control strains NCTC 6571 and NCTC 10442 were used in sensitivity testing, as specified by BSAC.

PCR

A 200-µl sample of blood and broth from each positive blood culture was lysed and DNA was purified according to the manufacturer's instructions with the Generation® DNA Purification Capture Column™ kit (Genta Corporation, Minneapolis, MN, USA). Oligonucleotide primers and fluorescence-labelled probes were based on published sequences of the mecA gene and designed for amplification and sequence-specific detection of a 98-bp fragment within the mecA gene. The primers used were as follows: MecA-F, 5'-
CAAGATATGAAAGTGTAATATTGTG-3' and MecA-R, 5'-ACTGCTTAATTGGAGGCAC-3'. The Cy-5-labelled probe sequence used for detection of the amplified product was as follows: MecA-P, 5'-AAA CAACGATAGATCATCATGAT-[Cy5]-3'. PCR was performed on the LightCycler Device (Biogene; Kimbleton, Cambs), that combines rapid thermal cycling and probe-specific detection of the amplified product. The amplification mixture consisted of 5 µl of 2× Taq-based Master Mix containing 3 mM MgCl₂, 0.5 µl each of mecA primers (0.4 µM final concentration), 1 µl of Cy-5-labelled probe MecA-P (0.2 µM final concentration), 0.5 µl of 1× SYBR Green I and 2 µl of template DNA. The cycling profile consisted of an initial denaturation step at 95°C for 10 s, followed by 50 cycles of 95°C for 0 s, 57°C for 1 s and 74°C for 1 s. Fluorescence acquisition to determine the melting temperatures of both the product and the probe were performed at the end of the amplification cycle.

**Conventional culture**

A sample from each positive blood culture was cultured on a blood agar plate. After incubation for 24 h, colonies were examined for purity and tested by latex agglutination.

**Antibiotic susceptibility testing**

Antibiotic disk susceptibility testing was performed following the recommendations of BSAC and the NCCLS. For BSAC disk testing, a suspension of each isolate was made to a turbidity of 0.5 MacFarlane standard and a 1 in 10 dilution was inoculated on to Columbia Agar (Oxoid) with sodium chloride (NaCl) 2% to achieve semi-confluent growth. A 1 µg oxacillin disk was applied to each plate and, after incubation at 30°C, zone diameters were measured at both 24 and 48 h. Zone sizes >15 mm were interpreted as indicating susceptibility. For NCCLS disk testing, a suspension of each isolate was made to a turbidity of 0.5 MacFarlane standard and this was inoculated on to Mueller-Hinton Agar (Oxoid). Zone diameters were measured after incubation at 35°C for 24 h. Zone sizes >18 mm were interpreted as indicating susceptibility. Any isolates with discrepant susceptibility testing results were forwarded to the PHLS Antibiotic Resistance Monitoring and Reference Laboratory, 6 Colindale Avenue, London for determination of MIC values by agar dilution.

**Results**

Sixty-seven isolates were tested, of which 49 were positive for the mecA gene. Disk susceptibility testing by the BSAC method detected 47 (96%) of 49 mecA-positive strains; the NCCLS method detected 46 of 49 mecA-positive strains (Table 1). Three mecA positive isolates had discrepant results by one or more method.

**Table 1. Comparison of oxacillin disk sensitivity testing of CNS by BSAC and NCCLS methods**

<table>
<thead>
<tr>
<th></th>
<th>BSAC method</th>
<th>NCCLS method</th>
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<tbody>
<tr>
<td>mecA result</td>
<td>Number of isolates tested</td>
<td></td>
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<tr>
<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Positive</td>
<td>47</td>
<td>2</td>
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</table>

These three isolates had methicillin MIC values of ≤2 mg/L, ≤2 mg/L and 4 mg/L, respectively, and would be classified as methicillin-sensitive by current BSAC criteria.

Incubation of oxacillin-sensitive strains for a total of 48 h, as specified by BSAC, did not result in any improvement in the sensitivity of the disk testing results.

All isolates lacking the mecA gene were sensitive by both methods of disk testing.

**Discussion**

CNS are a very heterogeneous group of organisms, and antibiotic sensitivity testing continues to be problematic. Several studies [8, 9] have indicated that an oxacillin breakpoint of >0.25 mg/L correlates better with mecA-associated resistance, and this resulted in a recent change to the NCCLS criteria for disc diffusion zone sizes. BSAC currently specify oxacillin breakpoints for staphylococci of 4 mg/L. Nonetheless, in this study, no real difference was apparent between either method when antibiotic disk sensitivity testing was employed.

The correlation between the presence of the mecA gene and phenotypic resistance to oxacillin in CNS remains less well defined than in S. aureus. Small numbers of mecA-negative CNS that are phenotypically methicillin resistant have been reported [10, 11], some of which is ascribed to β-lactamase hyperproduction [12]. There are also strains of mecA-positive CNS that appear to be methicillin sensitive on conventional susceptibility testing [13]. These isolates have oxacillin MIC values of 0.25–2 mg/L [8, 9, 13]. A study by Dickinson and Archer suggested that phenotypic expression of methicillin resistance in some mecA positive strains of CNS may be affected by transcriptional regulation of the mecA gene [14]. However, it is presently unclear if clinical infections with such isolates would fail to respond to standard β-lactam therapy.

Antibiotic disk sensitivity testing remains a cheap and readily available method for testing oxacillin susceptibility in CNS, and will detect the majority of methicillin-resistant strains. There appears to be no
significant difference in the sensitivity of the current antibiotic disk testing methods recommended by either BSAC or the NCCLS. In this small study, the clinical benefit of meca detection in CNS appears to be limited, especially when economic considerations are taken into account.

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References


Determination of Methicillin Resistance in CNS