Title
Use of VITEK 2 antimicrobial susceptibility profile to identify meC in methicillin-resistant
Staphylococcus aureus

Running title
VITEK 2 antimicrobial profiling to identify meC MRSA

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Abstract

The emergence of mecC MRSA poses a diagnostic challenge for clinical microbiology laboratories. Using the VITEK 2 system, we tested a panel of 896 Staphylococcus aureus isolates, and found that an oxacillin sensitive / cefoxitin resistant profile had a sensitivity of 88.7% and a specificity of 99.5% for the identification of mecC MRSA isolates. The presence of the mecC gene, determined by bacterial whole-genome sequencing, was used as the gold standard. This profile could provide a zero-cost screening method for identification of mecC positive MRSA strains.
Methicillin resistance in staphylococci is mediated by an altered penicillin-binding protein (PBP2a), which confers resistance to β-lactam antibiotics, and is encoded by the mecA gene on the mobile element, staphylococcal cassette chromosome mec (SCCmec) (1-2). The identification of methicillin-resistant Staphylococcus aureus (MRSA) in diagnostic microbiology laboratories can be achieved by a range of methods, including antimicrobial susceptibility testing, detection of PBP2a by latex agglutination tests, and the molecular detection of the mecA gene (3-6).

The description of MRSA isolates from the United Kingdom (UK) and Denmark that harboured a divergent mecA homologue termed mecC (formerly mecALGA251)(7) within a novel SCCmec XI element was of particular concern because these produced negative results, both by a latex agglutination test and by a polymerase chain reaction (PCR) assay for mecA (8). PCR assays are negative because of divergence in the primer-binding sites, a problem that was rectified by the development of new primers (9-11). Since its original description, mecC MRSA has been reported from a number of countries including France (12), Germany (13-14), the Netherlands (15), Switzerland (16), Republic of Ireland (17), Norway (18), Belgium (9) and Sweden (19), and appears to be increasing in prevalence in Denmark (20), highlighting the importance of identifying these isolates. mecC MRSA is capable of causing a range of infections, and appears to be predominantly community-acquired (20). In addition to humans, mecC MRSA has also been found in a range of host species (8-9, 18), with evidence of animal-to-human transmission (21).

Routine diagnostic tests do not, however, provide a mechanism for the identification of mecC, which still requires confirmation using PCR assays that are currently only available at reference laboratories (10-11). The availability of a simple method to identify mecC MRSA could be used to monitor changes in its distribution and prevalence over time. We made an anecdotal observation, based initially on a small number of strains, that mecC positive MRSA isolates were susceptible to oxacillin, but resistant to cefoxitin when tested using the Staph AST-P620 card on the VITEK 2 automated antimicrobial susceptibility testing system (bioMérieux, Marcy l’Étoile, France). This profile differed from the oxacillin resistant/cefoxitin resistant profile that is usually observed with mecA positive MRSA isolates.

To test this observation, we assessed the VITEK 2 susceptibility profile and mec gene status of a collection of 896 S. aureus isolates which were sequenced using the Illumina HiSeq platform at the Wellcome Trust Sanger Institute (Table 1). Genome sequencing was used as the gold standard for
determination of mec gene status. Clinical S. aureus isolates were collected as part of routine care and processed at the Cambridge Microbiology and Public Health Laboratory between 2006 and 2012. The isolates included in this study comprised MRSA screening and clinical isolates; 455 were MRSA (mecA positive) and 379 were methicillin-susceptible S. aureus, MSSA (mecA/mecC negative). We also included 62 mecC positive MRSA isolates, five of which were collected in Cambridge and 57 which were originally described by Garcia-Alvarez et al. [8].

We found that of the 455 mecA MRSA isolates, 98.0% were resistant to both oxacillin and cefoxitin (R/R), 1.1% were resistant to oxacillin but susceptible to cefoxitin (R/S), and 0.9% were susceptible to oxacillin but resistant to cefoxitin (S/R) (Table 1). None of the mecA MRSA isolates were susceptible to both oxacillin and cefoxitin. Of the 62 mecC MRSA isolates, 88.7% were susceptible to oxacillin but resistant to cefoxitin (S/R), 11.3% were resistant to both oxacillin and cefoxitin (R/R), and none were susceptible to both antimicrobials. Of the 379 mecA/mecC negative MSSA isolates, 1.1% were resistant to oxacillin but not to cefoxitin (R/S), none were susceptible to oxacillin and resistant to cefoxitin (S/R), and 98.8% were susceptible to both antimicrobials (S/S).

These results generate a sensitivity of 88.7% and a specificity of 99.5% for the identification of mecC MRSA based on the S/R profile in a population of both MRSA and MSSA (Table 2). Furthermore, the specificity and sensitivity of identification of mecA/mecC negative MSSA, on the basis of susceptibility to both oxacillin and cefoxitin (S/S) is 98.9% (4 false positives out of 379 MSSA tested) and 100% (no false negatives) respectively. A recent publication from UK Staphylococcal Reference Laboratory estimated the human mecC MRSA prevalence rate, as a proportion of phenotypic MRSA, to be 0.5% (5/995) [15]. At this prevalence rate, the probability that an oxacillin-susceptible/cefoxitin-resistant profile is a mecC MRSA is 47% (the positive predictive value) and the probability of a non-S/R MRSA not being mecC is 99.9% (the negative predictive value). The low prevalence of mecC would mean that about half the S/R results will be mecA MRSA. If confirmation of mecC status was required, only a relatively small number of isolates would require further testing by a combined mecA/mecC PCR assay. The high negative predictive value would enable the correct identification of the vast majority of mecA MRSA. The perfect specificity of the oxacillin-susceptible/cefoxitin-susceptible profile as a test for MSSA status ensures that no MRSA (mecA or mecC) would be wrongly identified as MSSA. The effect of prevalence rate on the interpretation of tests that do not have perfect sensitivity and specificity highlights the need for data from a formal prevalence survey of mecC MRSA. The atypical S/R profile of mecC MRSA isolates is likely to be explained by the findings of Kim et al. showing that the mecC encoded PBP2a has a higher relative affinity for oxacillin.
as compared to cefoxitin, therefore resulting in higher levels of resistance to cefoxitin than oxacillin (22).

Our findings suggest that in diagnostic laboratories where antimicrobial susceptibility testing is routinely performed using the VITEK 2 system, this method could provide a zero-cost screening method for identification of mecC positive MRSA strains, and could potentially be used to monitor changes in the prevalence of mecC positive MRSA over time. It does, however, require examination of the uncorrected VITEK 2 susceptibility results, since the instrument is programmed to override the raw data and report an oxacillin/cefoxitin S/R profile as R*/R, with an explanatory comment to indicate why this has occurred. This highlights one of the limitations of the ‘expert rules’, which result in automatic amendment of antimicrobial susceptibility data, and the need to educate technologists to examine the uncorrected data to identify possible mecC MRSA for confirmatory testing. Further studies to determine whether our findings can be reproduced using other phenotypic antimicrobial susceptibility methods are in progress.

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Transparency declarations
None to declare.

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### Table 1. Results of VITEK 2 antimicrobial susceptibility testing of Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Identity of S. aureus isolate*</th>
<th>Number</th>
<th>VITEK 2 Antimicrobial Susceptibility Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oxacillin S Cefoxitin R (S/R)</td>
</tr>
<tr>
<td>MRSA mecC positive</td>
<td>62</td>
<td>55/62 (88.7%)</td>
</tr>
<tr>
<td>MRSA mecA positive</td>
<td>455</td>
<td>4/455 (0.9%)</td>
</tr>
<tr>
<td>MSSA mecA and mecC negative</td>
<td>379</td>
<td>0/379 (0%)</td>
</tr>
</tbody>
</table>

*As determined by bacterial whole-genome sequencing

S = susceptible; R = resistant; MRSA = methicillin resistant Staphylococcus aureus; MSSA = methicillin-susceptible Staphylococcus aureus
Table 2. Diagnostic performance of VITEK 2 antimicrobial profiling to identify mecC MRSA

<table>
<thead>
<tr>
<th></th>
<th>Oxacillin S / Cefoxitin R</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>55</td>
<td>N/A</td>
</tr>
<tr>
<td>False negative</td>
<td>7</td>
<td>N/A</td>
</tr>
<tr>
<td>True negative</td>
<td>830</td>
<td>N/A</td>
</tr>
<tr>
<td>False positive</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>Sensitivity*</td>
<td>88.7%</td>
<td>77.5 – 95.0%</td>
</tr>
<tr>
<td>Specificity*</td>
<td>99.5%</td>
<td>98.7 – 99.8%</td>
</tr>
<tr>
<td>Likelihood ratio positive</td>
<td>185</td>
<td>69.3 - 594</td>
</tr>
<tr>
<td>Likelihood ratio negative</td>
<td>0.11</td>
<td>0.06 – 0.23</td>
</tr>
</tbody>
</table>

MRSA = methicillin-resistant Staphylococcus aureus; S = susceptible; R = resistant; 95% CI = 95% confidence interval; N/A = not applicable

*The sensitivity is the proportion of true positives testing positive and the specificity is the proportion of true negatives testing negative. True positives were defined as isolates possessing a mecC gene as determined by bacterial whole-genome sequencing. All other isolates were defined as true negatives.