Evaluation of the Clearview Exact PBP2a, a new immunochromatographic assay, for the detection of low-level methicillin-resistant Staphylococcus aureus (LL-MRSA)

Rapid assay for the detection of PBP2a

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Abstract

We evaluated the performance of a new immunochromatographic assay (ICA), the Clearview Exact PBP2a, for rapid detection of the penicillin-binding protein PBP2a in a challenge set of Staphylococcus aureus strains showing MIC to oxacillin ≤ 16 mg/l.

Sensitivity and specificity of ICA were 96.6% and 100%, respectively.

Detection of methicillin resistance Staphylococcus aureus (MRSA) has always been problematic and has been recently complicated by the emergence of hetero- or low-level methicillin-resistant strains (3,6,8,11). In Belgium, low-level (LL)-MRSA represented 1% of all MRSA isolates collected during the last national surveillance conducted in 2011 (O. Denis, Personal data). Identification of the mecA gene or its product, the penicillin binding protein (PBP) 2a, is considered the gold standard for demonstration of methicillin resistance. Although genotypic testing is high speed, sensitive and specific, it is costly and requires technical and financial resources not available in many laboratories. Rapid and easy to perform agglutination tests that detect the presence of PBP2a have been developed, showing excellent performance compared to phenotypic methods (1,7,9). The aim of this study was to evaluate the performance of a new immunochromatographic assay (ICA), the Clearview Exact PBP2a, for detection of methicillin resistance in LL-MRSA isolates and to compare the results obtained with those of other phenotypic methods, ie [1] disk diffusion with cefoxitin 30 µg (FOX) (NeoSensitabs, Rosco, Taastrup, Denmark), [2] MICs determination by agar dilution, [3] the Vitek 2 system (bioMérieux, Marcy l’Etoile, France) and [4] the MRSA Screen latex agglutination test (LAT) (bioMérieux, Marcy l’Etoile, France).
One hundred and eight *S. aureus* strains were selected from the Belgian MRSA Reference Laboratory, including 58 LL-MRSA (*mecA* positive, oxacillin MIC ranging from 0.06 to 16 mg/l) and 50 methicillin-susceptible *S. aureus* (MSSA) (*mecA* negative, oxacillin MICs ranging from 0.12 to 1 mg/l). Identification and methicillin resistance were confirmed by triplex PCR for 16S rRNA, *mecA* and *nuc* genes (4).

Agar dilution and disk diffusion methods were performed as recommended by the CLSI guidelines (2). The Vitek 2, the ICA and the LAT were used according to the manufacturer’s instructions.

For the LL-MRSA isolates, the results of each technique are presented in Table 1. The sensitivity of the agar dilution method was very low (48.3%; 95% IC, 35.4 to 61.1%) with 30 LL-MRSA isolates showing oxacillin MICs ≤ 2 mg/l which were misclassified as susceptible following CLSI recommendations. In the Vitek 2 system, oxacillin MICs alone displayed the same results as the agar dilution, with only 27 MRSA isolates categorized correctly as resistant. The cefoxitin (FOX) screen included in the Vitek 2 AST card was more sensitive (64.3%; IC 95%, 51.8 to 76.8%), confirming that this antibiotic is a stronger inducer of the *mecA* gene expression than oxacillin, especially in heterogeneous MRSA (3,6,11). Despite combining the oxacillin MICs and FOX screen results, the performance of the Vitek 2 Advanced Expert System (AES), remained surprising low (69.6%; IC 95%, 57.6 to 81.6%). In addition, two MRSA strains displaying the small colony variant (SCV) phenotype did not grow in the AST card. Considering these results, the Vitek 2 system should be further optimized to accurately identify LL-MRSA. A higher sensitivity (84.5%; IC 95%, 75.2 to 93.8%) was obtained with disk diffusion using FOX-30. Only 9 of the 58 LL-MRSA isolates, showing an oxacillin MIC ≤ 0.5 mg/l, were falsely identified as MSSA.

Compared with studies previously published, the performance of disk diffusion was
not as good as expected probably because our evaluation included MRSA strains with lower oxacillin MICs (≤ 0.25 mg/l) (3,6,11). The LAT and the new ICA test performed equally well (sensitivity, 96.6%; IC 95%, 91.9 to 100%) and misclassified only two MRSA isolates with oxacillin MICs below the susceptible breakpoint. After cefoxitin induction, one additional isolate was detected, enhancing the sensitivity of both techniques to 98.3%.

The specificity of all methods was 100% with all methicillin susceptible strains (n=50) correctly identified.

As shown in other studies, classic routine tests often failed to detect LL-MRSA populations MRSA (3,6,11). Rapid latex agglutination tests for the PBP2a have been proved a useful adjunct to phenotypic methods for accurate identification of methicillin resistance in S. aureus.

Recently, Matsui et al. developed an in-house immunochromatographic test highly sensitive, accurate and rapid, showing results consistent with those of the LAT and PCR (5). This technique looked very promising for use in routine tests. The commercial ICA that we evaluated accurately identified methicillin resistance in our S. aureus isolates setting. The technique was rapid (a matter of minutes) and easy to use with no centrifugation or boiling step required.

In conclusions, the cefoxitin disk diffusion was the most reliable technique for classic routine susceptibility testing of oxacillin resistance. The LAT and the ICA were sensitive and specific methods for the detection of PBP2a and represented a reliable alternative to PCR detection of the mecA gene and a useful adjunct to phenotypic methods for LL-MRSA identification. Nevertheless, the evaluation of the ICA was limited to a particular set of isolates in our study. This assay should be further
evaluated in a large-scale study, including not only all classes of methicillin resistance but also genotypically diverse MRSA isolates.

Acknowledgments

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References


Table 1: Performance of different methods for detection of LL-MRSA isolates (n = 58)

<table>
<thead>
<tr>
<th>Method</th>
<th>N° of isolates tested</th>
<th>True positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Oxacillin MICs</td>
<td>58</td>
<td>28, 48.3</td>
<td>30, 51.7</td>
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<tr>
<td>FOX-30</td>
<td>58</td>
<td>49, 84.5</td>
<td>9, 15.5</td>
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<tr>
<td>Vitek 2 Oxacillin MICs</td>
<td>56</td>
<td>29, 51.8</td>
<td>27, 48.2</td>
</tr>
<tr>
<td>FOXScreen</td>
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<td>36, 64.3</td>
<td>20, 35.7</td>
</tr>
<tr>
<td>AESd</td>
<td>56</td>
<td>39, 69.6</td>
<td>17, 30.4</td>
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<tr>
<td>Slidex MRSA</td>
<td>58</td>
<td>56, 96.6</td>
<td>2, 3.4</td>
</tr>
<tr>
<td>Clearview</td>
<td>58</td>
<td>56, 96.6</td>
<td>2, 3.4</td>
</tr>
</tbody>
</table>

*a* True positive: MRSA accurately classified as MRSA

*b* False negative: MRSA misclassified as MSSA

*c* Two isolates showing SCV phenotype not growing in Vitek 2

*d* AES: Advanced Expert System