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Evaluation of Clearview Exact PBP2a, a New Immunochromatographic Assay, for Detection of Low-Level Methicillin-Resistant *Staphylococcus aureus* (LL-MRSA)

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We evaluated the performance of a new immunochromatographic assay (ICA), the Clearview Exact PBP2a, for rapid detection of penicillin-binding protein 2a (PBP2a) in a challenge set of *Staphylococcus aureus* strains showing MICs to oxacillin of ≤16 mg/liter. The sensitivity and specificity of the ICA were 96.6% and 100%, respectively.

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) has always been problematic and has been recently complicated by the emergence of heterogeneous or low-level (LL)-MRSA strains (3, 6, 8, 11). In Belgium, LL-MRSA represented 1% of all MRSA isolates collected during the last national surveillance, conducted in 2011 (O. Denis, unpublished data). Identification of the *mecA* gene or its product, penicillin binding protein 2a (PBP2a), is considered the gold standard for demonstration of methicillin resistance. Although genotypic testing is fast, 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 that of 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 (Inverness Medical Innovations, Scarborough, MA), for detection of methicillin resistance in LL-MRSA isolates and to compare the results with those of other phenotypic methods, i.e., (i) disk diffusion with 30 μg cefoxitin (FOX-30) (NeoSensitabs, Rosco, Taastrup, Denmark), (ii) MIC determination by agar dilution, (iii) the Vitek 2 system (bioMérieux, Marcy l'Etoile, France), and (iv) the MRSA screen latex agglutination test (LAT) (bioMérieux, Marcy l'Etoile, France).

One hundred eight *S. aureus* strains were selected from the Belgian MRSA Reference Laboratory, including 58 LL-MRSA strains (*mecA* positive, with oxacillin MICs ranging from 0.06 to 16 mg/liter) and 50 methicillin-susceptible *S. aureus* (MSSA) strains (*mecA* negative, with oxacillin MICs ranging from 0.12 to 1 mg/liter). Identification and methicillin resistance were confirmed by triplex PCR for 16S rRNA, *mecA*, and *nuc* (4). The 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% inhibitory concentration [IC_{95}], 35.4 to 61.1), with 30 LL-MRSA isolates showing oxacillin MICs of ≤2 mg/liter which were misclassified as susceptible when 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 (antibiotic susceptibility testing) card was more sensitive (64.3%; IC_{95}, 51.8 to 76.8),

TABLE 1 Performance of different methods for detection of LL-MRSA isolates

Assay	No. of isolates tested (*n* = 58)	True positive[a]		False negative[b]	
		No.	%	No.	%
Oxacillin agar dilution	58	28	48.3	30	51.7
Cefoxitin disk diffusion (30 μg)	58	49	84.5	9	15.5
Vitek 2					
Oxacillin MIC	56[c]	29	51.8	27	48.2
FOX screen	56	36	64.3	20	35.7
AES[d]	56	39	69.6	17	30.4
Slidex MRSA LAT	58	56	96.6	2	3.4
Clearview Exact PBP2a ICA	58	56	96.6	2	3.4

[a] True positive, MRSA accurately classified as MRSA.
[b] False negative, MRSA misclassified as MSSA.
[c] Two isolates showing SCV phenotype did not grow in Vitek 2.
[d] AES, Advanced Expert System.

confirming that this antibiotic is a stronger inducer of *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, which showed oxacillin MICs of ≤0.5 mg/liter, were falsely identified as MSSA. When compared with the results of studies previously published, the performance of disk diffusion was not as good as expected, probably because our

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evaluation included MRSA strains with lower oxacillin MICs (≤0.25 mg/liter) (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 fail to detect LL-MRSA populations of MRSA (3, 6, 11). Rapid latex agglutination tests for PBP2a have been proven 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 that is 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* isolate setting. The technique was rapid (a matter of minutes) and easy to use, with no centrifugation or boiling step required.

In conclusion, 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 genotypically diverse MRSA isolates as well.

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