Detection of Penicillin-Binding Protein 2a by Rapid Slide Latex Agglutination Test in Coagulase-Negative Staphylococci

In a recent article, Hussain et al. evaluated a commercial latex agglutination test (LA), the MRSA-Screen (Denka Seiken Co., Niigata, Japan), for rapid detection of penicillin-binding protein 2a (PBP2a) in *mecA*-positive and *mecA*-negative coagulase-negative staphylococci (CNS) (2). The sensitivity and specificity were 100 and 99.5% (2), respectively, although the MRSA-Screen was initially developed for rapid detection of PBP2a in methicillin-resistant *Staphylococcus aureus* (MRSA) (1). However, this was a retrospective study with selected CNS strains. Furthermore, they used oxacillin-induced colonies for the LA since without induction only 72 (57.6%) of 125 *mecA*-positive CNS gave a positive result and additionally required an extended reaction time, i.e., 3 to 15 min, for agglutination (2).

We recently presented a retrospective evaluation of the MRSA-Screen with 60 *mecA*-positive and 60 *mecA*-negative CNS, mainly (72%) *Staphylococcus epidermidis* (R. Zbinden, E. Rundler, V. Kaspar, and B. Berger-Bächi, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. C-234, 1999). In contrast to the results of the study of Hussain et al., all *mecA*-positive CNS showed positive agglutination after 3 min without induction; one isolate, i.e., *Staphylococcus lugdunensis*, revealed a false-positive result. Thereafter, we tested prospectively 80 unselected freshly isolated CNS. They were subsequently verified for the presence of the *mecA* gene by PCR (unpublished data). Thirty-eight *mecA*-positive CNS, of which 34 were *S. epidermidis*, were analyzed by the MRSA-Screen. Thirty-two were clearly positive, 4 were weakly positive, and 2 were negative after 3 min. However, one of the latter became positive after longer reaction time, i.e., 13 min, and the second was positive after induction. Therefore, we prospectively tested 30 freshly isolated CNS by the MRSA-Screen from the blood agar and from the edge of the inhibition zone of the oxacillin disk from the routinely used Mueller-Hinton susceptibility plate (unpublished data). Only 1 of 10 oxacillin disk-resistant CNS showed stronger agglutination from the induced oxacillin inhibition edge. Since an agglutination time of 10 min instead of 3 min was proposed (4), we also observed agglutination after 10 min for the oxacillin disk-susceptible strains. However, one *mecA*-negative CNS out of 20 oxacillin disk-susceptible CNS revealed a false-positive result after 10 min. Even if our results did not reflect the same importance of induction before using MRSA-Screen as observed by Hussain et al., we agree that induction may be helpful in order to avoid false-positive results due to extended reaction time.

The phenotypic detection of methicillin resistance in CNS is

problematic, as Hussain et al. have shown in an earlier report (3). *Staphylococcus cohnii*, *Staphylococcus saprophyticus*, *Staphylococcus warneri*, *Staphylococcus lugdunensis*, and *Staphylococcus xylosus* showed 94.6% false-positive oxacillin resistance according to the new NCCLS breakpoints in the agar dilution test. However, the new NCCLS interpretative guidelines correctly classify the vast majority of clinically significant CNS, i.e., *S. epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis* (3). The authors have now demonstrated that the MRSA-Screen is effective in detecting PBP2a in CNS and is better than the conventional susceptibility tests in classifying *mecA*-negative CNS as oxacillin-susceptible (2). We propose larger prospective studies with consecutive freshly isolated CNS, including phenotypically oxacillin-susceptible, *mecA*-positive CNS to evaluate the MRSA-Screen in the routine laboratory. If the correlation of the MRSA-Screen and *mecA* positivity is over 99% in such prospective studies under routine laboratory conditions, the easy rapid MRSA-Screen might replace the *mecA* PCR for CNS with phenotypically unclear methicillin susceptibility.

REFERENCES

1. Cavassini, M., A. Wenger, K. Jaton, D. S. Blanc, and J. Bille. 1999. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. 37:1591-1594.
2. Hussain, Z., L. Stoakes, S. Garrow, S. Longo, V. Fitzgerald, and R. Lannigan. 2000. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. J. Clin. Microbiol. 38:2051-2054.
3. Hussain, Z., L. Stoakes, V. Massey, D. Diagre, V. Fitzgerald, S. El Sayed, and R. Lannigan. 2000. Correlation of oxacillin MIC with *mecA* gene carriage in coagulase-negative staphylococci. J. Clin. Microbiol. 38:752-754.
4. Marriott, D. J. E., T. Karagiannis, J. L. Harkness, and P. Kearney. 1999. Further evaluation of the MRSA-Screen kit for rapid detection of methicillin resistance. J. Clin. Microbiol. 37:3783-3784.

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