JOURNAL OF CLINICAL MICROBIOLOGY, June 2011, p. 2269–2271 Vol. 49, No. 6
0095-1137/11/$12.00 doi:10.1128/JCM.02060-10

Performance of a New MicroScan WalkAway PC30 Panel and Disk Diffusion Method for Detection of Oxacillin Resistance in *Staphylococcus* spp.▽

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Received 12 October 2010/Returned for modification 17 December 2010/Accepted 21 March 2011

The performance of the MicroScan WalkAway PC30 panel for detection of oxacillin resistance was evaluated by use of a collection of 420 staphylococcus isolates. The addition of a cefoxitin test (4 mg/liter) to the oxacillin MIC determination increased its raw performance for *Staphylococcus aureus*; additional data were required for coagulase-negative staphylococci.

Detection of oxacillin (OXA) resistance (OR) in staphylococci is a daily challenge for clinical laboratories. Erroneous susceptibility results could lead to therapeutic failure or inadequate antimicrobial selection pressure (5, 18). Cefoxitin (FOX) testing is currently recommended and used for this purpose (2). We evaluated the performance of a new MicroScan WalkAway panel (Siemens, Sacramento, CA) that includes this compound and the disk diffusion method (DDM) for the detection of OR.

(This work was presented in part at the 20th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Vienna, Austria, 10 to 13 April 2010.)

A set of 420 nonduplicate isolates of *Staphylococcus aureus* ($n = 370$) and coagulase-negative staphylococci (CNS; $n = 50$) was collected from two nationwide studies through the French College de Bacteriologie-Virologie-Hygiene network, comprising isolates from cases of community-acquired skin and soft tissue infections (203 *S. aureus* isolates in 2006) and clinically significant bacteremia (167 *S. aureus* and 50 CNS isolates in 2007) (9, 10). Identification was performed using (i) specific *gyrB* PCR for *S. aureus*, (ii) mass spectrometry (Axima Assurance; Shimadzu) for CNS, and (iii) 16S RNA sequencing when the identification differed from that obtained at the participating-laboratory level (3, 4, 11). The detection of the *mecA* gene was performed as previously described (10). OR was detected by (i) the FOX DDM according to CLSI guidelines, (ii) the moxalactam (MOX) DDM using the French official criteria (OR is shown by a diameter of <23 mm), and (iii) the MicroScan WalkAway PC30 panel (Siemens, Sacramento, CA) (13, 14). This panel contained oxacillin in doubling dilutions from 0.25 to 2 μg/ml and an additional FOX test (4 μg/ml); each strain was categorized as OR if its oxacillin MIC was greater than 2 μg/ml for *S. aureus* and 0.5 μg/ml for CNS or if the FOX test was positive (i.e., yielded a growth). The panels were run automatically according to the manufacturer's instructions. Quality control strains (oxacillin-susceptible *S. aureus* ATCC 25923 and OR *S. aureus* [ORSA] ATCC 43300) were included in each sample set. All results were within the acceptable range provided by the 2008 CLSI guidelines (2). The contribution of the FOX test was statistically evaluated with the Fisher's exact test. A P value of ≤0.05 was considered to reflect significance. All computations were done with R Project software (http://www.r-project.org).

OR was detected by PCR in 22.7% of *S. aureus* (84/370) and 66% of CNS (33/50) isolates; the latter group included isolates of *S. epidermidis* (34/50), *S. haemolyticus* (8/50), *S. hominis* (5/50), *S. capitis* (2/50), and *S. schleiferi* (1/50). Considering PCR as the gold standard, the sensitivity, specificity, positive and negative predictive values, and numbers of *v*ery *m*ajor *e*rrors (VME; false susceptible) and *m*ajor *e*rrors (ME; false resistant) for the different tests and combinations are reported in Tables 1 (*S. aureus*) and 2 (CNS). For *S. aureus* isolates, the contribution of the FOX test to the PC30 panel performance was statistically nonsignificant ($P = 0.367$). Like 9 other ORSA isolates, the only member of the sequence type 80 (ST80) Panton-Valentine leucocidin-producing clone isolated from skin infections was not identified by the FOX-plus-MOX DDM combination (B. Lamy and J. W. Decousser, unpublished data).

OR is sometimes difficult to detect, especially when the *mecA* gene is heterogeneously expressed (5, 17). Oxacillin susceptibility should be tested in a culture medium containing 2%

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† The members of the College de Bacteriologie Virologie Hygiene Study Group are listed in the acknowledgments.

▽ Published ahead of print on 30 March 2011.

TABLE 1. Comparison of sensitivity, specificity, and positive and negative predictive values, including the 95% confidence intervals, as well as numbers of very major and major errors of the different tests for the 370 *S. aureus* strains[a]

Test and drug(s)	% Sensitivity[b] (95% CI)[f]	% Specificity[c] (95% CI)	% PPV (95% CI)	% NPV (95% CI)	No. of: VMEs[d]	No. of: MEs[e]
Disk diffusion						
FOX	75 (64.4; 83.8)	100 (98.7; 100)	100 (94.5; 100)	93.1 (89.7; 95.7)	21	0
MOX	88.1 (79.2; 94.1)	99.6 (98.1; 99.9)	98.6 (92.8; 99.9)	96.6 (93.8; 98.4)	10	1
MOX + FOX	88.1 (79.2; 94.1)	99.6 (98.1; 99.9)	98.6 (92.8; 99.9)	96.6 (93.8; 98.4)	10	1
PC30 panel						
OXA MIC	95.2 (88.3; 98.7)	100 (98.7; 100)	100 (95.4; 100)	98.6 (96.5; 99.6)	4	0
FOX test	97.6 (91.7; 99.7)	100 (98.7; 100)	100 (95.6; 100)	99.3 (97.5; 99.9)	2	0
OXA MIC + FOX test	98.8 (93.5; 99.9)	100 (98.7; 100)	100 (98.7; 100)	99.6 (98.1; 99.9)	1	0

[a] The different tests for the 370 *S. aureus* strains included cefoxitin (FOX) and moxalactam (MOX) alone or in combination in the disk diffusion method and the determination of oxacillin MIC (OXA MIC) and the FOX test alone or in combination in the PC30 panel. PPV, positive predictive value; NPV, negative predictive value.
[b] Percentage of the 84 *mecA*-positive isolates for which positive test results were obtained.
[c] Percentage of the 286 *mecA*-negative isolates for which negative test results were obtained.
[d] False susceptibility.
[e] False resistance.
[f] 95% exact binomial confidence intervals are shown.

NaCl or incubated at 30°C; this last solution is not adapted to automated antimicrobial-susceptibility testing systems. In 2002, Felten et al. demonstrated that FOX testing using DDM was the best-performing test for routine detection of OR in *S. aureus* (7). Additional reports confirmed the contribution of the FOX DDM, which was included in the French and U.S. guidelines (1, 2, 13, 22). The breakpoint varied according to the country; in France, the lower breakpoint for the FOX DDM was 24 mm, while it was 21 mm for the CLSI (2, 13). Additionally, an intermediate category of strains exhibiting a zone diameter between 25 and 26 mm was established, requiring a confirmatory test as *mecA* or PBP2a detection (13). These discrepancies may explain the gap between the sensitivity results in the study of Felten et al. and in this present work (100% versus 75%) (7). In our study, 14 ORSA isolates exhibited a zone diameter between 22 and 24 mm and 6 isolates a zone diameter between 25 and 26 mm. Concerning the CNS, the lack of performance of the FOX DDM was debated early on and has recently been reported as species dependent (8, 12). Therefore, performance reports should be based on accurate species identification and conclusions limited to the tested species. MOX has been shown to have a higher accuracy than FOX for the detection of OR in CNS (13, 19). Indeed, the combination of MOX with FOX increased the performance of DDM to detect OR in CNS compared to the use of FOX alone (100% sensitivity versus 90.9%) (Table 2).

Recently, a MIC value of 4 μg/ml was established for FOX to detect the presence of the *mecA* gene (21). In the study of Swenson et al., only 0.3% (1/312) of *mec*A-positive *S. aureus* isolates showed a FOX MIC inferior to this breakpoint (21). This variability in the level of the expressed resistance was previously reported for methicillin and attributed to a heterogeneous expression of low-affinity penicillin-binding protein (5). This breakpoint was added to the standard guidelines in the United States and Europe and allowed its inclusion in the panels or cards of automated systems (2, 6). As intended, their performances were significantly improved (15, 20, 23). Until recently, FOX was lacking from the Microscan panel, and misclassification of ORSA strains as susceptible was reported (16). The putative contribution of a FOX test was confirmed in

TABLE 2. Comparison of sensitivity, specificity, and positive predictive value and negative predictive value, including the 95% confidence intervals, as well as numbers of very major and major errors of the different tests for the 50 CNS strains[a]

Method and drug(s)	% Sensitivity[b] (95% CI)[f]	% Specificity[c] (95% CI)	% PPV (95% CI)	% NPV (95% CI)	No. of: VMEs[d]	No. of: MEs[e]
Disk diffusion						
FOX	90.9 (71.8; 96.6)	100 (80.5; 100)	100 (88.1; 100)	85 (58.1; 94.6)	3	0
MOX	100 (89.4; 100)	100 (80.5; 100)	100 (88.1; 100)	100 (80.5; 100)	0	0
MOX + FOX	100 (89.4; 100)	100 (80.5; 100)	100 (88.1; 100)	100 (80.5; 100)	0	0
PC30 panel						
OXA MIC	100 (89.4; 100)	94.1 (71.3; 99.8)	97.1 (84.7; 99.9)	100 (79.4; 100)	0	1
FOX test	90.9 (75.7; 98.1)	94.1 (71.3; 99.8)	96.8 (83.3; 99.9)	84.2 (60.4; 96.6)	3	1
OXA MIC + FOX test	100 (89.4; 100)	88.2 (63.5; 98.5)	94.2 (80.2; 99.3)	100 (78.2; 100)	0	2

[a] The different tests for the 50 CNS strains included cefoxitin (FOX) and moxalactam (MOX) alone or in combination in the disk diffusion method and the determination of oxacillin MIC (OXA MIC) and the FOX test alone or in combination in the PC30 panel. PPV, positive predictive value; NPV, negative predictive value.
[b] Percentage of the 33 *mecA*-positive isolates for which positive test results were obtained.
[c] Percentage of the 17 *mecA*-negative isolates for which negative test results were obtained.
[d] False susceptibility.
[e] False resistance.
[f] 95% exact binomial confidence intervals are shown.

our study: the number of VME decreased (4 to 1), and there was a trend of increasing sensitivity (98.8% versus 95.2%) for the combination compared to the sensitivity obtained with the OXA MIC alone, even though statistical significance was not reached ($P = 0.367$). Interestingly, the only representative strain of the ST80 ORSA clone was detected by the FOX test, although it was miscategorized by the FOX DDM, as previously described (16). The ability of the FOX test to detect this kind of low-level OR should be confirmed in a larger study. Concerning the CNS strains, the contribution of the FOX tests (DDM and panel PC30) was poor, as previously reported (21). However, the number of strains was insufficient to draw any conclusion, the limited power rendering irrelevant the meaning of the not-significant P value. Nevertheless, according to the confidence intervals of the calculated performances, a putative negative impact of this test could not be excluded. These preliminary results should be strengthened by a more powerful study that includes the species associated with the lower levels of performance of OR detection in the study of John et al. (i.e., *S. simulans*, *S. cohnii*, and *S. saprophyticus*) (12). To date, the published levels of performance of FOX MIC determination in CNS agree with our findings (12, 19).

In conclusion, the new PC30 panel is an accurate automated test for OR detection in clinically relevant *S. aureus* strains but additional data are required for CNS.

The ColBVH study group participants are C. Alba Sauviat, C. Auvray, C. Bartizel, I. Baudinat, E. Benabid, F. Bessis, H. Biessy, M. Bietrix, S. Bland, C. Bouquigny, B. Cartier, A. Cecille, G. Chambreuil, C. Chaplain, H. Chardon, N. Chioukh, H. De Montclos, I. Verdier, J. M. Delarbre, A. Gravet, D. Deligne, M. C. Demachy, V. Doat, B. Dubourdieu, S. Dubourdieu, C. Durand, Z. El Harrif-Heraud, F. Evreux, N. Fonsale, S. Gabriel, M. Gavignet, F. Geffroy, G. Grise, P. Guiet, J. Heurte, E. Heusse, J. L. Jacob, E. Jaouen, G. Khatib, J. P. Lafargue, R. Lamarcia, E. Laurens, A. Le Coustumier, E. Lecaillon, C. Lemble, S. Leotard, A. Mandjee, A. Marmonier, R. Martin, M. Menouar, O. Menouni-Foray, A. Michel, D. Neri, X. Palette, B. Pangon, C. Payen, D. Pierrejean, J. Pollet, H. Porcheret, M. Posson, A. Raoult, C. Rigal, R. Sanchez, Y. Scat, A. Secher, D. Simeon, J. P. Thellier, B. Tourrans, A. Vachee, M. Vasseur, A. Verhaeghe, M. Villemain, and L. Villeneuve.

The PC30 panels were kindly provided by Siemens.

This study was supported in part by a grant from Siemens.

J. W. Decousser has received speaker fees from Siemens.

REFERENCES

1. **Broekema, N. M., T. T. Van, T. A. Monson, S. A. Marshall, and D. M. Warshauer.** 2009. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mecA*-mediated resistance in *Staphylococcus aureus* in a large-scale study. J. Clin. Microbiol. **47:**217–219.
2. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing; 18th informational supplement. CLSI M100-S17. CLSI, Wayne, PA.
3. **De Buyser, M. L., A. Morvan, F. Grimont, and N. El Solh.** 1989. Characterization of staphylococcus species by ribosomal RNA gene restriction patterns. J. Gen. Microbiol. **135:**989–999.
4. **Decousser, J. W., C. Bartizel, M. Zamni, N. Fadel, and F. Doucet-Populaire.** 2007. *Clostridium symbiosum* as a cause of bloodstream infection in an immunocompetent patient. Anaerobe **13:**166–169.
5. **de Lencastre, H., A. M. Sa Figueiredo, C. Urban, J. Rahal, and A. Tomasz.** 1991. Multiple mechanisms of methicillin resistance and improved methods for detection in clinical isolates of *Staphylococcus aureus.* Antimicrob. Agents Chemother. **35:**632–639.
6. **European Committee on Antimicrobial Susceptibility Testing.** 27 April 2010, accession date. Breakpoint tables for interpretation of MICs and zone diameters. Version 1.3, January 5, 2011. European Committee on Antimicrobial Susceptibility Testing, Basel, Switzerland. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_breakpoints_v1.3_pdf.pdf.
7. **Felten, A., B. Grandy, P. H. Lagrange, and I. Casin.** 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J. Clin. Microbiol. **40:**2766–2771.
8. **Frigatto, E. A. M., A. M. O. Machado, A. C. G. Pignatari, and C. Gales.** 2005. Is the cefoxitin disk test reliable enough to detect oxacillin resistance in coagulase-negative Staphylococci? J. Clin. Microbiol. **43:**2028–2029.
9. **Gallon, O., et al.** 2009. In vitro activity of daptomycin against staphylococci isolated from bacteraemia and community-onset skin and soft tissues infection in France: data from two nationwide studies. Eur. J. Clin. Microbiol. Infect. Dis. **28:**1209–1215.
10. **Gallon, O., et al.** 2010. Antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated in 2007 from French patients with bloodstream infections: goodbye hVISA, welcome Geraldine? J. Antimicrob. Chemother. **65:**1297–1316.
11. **Gravet, A., et al.** 2010. The use in routine of mass spectrometry in a hospital microbiology laboratory. Pathol. Biol. **59:**19–25. (In French.)
12. **John, A. J., et al.** 2009. Comparison of three phenotypic techniques for detection of methicillin resistance in *Staphylococcus* spp. reveals a species-dependent performance. J. Antimicrob. Chemother. **63:**493–496.
13. **Join-Lambert, O. F., et al.** 2007. Comparison of cefoxitin and moxalactam 30μg disc diffusion methods for detection of methicillin resistance in coagulase-negative staphylococci. J. Antimicrob. Chemother. **59:**763–766.
14. **Jorgensen, J. H., and M. J. Ferraro.** 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis. **49:**1749–1755.
15. **Junkins, A. D., et al.** 2009. BD Phoenix and Vitek 2 detection of *mecA*-mediated resistance in *Staphylococcus aureus* with cefoxitin. J. Clin. Microbiol. **47:**2879–2882.
16. **Kaase, M., B. Barras, S. Friedrich, F. Szabados, and S. G. Gatermann.** 2009. Performance of Microscan WalkAway and Vitek 2 for detection of oxacillin resistance in a set of methicillin-resistant *Staphylococcus aureus* isolates with diverse genetic backgrounds. J. Clin. Microbiol. **47:**2623–2625.
17. **Kloos, W. E., and T. L. Bannerman.** 1999. *Staphylococcus* and *Micrococcus*, p. 264–282. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, DC.
18. **Lode, H. M.** 2009. Clinical impact of antibiotic-resistant Gram-positive pathogens. Clin. Microbiol. Infect. **15:**212–217.
19. **Pupin, H., et al.** 2007. Evaluation of moxalactam with the BD Phoenix system for detection of methicillin resistance in coagulase-negative staphylococci. J. Clin. Microbiol. **45:**2005–2008.
20. **Roisin, S., C. Nonhoff, O. Denis, and M. J. Stuelens.** 2008. Evaluation of new Vitek 2 card and diffusion method for determining susceptibility of *Staphylococcus aureus* to oxacillin. J. Clin. Microbiol. **46:**2525–2528.
21. **Swenson, J. M., et al.** 2009. Correlation of cefoxitin MICs with the presence of *mecA* in *Staphylococcus* spp. J. Clin. Microbiol. **47:**1902–1905.
22. **Swenson, J. M., F. C. Tenover, and the Cefoxitin Disk Study Group.** 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J. Clin. Microbiol. **43:**3818–3823.
23. **Torres, E., S. Perez, R. Villanueva, and G. Bou.** 2008. Evaluation of the Vitek AST-P559 card for detection of oxacillin resistance in *Staphylococcus aureus.* J. Clin. Microbiol. **46:**4114–4115.