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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[▽]

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Of 104 genotypically diverse methicillin-resistant *Staphylococcus aureus* (MRSA) isolates tested with the MicroScan WalkAway (Pos MIC 24 panel) and Vitek 2 (AST-P549 card) systems, 7 and 6 isolates, respectively, showed an oxacillin MIC of ≤2mg/liter. Most of these MRSA isolates were community acquired. However, if the cefoxitin screen of AST-P549 was also considered, MRSA detection failed for only one isolate.

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased over the last years. Reliable detection of MRSA is important since a false report of a patient's isolate as methicillin susceptible would result in inadequate therapy with probably fatal consequences (2). Whereas MRSA infections formerly occurred almost exclusively in hospitalized patients, community-acquired MRSA (cMRSA) isolates have been reported recently in patients without any previous contact with the health care system (7).

Many laboratories rely on automatic susceptibility testing methods that use oxacillin MIC testing, oxacillin breakpoint detection in the presence of salt, or cefoxitin MIC testing as markers for the presence of methicillin resistance. Many studies have investigated the detection of MRSA by the Vitek 2 system (3, 4, 8, 11, 12, 13, 15, 17); however, data for the performance of the MicroScan WalkAway system in MRSA detection are scarce (17).

Most studies evaluating the performance of Vitek 2 used consecutive clinical strains (3, 8, 11, 12, 15), but this approach may be biased by the overrepresentation of locally predominant clones and may not predict performance in other geographical areas. We therefore used a collection of MRSA strains with distinct pulsed-field gel electrophoresis (PFGE) patterns to study MRSA detection using the MicroScan WalkAway and Vitek 2 systems.

From 1998 to 2006, noncopy MRSA isolates (n = 1,516), initially identified by oxacillin screening agar or Vitek, from four hospitals in the Bochum area were collected and typed by PFGE as described previously (5). Of these, 120 isolates with different PFGE patterns were chosen. The patterns were interpreted according to the criteria of Tenover et al. (18), and isolates grouped into PFGE types and subtypes.

For susceptibility tests, isolates from frozen storage were subcultured twice on Columbia blood agar at 37°C in 5% CO_2 before being tested with the Vitek 2 system using the AST-P549 card and the MicroScan WalkAway system using the Pos MIC 24 panel.

Whenever results for oxacillin in the Vitek 2 or MicroScan WalkAway system or for the cefoxitin screen in the Vitek 2 system were not indicative of MRSA, a *mecA* PCR was performed from colonies growing on purity control plates of both automatic systems and a *S. aureus*-specific PCR for SA442 (16) was used as an internal positive control. In addition, the Panton-Valentine leukocidin (PVL)-coding genes *lukS-PVL* and *lukF-PVL* were detected by PCR (9). SCC*mec* typing (10) and *spa* typing (6) were performed as described previously.

Loss of *mecA* during storage of isolates could be demonstrated in 16 of 120 isolates by PCR (14), a proportion that is similar to that described before (19). Of the remaining 104 true MRSA isolates, 95 were detected as MRSA with both automatic systems.

An oxacillin MIC of ≤2 mg/liter was measured for six isolates with the Vitek 2 test and for seven isolates with the WalkAway test (Table 1); thus, those isolates would not have been detected as MRSA based on oxacillin MICs alone. Microdilution performed according to CLSI methods (1) showed resistant oxacillin MICs for all but one of these isolates, whereas by Etest on Mueller-Hinton agar with 2% NaCl, oxacillin MICs of ≥4 mg/liter were found for only two isolates. Microcolonies were the only indication for MRSA in most of the remaining strains, demonstrating the challenge of detecting MRSA in those isolates. The cefoxitin screen incorporated in the Vitek 2 AST-P549 card was positive for five of six isolates not detected by oxacillin MIC. Thus, cefoxitin testing together with oxacillin MIC testing clearly leads to better MRSA detection. Cefoxitin MICs of ≥16 mg/liter and ≥4 mg/liter were also found by microdilution and Etest.

Intentionally, no proportions of failed MRSA detection in relation to all MRSA isolates tested are given in this study because such figures would be misleading, since in our collection, rarely occurring strains are overrepresented. The nine isolates with problematic MRSA detection with either the Vitek 2 or WalkAway system were from PFGE subgroups that represent only 1.2% of our MRSA strain collection. MRSA

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TABLE 1. All test results for MRSA isolates with negative cefoxitin screen in Vitek 2 or oxacillin MIC of ≤2 mg/liter in Vitek 2 or MicroScan WalkAway assay[a]

Isolate	Cefoxitin screen in Vitek 2	MIC (mg/liter) of:						PFGE type	*spa* type	PVL	SCC*mec* type	Resistance phenotype(s)
		Oxacillin in Vitek 2	Oxacillin in WalkAway	Oxacillin by Etest	Oxacillin by microdilution	Cefoxitin by Etest	Cefoxitin by microdilution					
877	+	2	>2	6*	>16	8*	>16	16-3	t044	−	IV	CIP
1459	+	2	2	4*	16	6*	16	27-1	t044	+	IV	ERY, TET, FA
1662	+	>2	2	1.5*	16	4*	>16	27-0	t040	+	IV	TET, FA
1883	+	2	2	1*	>16	8*	>16	46-1	t105	+	nt	CIP, GEN
2336	−	2	2	1*	>16	8*	>16	13-13	t4861	−	nt	ERY, SXT, TET, CIP, FA, SXT
2449	+	2	2	0.5	16	8	>16	27-5	t044	+	IV	ERY, TET, FA
2582	+	1	0.5	0.125*	0.5	6*	16	44-12	t4860	−	IV	CIP
2748	−	>2	>2	1.5*	16	4	16	13-4	t008	−	IV	ERY, CIP, FOF
2757	+	>2	2	0.5*	8	8*	16	53-0	t355	+	V	GEN, ERY

[a] *, microcolonies visible; nt, not typeable; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline; FA, fusidic acid; GEN, gentamicin; SXT, sulfamethoxazol-trimethoprim; FOF, fosfomycin.

detection was not problematic for most of the PFGE groups studied, especially not for PFGE group 35, which appeared in 2001 and accounted for 56.5% of all MRSA isolates in 2006.

In previous studies using older Vitek cards without a cefoxitin screen, 27 of 27 (11), 195 of 197 (12), 18 of 18 (3), 61 of 61 (8), and 202 of 203 (15) MRSA isolates were detected by Vitek 2. The better performance in those studies compared to our work is explainable since consecutive isolates were used in those, whereas in our study, high genetic diversity was secured and rarely occurring PFGE types were intentionally overrepresented. When challenge strains were used in previous studies, MRSA detection failed in 5 of 85 (4) and 7 of 79 (17) isolates. The latter study (17) is the only one examining MRSA detection by the MicroScan WalkAway system, and it found misclassification as methicillin susceptible for 9 of 79 MRSA challenge strains. In a recent study using the Vitek 2 system with the AST-P549 card, MRSA detection failed for 4 of 157 MRSA strains (13).

In four of the nine isolates with problematic MRSA detection results, a combination of PVL and SCC*mec* type IV or V was found, typical for cMRSA. Two additional isolates had *spa* types t044 and t008, which are associated with cMRSA of clonal lineages ST80 and ST8. Low-level oxacillin resistance in cMRSA isolates of clonal lineage ST80 has been reported previously by Witte et al. (20).

The emergence of cMRSA requires reliable detection of methicillin (meticillin) resistance in this pathogen and argues for an additional cefoxitin screen in automatic susceptibility testing.

Since all cMRSA isolates in our study showed an increased oxacillin MIC of ≥2 mg/liter with both automatic systems, an expert rule could also be programmed to recommend additional testing for isolates with a MIC of ≥2 mg/liter. As we did not test methicillin-susceptible isolates in our study, we do not know if such an expert rule would result in an unacceptably high number of unnecessary additional tests.

In conclusion, the majority of MRSA isolates from our area can be detected by oxacillin MIC determination either with the AST-P549 card in the Vitek 2 system or with the Pos MIC 24 panel in the WalkAway MicroScan system. However, some cMRSA isolates can be missed by using an oxacillin MIC threshold of ≥4 mg/liter alone.

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