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Rapid Recognition of Methicillin-Resistant *Staphylococcus aureus* by Use of Automated Test Systems

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The ability to rapidly recognize methicillin-resistant *Staphylococcus aureus* by use of two automated instrument systems, the MS-2 system (Abbott Laboratories, Diagnostics Division, Irving, Tex.) and the AutoMicrobic system (Vitek Systems, Hazelwood, Mo.), was evaluated on a collection of 95 methicillin-resistant *S. aureus* isolates recovered from at least six geographical areas of the United States. Isolates were simultaneously tested with both systems, and the results were compared with MIC tests performed by the National Committee for Clinical Laboratory Standards agar dilution method. Methicillin-resistant *S. aureus* isolates were defined as those with a methicillin MIC ≥ 8 μg/ml by the reference procedure. Overall, with the AutoMicrobic system, 94.7% of 95 methicillin-resistant *S. aureus* isolates were detected, and with the MS-2 system, 91.6% of the isolates were detected. Isolates with methicillin MICs ≥ 32 μg/ml were readily detected with both systems (41 of 42 isolates). Of 53 isolates from three locales with methicillin MICs of 8 or 16 μg/ml, 90.6% (48) were detected by the AutoMicrobic system, whereas 86.8% (46) were detected by the MS-2 system. A program update which has been added to the MS-2 system prints a warning message indicating possible methicillin-resistant *S. aureus* with isolates which demonstrate multiple antibiotic resistance (greater than or equal to four drugs other than methicillin). This warning message would have provided presumptive recognition of six of eight isolates with discrepant results for methicillin by the MS-2 system.

Methicillin-resistant (MR) *Staphylococcus aureus* has become a rapidly emerging clinical and epidemiological problem in U.S. hospitals in recent years (4, 10, 18, 23). Although MR *S. aureus* has been considered primarily a problem of large, tertiary-care hospitals (1, 4, 10, 12), infections due to such strains have also been encountered in smaller, primary-care hospitals (10) and in community-acquired infections (13, 19). Difficulties in recognizing MR *S. aureus* by in vitro test methods have been previously described (2, 3, 8, 11, 24). In particular, certain rapid instrument test systems have been reported to be unreliable for detecting MR *S. aureus* isolates (2, 5–7). The purpose of this study was to evaluate the current version of one of the instruments, the MS-2 system (Abbott Laboratories, Diagnostics Division, Irving, Tex.), in parallel with a second instrument, the AutoMicrobic system (Vitek Systems, Hazelwood, Mo.), which has not been extensively studied with MR *S. aureus* strains.

MATERIALS AND METHODS

Test isolates. A group of MR *S. aureus* isolates recovered from patients in our teaching hospitals in San Antonio, Tex., and from at least six other geographical areas of the United States, including Atlanta, Ga.; Charlottesville, Va.; Houston, Tex.; Rochester, Minn.; Salt Lake City, Utah; and St. Louis, Mo., was employed in this study. Several additional strains were also obtained from Abbott Diagnostics Division, Irving, Tex., which had in turn been collected by them from a number of sites in the United States. Each isolate was identified and characterized as MR *S. aureus* in the individual laboratories before this study. Testing of methicillin-susceptible (MS) *S. aureus* isolates from our laboratories was also incorporated into these studies.

Reference susceptibility methods. All isolates used in this study were initially tested by the reference agar dilution method, as described in the National Committee for Clinical Laboratory Standards (NCCLS) standard PSM-7 (16). Test conditions included use of unsupplemented Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.), inoculation with a multipoint replicator, and incubation at 35°C for 16 to 20 h. MICs to methicillin and oxacillin were determined for all strains. Methicillin resistance in this study was considered as an MIC ≥ 8 μg/ml, and oxacillin resistance was considered as an MIC ≥ 4 μg/ml (16). All isolates which appeared to be resistant to either methicillin or oxacillin, but not to both drugs, were subsequently retested with Mueller-Hinton agar supplemented with 2% NaCl (25). Five of these strains were also tested exactly as suggested by Thornsberry and McDougal (25), using divalent cation–2% NaCl-supplemented Mueller-Hinton broth (Difco) in microdilution trays, with incubation at 35°C for 24 h.

MS-2. A standard MS-2 clinical instrument, in which computer program version 03.02 was incorporated, was used in this study. MS-2 susceptibility tests were performed exactly as directed by the manufacturer. In the MS-2, a 5-μg methicillin elution disk is utilized in the test cuvette, and methicillin resistance is considered as an MIC > 5 μg/ml.

AMS. A standard AMS instrument (model 120) with program version p11.ROF, was used for this study. Susceptibility tests were performed exactly as recommended by the manufacturer with a single lot of AMS gram-positive susceptibility cards. In the AMS, a single test well containing 2 μg of oxacillin per ml is employed, and oxacillin resistance is considered as an MIC > 2 μg/ml.

RESULTS

The results of the NCCLS reference agar dilution susceptibility tests of 136 isolates of *S. aureus* are shown in Table 1.

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TABLE 1. Results of methicillin and oxacillin reference agar dilution MICs on 136 isolates of *S. aureus*

Tests yielding the following susceptibility categories:	No. of isolates (%)	Category of *S. aureus*
Agreement		
Methicillin MIC ≥ 8 μg/ml Oxacillin MIC ≥ 4 μg/ml	95 (69.8%)	MR
Methicillin MIC ≤ 4 μg/ml Oxacillin MIC ≤ 2 μg/ml	27 (19.8%)	MS
Overall agreement	122/136 (89.7%)	
Disagreement[b]		
Methicillin MIC ≥ 8 μg/ml Oxacillin MIC ≤ 2 μg/ml	11 (8.1%)	MR
Methicillin MIC ≤ 4 μg/ml Oxacillin MIC ≥ 4 μg/ml	3 (2.2%)	OR[a]
Overall disagreement	14/136 (10.3%)	

[a] Oxacillin-resistant.
[b] Retesting of these isolates with NaCl-supplemented Mueller-Hinton agar provided agreement; see text for details.

TABLE 2. Reference agar dilution MICs of 95 MR *S. aureus* used in comparative tests with the MS-2 and AMS systems

Methicillin MIC (μg/ml)[a]	No. of isolates	Oxacillin MIC (μg/ml)[b]	No. of isolates
1	0	1	1
2	0	2	7
4	0	4	10
8	12	8	12
16	41	16	7
32	17	32	14
64	9	64	24
>64	16	>64	20

[a] Median methicillin MIC, 16 μg/ml.
[b] Median oxacillin MIC, 32 μg/ml.

Categorization of isolates as either susceptible or resistant to the two semisynthetic penicillins was uniformly achieved with 89.7% of the 136 isolates, i.e., the majority of test strains were either susceptible or resistant to both methicillin and oxacillin. However, with 14 of the isolates (10.3%), there was a susceptibility category difference between the two drugs which was based on the MIC criteria described above. Most of these isolates (11 of 14) appeared to be resistant to methicillin (MIC ≥ 8 μg/ml) but susceptible to oxacillin (MIC ≤ 2 μg/ml), whereas three isolates appeared to be resistant to oxacillin but susceptible to methicillin. Retesting of these 14 isolates with 2% NaCl-supplemented Mueller-Hinton agar or broth provided full agreement, with all 14 strains demonstrating resistance to both methicillin and oxacillin (median methicillin MIC, 32 μg/ml; median oxacillin MIC, 32 μg/ml).

A group of 95 MR *S. aureus* and 25 MS *S. aureus* isolates from six of the geographical areas (Charlottesville, Houston, Irving, Rochester, Salt Lake City, and San Antonio) were selected for simultaneous parallel testing in the MS-2 and AMS systems. The agar dilution MICs of the 95 MR *S. aureus* isolates to both methicillin and oxacillin are defined in Table 2. These isolates were selected because they represented various references MICs to the two antibiotics and because they were derived from different geographical areas of the United States.

Rapid recognition of MR *S. aureus* was achieved with either system for the majority of isolates (Table 3). The overall rate of resistance detection was somewhat higher with the AMS (94.7%) than the MS-2 (91.6%). However, the program feature recently (1982) added to the MS-2, which prints a warning message indicating the possibility of MR *S. aureus* based on multiple antibiotic resistance (but apparent methicillin susceptibility), would have presumptively signaled the presence of an additional six MR *S. aureus* isolates, which would have elevated the overall recognition to 97.9%. Both instruments were highly accurate when testing strains with marked resistance to methicillin, i.e., methicillin MICs ≥ 32 μg/ml by the NCCLS reference agar dilution test. Lower detection rates were achieved with both instruments when strains of marginal methicillin resistance (methicillin MIC, 8 or 16 μg/ml) were tested. Three of the isolates with marginal methicillin resistance (from Houston) were retested with the MS-2 in replicates on 7 different days. The MS-2 reported them as being resistant in 54% of 50 tests. Only 1 of 25 MS *S. aureus* isolates was reported as being resistant to methicillin by the MS-2, and only one isolate (a different one) was reported to be falsely resistant to oxacillin by the AMS.

DISCUSSION

Infections due to MR *S. aureus* have recently become a serious problem in many areas of the United States (4, 10, 14). It is essential both for appropriate selection of antimicrobial agents for therapy and for hospital infection control that such strains be quickly and accurately recognized. Several factors have complicated detection of MR *S. aureus*, including lack of a universally accepted definition of the level of resistance which defines MR *S. aureus*. The MIC correlate cited for definition of methicillin resistance by Food and Drug Administration disk diffusion testing is a methicillin MIC > 3 μg/ml or an oxacillin MIC > 0.6 μg/ml (9). It is suggested in the more recent NCCLS disk susceptibility document M2-A2S2 (15) that the MIC correlates which define resistance should be 16 μg/ml or greater for methicillin and 8 μg/ml or greater for oxacillin. Similar differences exist between the older proposed NCCLS standards for interpretation of MIC test results. The NCCLS standard PSM-7 (16) defined a methicillin MIC > 4 μg/ml or an oxacillin MIC > 2 μg/ml as "very resistant." Most recently, the MIC levels for "resistance" have been raised for methicillin to an MIC > 8 μg/ml and for oxacillin to an MIC > 2 μg/ml in the NCCLS dilution test standard M7-T (17).

The changing MIC criteria for the definition of MR *S. aureus* have caused confusion among microbiologists and have made it difficult for manufacturers of automated instru-

TABLE 3. Comparison of MS-2 and AMS results on 95 MR *S. aureus* isolates

Test system	% *S. aureus* isolates detected by the following criteria (no. detected/total no. tested):		
	All MR *S. aureus* isolates	Methicillin MICs ≥32 μg/ml	Methicillin MICs 8 or 16 μg/ml
MS-2[a]	91.6 (87/95)	97.6 (41/42)	86.8 (46/53)
AMS	94.7 (90/95)	100.0 (42/42)	90.6 (48/53)

[a] Results do not include isolates detected only by warning message (see text for details).

ments or susceptibility test devices to conform to the most current recommendations. Perhaps even more problematic than the definition of MR *S. aureus* have been the technical difficulties involved in recognizing these strains. The term "heteroresistance" has been used to describe the fact that both susceptible and resistant subpopulations are present in such strains (20, 22, 25). In vitro test methods must be able to recognize the slower-growing, resistant component of the culture in the presence of the more rapidly growing, susceptible population that is also present (22). Certain automated instruments, namely the MS-2 and the Autobac I (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.) have been reported to have considerable difficulty in testing MR *S. aureus* (2, 5–7), whereas the AMS recently has been shown to detect 96% of a group of oxacillin- and methicillin-resistant staphylococci (21).

In the present study, the definition of MR *S. aureus* was that described in the NCCLS standard PSM-7 (16), since this study was initiated before the publication of the more recent standard M7-T (17) and both instruments were programmed to define resistance by the previous guidelines. Based on these criteria, both instruments performed well in this study (>90% accuracy), especially if high-level methicillin resistance (agar dilution MIC $\geq$ 32 μg/ml) was characteristic of the strain tested. Strains with lower level resistance (methicillin MICs, 8 or 16 μg/ml) were less often recognized by the instruments, although they were successfully detected >85% of the time by both instruments. It should be noted that use of the more recent definition of MR *S. aureus* (17) would cause the strains with a methicillin MIC of 8 μg/ml to be classified as methicillin susceptible.

The results obtained in our study are significantly more favorable than those described previously for the MS-2 (2, 5, 6) and only slightly less impressive than recently reported for the AMS (21). A possible explanation for the improved results reported here for the MS-2 is that Abbott Laboratories made a change in the analysis program used with staphylococcal susceptibility tests in June 1982 (program version 03.01 and later 03.02). This change eliminated the intermediate results category for methicillin (MICs which would correlate to 5 to 15 μg/ml) and added a message of "CAUTION: MULTIPLE RESISTANCE. POSSIBLE METH/OXA RESISTANCE." This warning message now occurs if a staphylococcus appears to be susceptible to methicillin but is resistant to penicillin plus any three of the following: cefoxitin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, tetracycline. The principle that MR *S. aureus* are often multiply resistant has been well established in previous studies (12, 14). In our study, this program revision was utilized, whereas earlier studies, in which lower rates of detection of MR *S. aureus* isolates have been reported (2, 5, 6) preceded this software change, i.e., an earlier program version was utilized. Use of the newer MS-2 program allowed us to detect >90% of 95 MR *S. aureus* isolates tested based on a resistant methicillin test result, and it would have enabled presumptive recognition of six of the other remaining isolates by use of the caution message indicating multiple resistance. The test strains used in this study included isolates kindly provided by the authors of two of the previous reports on the MS-2 (5, 6).

It is not clear whether the results reported recently by Stotler and Meyer (21) for the AMS were obtained with the same programming available to us for our study (program revision p11.ROF). However, our findings confirm the impression that the AMS performs very well in recognizing the presence of MR *S. aureus* of high or low resistance levels. In addition, the AMS was found by Stotler and Meyer (21) to also reliably detect methicillin resistance among coagulase-negative staphylococci.

Initial results of reference agar dilution susceptibility tests suggested that approximately 10% of our isolates were resistant to one of the two penicillins, but not both. However, retesting of these isolates with NaCl-supplemented Mueller-Hinton broth or agar in a manner similar to the recent recommendations of Thornsberry and McDougal (25) demonstrated resistance to both drugs by all 14 isolates. It is possible that supplementation of the test media used in the MS-2 and AMS instruments with divalent cations and NaCl might further enhance detection of MR *S. aureus*. Such a maneuver might also improve cephalothin susceptibility results on MR *S. aureus* isolates, as suggested by Thornsberry and McDougal (25). In the present study, 92 of 95 MR *S. aureus* isolates were reported with the MS-2 as being either susceptible or intermediate to cephalothin, whereas 50 of 95 strains were reported with the AMS as being either very susceptible or moderately susceptible to cephalothin.

Neither of these instruments may presently be ideal for use in hospitals which harbor endemic MR *S. aureus* strains of marginal resistance. In such a setting, it is likely that repeated testing of the same strain occurs in the form of separate patient isolates. When we tested three MR *S. aureus* isolates from Houston which showed marginal resistance by the standard agar dilution test (methicillin MIC, 8 and 16 μg/ml), we achieved a rate of detection with the MS-2 of only 54%. This level of performance is similar to that reported by Carlson et al. (6) with these same isolates. Thus, hospitals known to have isolates of marginal resistance might benefit from an alternate susceptibility test procedure, such as that described by Thornsberry and McDougal (25).

In conclusion, both the MS-2 and AMS systems, in which current computer program versions are used, performed reliably in the detection of MR *S. aureus* challenge strains from several geographical areas of the United States. The level of accuracy achieved by both instruments is commendable in light of the technical intricacies involved in detection of methicillin-heteroresistant strains and the lack of agreement on the exact definition of MR *S. aureus*. The ability to reliably detect MR *S. aureus* in a 4- to 6-h test period could significantly facilitate patient care and help to prevent further transmission of such strains within a hospital.

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