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Reliability of the MS-2 System in Detecting Methicillin-Resistant *Staphylococcus aureus*

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The MS-2 system (Abbott Diagnostics, Division of Abbott Laboratories, Dallas, Tex.) is an automated system capable of rapid antimicrobial susceptibility testing. However, the short incubation periods used by the device may adversely affect its ability to detect slowly growing resistant organisms. Shortly after the introduction of the MS-2 system into the University of Mississippi Medical Center clinical microbiology laboratory, we noted discrepancies between the MS-2 and the disk diffusion susceptibility reports when methicillin-resistant *Staphylococcus aureus* isolates were tested. Subsequently, we determined the susceptibilities of 75 such isolates by the MS-2 and Kirby-Bauer disk diffusion methods and measured the minimum inhibitory concentrations of methicillin, oxacillin, and cephalothin for 33 of the 75 isolates by standardized agar dilution techniques. There was only 47% overall agreement between the MS-2 and disk diffusion methods when methicillin was tested and 15% agreement when cephalothin was the test drug. There was 93% or more overall agreement between the two methods when other antimicrobial agents were tested. The minimum inhibitory concentration of methicillin was ≥16 μg/ml for all 33 isolates evaluated by the agar dilution method. A comparison of the MS-2 and agar dilution results revealed an overall agreement of 49% when the susceptibilities to methicillin were determined. The MS-2 system reported that multiple methicillin-resistant *S. aureus* isolates obtained from a single patient were either resistant, intermediate, or sensitive to methicillin. Inconsistent results were also obtained when a single isolate was tested simultaneously in 10 cuvette cartridges. We conclude that the MS-2 system does not reliably detect methicillin and cephalothin resistance among *S. aureus*.

Automated systems that rapidly identify bacteria and determine susceptibilities to antimicrobial agents represent a major technical advance in clinical microbiology. However, such automated devices do have some drawbacks. For instance, certain drug-organism combinations yield spurious susceptibility results when tested in automated systems (1, 12, 14). For example, in 1978 Cleary and Maurer (8) reported that the Autobac 1 system frequently gave incorrect results when the susceptibilities of methicillin-resistant *Staphylococcus aureus* (MRSA) to methicillin and clindamycin were tested. Recently, Barnes et al. (1) and Thornsberry et al. (14) evaluated the MS-2 system, but they did not comment on the ability of the device to detect methicillin resistance among strains of *S. aureus*.

After the introduction of the MS-2 system into the microbiology laboratory of the University Hospital at the University of Mississippi Medical Center in October 1980, we frequently observed discrepancies between the antimicrobial susceptibility results obtained when MRSA were tested by the MS-2 and disk diffusion methods. Therefore, we conducted a study to determine the frequency and type of discrepancies that occurred when the antimicrobial susceptibility of MRSA was determined by the MS-2, disk diffusion, and agar dilution methods.

MATERIALS AND METHODS

Isolates. Multiply resistant strains of *S. aureus* (i.e., resistant to penicillin, ampicillin, gentamicin, erythromycin, and clindamycin) were recovered since January 1978 from patients at the University of Mississippi Medical Center. All multiply resistant *S. aureus* isolated since July 1979 that were tested by disk diffusion methods performed at 30°C were MRSA. The results of antimicrobial susceptibility tests performed on 75 MRSA isolates recovered from 75 persons since October 1980 were available for analysis.

Susceptibility testing methods. MS-2 antimicrobial susceptibility tests were performed on all 75 isolates within 1 or 2 days of recovery from clinical specimens. The MS-2 tests were performed at 35°C according to recommendations of the manufacturer.

Disk diffusion tests were performed on all 75 isolates within 7 days after the MS-2 results were available. Disk diffusion tests were done at 30°C by standardized

techniques (4). Each isolate was then suspended in brain heart infusion broth (Difco Laboratories) and stored at −70°C.

Subsequently, 33 representative isolates were selected, and the minimum inhibitory concentrations (MICs) of methicillin, oxacillin, and cephalothin for the isolates were measured by an agar dilution method.

Before agar dilution tests were performed, the frozen organisms were thawed, streaked onto blood agar and DNase test agar plates, and incubated at 36°C for 18 to 24 h. Agar dilution tests were performed at 35°C, by the method described in the *Manual of Clinical Microbiology* (15), with methicillin sodium standard powder (lot D 0691) and oxacillin sodium standard powder (lot M 9540) provided by Bristol Laboratories and cephalothin sodium standard powder (lot SI-698-9F) from Eli Lilly Research Laboratories. Inoculated plates were read at 24 and 48 h. A quality control strain (*S. aureus* ATCC 25923) of known susceptibility was included in tests performed by all three methods.

Some patients had MRSA isolated from several sites (e.g., nose, sputum, and wound) on multiple occasions while they were hospitalized. The MS-2 results of 14 MRSA isolated from one such patient were compared. These tests were performed on different days during a 20-day period.

Also, a single isolate from the patient mentioned above was inoculated into 10 MS-2 cuvette cartridges and simultaneously tested for sensitivities to 10 antimicrobial agents.

RESULTS

All 75 isolates were coagulase positive, and all 33 isolates grown on DNase test agar gave positive reactions. Disk diffusion tests indicated that all 75 isolates were resistant to methicillin, oxacillin, and nafcillin and 63 (84%) were resistant to cephalothin. All isolates grew to the edges of the methicillin, oxacillin, and nafcillin disks. A light growth of small colonies within a larger zone of inhibition was noted around cephalothin disks at 24 h and was even more apparent at 48 h. The small colonies usually grew to the edge of the cephalothin disk although a few isolates had zone diameters of ≤14 mm. Table 1 compares the results of the MS-2 and disk diffusion methods for the 75 isolates. There was 93% or more overall agreement between the MS-2 and disk diffusion methods when chloramphenicol, clindamycin, erythromycin, gentamicin, and tetracycline were tested. There was only 46.7% overall agreement when methicillin was tested; 21.3% of the isolates were reported as sensitive and 32% as intermediately sensitive to methicillin by the MS-2 system. When cephalothin was the test drug, there was only 14.7% overall agreement between the two methods.

Each of the 33 isolates tested by the agar dilution method had a methicillin MIC of ≥16 μg/ml at 24 and 48 h. A comparison of the results of the agar dilution and MS-2 methods revealed an overall agreement of 49%, with 15% very major and 36% minor discrepancies when methicillin was tested. When cephalothin was the test drug, there was only 6.1% overall agreement, with 81.8% very major and 12.1% minor discrepancies.

The 48-h agar dilution MICs of methicillin, oxacillin, and cephalothin for the isolates reported by the MS-2 system as being resistant, intermediate, and sensitive to methicillin are shown in Fig. 1. Each of the three groups of isolates had a median methicillin MIC of 64 μg/ml and a median oxacillin MIC of 128 μg/ml. The median cephalothin MIC was 64 μg/ml for the isolates reported as being resistant and 32 μg/ml for isolates reported as being intermediate or sensitive to methicillin. For 27 (82%) of the isolates, the 24-h methicillin MICs were within 1 $\log_2$ dilution of the 48-h reading. In six instances, the 24-h methicillin MICs were 2 to 3 $\log_2$ dilutions (four and two isolates, respectively) lower than the 48-h readings. The ATCC control strain always had methicillin, oxacillin, and cephalothin MICs of <2 μg/ml at 24 and at 48 h.

A comparison of the results of the disk diffusion and agar dilution methods revealed 100% agreement between the two techniques when methicillin or oxacillin was tested. With cephalothin, there was 85% overall agreement, with 6% very major and 9% minor discrepancies

TABLE 1. Comparison of the MS-2 and disk diffusion results for 75 isolates

Antimicrobial agent	Agreement (%)	Discrepancies (%)[a]		
		Very major	Major	Minor
Cephalothin	14.7	81.3	0.0	4.0
Chloramphenicol	97.3	1.3	1.3	0.0
Clindamycin	98.7	0.0	1.3	0.0
Erythromycin	100.0	0.0	0.0	0.0
Gentamicin	96.0	1.3	2.7	0.0
Methicillin	46.7	21.3	0.0	32.0
Tetracycline	93.3	4.0	1.3	1.3

[a] Very major, susceptible by the automated method and resistant by disk diffusion; major, resistant by the automated method and sensitive by disk diffusion; minor, intermediate by either the automated or disk diffusion method.

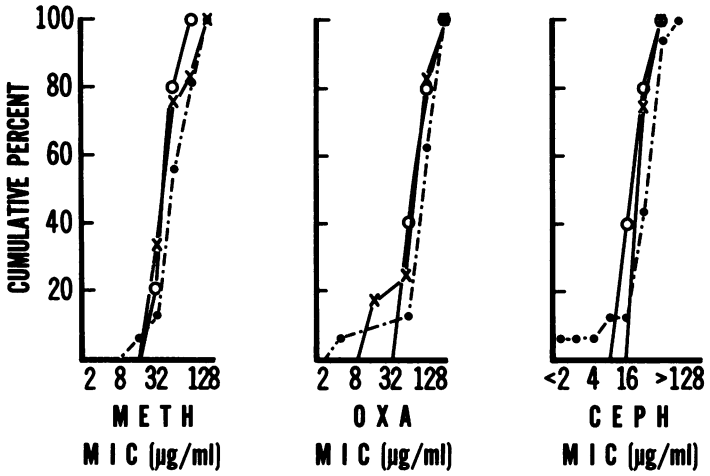

FIG. 1. Agar dilution MIC of methicillin (meth), oxacillin (oxa), and cephalothin (ceph) for MRSA reported as being resistant (16 isolates), intermediate (12 isolates), and sensitive (5 isolates) to methicillin by the MS-2 system. Symbols: ●, resistant; ×, intermediate; ○, sensitive.

between the disk diffusion and agar dilution methods.

The results of the MS-2 susceptibility tests performed on multiple isolates from a single patient are shown in Table 2. All 14 isolates recovered from different sites were multiply resistant *S. aureus* (i.e., resistant to penicillin, kanamycin, gentamicin, erythromycin, clindamycin, and ampicillin). However, these 14 isolates were reported by the MS-2 system as being

TABLE 2. MS-2 results on multiple MRSA isolates from a single patient

Source and date[a]	Antibiotic susceptibility to[b]:									
	T	P	M	Ka	G	E	CL	CH	CE	A
Blood culture										
2/9/81	S[c]	R	S	R	R	R	R	S	S	R
2/10/81	S	R	I	R	R	R	R	S	S	R
Sputum										
2/1/81[d]	R	R	I	R	R	R	R	S	S	R
2/6/81	S	R	R	R	R	R	R	R	S	R
2/9/81	S	R	R	R	R	R	R	R	S	R
2/14/81	R	R	S	R	R	R	R	I	S	R
Urine										
2/4/81[d]	S	R	S	R	R	R	R	S	S	R
2/9/81	S	R	S	R	R	R	I	S	S	R
2/10/81	S	R	R	R	R	R	R	S	S	R
2/10/81	S	R	S	R	R	R	R	S	I	R
Nose										
2/5/81	S	R	R	R	R	R	R	R	S	R
2/18/81	R	R	R	R	R	R	R	R	S	R
2/19/81	S	R	R	R	R	R	R	R	S	R
2/24/81	S	R	R	R	R	R	R	R	I	R

[a] All dates refer to 1981.

[b] T, Tetracycline; P, penicillin; M, methicillin; Ka, kanamycin; G, gentamicin; E, erythromycin; CL, clindamycin; CH, chloramphenicol; CE, cephalothin; A, ampicillin.

[c] S, Sensitive; I, intermediate; R, resistant.

[d] Confirmed as MRSA by disk diffusion methods.

TABLE 3. Results of 10 MS-2 susceptibility tests performed simultaneously on a single MRSA isolate

Cuvette cartridge	Antibiotic susceptibility to[a]:									
	T	P	M	K	G	E	CL	CH	CE	A
1	S	R	R	R	R	R	R	S	S	R
2	S	R	I	R	R	R	R	S	S	R
3	S	R	I	R	R	R	R	S	S	R
4	S	R	R	R	R	R	R	S	S	R
5	S	R	R	R	R	R	R	S	S	R
6	S	R	I	R	R	R	R	S	S	R
7	S	R	S	R	R	R	R	S	S	R
8	S	R	I	R	R	R	R	S	S	R
9	S	R	I	R	R	R	R	S	S	R
10	S	R	R	R	R	R	R	S	S	R

[a] See Table 2 footnotes.

either resistant, intermediate, or sensitive to methicillin. Even isolates recovered from the same source on the same day (e.g., two urine cultures obtained on February 10) were reported as having differing susceptibilities to methicillin. Disk diffusion susceptibility tests performed on 2 of the 14 isolates confirmed that they were resistant to methicillin.

Table 3 shows the results of 10 MS-2 susceptibility tests performed simultaneously on a single MRSA isolate recovered from one patient (e.g., 10 cuvette cartridges were inoculated with the same organism). This single isolate was reported as being resistant (four cartridges), intermediate (five cartridges), and sensitive (one cartridge) to methicillin.

DISCUSSION

The MS-2 system is a computerized, automated device designed to determine the antimicrobial susceptibility of aerobic, nonfastidious bacteria. Susceptibilities are established by comparing the growth curve of an organism incubated in the presence of an antimicrobial agent with the growth curve of the same organism incubated in broth without antimicrobial agents. The MS-2 and similar systems such as the Autobac 1 use incubation times that are much shorter than those of more established methods. Specifically, the MS-2 system usually incubates *S. aureus* isolates for approximately 4 h, whereas disk diffusion and agar dilution methods require 18- to 24-h incubation times (12). As a result, such automated systems may not detect resistant organisms that grow slowly.

For this reason, it is important to note that most strains of MRSA are heteroresistant to methicillin; i.e., only a fraction of the daughter cells derived from a single colony are phenotypically resistant to methicillin, whereas the majority are sensitive to methicillin. Since the daughter cells that comprise the resistant subpopulation grow slowly, Thornsberry et al. (14) predicted that automated systems might not detect MRSA reliably.

The inability of automated systems to identify MRSA would be of little consequence if such isolates were seldom recovered from clinical material. Although a few outbreaks of nosocomial MRSA infections were reported between 1967 and 1970, several studies conducted before 1973 suggested that MRSA is uncommon in this country (2, 5, 7). However, by September 1980, outbreaks of nosocomial MRSA had been reported from hospitals in 15 cities in the United States (6). Furthermore, two recent surveys (16; J. M. Boyce and W. Causey, Abstr. Assoc. Pract. Infect. Control, abstr. no. 14, 1981) show that university and community teaching hospitals in many more cities have isolated MRSA from one or more patients in recent years. In addition, numerous smaller community and municipal hospitals located near the affected teaching hospitals have recently had experience with MRSA, presumably because of intramural transmission of the agent. As these organisms become more and more prevalent, the use of antimicrobial susceptibility tests that reliably detect MRSA will become increasingly important.

Proposed techniques for improved detection of heteroresistant strains of *S. aureus* include: (i) incubation of susceptibility tests at 30 to 35°C; (ii) use of media containing 5% NaCl; (iii) increased inoculum size; and (iv) incubation of tests for 48 h (3, 8, 9). Several investigators have shown that disk diffusion tests performed at 30 or 35°C (9, 13) reliably detect MRSA.

Semiautomated broth microdilution methods performed in standard medium (Mueller-Hinton broth without added NaCl) at 35°C may not be reliable if read at 24 h but can accurately identify MRSA after a 48-h incubation period (3). Jones et al. (10) recently evaluated a newly developed microdilution susceptibility test system that uses a special medium formulation and incubation of

tests for 15 to 18 h at 35°C and found that several challenge strains of MRSA were appropriately categorized as methicillin resistant.

As mentioned above, automated systems that use very short incubation periods may fail to detect highly methicillin-resistant subpopulations of bacterial cells that grow slowly. For example, Cleary and Maurer (8) showed that 79% of 57 MRSA isolates were recorded as sensitive or intermediately sensitive to methicillin when tested by the Autobac 1 system, which uses a 3-h incubation period. The use of broth containing 5% NaCl and a reduction of incubation temperature from 36 to 32°C did not affect the results. Prolonged (5 h) incubation of tests improved the Autobac 1 results somewhat, but there was still only 44% overall agreement between the Autobac 1 and disk diffusion methods.

When we tested fresh clinical isolates of MRSA with the MS-2 system, 53.3% of the isolates were reported as intermediate or sensitive to methicillin (Table 1). Since all 75 isolates incubated at 30°C grew to the edges of the methicillin and oxacillin disks and all 33 isolates tested by the agar dilution method had a methicillin MIC of ≥16 μg/ml, we have no doubt that these multiply resistant staphylococci are methicillin resistant.

The median agar dilution MIC of methicillin was identical for groups of isolates reported by the MS-2 system as resistant, intermediate, or sensitive to methicillin. This finding suggests that the variable MS-2 results cannot be explained on the basis of interstrain differences in the levels of methicillin resistance. When several MRSA isolates that presumably represented a single strain were tested (all were from a single patient), variable methicillin susceptibility results were also reported (Table 2). The MS-2 system even reported inconsistent results when a single isolate was tested. That is, when the same isolate was tested simultaneously in 10 cartridges, some channels reported that the strain was resistant whereas others reported that it was intermediate or sensitive to methicillin (Table 3).

The MS-2 system reported that 96% of isolates were sensitive to cephalothin; however, only 12 (16%) were intermediate or susceptible to cephalothin by disk diffusion tests performed at 30°C. Although the incubation temperature used in this study may be responsible for the low proportion of isolates that appear susceptible to cephalothin by the disk diffusion method, it is also possible that this finding is peculiar to the organisms from the University Hospital at the University of Mississippi and that strains from other institutions may be susceptible to cephalothin if they are tested at 30°C. In a majority of the studies in which MRSA isolates have appeared susceptible to cephalothin by disk diffusion techniques, an incubation temperature of 35 or 37°C was used. However, Peacock et al. (11) recently reported that only 28% of the MRSA isolates they tested at 35°C were sensitive to cephalothin by the disk diffusion method, whereas 32% were sensitive by agar dilution tests. Interestingly, Yourassowsky et al. (17) recently showed that *S. aureus* that is resistant to methicillin (and relatively resistant to cephalosporins) could not be differentiated clearly from fully sensitive strains of *S. aureus* when exposed to various cephalosporin drugs in an MS-2 system. The authors concluded that the early (4-h) cephalosporin susceptibility results obtained when MRSA isolates were tested in automated devices must be interpreted with caution.

We conclude that the MS-2 system does not reliably detect strains of *S. aureus* resistant to methicillin and cephalothin. Strains of *Staphylococcus epidermidis* were not included in our study, and further studies are needed to determine whether a similar problem exists when methicillin-resistant strains of *S. epidermidis* are tested by the MS-2 method. Strains of multiply resistant *S. aureus* that appear sensitive to methicillin when tested in the MS-2 system should also be tested by a method that reliably detects methicillin-resistant staphylococci, e.g., standard disk diffusion susceptibility tests performed at 30 or 35°C. In hospitals where confirmed MRSA have been identified and where the MS-2 system is the only method routinely used to determine antimicrobial susceptibilities, clinical microbiologists should inform clinicians that methicillin and cephalothin sensitivity reports may not be valid when MRSA is tested.

Although it is not the purpose of this paper to discuss therapy, it should be understood that cephalothin and other β-lactam antibiotics are not considered appropriate therapy for MRSA infections, regardless of the results of susceptibility tests.

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