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Interlaboratory Variation of Antibiograms of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Strains with Conventional and Commercial Testing Systems

KENNETH E. ALDRIDGE,[1,2]* AILLEEN JANNEY,[2] CHARLES V. SANDERS,[1] AND ROBERT L. MARIER[1]

Departments of Medicine[1] and Pathology,[2] Louisiana State University Medical Center, New Orleans, Louisiana 70112

Received 15 April 1983/Accepted 22 August 1983

Laboratory-prepared (conventional) and commercial susceptibility testing systems were compared by using a group of methicillin-resistant (MR) and methicillin-susceptible (MS) strains of *Staphylococcus aureus*. A group of 25 MR and 15 MS *S. aureus* strains were coded and tested blindly by disk diffusion, agar dilution, broth microdilution, Sensititre, Micro-Media, Sceptor, API 3600S, MicroScan, Autobac I, and MS-2 systems. All systems were incubated at 35°C and read with either a manual or automated reader at the recommended times. Where applicable, systems were also read at 48 h. Among the conventional assays, the broth and agar dilution methods were comparable, both detecting 88% of the MR strains at 24 h and detecting 92 and 96%, respectively, at 48 h. The disk diffusion method was less efficient, detecting only 36 and 72% at 24 and 48 h, respectively. Detection of cephalothin resistance was low for all systems at both time periods, with agar dilution and disk diffusion being the most and least efficient, respectively. Some variability was also seen with detection of resistance to clindamycin and gentamicin. Among the MS strains, variability among the conventional systems occurred with methicillin, gentamicin, ampicillin, and penicillin. Comparison of the commercial systems with manual readers with the broth microdilution method (reference method) showed that for MR strains, the Sceptor system gave identical results at 24 and 48 h. Sensititre detected 68 and 88% of the MR strains, whereas Micro-Media was least effective detecting 12 and 80% at 24 and 48 h, respectively. None of the commercial systems detected cephalothin resistance well, with only one strain being indicated by the Sceptor and Sensititre systems at 48 h. Slight differences were also seen among the systems with clindamycin and gentamicin. With regard to the MS strains, variability among the systems was seen with methicillin, penicillin, ampicillin, clindamycin, and gentamicin. Among commercial systems with automated readers, the API system detected a greater number of MR strains than did the reference method at 24 and 48 h, 96 and 100%, respectively. The MicroScan method was comparable to the reference method detecting 80 and 88% of the MR strains at both time periods, respectively. Both Autobac I and MS-2 were much less effective in detecting MR strains, noting only 32 and 16%, respectively, at the 3- to 6-h readings. Poor detection of cephalothin resistance among MR strains was evident in all systems. Variability also occurred among the systems with clindamycin, gentamicin, and ampicillin. A single strain of the MR group was reported to be vancomycin resistant by the API system. Among the MS group, the greatest variability was seen with methicillin. Less variability occurred with penicillin, ampicillin, gentamicin, and vancomycin.

The introduction of penicillin as antistaphylococcal therapy was highly successful until resistance due to β-lactamases was encountered. Subsequently, the β-lactamase-resistant, semisynthetic penicillin methicillin was introduced for therapy against the penicillinase-producing strains. In 1961, only 2 years after the introduction of this compound, Jevons reported the emergence of methicillin-resistant (MR) *Staphylococcus aureus* (20). Later reports throughout Europe indicated outbreaks of infection due to MR *S. aureus* (9, 11, 21, 23, 32, 39)

with reports of greater than 30% of all nosocomial blood isolates exhibiting methicillin resistance (19, 24). In the United States, the first report of an MR *S. aureus* outbreak was from the Boston City Hospital in 1968 (2), with other outbreaks later indicated in other locations (12, 25, 33, 35).

Even today, MR *S. aureus* is somewhat difficult to recognize in the laboratory. Often it is identified only retrospectively, after treatment failure. Not only are MR *S. aureus* isolates resistant to a variety of antibiotics, but they have a number of biochemical characteristics that differ from their methicillin-susceptible (MS) counterparts (17, 36). Within a given MR *S. aureus* population, the percentage of cells that are resistant to methicillin can be extremely small, thus requiring a longer incubation for detection. The expression of this heterogeneity is influenced by temperature, with higher temperatures suppressing methicillin resistance. Extending the incubation time and increasing the osmolality of the test medium also increase detection of MR *S. aureus*.

Although the growth characteristics of MR *S. aureus* have been defined, no biochemical or serological test can be used to detect MR *S. aureus* without the aid of antimicrobial susceptibility testing. Although disk diffusion and antibiotic dilution methods have been used, no one method has been reported to be consistently reliable. Antibiograms of MR *S. aureus* show variable resistance to penicillins, cephalosporins, macrolides, tetracyclines, and aminoglycosides (31). The purpose of this study was to compare the antibiograms for MR and MS *S. aureus* of both laboratory-prepared reference methods and various commercially available systems.

MATERIALS AND METHODS

Organisms. Forty clinical isolates of *S. aureus* were used in this study. Identification of each strain showed gram-positive cocci in clusters that were both catalase and tube-coagulase positive. Each organism was stored on tryptic soy agar slants at 4°C until testing.

Antibiotics. Laboratory standard powders used in this study were obtained from the following manufacturers: ampicillin and methicillin, Beecham Laboratories, Bristol, Tenn.; cephalothin and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; penicillin G, Pfizer Inc., New York, N.Y.; clindamycin, The Upjohn Co., Kalamazoo, Mich.; gentamicin, Schering Corp., Kenilworth, N.J. All powders were stored at −20°C until use and were incorporated into test media on the day of rehydration.

Characterization of test strains. Although initially screened by the disk diffusion method at 24 h, all organisms used in this study were quantitatively tested for methicillin resistance by the broth microdilution method (30). Three variables were used that have been shown to enhance detection of methicillin resistance: incubation at 30°C, extended incubation at 35°C, and increased inoculum size at 35°C. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was used to prepare serial twofold dilutions of methicillin with a range of 256 to 0.125 μg/ml. Two sets of plates were inoculated with the standard inoculum (10^5 CFU/ml) of each organism and one set of plates was inoculated with a high inoculum (10^8 CFU/ml). One plate with the standard inoculum and the plate with the high inoculum were incubated at 35°C. The second plate with the standard inoculum was incubated at 30°C. The minimal inhibitory concentrations (MICs) of the drug were read at 24 and 48 h for all plates. Organisms against which an MIC of ≥4 μg/ml was found on all three plates were considered MR. Those against which MICs of ≤2 μg/ml were found were considered MS. Each strain was then selected at random and given a code number that was not broken until all test systems were complete. This population of coded organisms, 25 MR and 15 MS *S. aureus*, was submitted blindly to various laboratories and run by the various systems described.

Conventional systems tested. Each coded isolate was tested by the following standard methods: broth microdilution, agar dilution, and disk diffusion. The broth microdilution system was carried out as previously described (30). The agar dilution method was carried out by preparing twofold dilutions (256 to 0.125 μg/ml) of each antibiotic in melted and cooled (55°C) Mueller-Hinton agar (Difco). Each dilution was poured into petri dishes, allowed to solidify, and inoculated by means of a Steers replicator device delivering ca. 10^5 CFU of each isolate to the surface of each plate (13, 38). The disk diffusion assay was performed with each antibiotic by the method of Bauer et al. (5), and zone size interpretation followed recommendations of the National Committee for Clinical Laboratory Standards (29). Oxacillin disks were used for detecting methicillin resistance in the disk diffusion assay. Small-colony variants appearing within the zone of inhibition around the oxacillin disk, were considered indicative of methicillin resistance which occurred in the majority of the cases. All test systems were incubated at 35°C and read at 24 and 48 h.

Commercial systems tested. Each coded strain was tested by the following methods according to the manufacturers' recommendations: Sensititre (Gibco Diagnostics, Lawrence, Mass.); Micro-Media (Micro-Media Systems, Campbell, Calif.); and Sceptor (Johnston Laboratories, Cockeysville, Md.); API 3600S (Analytab Products, Plainview, N.Y.); MicroScan (American MicroScan, Campbell, Calif.); Autobac I (General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.); and MS-2 (Abbott Laboratories, Irving, Tex.). The following clinical laboratories in New Orleans, La. performed interlaboratory testing of the methods: Sensititre and API 3600S, Charity Hospital; Micro-Media, Tulane University Hospital; Sceptor, East Jefferson Hospital; MicroScan, Division of Infectious Diseases, Louisiana State University Medical Center; Autobac I, Touro Infirmary Hospital. MS-2 was tested at the Veterans Administration Hospital, Nashville, Tenn. Where applicable, in addition to the 24-h reading, plates were read again at 48 h. MS-2 and Autobac I were read only once after 3 to 6 h of incubation.

β-Lactamase assay. β-Lactamase was detected with a penicillin G-impregnated disk (Marion Laboratories,

TABLE 1. Comparison of conventional methods testing MR *S. aureus* (n = 25)[a]

Antibiotic	Resistance cutoff[b]	No. of *S. aureus* organisms reported by:											
		Broth microdilution				Agar dilution				Disk diffusion			
		24 h		48 h		24 h		48 h		24 h		48 h	
		S	R	S	R	S	R	S	R	S	R	S	R
Methicillin	4	4	21	2	23	4	21	1	24	16	9	7	18
Penicillin	0.25	0	25	0	25	0	25	0	25	0	25	0	25
Cephalothin	32	23	2	20	5	20	5	11	14	25	0	25	0
Clindamycin	4	2	23	2	23	2	23	2	23	1	24	1	24
Gentamicin	8	17	8	17	8	14	11	12	13	17	8	17	8
Vancomycin	4	25	0	25	0	25	0	25	0	25	0	25	0
Ampicillin	0.25	0	25	0	25	0	25	0	25	0	25	0	25

[a] S, Susceptible; R, resistant.
[b] Concentrations (micrograms per milliliter) were greater than or equal to the values given.

Kansas City, Mo.). A drop of sterile water was added to each dehydrated disk; three to five colonies were picked with an applicator stick and added to the disk. After 1 h at room temperature, each disk was read according to the following criteria: yellow color around the organisms indicated breakdown of penicillin G by β-lactamase; blue color indicated the absence of β-lactamase. Control strains for β-lactamase production were *S. aureus* ATCC 29213 (positive) and *S. aureus* ATCC 25923 (negative).

Reference method and cut off concentrations. Conventional broth microdilution was chosen as the reference method, as it has been shown to correlate well with the results of the collaborative study of the World Health Organization (13). Where applicable, resistance to each of the antibiotics was interpreted by using the following concentrations (micrograms per milliliter): methicillin, ≥4; penicillin, ≥0.25; cephalothin, ≥32; clindamycin, ≥4; gentamicin, 8; vancomycin, ≥4; and ampicillin, ≥0.25.

RESULTS

Comparison of conventional methods. Table 1 shows the results of the conventional systems used in the study. At 24 h, both the broth and agar dilutions were equal in detecting MR *S. aureus* (84% detected); the disk diffusion method showed only 36%. At 48 h, the agar dilution and broth microdilution each detected over 90% MR *S. aureus* (96 and 92%, respectively); the agar diffusion was the least effective, detecting 72%.

Cephalothin resistance was detected in 20, 8, and 0% of the MR *S. aureus* by the agar dilution, broth microdilution, and disk diffusion methods, respectively, at 24 h. At 48 h, the agar dilution method detected 56%, whereas the broth microdilution detected 20% resistance to cephalothin. At 48 h, disk diffusion failed to detect any cephalothin resistance.

All three conventional methods were in complete agreement, showing all the MR *S. aureus* strains to be resistant to penicillin and ampicillin and susceptible to vancomycin. The broth microdilution and agar dilution indicated 92% of strains resistant to clindamycin at 24 and 48 h, whereas the disk diffusion showed 96% resistance at both times. Detection of gentamicin resistance showed good agreement between broth microdilution and disk diffusion (32% resistance detected at 24 and 48 h). The agar dilution showed 44 and 52% resistance at 24 and 48 h, respectively.

Table 2 compares results with the three conventional methods against the 15 strains of MS

TABLE 2. Comparison of conventional methods testing MS *S. aureus* (n = 15)[a]

Antibiotic	Resistance cutoff[b]	No. of *S. aureus* organisms reported by:											
		Broth microdilution				Agar dilution				Disk diffusion			
		24 h		48 h		24 h		48 h		24 h		48 h	
		S	R	S	R	S	R	S	R	S	R	S	R
Methicillin	4	12	3	12	3	14	1	12	3	15	0	15	0
Penicillin	0.25	3	12	3	12	2	13	2	13	2	13	2	13
Cephalothin	32	15	0	15	0	15	0	15	0	15	0	15	0
Clindamycin	4	15	0	15	0	15	0	15	0	15	0	15	0
Gentamicin	8	15	0	13	2	15	0	15	0	15	0	15	0
Vancomycin	4	15	0	15	0	15	0	15	0	15	0	15	0
Ampicillin	0.25	3	12	3	12	1	14	1	14	2	13	2	13

[a] S, Susceptible; R, resistant.
[b] Concentrations (micrograms per milliliter) were greater than or equal to the values given.

TABLE 3. Comparison of commercial systems with the reference method in the detection of MR *S. aureus* isolates (n = 25)[a]

Antibiotic	Resistance cutoff[b]	No. of MR *S. aureus* organisms detected by:													
		Broth microdilution		Sensititre		Micro-Media		Sceptor		API 3600S		MicroScan		Autobac I	MS-2
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	6 h	6 h
Methicillin	4	21	23	17	22	3	20	21	23	24	25	20	22	8	4
Penicillin	0.25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Cephalothin	32	2	5	0	1	0	0	0	1	0	3	0	6	0	2
Clindamycin	4	23	23	23	23	24	24	24	24	24	24	23	23	2	23
Gentamicin	8	8	8	7	8	8	8	8	8	8	8	6	6	8	7
Vancomycin	4	0	0	0	0	0	0	—[c]	—	1	1	0	0	0	—
Ampicillin	0.25	25	25	25	25	25	25	25	25	—	—	25	25	24	24

[a] Results are expressed as the number of isolates against which MICs indicate resistance at 24 and 48 h. Autobac I and MS-2 readings were made at 3 to 6 h.

[b] Concentrations (micrograms per milliliter) were greater than or equal to the values given.

[c] Dashes indicate that a particular antibiotic was not tested by a system.

S. aureus. Variability of the antibiograms was greater among the MS *S. aureus* than among the MR *S. aureus* when methicillin, penicillin, and ampicillin were compared. At both 24 and 48 h, 20% of the MS *S. aureus* were identified as MR *S. aureus* by the broth microdilution method, whereas the agar dilution technique indicated 7% at 24 h, and 20% at 48 h were MR *S. aureus*. At both 24 and 48 h, the disk diffusion method indicated all 15 strains to be MS *S. aureus*.

The broth microdilution method indicated 80% of the MS *S. aureus* strains to be resistant to penicillin at 24 and 48 h. Comparatively, both the agar dilution and the disk diffusion methods showed 87% to be resistant at both time periods. The broth microdilution method showed 80% of the MS *S. aureus* to be resistant to ampicillin at 24 and 48 h; the agar dilution method showed resistance in 87% of the strains at both time periods. No resistance was detected to cephalothin, clindamycin, or vancomycin by any of the methods. Against gentamicin, all methods showed each strain to be susceptible, except at 48 h, when two strains were resistant by the broth microdilution method.

Comparison of systems read manually. Tables 3 and 4 show the results of the various commercial systems when testing the 25 strains of MR *S. aureus*. In regard to methicillin resistance, the Sceptor system results were identical to the broth microdilution results; both detected 84 and 92% of MR *S. aureus* at 24 and 48 h, respectively. Sensititre detected 68 and 88% at 24 and 48 h, respectively, whereas Micro-Media detected 12 and 80% at the same time periods. At 24 h, none of the commercial systems detected any resistance to cephalothin, compared with 8% for the broth microdilution method. At 48 h, both the Sceptor and Sensititre systems detected one strain, whereas the Micro-Media system detected none. The Sensititre system and the broth microdilution technique both detected 92% resistance to clindamycin at 24 and 48 h. Both the Sceptor and Micro-Media systems detected

TABLE 4. Comparison of commercial systems with the reference method in the detection of MS *S. aureus* isolates (n = 15)[a]

Antibiotic	Resistance cutoff[b]	No. of MS *S. aureus* organisms detected by:													
		Broth microdilution		Sensititre		Micro-Media		Sceptor		API 3600S		MicroScan		Autobac I	MS-2
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	6 h	6 h
Methicillin	4	3	3	0	1	0	0	1	4	6	9	0	1	0	0
Penicillin	0.25	12	12	13	13	12	12	15	15	13	13	13	13	14	13
Cephalothin	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clindamycin	4	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Gentamicin	8	0	2	1	2	0	0	0	0	0	0	2	2	0	0
Vancomycin	4	0	0	0	0	0	0	—[c]	—	0	1	0	0	0	—
Ampicillin	0.25	12	12	13	14	12	12	11	13	—	—	12	13	13	12

[a] Results are expressed as the number of isolates against which MICs indicate resistance at 24 and 48 h. Autobac I and MS-2 readings were made at 3 to 6 h.

[b] Concentrations (micrograms per milliliter) were greater than or equal to the values given.

[c] Dashes indicate that a particular antibiotic was not tested by a system.

96% at these time periods. Gentamicin resistance was 32% for all systems at 24 and 48 h, except at 24 h, the Sensititre system showed 28% resistance. All systems showed 100% resistance to penicillin and ampicillin, and 100% susceptibility to vancomycin at both 24 and 48 h.

Table 4 indicates the antibiogram results of the MS *S. aureus* with the various systems. Micro-Media was the only system to detect no resistance to methicillin at both time periods. Sensititre showed no resistance at 24 h but detected one strain as resistant at 48 h. Sceptor showed one and four strains to be resistant at 24 and 48 h, respectively. The reference method called three strains resistant at both time periods.

Detection of penicillin resistance was identical (80% resistance at both 24 and 48 h) for Micro-Media and the broth microdilution method. The Sensititre system showed 87% resistance at both time periods, whereas Sceptor reported all strains to be resistant, including the American Type Culture Collection control.

Both the Micro-Media and Sceptor systems showed all the MS *S. aureus* strains to be gentamicin sensitive, whereas the Sensititre system reported one strain at 24 h and two strains at 48 h resistant to the same antibiotic. The reference method indicated no resistance at 24 h and two strains resistant at 48 h.

Both the reference method and the Micro-Media system detected 80% resistance to ampicillin at both time periods. At 24 and 48 h the Sensititre system showed 87 and 93%, respectively; the Sceptor system indicated 73 and 87%, respectively.

All MS *S. aureus* were shown to be susceptible to clindamycin by all systems except the Micro-Media system, which indicated one resistant strain at both time periods. All strains were shown to be susceptible to cephalothin and vancomycin; Sceptor, which does not test vancomycin, was excluded.

Comparison of systems with automated readers. Tables 3 and 4 show the results of the systems with automated readers. The API system was more sensitive than the broth microdilution method at both time periods in detecting methicillin resistance. The API was the only system to detect all 25 strains of MR *S. aureus*. At 24 and 48 h, respectively, the MicroScan system reported 80 and 88% of the MR *S. aureus* strains to be resistant. The least acceptable results were from Autobac I and MS-2, which reported 32 and 16% resistance at a 3- to 6-h reading. API 3600S, MicroScan, and Autobac I showed no strains resistant to cephalothin at the initial reading. At 48 h, the API reported three strains resistant, and the MicroScan reported six strains resistant. MS-2 reported two strains resistant at its reading. Clindamycin results were comparable for all systems except Autobac I, which showed only two resistant strains. Gentamicin results for API 3600S and Autobac I were identical to those of the reference method, whereas MicroScan and MS-2 detected six and seven resistant strains, respectively. All strains were reported to be resistant to penicillin and ampicillin, except a single strain which was resistant to ampicillin by Autobac I and MS-2. Similarly, all strains were susceptible to vancomycin, except a single resistant strain reported by API 3600S.

With regard to methicillin and the MS *S. aureus* strains, resistance was highest for the API system, which showed six and nine strains resistant at 24 and 48 h, respectively. The reference method reported three strains resistant at both time periods, whereas the MicroScan system reported one strain at 48 h. No resistance was detected by the Autobac I and MS-2 systems. The reference method and the MicroScan system both reported two strains resistant to gentamicin, whereas the other systems reported none. Ampicillin and penicillin results were comparable among all systems. A single strain was reported to be vancomycin resistant by the API system, whereas all other methods showed no resistance. No resistance to cephalothin or clindamycin was reported by any of the systems.

Comparative correlation with reference method. Tables 5 and 6 indicate the percent agreement of the results when each system was compared with the broth microdilution method. The greatest variability in results was with methicillin, for testing both the MR and MS *S. aureus*. The agar dilution and Sceptor assays gave the best correlation, producing 92 and 100% agreement at 24 and 48 h, respectively. Sensititre, API 3600S, and MicroScan were all comparable at both time periods. MS-2 and Autobac-1 gave the least correlation of the commercial methods as did the disk diffusion of the conventional methods. Among the MS *S. aureus* strains, no method gave 100% correlation with the reference method. The MS-2 system gave the least correlation (53%), whereas all the other systems were approximately equal in their correlation. In contrast to the MR *S. aureus* strains, only Senstitre showed an increase in correlation at the 48-h reading with the MS *S. aureus* strains. Interestingly, the correlation was lower for the agar dilution, Sceptor, API 3600S, and MicroScan with the extended incubation.

Correlation of cephalothin susceptibility for the MR *S. aureus* was generally better at 24 h than at 48 h. At 24 h, the disk diffusion, Sensititre, Micro-Media, API 3600S, MicroScan, Autobac I, and Sceptor systems agreed 96% of the time. Each system read at 48 h showed a reduc-

TABLE 5. Agreement of each system with the reference broth microdilution method by using MR *S. aureus* strains (n = 25)

System (h of incubation)	% Agreement						
	Methicillin	Penicillin	Cephalothin	Clindamycin	Gentamicin	Vancomycin	Ampicillin
Agar dilution							
24	92	100	80	100	88	100	100
48	100	100	68	100	84	100	100
Disk diffusion							
24	52	100	96	100	100	100	100
48	72	100	80	100	100	100	100
Sensititre							
24	84	100	96	100	100	100	100
48	92	100	80	100	100	100	100
Micro-Media							
24	20	100	96	100	100	100	100
48	68	100	84	100	100	100	100
Sceptor							
24	92	100	96	100	100	—[a]	100
48	100	100	88	100	100	—	100
API 3600S							
24	88	100	96	96	100	96	100
48	88	100	76	96	100	96	100
MicroScan							
24	88	100	96	100	100	100	100
48	88	100	76	100	100	100	100
Autobac I							
3 to 6	52	92	96	16	100	100	96
MS-2							
3 to 6	36	100	88	100	100	—	100

[a] —, Not tested.

tion in correlation. The agar dilution method gave the least correlation at both time periods. No resistance was found among the MS *S. aureus* strains; thus, each system showed 100% correlation.

Among the MR *S. aureus* strains, detection of clindamycin resistance showed extremely poor correlation with the reference method, whereas gentamicin results from the agar dilution method showed less than 90% correlation. Among the MS *S. aureus* strains several of the assays had results for penicillin G and ampicillin that did not correlate. Poor correlation was seen with the reference method and the Autobac I with regard to penicillin G results.

Comparison of additional incubation. Table 7 shows the percent of MR *S. aureus* and MS *S. aureus* isolates that switched from susceptible to resistant after an additional 24 h of incubation. Among the MR *S. aureus* strains, all systems tested detected more methicillin resistance after extended incubation; Micro-Media had the highest increase (68%). Increased detection with disk diffusion was indicated primarily by the appearance of small-colony variants within the zone of inhibition. Resistance to cephalothin was also increasingly detected with additional incubaton; agar dilution and MicroScan systems had the largest increases. The only other increase in detection was with gentamicin resistance by the agar dilution method.

Disturbingly, some MS *S. aureus* isolates did shift into the resistant category with an additional 24 h of incubation. This shift was most evident with methicillin, which was shown to change in most of the systems. Sceptor and API 3600S had a significant increase in resistant MICs (20%). The broth microdilution and Sensititre showed increases in gentamicin resistance, whereas increases in resistance to ampicillin were seen with Sensititre, Sceptor, and MicroScan. At 48 h, the API 3600S indicated a single strain of MS *S. aureus* to be vancomycin resistant. Interestingly, no increases in resistance were seen with

TABLE 6. Agreement of each system with the reference broth microdilution method by using MS *S. aureus* strains (n = 15)

System	% Agreement						
	Methicillin	Penicillin	Cephalothin	Clindamycin	Gentamicin	Vancomycin	Ampicillin
Agar dilution							
24	93	93	100	100	100	100	87
48	87	93	100	100	100	100	87
Disk diffusion							
24	87	93	100	100	100	100	93
48	87	93	100	100	100	100	93
Sensititre							
24	80	93	100	100	100	100	93
48	87	93	100	100	100	100	93
Micro-Media							
24	80	100	100	100	100	100	93
48	80	100	100	100	100	100	93
Sceptor							
24	87	100	100	100	100	—[a]	87
48	80	100	100	100	100	—	93
API 3600S							
24	80	100	100	100	100	100	—
48	60	100	100	93	100	93	—
MicroScan							
24	80	100	100	100	100	100	100
48	73	100	100	100	100	100	100
Autobac I							
3 to 6	80	60	100	100	100	100	100
MS-2							
3 to 6	53	100	100	100	100	—	100

[a] —, Not tested.

the disk diffusion and Micro-Media systems.

β-Lactamase production. Of the 40 strains, 38 produced β-lactamase. The two negative strains were the *S. aureus* ATCC 25923 and a clinical MS *S. aureus* strain. Only two systems called one or both of these organisms resistant according to our cutoff concentrations. The Sceptor system called both strains resistant, whereas Autobac I called the American Type Culture Collection strain resistant and the clinical strain sensitive.

DISCUSSION

The movement towards more quantitative testing results for antimicrobial susceptibility testing has not been without problems. Lack of a standardized method, establishment of cutoff points, and interpretation of results by physicians have been major difficulties. In addition, certain strains of organisms present special problems in determining their true antibiograms. Among these organisms are the *Enterobacter* species that have inducible β-lactamases, *Pseudomonas aeruginosa* with media supplementation, and MR *S. aureus*. Because of the importance of and increase in MR *S. aureus* infections, especially of nosocomial origin, reliable systems for detecting MR *S. aureus* isolates are highly desirable (16). Perhaps one of the most important factors in the spread of MR *S. aureus* infections is that many MR *S. aureus* are missed in the laboratory and are recognized only retrospectively. Factors contributing to this lack of detection of MR *S. aureus* isolates are the degree of heterogeneity exhibited at 35°C and the slow growth rate of the resistant organisms in the presence of methicillin (3). The present study addressed the latter problem by testing both laboratory-prepared and commercial systems for their ability to detect methicillin resistance among *S. aureus*.

The purpose of the study was to accurately characterize a population of *S. aureus* strains containing both MR and MS organisms. The

TABLE 7. Comparison of the increase in resistance with an additional 24-h incubation[a]

Organism class	Antibiotic	% Increase in resistance							
		Broth microdilution	Agar dilution	Disk diffusion	Sensititre	Micro-Media	Sceptor	API 3600S	MicroScan
MR *S. aureus*	Methicillin	8	12	36	20	68	8	4	8
	Cephalothin	12	36	0	4	0	4	12	24
	Gentamicin	0	8	0	0	0	0	0	0
MS *S. aureus*	Methicillin	0	13	0	6	0	20	20	7
	Gentamicin	13	0	0	6	0	0	0	0
	Vancomycin	0	0	0	0	0	0	7	0
	Ampicillin	0	0	0	6	0	14	—[b]	7

[a] Only antibiotics that showed a change in resistance are reported.
[b] —, Not tested.

coded isolates were then tested by a variety of methods, including that used to characterize the organisms. All systems varied in detecting methicillin resistance as well as resistance to other antibiotics.

When the results from the three noncommercial methods for MR *S. aureus* antibiograms were compared, the greatest variability was found with methicillin, cephalothin, and gentamicin. Both the broth and agar dilution methods were comparable and superior to the disk diffusion method for detecting methicillin resistance at both time periods. With regard to cephalothin, the agar dilution was superior to the broth microdilution and disk diffusion. Because of therapeutic failures, all MR *S. aureus* isolates should be considered resistant to cephalosporins (1, 28). Detection of cephalothin resistance by these methods was less satisfactory than for methicillin resistance. Rylander et al. (35) reported poor correlation between agar and broth methods in detecting cephalosporin resistance among both gram-positive and gram-negative organisms. The present study supports their results in that more cephalosporin resistance was detected by using the agar dilution method. Likewise, more gentamicin resistance was detected with the agar dilution method, whereas the broth and disk diffusion methods showed identical results at both times.

Comparatively, the three noncommercial methods showed some variability of antibiograms among MS *S. aureus* strains. Again, the methicillin susceptibilities had the greatest variability, with broth and agar dilution methods indicating three resistant MICs at 48 h, whereas disk diffusion indicated none. Interestingly, at the time of initial characterization, against all three strains, the MIC was 2 μg/ml. During the study, the MIC against all three was 4 μg/ml both at 24 and 48 h. Given that acceptable results for reproducibility are ±1 $\log_2$ dilution, a value of 4 μg/ml would be acceptable for an MIC against a strain of which the initial value was 2 μg/ml. This one-dilution increase in the MIC, however, shifts these organisms from the susceptible to the resistant category. Other variability among the systems showed the broth microdilution method indicated two MS *S. aureus* strains to be gentamicin resistant that were susceptible by all other methods. With regard to ampicillin, the broth microdilution method detected the least resistance; the agar dilution method the most.

All the commercial systems were comparable to the broth microdilution method in their ability to detect MR *S. aureus* at 48 h. The 24-h readings showed that Sceptor detected more methicillin resistance than did Sensititre, which was better than Micro-Media. At 24 h, both the broth microdilution and Sceptor missed 16% of MR *S. aureus*, whereas Sensititre and Micro-Media missed 32 and 88%, respectively.

The detection of MR *S. aureus* strains by the API and MicroScan systems was comparable to that of the reference method. The API method was the only system to detect all MR strains with regard to methicillin resistance. Autobac I and MS-2 showed the least ability to detect methicillin resistance. Cephalothin susceptibilities varied among the systems; only the reference method and MS-2 detected cephalothin resistance at the initial readings. At the 48-h reading, the reference method, MicroScan, and API 3600S reported the most cephalothin-resistant strains. Agreement varied only slightly with the other antibiotics tested except for clindamycin resistance with the Autobac I.

Interestingly, all of the commercial systems except MicroMedia detected at least one strain among the MS *S. aureus* isolates to have a resistant MIC in 48 h. At the recommended reading times, however, Sensititre, Micro-Media, MicroScan, Autobac I, and MS-2 showed no methicillin resistance among the MS *S. aureus*. Against all strains reported as resistant, the MIC was 2 μg/ml upon characterization. For response to penicillin G all systems except Scep-

tor were comparable. The Sceptor system reported all isolates resistant at both time periods, even the American Type Culture Collection strain included as an internal control. All systems were comparable for clindamycin results except for one strain with the Micro-Media system. A slight amount of variability in antibiograms also occurred with gentamicin and ampicillin.

Among MR *S. aureus*, when all the systems were compared for agreement with the reference method, the greatest variability occurred with regard to methicillin and cephalothin. Among the MS *S. aureus*, the agreement of the systems with the reference methods was good for most systems. Greatest variability occurred with methicillin and ampicillin.

Caution should be used in the interpretation of MIC results for β-lactamase-sensitive penicillins such as penicillin and ampicillin without the aid of a β-lactamase production test. A previous report has indicated that strains of *S. aureus* with penicillin MICs of ≤0.05 μg/ml can produce β-lactamase (15). National Committee for Clinical Laboratory Standards standards recommend that any β-lactamase-positive strains of *S. aureus* be reported as resistant to the β-lactamase-sensitive penicillins (29). All of the commercial systems in this study recommend use of a β-lactamase detection for test *S. aureus* strains.

The variability among different quantitative systems is well documented in previous literature. Jackson and Finland (18), who compared broth and agar dilutions, noted a wide range of results. Their results were influenced by inoculum size, incubation time, and definition of endpoints. In the international collaborative study of Ericsson and Sherris (13), poor correlation was noted between broth and agar methods when β-lactamase-producing *S. aureus* was tested. They also reported a significant variation in results from different laboratories, although they were using the same media, organisms, and antibiotics. Kluge (26) reported significant discrepancies among agar and broth dilution methods in testing MR *S. aureus* against methicillin, oxacillin, and cefamandole. Barry and Badal (3), in a study similar to ours, also noted overlap between MR and MS *S. aureus* strains when using the broth microdilution method. They also reported that extended incubation enhanced the detection of MR *S. aureus*.

In a previous study, Gavan et al. (14) reported that the Sensititre correlated with the broth microdilution method within ±1 dilution 87.6% of the time. With regard to the beta-lactam antibiotics and staphylococci, greater than 13% of the Sensititre MICs differed by ≥2 dilutions from the broth microdilution method. No mention was made of testing MR *S. aureus* strains. In a previous study reported by Barry et al. (4), the Micro-Media system gave results within ±1 dilution at least 96% of the time. All results for the *S. aureus* tested were within ±1 dilution; however, none of these were MR *S. aureus*. Jones et al. (22) reported that only 7.8% of the staphylococcal isolates tested by the Sceptor system were outside of the ±1 dilution limits when compared with results from the broth microdilution. Some variation was reported when MR *S. aureus* were examined by the two methods. Blair and Cleary (6) have recently reported an 82% correlation between the Sceptor system and disk diffusion for methicillin resistance. Interestingly, against those organisms reported as susceptible by Sceptor, the MIC was 4 μg/ml, and the organisms displayed a multiresistance pattern. In our study, they would have been called resistant.

Only a small percentage of methicillin resistance among MR *S. aureus* was detected by Autobac I and MS-2. Lindsey and Barnes (27) reported that a comparison of each system to the disk diffusion method showed discrepancies with a variety of organisms, especially with the penicillins and cephalothin. Carlson et al. (8) reported 65 of 100 MR *S. aureus* strains showing resistance by the agar diffusion method were indicated as susceptible by the MS-2. Boyce et al. (7) reported only 47% agreement between MS-2 and disk diffusion in detecting methicillin resistance of MR *S. aureus* and only 15% agreement of cephalothin results with the same organisms. Interestingly, in the same study, when a single strain of MR *S. aureus* was set up in 10 separate tests simultaneously, the methicillin results showed four were resistant, five were intermediate, and one was susceptible. Cleary and Maurer (10) reported that 70% of 57 MR *S. aureus* isolates were reported susceptible to methicillin by Autobac I. In addition, they reported 21% of the clindamycin susceptibilities to disagree with disk diffusion results without prolonged incubation. Eriquez and Becker (Program Abstr. Int. Symp. Rapid Methods Automation Microbiol., 3rd, Washington, D.C., abstr. no. 161, 1981) have reported the API 3600S to detect 100% of MR *S. aureus* strains tested when compared with the disk diffusion and broth dilution susceptibility methods.

Regardless of the method used, the key to detecting MR *S. aureus* may be in the multiresistant pattern exhibited by these organisms. In our experience, the great majority of the MR *S. aureus* have a resistance pattern to ampicillin, chloramphenicol, erythromycin, clindamycin, and penicillin. The cephalosporins appear to be effective, but additional incubation will increase resistance. On the other hand, most of the MS *S. aureus* strains are resistant only to ampicillin

and penicillin, with occasional resistance to one of the other antibiotics. Although at 24 h some strains of MR *S. aureus* will appear to be susceptible to the β-lactamase-resistant penicillins and cephalosporins, the multiresistant pattern to the other antibiotics is evident and should be a clue to MR *S. aureus* detection. In the present study, the strains of MR *S. aureus* were resistant to penicillin G, ampicillin, and clindamycin by the broth microdilution method. Eight other strains were also resistant to gentamicin. Among MS *S. aureus*, resistance was seen primarily with penicillin G and ampicillin. These organisms can then be reincubated to verify resistance.

The results of this study indicate that at the recommended reading times for each system (18 to 24 h), some systems are better than others in detecting MR *S. aureus*. Also, the results of each system examined varied, and no system gave 100% detection of MR *S. aureus* at the initial reading. In addition, some overlap was seen in MIC endpoints between MR and MS *S. aureus*. All systems examined detected more MR *S. aureus* with an additional 24-h incubation. It is not known whether the variability in detecting MR *S. aureus* is a function of the metabolic state of the organism or suboptimal growth conditions.

Other studies have shown that MR *S. aureus* isolates are multiresistant to various antibiotic classes and that this mode of detection could serve as a better predictor of methicillin resistance than methicillin MICs (2, 10, 37). As with rapid tests for β-lactamase production, elucidation of the mechanism of methicillin resistance among *S. aureus* could lead to a more rapid and reliable means of detecting MR *S. aureus*.

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