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Clinical Laboratory Evaluation of the Abbott MS-2 Automated Antimicrobial Susceptibility Testing System: Report of a Collaborative Study

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The MS-2 system (Abbott Diagnostics, Division of Abbott Laboratories, Dallas, Tex.) was evaluated for its efficacy in determining the susceptibilities of both clinical and selected challenge (nonfastidious, facultative, and aerobic) isolates. The MS-2 results were compared with standard Kirby-Bauer disk diffusion and microdilution results by using fresh clinical isolates. For gram-positive isolates other than enterococci, overall agreement between MS-2 and reference results was 93 to 98%. With enterococci, MS-2 agreement with disk diffusion was 68% but with microdilution was 86% (agreement between disk diffusion and microdilution was 73%). The main discrepancies with enterococci were with cephalothin, penicillin, gentamicin, and kanamycin. With clinical gram-negative isolates, the overall agreement was 91 to 93%, with most discrepancies occurring with *Enterobacter* spp. and β-lactam antibiotics (MS-2 versus disk diffusion, 84%; MS-2 versus microdilution, 84%; disk diffusion versus microdilution, 87%) and with *Serratia* spp. and colistin (false-susceptible results). The agreement of MS-2 results with established reference antibiograms of a special collection of challenge strains was 91 to 97% for the gram-positive cocci and 86 to 98% for the gram-negative strains. (With *Enterobacter* spp., agreement was 86%, but was >90% for all other organism groups.) Of the 98 finite MS-2 minimum inhibitory concentrations (MICs) that could be directly compared with microdilution MICs, 77 (79%) were within ±1 well of the geometric mean microdilution MIC. MS-2 analysis time ranged from 2.8 to 6.5 h (mean, 4.2 h). On the basis of these results, we conclude that the MS-2 can be expected to yield rapid and accurate results with most nonfastidious, facultative, and aerobic pathogens.

Within the last decade, interest in developing and using automated instrumentation in the clinical microbiology laboratory has increased. In most instances, such instrumentation has been developed for antimicrobial susceptibility testing (2, 5, 7). One recently developed system is the MS-2 (Abbott Diagnostics, Division of Abbott Laboratories, Dallas, Tex.). In the MS-2 system, a test organism is inoculated into a cartridge with 11 cuvettes; 1 serves as an untreated growth control and the other 10 contain antimicrobial agents. Growth is monitored turbidimetrically at 5-min intervals, and susceptibility is determined automatically by computer comparison of growth kinetics in the presence of an antimicrobial agent with kinetics in the untreated control, i.e., a comparison of growth curves.

This is a report of a collaborative evaluation of the MS-2 system. Performance of the system was compared with that of two reference methods—the standardized agar disk diffusion and the broth microdilution with both fresh clinical isolates and a specific collection of organisms selected to challenge the system with drug-organism combinations that are most important clinically.

MATERIALS AND METHODS

Collaborative study. The collaborating investigators (see Table 1, footnotes) developed a protocol for evaluating the MS-2 system that was divided into four phases. In phase I, the investigators familiarized themselves with the MS-2 system and its operation and checked all three methods by testing each of 18 selected organisms by each method. In phase II, each laboratory tested 200 fresh isolates obtained from its own clinical specimens, selected to ensure that a predetermined number of each species was tested. The actual distribution of organisms tested initially in phase II is shown in Table 1.

In phase III, a special set of 179 challenge strains

TABLE 1. *Distribution of clinical isolates in phase II testing*

Organism	CDC[a]	Mayo[b]	UNC[c]	UW[d]
E. coli	54	45	40	43
Enterobacter spp.	23	23	23	21
Klebsiella spp.	20	20	20	20
Other gram-negative organisms (*Serratia* spp., *Providencia* spp., etc.)	28	29	27	30
P. mirabilis	18	18	18	18
Indole-positive *Proteus* spp.	12	12	13	12
P. aeruginosa	22	21	20	20
Enterococci	14	15	8	15
Nonenterococcal group D streptococci	1	0	7	0
S. aureus	27	24	22	25
S. epidermidis	12	11	12	12
Total	231	218	210	216

[a] CDC, Center for Disease Control, Atlanta, Ga.
[b] Mayo, The Mayo Clinic, Rochester, Minn.
[c] UNC, University of North Carolina, Chapel Hill.
[d] UW, University of Washington, Seattle.

was selected by one of us (C.T.) to be tested in each of the four laboratories. These challenge strains included organisms that were both susceptible and multiply resistant to a variety of clinically important antimicrobial agents. Isolates were deliberately selected without regard to their normal frequency of clinical occurrence to provide a severe test of the instrument. Numbers were assigned to these organisms, and a blind-coded set was sent to each of the four laboratories. Strains in this collection were chosen so that the system would be challenged with most of the known organism-antibiotic resistance patterns of clinical importance. Resistance patterns of these organisms are summarized in Tables 2 and 3. Any new susceptibility system should be able to differentiate such resistant strains from susceptible isolates of the same species.

Problem combinations encountered in the initial phase II and III studies were noted and corrected by the manufacturer where possible. Such changes included alterations in the cartridge design, alteration of disk concentrations for ampicillin and kanamycin, and modification of the analytic algorithm to improve the accuracy of results on enterococci. Phase IV consisted of repeat testing of the phase III challenge organisms with such system improvements incorporated in the MS-2. After phase IV, phase II was repeated in two of the four laboratories to confirm that the modification system yielded acceptable results with the fresh clinical isolates as well as with the challenge strains. (See Tables 7 to 12 for data from these two laboratories.)

All organisms were tested with three methods: (i) the MS-2 system, (ii) a standardized agar disk diffusion method (4), and (iii) a broth microdilution method (6). The same lots of media and antimicrobial agents were used by all investigators. Inocula for the three tests were prepared from several well-isolated colonies from an overnight blood agar plate. For the MS-2 inoculum, organisms were suspended directly in 4.0 ml of saline to obtain a turbidity equivalent to a 0.5 McFarland standard. The MS-2 cartridge was inoculated with an appropriate volume of this saline suspension (see below).

Organisms from the same colonies were, at the same time, introduced into a tube of Trypticase soy broth (BBL Microbiology Systems), which was incubated at 35°C until visible turbidity had developed (1 to 3 h). Turbidity was adjusted to match the 0.5 McFarland standard, and this suspension was used to inoculate the agar disk diffusion plate. A further 1:10 dilution of the Trypticase soy broth suspension was made for use as the inoculum for the microdilution test.

MS-2 system. The MS-2 is an automated system designed to perform antimicrobial susceptibility testing by comparing growth kinetics of an organism in the presence of an antimicrobial agent with growth kinetics of an untreated control (5). Components of the system (Fig. 1) include the cuvette cartridge, the disk loader-sealer, a control module (computer), and one or more analysis modules.

Antimicrobial agents for the MS-2 are supplied as filter paper elution disks which are automatically dispensed and sealed into the bottom of the appropriate cuvette chambers by using the loader-sealer unit. Contents of antimicrobial agents in the elution disks are shown in Table 4. The cuvette cartridge has 11 lower chambers (cuvettes), one of which serves as a growth control, with the other 10 each containing individual antimicrobial agents. The upper part of the cartridge is a chamber in which the culture grows into logarithmic phase before being automatically distributed into

TABLE 2. *Resistance patterns of gram-positive isolates tested in phases III and IV*

Organism (no. tested)	No. of strains resistant to:										No. of multiresistant isolates
	Amp[a]	Ceph	Chlor	Clind	Eryth	Gent	Kana	Meth	Pen	Tetra	
S. aureus (21)	NT[b]	0	6	5	11	0	4	2	16	7	13
S. epidermidis (21)	NT[b]	1	2	7	8	5	10	7	15	5	11
Enterococci (16)	0	13	0	13	2	15	16	14	14	13	16
Other group D streptococci (5)	0	0	0	0	0	0	4	2	2	3	4
Total (63)	0[c]	14	8	25	21	20	34	25	47	28	44

[a] Amp, ampicillin; Ceph, cephalothin; Chlor, chloramphenicol; Clind, clindamycin; Eryth, erythromycin; Gent, gentamicin; Kana, kanamycin; Meth, methicillin; Pen, penicillin; Tetra, tetracycline.
[b] Ampicillin not tested (NT) against staphylococci; see Pen data for β-lactamase producers.
[c] Total of 21 isolates tested with ampicillin.

TABLE 3. *Resistance patterns of gram-negative isolates tested in phases III and IV*

Organism (no. tested)	No. of strains resistant to:									No. of multi-resistant isolates
	Amp[a]	Carb	Ceph	Chlor	Col	Gent	Kana	Nitro	Tetra	
E. coli (21)	9	8	3	2	0	3	6	0	7	9
P. aeruginosa (29)	29	12	29	28	0	9	24	29	28	29
Proteus spp.[b] (22)	9	2	9	6	20	2	3	22	19	22
Enterobacter spp. (20)	18	5	19	3	3	1	3	4	3	20
Klebsiella pneumoniae (10)	9	10	0	0	0	0	1	0	1	9
Serratia spp. (9)	6	1	8	2	9	1	2	8	5	9
Other[c] (5)	2	2	4	3	1	0	1	2	4	5
Total (116)	82	40	72	44	33	16	40	65	67	103

[a] Amp, ampicillin; Carb, carbenicillin; Ceph, cephalothin; Chlor, chloramphenicol; Col, colistin; Gent, gentamicin; Kana, kanamycin; Nitro, nitrofurantoin; Tetra, tetracycline.

[b] *P. mirabilis* (14), *P. vulgaris* (4), *P. rettgeri* (3), and *P. morganii* (1). (The latter two species have been reclassified since inception of this study.)

[c] *Salmonella typhi* (2), *Shigella dysenteriae* (1), *Providencia stuartii* (1), and *Acinetobacter calcoaceticus* (1).

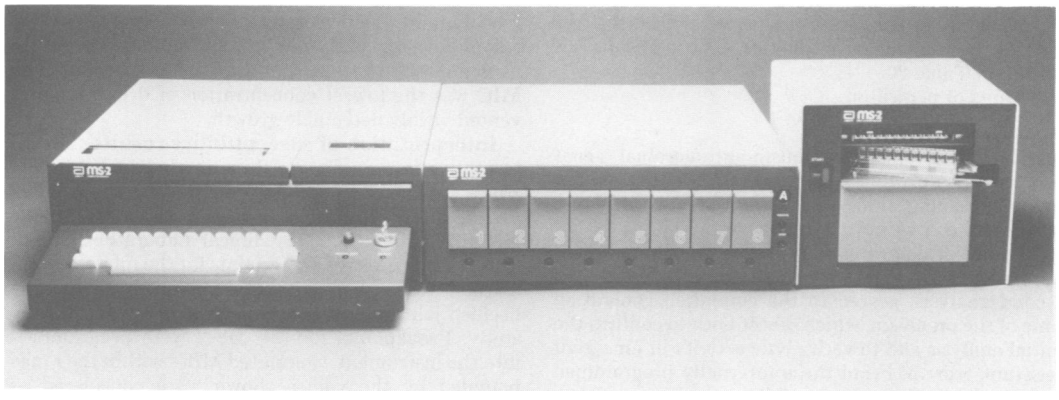

FIG. 1. *The MS-2 system. From left to right are the control module, the analysis module, and the disk loader-sealer. In the opening of the loader-sealer is a cuvette cartridge.*

each of the 11 test cuvettes, i.e., before contact with the antimicrobial agents. Only when the growing culture is distributed into the individual cuvettes are the antibiotics eluted into the broth. Before the initiation of this collaborative study, panels of drugs to be tested with gram-positive cocci (staphylococci and streptococci) and with gram-negative bacilli had been selected.

The computerized control module stores the information entered by the operator concerning the test organism (source, culture number, etc.), controls the sequence of events leading to analysis, accesses and stores the data, computes the evaluation of susceptibility, and prints out the final report.

Each analysis module provides positions for eight cartridges and contains individual electro-optical systems for monitoring turbidity within every cuvette in each cartridge at 5-min intervals. Each module also provides a constant temperature (35°C) and continual agitation throughout the test. After the cartridge is inserted into the analysis module, growth in the upper chamber is monitored by the instrument at 5-min intervals, and when the culture has shown a clear-cut increase in optical density (change of at least 0.008), indicating entry into logarithmic-phase growth, it is automatically transferred into each of the 11 lower cuvettes by the introduction of pulses of air pressure into the upper growth chamber.

After the culture is transferred into the lower cuvettes, the antibiotics are eluted into the broth, and the antibiotic action, if any, occurs. The turbidity in each cuvette is read every 5 min, and all values are stored in the computer memory.

As logarithmic growth proceeds, growth rate constants are internally computed and verified, and rate constants of the untreated control and growth curves from each of the antimicrobial test chambers are compared. Any antimicrobial agent causing a reduction of growth rate to less than 10% of the control value is considered to be effective; i.e., the organism is susceptible. In addition, a susceptible interpretation is assigned to any combination where clear-cut lysis of the organism has occurred as defined by a progressive negative rate constant (clearing of the bacterial sus-

TABLE 4. *Content of antimicrobial agents in elution disks used in the MS-2 system*

Drug	Content in panel (μg):	
	Gram-positive	Gram-negative
Ampicillin	2.5	5
Carbenicillin (low)[a]		16
Carbenicillin (high)[a]		100[b]
Cephalothin	10	10
Chloramphenicol	9	9
Clindamycin	1[c]	
Colistin		6
Erythromycin	3	
Gentamicin	4	4
Kanamycin	8	8
Methicillin	5[c]	
Nitrofurantoin		30
Penicillin	2[c,d]	

[a] Two levels of carbenicillin were used in the MS-2: a high range for *P. aeruginosa* and a low range for other gram-negative bacilli.

[b] *Pseudomonas* spp. only.

[c] Ancillary studies were performed with disks containing less of these antibiotics. Those results are shown in Table 20.

[d] Units of penicillin.

pension). Conversely, organism-antimicrobial agent combinations exhibiting a growth rate either similar to or exceeding that of the untreated control are designated as resistant.

The initial determination of rate constants occurs during logarithmic-phase growth and is followed by a second analysis, geared to the computed generation time of the organism, which serves both to confirm the initial analysis and to verify lytic activity or emergent resistant growth. From these internally programmed computer functions, susceptibility results are determined, and the antibiogram is automatically printed out as a category (susceptible or resistant) or as a minimal inhibitory concentration (MIC).

Results for each antimicrobial agent are printed as follows: (i) susceptible ("S"), MIC less than a specific concentration (e.g., <4 μg/ml); (ii) resistant ("R"), MIC greater than a specific concentration (e.g., >12 μg/ml); or (iii) a finite computed intermediate ("I") MIC (bounded by the two threshold concentrations in i and ii above [e.g., 6.5 μg/ml]). These threshold values are shown in Tables 5 and 6.

To perform a test with the MS-2 system, the appropriate battery of disks (Table 4) is loaded and sealed into a cuvette cartridge, and 15 ml of modified (starch omitted) Sensitest broth (Oxoid) is placed into the upper chamber. Inoculum is prepared from an overnight plate, as previously described, either 200 μl (for gram-negative organisms) or 400 μl (for gram-positive organisms) of the adjusted saline suspension is added to the broth in the upper chamber, and the loading port is sealed. After entering appropriate specimen information via the computer keyboard, the operator inserts the cuvette cartridge into its designated position in the analysis module. This sequence is repeated until all positions available in the analysis modules are filled. The remaining functions in the test—incubation, agitation, turbidity readings, susceptibility determinations, and reporting—are all performed automatically. Final analyses of different isolates occur at different times, and as one culture is completed, another cartridge can be inserted into that position without waiting until all tests in that module are completed. For much of this study, two analysis modules were used with a single control module.

Agar disk diffusion method. The standardized agar disk diffusion tests were performed by the method of Bauer et al. (1) as modified and described by the National Committee for Clinical Laboratory Standards (4).

Broth microdilution method. Broth microdilution trays were prepared in each laboratory by using a commercially available dispenser (MIC 2000; Dynatech Laboratories). Each well of the trays was filled with 0.1 ml of the Sensitest broth-antimicrobial agent mixture. Filled trays were sealed in plastic bags and stored at −70°C for a maximum of 2 weeks. They were removed as needed, thawed, and inoculated with the test organism using the MIC 2000 inoculator. The final inoculum in each well was approximately 5×10^5 colony-forming units per ml. Inoculated trays were incubated for 18 to 24 h at 35°C before reading. The MIC was the lowest concentration of drug that prevented visibly detectable growth.

Interpretation of susceptibility results. The interpretive criteria for the three methods used in this study are shown in Tables 5 and 6. The breakpoints for the disk diffusion zones were as described by the National Committee for Clinical Laboratory Standards (4). Interpretive breakpoints for the microdilution method were either those used previously (7) or were decided jointly by the investigators in advance of the study. Breakpoints for the MS-2 were programmed into the instrument. Computed MICs within the range bounded by the values shown were considered as intermediate results. In some cases, the MS-2 programming was designed to override an intermediate result if specific biological events were detected, e.g., lysis or late-emergent outgrowth.

Three categories were established for comparing the data from the MS-2 with those of the two reference systems: discrepancies were categorized as minor (involving an intermediate result), major (resistant by MS-2 and susceptible by the reference method), or very major (susceptible by MS-2 and resistant by the reference method). For examination and resolution of any discrepancies encountered during testing, growth curves were generated from the recorded test data by plotting optical density versus time (Fig. 2).

Reference antibiogram. With many of the organisms in the phase III challenge collection, disparate results were obtained from the two standard reference methods in one or more laboratories. Consequently, it was deemed necessary to establish a reference antibiogram to serve as a fixed index for comparing accuracy. All results obtained by disk diffusion and microdilution in each of the four collaborating laboratories were tabulated and reviewed to define the most probable correct answer for each organism-antibiotic combination. Each combination was tested by disk diffusion

TABLE 5. *Interpretive criteria for disk diffusion, microdilution, and MS-2 tests with gram-positive organisms*

Drug	Kirby-Bauer disk diffusion zone diam (mm)			Microdilution MIC (μg/ml)			MS-2 MIC (μg/ml)		
	R[a]	I	S	R	I	S	R	I	S
Cephalothin	≤14	15–17	≥18	≥32	16	≤8	≥30	29.9–10.1	≤10
Clindamycin	≤14	15–16	≥17	≥8	4	≤2	≥3	2.9–1.1	≤1
Erythromycin	≤13	14–17	≥18	≥8	4	≤2	≥9	8.9–3.1	≤3
Gentamicin	≤12		≥13	≥16	8	≤4	≥12	11.9–4.1	≤4
Methicillin	≤9	10–13	≥14	≥16	8	≤4	≥15	14.9–5.1	≤5
Penicillin[b]	≤20	21–28	≥29	>0.25		≤0.25	≥6	5.9–2.1	≤2[c]
Penicillin[d]	≤11	12–21	≥22	≥4	2	≤1.0	≥6	5.9–2.1	≤2[c]
Tetracycline	≤14	15–18	≥19	≥16	8	≤4	≥15	14.9–5.1	≤5
Kanamycin	≤13	14–17	≥18	≥32	16	≤8	≥24	23.9–8.1	≤8
Chloramphenicol	≤12	13–17	≥18	≥32	16	≤8	≥27	26.9–9.1	≤9
Ampicillin[e]	≤11	12–13	≥14	≥32	16	≤8	≥7.5	7.4–2.6	≤2.5

[a] R, resistant; I, intermediate; S, susceptible.
[b] Staphylococci.
[c] Units of penicillin.
[d] Streptococci.
[e] Enterococci only.

TABLE 6. *Interpretive criteria for disk diffusion, microdilution, and MS-2 tests with gram-negative organisms*

Drug	Kirby-Bauer disk diffusion zone diam (mm)			Microdilution MIC (μg/ml)			MS-2 MIC (μg/ml)		
	R[a]	I	S	R	I	S	R	I	S
Ampicillin	≤11	12–13	≥14	≥32	16	≤8	≥15	14.9–5.1	≤5
Carbenicillin[b]	≤13	14–16	≥17	≥256	128	≤64	≥300	299–101	≤100
Carbenicillin[c]	≤17	18–22	≥23	≥64	32	≤16	≥48	47.9–16.1	≤16
Cephalothin	≤14	15–17	≥18	≥64	32	≤16	≥30	29.9–10.1	≤10
Chloramphenicol	≤12	13–17	≥18	≥32	16	≤8	≥27	26.9–9.1	≤9
Colistin	≤8	9–10	≥11	≥16	8	≤4	≥18	17.9–6.1	≤6
Gentamicin	≤12		≥13	≥16	8	≤4	≥12	11.9–4.1	≤4
Kanamycin	≤13	14–17	≥18	≥32	16	≤8	≥24	23.9–8.1	≤8
Nitrofurantoin	≤14	15–16	≥17	≥128	64	≤32	≥90	89.9–30.1	≤30
Tetracycline	≤14	15–18	≥19	≥16	8	≤4	≥15	14.9–5.1	≤5

[a] See Table 5, footnote *a*.
[b] *Pseudomonas* spp.
[c] Other gram-negative organisms.

and microdilution at two different times in each of the four laboratories, thereby yielding a total of 16 results for each combination. With these data, and without consideration of results obtained from the MS-2, a reference antibiogram was established for most of the more than 1,600 organism-antibiotic test combinations in the phase III collection. In some cases, however, no clear-cut result was obtained from the multiple manual testing. In these cases, several of the investigators defined the most probable correct answer by scientific and clinical judgment based on the relationship of results of the test strain to those of sensitive members of the species, evidence for the presence of resistance determinants, and experience with treatment of infections due to similar organisms with the specific antibiotic. This antibiogram served as a reference against which the accuracy of any of the three test methods could be compared. It should be noted that this reference base was available only for test results on phase III strains (phases III and IV testing). The reference antibiogram could not be used for phase II strains, which were clinical isolates tested only in the laboratory of origin.

Antimicrobial agents, disks, and culture media. Single lots of these materials were obtained and distributed to each investigator to provide matched reagents in each of the laboratories. Disks and culture media used in the diffusion tests were obtained from BBL Microbiology Systems.

RESULTS

As indicated above, the initial phase II and III studies revealed some problems in the MS-2 system, and changes were made to correct as many as possible. For example, some computer software and analytical programs were changed, the cuvette cartridge design was modified to

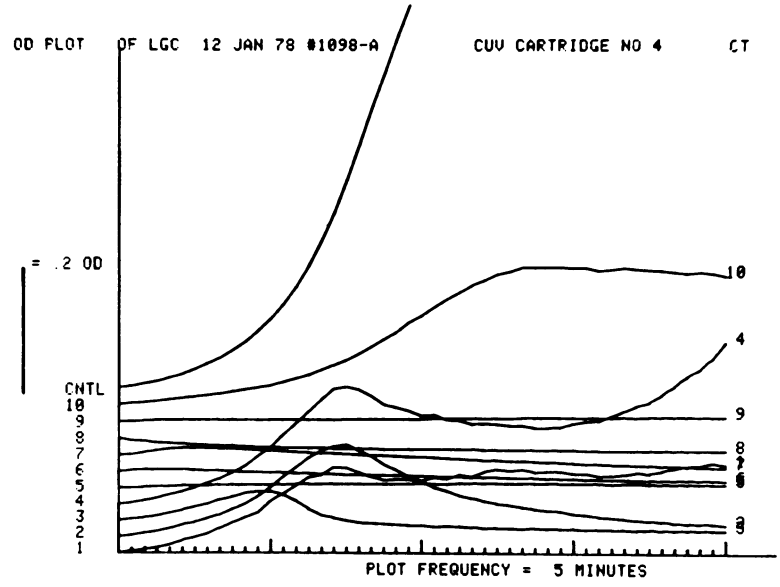

FIG. 2. *MS-2-generated 4-h growth curves for a multiresistant strain of S. aureus. OD, optical density; LGC, lower growth chamber; CNTL, control curve; 1 to 10, curves in 10 antimicrobial agents.*

permit freer exchange of air, and the contents of penicillin, methicillin, and clindamycin in elution disks were decreased.

After these system modifications, phase II (clinical isolates) was repeated in two of the collaborating laboratories. The efficacy of the different-content elution disks was also verified in two of the laboratories. Phase III strains (challenge strains) were retested in four laboratories.

Phase II. In the initial phase II studies (before system modifications), correlations of MS-2 results with disk diffusion and microdilution for the gram-positive cocci (Table 1) were 86.9 and 92.9%, respectively, and the correlations for gram-negative organisms were 87.6 and 85.8%, respectively.

The phase II results obtained by the two laboratories after system modifications are shown in Tables 7 to 12. Agreement between results of each method and the type of discrepancy is tabulated by organism and antimicrobial agent. In these tables, the numbers and types of discrepancies are listed as very major, major, or minor for the total number of organisms tested (e.g., 0,3,0/25 means no very major, three major, and no minor discrepancies for 25 organisms tested).

Phase II agreement between MS-2 and disk diffusion results for the gram-positive cocci (Table 7) was good for *Staphylococcus aureus* (overall, 97.9%). Three reports of susceptibility by the MS-2 and resistance by diffusion were noted with penicillin. These were probably weakly β-lactamase-positive strains. Similarly, very major errors with erythromycin were probably inducibly resistant strains. Lower accord was observed between enterococci and gentamicin, kanamycin, penicillin, cephalothin, and erythromycin. With all of these combinations except enterococci-erythromycin, the discrep-

TABLE 7. *Phase II: MS-2 versus disk diffusion results for gram-positive organisms*[a]

Drug	No. of organisms[b]:			Full agreement by drug (%)
	S. aureus	*S. epidermidis*	Enterococci	
Cephalothin	0,0,0/42	0,0,0/24	0,3,19/31	77.3
Clindamycin	0,0,0/42	0,0,0/24	3,0,0/31	96.9
Erythromycin	3,0,0/42	0,0,0/24	9,0,4/31	83.5
Gentamicin	0,0,0/42	0,4,0/24	0,16,0/31	79.4
Methicillin	0,0,0/42	0,0,5/24	0,0,0/31	94.8
Penicillin	3,0,1/42	4,0,2/24	0,1,28/31	59.8
Tetracycline	0,0,0/42	0,0,0/24	0,0,0/31	100.0
Kanamycin	0,0,1/42	0,0,0/24	1,0,12/31	85.6
Chloramphenicol	0,0,0/42	0,0,0/24	0,0,1/31	99.0
Ampicillin	NT[c]	NT	0,0,1/31	96.8
Full agreement by organism (%)	97.9	93.0	68.4	86.6[d]

[a] The second method is the reference.

[b] Numbers indicate very major, major, and minor discrepancies, respectively, per total number of organisms. These results were obtained in two laboratories, and thus the totals do not agree with those in Table 1 (see the text).

[c] NT, Not tested.

[d] Overall full agreement.

TABLE 8. *Phase II: MS-2 versus microdilution results for gram-positive organisms*[a]

Drug	No. of organisms[b]:			Full agreement by drug (%)
	S. aureus	*S. epidermidis*	Enterococci	
Cephalothin	0,0,0/42	1,0,0/24	0,7,11/31	80.4
Clindamycin	0,0,0/42	0,0,0/24	1,0,1/31	97.9
Erythromycin	1,0,1/42	0,0,0/24	9,0,0/31	88.6
Gentamicin	0,0,0/42	0,2,0/24	1,0,8/31	88.6
Methicillin	0,0,0/42	0,0,3/24	0,0,0/31	96.9
Penicillin	2,2,0/42	2,1,0/24	1,3,0/31	88.6
Tetracycline	1,0,0/42	0,1,0/24	0,0,0/31	97.9
Kanamycin	0,0,1/42	0,0,0/24	1,0,0/31	97.9
Chloramphenicol	0,0,0/42	0,0,0/24	0,0,0/31	100.0
Ampicillin	NT[c]	NT	0,1,1/31	93.5
Full agreement by organism (%)	97.9	95.4	85.5	93.0[d]

[a, b, c, d] See Table 7, footnotes *a*, *b*, *c*, and *d*.

TABLE 9. *Phase II: microdilution versus disk diffusion results for gram-positive organisms*[a]

Drug	No. of organisms[b]:			Full agreement by drug (%)
	S. aureus	*S. epidermidis*	Enterococci	
Cephalothin	0,0,0/42	0,1,0/24	2,1,12/31	83.5
Clindamycin	0,0,0/42	0,0,0/24	1,0,1/31	97.9
Erythromycin	1,0,1/42	0,0,0/24	0,0,4/31	93.8
Gentamicin	0,0,0/42	1,3,0/24	0,15,7/31	73.2
Methicillin	0,0,0/42	0,0,1/24	0,0,0/31	98.9
Penicillin	2,0,1/42	3,0,3/24	0,1,27/31	61.8
Tetracycline	0,1,0/42	1,0,0/24	0,0,0/31	97.9
Kanamycin	0,0,0/42	0,0,0/24	0,0,12/31	87.6
Chloramphenicol	0,0,0/42	0,0,0/24	0,0,1/31	99.0
Ampicillin	NT[c]	NT	0,0,0/31	100.0
Full agreement by organism (%)	98.4	94.0	72.9	88.6[d]

[a, b, c, d] See Table 7, footnotes *a*, *b*, *c*, and *d*.

ancies were due to the high frequency of false-susceptible or intermediate results obtained with these drugs against enterococci in the disk diffusion test when the MS-2 and microdilution methods recorded them as resistant.

A comparison of MS-2 results with microdilution data (Table 8) showed a much better correlation for the enterococcus group, with cephalothin and gentamicin discrepancies shifting into the minor category, and a very substantial (29%) increase in penicillin accord.

When results of disk diffusion were compared directly with microdilution findings (Table 9), it was apparent that substantial differences existed between the two conventional reference methods with regard to susceptibility of enterococcal isolates. Full accord between the two reference methods for the enterococci was only 73%, showing high levels of minor discrepancies with cephalothin, penicillin, and kanamycin and a high level of major discrepancies for gentamicin, apparently due to false-susceptible results from the disk diffusion method. As noted in Tables 5 and 6, disk diffusion breakpoints for gentamicin used in these studies were from the first edition of the National Committee for Clinical Laboratory Standards (4) (recently a second edition has been published containing different gentamicin breakpoints); these may have contributed to the level of discrepancies noted between the reference methods.

In summary, phase II MS-2 results with gram-positive cocci showed a high level of full accord with reference methods (93 to 98%) for the staphylococci, but for enterococcal isolates, MS-2 results compared more closely with microdilution results than with those from the disk diffusion method.

With the gram-negative phase II organisms, a comparison of MS-2 versus disk diffusion results (Table 10) showed excellent accord for *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus mirabilis*, and indole-positive *Proteus* spp. Full accord was below 90% only for *Enterobacter* isolates and other gram-negative organisms, primarily *Serratia* isolates (Table 1). Examination of the data showed that the very major discrepancies with *Enterobacter* spp. occurred specifically with ampicillin and cephalothin, showing high agreement with all other antimicrobial agents tested. Discrepancies in the "other" category were mostly false-susceptible results on *Serratia* spp. tested with colistin. Aside from these three specific areas, other discrepancies were primarily minor and were scattered throughout the organism-antimicrobial agent combinations.

Similar findings were observed in comparing results of the MS-2 with microdilution (Table 11). Very major discrepancies with *Enterobacter* spp.-ampicillin-cephalothin combination were significantly reduced, whereas the *Serratia* spp.-colistin results were unchanged. Full accord in these areas remained below that obtained for all other organism groups. Except for ampicillin and cephalothin, agreement for all other antimicrobial agents was 90% or greater.

When disk diffusion results were compared with those from microdilution (Table 12), exactly the same problem areas as described above were encountered, with the difference being that very major discrepancies were several percent lower and minor discrepancies were somewhat higher. Overall full accord between the two reference methods (92.1%) was essentially identical

TABLE 10. *Phase II: MS-2 versus disk diffusion results for gram-negative organisms*

Drug	No. of organisms[a]: *E. coli*	*P. aeruginosa*	*Klebsiella* spp.	*P. mirabilis*	Other *Proteus* spp.[b]	*Enterobacter* spp.	Other gram-negative organisms[c]	Full agreement by drug (%)
Ampicillin	0,2,1/61	0,0,0/36	0,0,4/39	0,0,0/34	1,3,6/30	22,1,3/44	3,0,5/48	82.5
Carbenicillin	0,0,0/61	1,2,0/36	0,0,2/39	0,0,0/34	0,3,0/30	1,0,1/44	3,0,10/48	92.1
Cephalothin	0,1,8/61	1,0,0/36	1,0,1/39	0,1,0/34	0,1,0/30	14,1,5/44	1,0,2/48	87.3
Chloramphenicol	0,1,1/61	0,2,0/36	0,0,0/39	0,0,0/34	0,1,2/30	0,0,1/44	1,0,1/48	96.6
Gentamicin	0,1,3/61	0,0,0/36	0,0,0/39	0,0,0/34	0,0,0/30	0,1,0/44	0,1,1/48	97.6
Kanamycin	0,0,4/61	3,0,2/36	0,0,0/39	0,0,0/34	1,1,0/30	0,0,6/44	1,2,3/48	92.1
Colistin	0,0,0/61	0,0,0/36	0,0,0/39	0,0,0/34	0,0,1/30	0,0,0/44	11,1,2/48	94.9
Tetracycline	0,0,1/61	0,0,2/36	0,0,0/39	0,0,0/34	0,0,3/30	0,1,4/44	1,1,5/48	93.8
Tobramycin	0,4,0/61	1,0,2/36	0,0,0/39	0,0,0/34	0,1,0/30	0,0,1/44	0,0,5/48	95.2
Full agreement by organism (%)	95.1	95.1	97.7	99.7	91.1	84.3	86.1	92.5[d]

[a] See Table 7, footnote *b*.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] Overall full agreement.

TABLE 11. *Phase II: MS-2 versus microdilution results for gram-negative organisms*

Drug	No. of organisms[a]: *E. coli*	*P. aeruginosa*	*Klebsiella* spp.	*P. mirabilis*	Other *Proteus* spp.[b]	*Enterobacter* spp.	Other gram-negative organisms[c]	Full agreement by drug (%)
Ampicillin	0,1,0/61	0,0,0/35	1,0,2/39	0,0,0/35	1,2,4/31	14,1,5/44	4,1,10/48	84.3
Carbenicillin	0,0,2/61	2,2,2/35	2,0,0/39	0,0,0/35	0,3,0/31	0,0,1/44	4,1,10/48	90.1
Cephalothin	0,2,6/61	1,0,0/35	0,1,1/39	1,1,1/35	1,3,2/31	4,1,5/44	1,0,0/48	89.4
Chloramphenicol	1,0,3/61	0,0,1/35	0,0,0/39	1,0,4/35	0,0,4/31	0,0,3/44	5,0,4/48	91.1
Gentamicin	0,0,2/61	0,0,2/35	0,0,0/39	0,0,0/35	1,0,1/31	1,1,1/44	3,0,2/48	95.2
Kanamycin	0,1,3/61	3,0,2/35	0,0,0/39	0,0,2/35	1,1,0/31	3,0,3/44	2,2,1/48	91.8
Colistin	0,0,0/61	0,0,0/35	0,0,0/39	0,0,0/35	0,0,1/31	2,0,1/44	11,0,1/48	94.5
Tetracycline	0,0,2/61	0,0,2/35	0,0,0/39	0,0,1/35	0,0,0/31	4,0,6/44	2,1,5/48	92.2
Full agreement by organism (%)	95.3	93.9	97.8	96.1	89.9	84.1	81.8	91.1[d]

[a] See Table 7, footnote *b*.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] Overall full agreement.

to that obtained when MS-2 results were compared with those of either method alone (92.5, 91.1%).

Phase IV results. Since phase IV results were representative of MS-2 performance with the phase III challenge strains after final system improvements had been incorporated, only the phase IV results will be considered in detail here.

In evaluating results obtained by the three test methods in phase IV, we compared the findings obtained by each method with those obtained by the reference antibiogram since, as previously noted, results produced by the two conventional reference methods for many of these strains were in conflict. Consequently, the reference antibiogram was used as the reference to compare the accuracy of each system.

MS-2 results for the challenge strains in the Phase IV study are summarized in Tables 13 and 14. Accord between MS-2 results for gram-positive organisms and the reference antibiogram is shown in Table 13. By organism group, full accord with the MS-2 ranged from a low of 91.2% (for nonenterococcal group D streptococci) to 96.8% for *S. aureus* isolates. As a function of the specific antimicrobial agent tested, results ranged from 91.4% with penicillin and clindamycin to 100% for ampicillin. Overall full accord for all combinations was 95.2%.

Similar data for the gram-negative organisms studied in phase IV are shown in Table 14. As previously described for the phase II results, very major discrepancies were noted with some strains of *Enterobacter* and the β-lactam anti-

TABLE 12. *Phase II: microdilution versus disk diffusion results for gram-negative organisms*[a]

Drug	No. of organisms[b]:							Full agreement by drug (%)
	E. coli	*P. aeruginosa*	*Klebsiella* spp.	*P. mirabilis*	Other *Proteus* spp.[c]	*Enterobacter* spp.	Other gram-negative organisms[d]	
Ampicillin	0,0,0/61	0,0,0/35	0,1,4/39	0,0,0/35	0,2,9/31	5,0,3/44	0,1,12/48	87.4
Carbenicillin	0,0,2/61	0,1,2/35	0,0,2/39	0,0,0/35	0,0,0/31	0,0,0/44	3,3,4/48	94.2
Cephalothin	0,0,5/61	0,0,0/35	1,0,2/39	0,1,1/35	2,0,2/31	9,0,10/44	1,0,2/48	87.7
Chloramphenicol	0,2,2/61	0,0,1/35	0,0,0/39	0,1,5/35	0,0,4/31	0,1,2/44	0,5,5/48	90.4
Gentamicin	0,0,1/61	0,0,2/35	0,0,0/39	0,0,0/35	0,1,1/31	0,1,1/44	0,4,0/48	96.2
Kanamycin	1,0,3/61	0,0,4/35	0,0,0/39	0,0,3/35	0,0,0/31	0,1,4/44	1,3,4/48	91.8
Colistin	0,0,0/61	0,0,0/35	0,0,0/39	0,0,0/35	0,0,0/31	0,2,1/44	1,1,3/48	97.3
Tetracycline	0,0,2/61	0,0,2/35	0,0,0/39	0,0,0/35	0,0,3/31	0,4,2/44	0,1,9/48	92.1
Full agreement by organism (%)	96.3	95.7	96.8	96.1	90.3	86.9	83.6	92.1[e]

[a] The disk diffusion test is the reference.
[b] See Table 7, footnote *b*.
[c] See Table 3, footnote *b*.
[d] See Table 3, footnote *c*.
[e] Overall full agreement.

TABLE 13. *Phase IV: discrepancies between MS-2 and reference antibiogram results for gram-positive organisms*

Drug	No. of organisms[a]:				Full agreement by drug (%)
	S. aureus	*S. epidermidis*	Enterococci	Other	
Ampicillin	NT[b]	NT	0,0,0/63	0,0,0/17	100.0
Cephalothin	0,1,0/83	1,0,0/80	0,0,0/63	0,0,0/17	99.2
Chloramphenicol	1,0,0/83	0,1,0/80	0,0,0/63	0,0/0/17	99.2
Clindamycin	0,0,0/83	4,0,0/80	17,0,0/63	0,0,0/17	91.4
Erythromycin	0,0,6/83	2,0,0/80	7,0,0/63	0,0,0/17	93.8
Gentamicin	0,0,0/83	1,0,0/80	2,1,1/63	0,2,2/17	96.3
Kanamycin	1,0,0/83	1,4,0/80	0,0,0/63	1,0,3/17	95.9
Methicillin	0,4,3/83	2,0,4/80	0,0,3/63	0,1,1/17	92.6
Penicillin	0,0,0/83	17,0,0/80	0,0,0/63	4,0,0/17	91.4
Tetracycline	7,0,1/83	2,0,0/80	0,0,0/63	0,0,1/17	95.5
Full agreement by organism (%)	96.8	94.6	95.1	91.2	95.2[c]

[a] See Table 7, footnote *b*. The total number of organisms represents only those strains for which results were obtained in all four laboratories and thus may not agree with the results in Table 1.
[b] NT, Not tested.
[c] Overall full agreement.

biotics and with *Serratia* spp. and colistin. Examining MS-2 growth curves showed that these discrepancies resulted from late-emerging (delayed) resistance in these particular combinations (this will be discussed later in more detail). Full accord for the combinations noted above was in the 86 to 88% range, but for all other gram-negative organism groups and antibiotics tested, full accord was found to be in the range of 90 to 97%. Overall agreement for the gram-negative organism-antibiotic combinations tested was 92.4%.

In earlier stages of investigation of phase III challenge organisms, several strains of *S. aureus* selected for possession of inducible resistance to erythromycin were found to yield susceptible results in the MS-2 system. This problem had previously been described in the Autobac I system (8). Studies in the Abbott laboratories ascertained that this problem could be effectively resolved by adding subinhibitory levels of erythromycin to the MS-2 medium before inoculating the upper growth chamber. By adding inducer, the problem of the major discrepancies with these isolates was completely eliminated (Table 15). The results for staphylococci shown in Table 13 (see also Table 17) were also obtained with the use of erythromycin inducer.

A summary of the phase IV results is shown in Table 16. The overall accuracy of the MS-2

TABLE 14. *Phase IV: discrepancies between MS-2 and reference antibiogram results for gram-negative organisms*

Drug	No. of organisms[a]: *E. coli*	*P. aeruginosa*	*Klebsiella* spp.	*P. mirabilis*	Other *Proteus* spp.[b]	*Enterobacter* spp.	Other gram-negative organisms[c]	Full agreement by drug (%)
Ampicillin	0,8,0/80	1,0,0/112	0,4,0/40	0,0,1/48	0,1,2/38	26,2,2/79	1,5,6/60	87.1
Carbenicillin (low)[d]	0,0,0/80		1,0,1/40	0,0,0/48	0,5,1/38	4,4,1/79	1,4,0/60	93.6
Carbenicillin (high)[d]		5,5,5/112						86.5
Cephalothin	0,6,0/80	0,0,1/112	0,0,0/40	0,0,0/48	0,0,3/38	26,0,1/79	2,0,0/60	91.5
Chloramphenicol	0,1,3/80	2,0,4/112	0,0,0/40	0,0,4/48	1,0,2/38	0,0,2/79	2,0,1/60	95.2
Colistin	0,1,0/80	0,0,2/112	0,0,1/40	0,0,0/48	2,1,4,38	3,0,1/79	31,0,0/60	90.0 (96.4)[e]
Gentamycin	0,0,0/80	0,0,2/112	0,4,1/40	0,0,0/48	0,1,1/38	4,0,0/79	0,0,0/60	97.2
Kanamycin	0,7,0/80	6,1,8/112	0,0,1/40	0,0,0/48	0,1,0/38	0,0,1/79	0,0,0/60	94.5
Nitrofurantoin	0,0,0/80	1,0,3/112	0,2,9/40	2,0,1/48	0,0,5/38	0,4,17/79	0,0,1/60	89.5
Tetracycline	0,0,2/80	4,2,0/112	0,0,1/40	0,0,0/48	1,0,1/38	0,0,3/79	0,0,5/60	95.9
Full agreement by organism (%)	96.1	94.8	93.0	98.2	90.7	86.0	89.1 (94.8)[e]	92.4[f] (93.3)[e]

[a] See Table 7, footnote *b*.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] See Table 4, footnote *a*.
[e] Value within parentheses indicates percent agreement if *Serratia*-colistin combination is removed from data base.
[f] Overall full agreement.

TABLE 15. *Effect of erythromycin inducer on MS-2 susceptibility results obtained with S. aureus and erythromycin*

Inducer[a]	No. of strains	Discrepancies (%)		
		Very major	Major	Minor
No	84	26.1%	0	6%
Yes	83	0	0	7.2%

[a] 0.02 μg of erythromycin per ml of broth.

TABLE 16. *Phase IV: overall full agreement of three test methods versus the reference antibiogram*

Test method	Agreement (%)	
	Gram-positive organisms	Gram-negative organisms
MS-2	95.2	92.4
Disk diffusion	91.0	96.8
Microdilution	96.2	94.4

system with gram-positive isolates was 95.2% and with gram-negative organisms was 92.4% when compared with the reference antibiogram. Comparable values for the disk diffusion method were 91.0 and 96.8%, respectively, and for the microdilution method, 96.2 and 94.4%, respectively.

MS-2 MIC results. As noted previously, the MS-2 system produces susceptibility results in both qualitative (resistant, intermediate, and susceptible) and quantitative (MIC) modes.

To evaluate the accuracy of MICs obtained from the MS-2, we compared them with MICs from the microdilution method.

The "true" MIC was determined from the eight microdilution endpoints obtained for each organism-antibiotic combination in the four laboratories. Agreement between the MS-2 and microdilution MICs was defined as one of the following categories: (i) the MS-2 MIC indicated a susceptible threshold value ($< X$) and the microdilution endpoints were also less than X, e.g., MS-2 MIC <4 μg/ml and microdilution MIC = 2 μg/ml; (ii) the MS-2 MIC gave a resistant threshold value ($> Y$) and microdilution values were also greater than Y, e.g., MS-2 MIC >12 μg/ml and microdilution MIC = 16 μg/ml; or (iii) the MS-2 results were finite computed MICs, and the geometric mean ($\log_2$) of MS-2 values was within ±1.0 twofold increment of the geometric mean of microdilution values (agreement to within ± one well), e.g., MS-2 geometric mean = 3.6 and microdilution geometric mean = 4.1. Any set of data that failed to meet one of the above criteria was considered to be in disagreement. These data are shown in Tables 17 (gram-positive cocci) and 18 (gram-negative bacilli).

By organism, the overall MIC agreement ranged from a low of 89% for *Enterobacter* spp. to a high of 100% for the nonenterococcal group D streptococci. By specific antibiotic, the overall MIC agreement was greater than 90% for all drugs except carbenicillin tested against *P. aeruginosa* (82%) and penicillin (85%) when

TABLE 17. *Phase IV: agreement between MICs determined by MS-2 and microdilution for gram-positive cocci*

Drug	Agreement (%)				
	S. aureus	*S. epidermidis*	Enterococci	Nonenterococcal group D streptococci	Overall
Ampicillin	NT[a]	NT	100	100	100
Cephalothin	100	100	93	100	98
Chloramphenicol	100	100	100	100	100
Clindamycin[b]	100	95	67	100	90
Erythromycin	100[c]	100	87	100	97
Gentamicin	100	100	100	100	100
Kanamycin	95	100	100	100	98
Methicillin	90	95	100	100	95
Penicillin G[c]	80	80	93	100	85
Tetracycline	100	100	100	100	100
Overall	96	97	94	100	96[d]

[a] NT, Not tested.
[b] Original high level disks.
[c] Inducer added.
[d] Composite full agreement.

TABLE 18. *Phase IV: agreement between MICs determined by MS-2 and microdilution for gram-negative bacilli*

Drug	Agreement (%)							
	E. coli	*Klebsiella* spp.	*Enterobacter* spp.	*P. mirabilis*	Other *Proteus* spp.[a]	*P. aeruginosa*	Other[b]	Overall
Ampicillin	95	100	63	100	100	100	93	92
Carbenicillin (low)[c]	100	100	89	100	75		93	94
Carbenicillin (high)[c]						82		82
Cephalothin	95	100	84	100	100	100	100	96
Chloramphenicol	100	100	100	92	100	93	93	96
Colistin	100	100	95	100	88	100	100	98
Gentamicin	100	90	95	100	100	96	100	97
Kanamycin	95	100	100	100	100	96	100	98
Nitrofurantoin	100	90	74	100	75	93	93	90
Tetracycline	100	100	100	100	100	93	100	98
Overall	98	98	89	99	93	95	97	95[d]

[a] See Table 3, footnote *b*.
[b] See Table 3, footnote *c*.
[c] See Table 4, footnote *a*.
[d] Composite full agreement.

tested with gram-positive cocci. Overall composite agreement of MICs for all phase IV organism-antimicrobial agent combinations was 95 to 96%. When individual organism-antimicrobial agent combinations were examined, those showing agreement of <90% were clindamycin and enterococci (67%), erythromycin and enterococci (87%), penicillin and *S. aureus* (80%), penicillin and *Staphylococcus epidermidis* (80%), ampicillin and *Enterobacter* spp. (63%), carbenicillin and *Enterobacter* spp. (89%), carbenicillin and other *Proteus* spp. (75%), carbenicillin and *P. aeruginosa* (82%), cephalothin and *Enterobacter* spp. (84%), nitrofurantoin and *Enterobacter* spp. (74%), and nitrofurantoin and other *Proteus* spp. (75%).

Since the great majority of strains tested showed either clear-cut resistance or clear-cut susceptibility to a given antimicrobial agent, most of the MS-2 MIC results fell into categories i or ii above, i.e., a comparison of threshold values. Consequently, an additional specific analysis was made of the category iii results, i.e., those in which finite MICs were computed by the MS-2 for organism-antimicrobial agent combinations having intermediate levels of suscep-

tibility. This occurred in less than 10% of the combinations tested. For each such combination, the geometric mean (log_2) of the four MS-2 MICs was compared to the geometric mean of eight microdilution MICs, and the level of agreement to within ± one dilution was determined. The results of this analysis are shown in Table 19. Most of the discrepancies noted were due to intermediate MICs on resistant strains.

Improved disk concentrations. After completing phase IV, an additional ancillary study was performed in two of the collaborative laboratories (Center for Disease Control and the University of Washington) to validate findings by the manufacturer that reduced MS-2 disk concentrations for penicillin, clindamycin, and methicillin provided a substantial reduction in discrepancies noted with gram-positive cocci (Table 13). All gram-positive organisms in the challenge collection were retested with the reduced disk concentrations, and the results are summarized in Table 20. The data showed that the 8% rate of very major discrepancies previously observed with penicillin and clindamycin was reduced to less than 1% by use of the new disk concentrations, and the high level of accord with *S. aureus* was not affected.

Interlaboratory reproducibility. The level of category agreement between participating laboratories for all three susceptibility test procedures was examined and compared by using phase IV results with the challenge strains (Table 21). Total agreement, i.e., identical results in all four laboratories, ranged from 83.6% (microdilution with gram-negative bacilli) to 92.4% (MS-2 with gram-positive cocci). When examined for agreement in three of four laboratories, the values were substantially higher at 95% or greater. Although there were slight differences in the reproducibilities of the three methods, these were not considered to be significant. We concluded that reproducibility of results from the MS-2 system was at least equal to that of the reference methods when using lot-matched reagents.

Analysis time. An analysis was made of actual time needed to obtain susceptibility results with the MS-2 for all testing done in phase IV. Since analysis time in the MS-2 system is a function of biological growth rate rather than clock time, a range of values was observed (Table 22). The composite average test time for all challenge strains in all four laboratories was 4.2 h.

DISCUSSION

The MS-2 has certain similarities to the Autobacl system (7) in that tests are performed in a cuvette cartridge having multiple individual chambers, single concentrations of antimicrobial agents are eluted into the broth from impregnated paper disks, and tests are performed on pure cultures in 3 to 6 h to obtain results on the same day that susceptibility tests are initiated.

The MS-2 system differs in the method in which these analyses are made. Growth (turbidity) in each cuvette chamber is monitored at 5-min intervals, and the data are stored in the computer. From these data, growth curves are generated by the computer for each organism-drug combination and also for the organism in the absence of antimicrobial agent (growth control).

The growth curves can be visually displayed in research models of the MS-2 system, but in the clinical instrument they are generated and compared internally. After the susceptibility analyses are made by the computer, the results are automatically printed out. Isolates are categorized as susceptible if essentially complete inhibition or lysis has occurred and as resistant if growth response approximates that of the untreated control. Between these qualitative thresholds, MICs are automatically calculated and reported.

Other differences are that organisms are permitted to start growing before they are brought into contact with the antimicrobial agents, and the final susceptibility results are automatically determined when adequate reaction between the organism and the drugs has occurred.

In a previous collaborative study (7), an automated testing system was evaluated essentially from data collected by using only recent clinical isolates from within the United States. As a consequence, certain important antibiotic-organism resistance patterns were poorly represented because of their rarity in this country. For that reason, we developed the set of selected challenge strains (phase III strains) for the present study. This collection was very useful both to the manufacturer and to the evaluators in defining potential problem areas with the new system (e.g., the need for an erythromycin inducer in tests with certain staphylococci). We strongly recommend that a similar specific collection of challenge organisms should be included in any future evaluation of susceptibility testing systems.

When discrepancies or other problems occurred during testing, printed growth curves from that test were referred to for clarification. In each discrepancy situation, such growth curves clearly indicated why the MS-2 made a particular determination. This proved to be extremely useful in resolving discrepancies, cor-

TABLE 19. *Comparison of MS-2 finite MICs with geometric mean microdilution MICs*[a]

Drug	Organism	No. of MICs			
		Total MS-2	Within	Below	Above
Ampicillin	*Enterobacter* spp.	8	3	5[b]	
	Serratia spp.	5	5		
	Proteus spp.	1	1		
	Klebsiella spp.	1	1		
Carbenicillin	*Klebsiella* spp.	1	1		
	Proteus spp.	2	1		1[c]
	Enterobacter spp.	2	1		1[c]
	Pseudomonas spp.	2	1		1[c]
Cephalothin	*Enterobacter* spp.	5	2	3[b]	
	Proteus spp.	1	1		
	S. epidermidis	1	1		
	Enterococci	2	2		
Chloramphenicol	*E. coli*	1	1		
	Proteus spp.	1	1		
	Acinetobacter spp.	1	1		
	Pseudomonas spp.	3	2	1[b]	
	S. epidermidis	1	1		
Clindamycin	*S. epidermidis*	1		1[b]	
Colistin	*Proteus* spp.	1		1[b]	
	Enterobacter spp.	1		1[b]	
Gentamicin	*Pseudomonas* spp.	1		1[b]	
	Enterococci	6	6		
	Group D streptococci	3	3		
Kanamycin	*Pseudomonas* spp.	1	1		
	Group D streptococci	1	1		
	S. aureus	1			1[d]
Methicillin	*S. epidermidis*	6	5		1[d]
Nitrofurantoin	*Enterobacter* spp.	11	11		
	Proteus spp.	7	6	1[b]	
	Pseudomonas spp.	2		2[b]	
	Klebsiella spp.	5	5		
Penicillin	*S. epidermidis*	6	6		
	S. aureus	2	2		
	Enterococci	2	2		
Tetracycline	*Pseudomonas* spp.	2	2		
	Serratia spp.	1	1		
Totals		98	77	16	5

[a] Total number of MS-2 MICs compared with microdilution MICs (geometric mean ± one well).
[b] Intermediate MICs on resistant strains.
[c] Intermediate MICs on susceptible strains.
[d] Five-tube or greater variance in microdilution MICs.

recting problem areas, and in understanding the actual kinetics of specific organism-antimicrobial agent interactions.

Comparison of phase II MS-2 results with those from disk diffusion showed a significant level of discrepancies when enterococci were tested against penicillin, cephalothin, gentamicin, and kanamycin (Table 7). The discrepancies

TABLE 20. *Ancillary study of lower content of penicillin, methicillin, and clindamycin disks: agreement of MS-2 and reference antibiogram*

Organism or antibiotic	No. of organisms tested	Disk concn (μg)		Full accord (%)	Discrepancies (%)		
		New	Old		Very major	Major	Minor
Organism							
S. aureus	42			96.7	1.6	0.3	1.4
S. epidermidis	40			97.2	1.3	0.8	0.7
Group D streptococci							
Enterococcal	32			97.6	1.7	0.6	0.1
Nonenterococcal	8			94.4	0.7	1.4	3.5
Antibiotic							
Clindamycin[a]	122	0.5	1.0	98.4	0.8	0	0.8
Methicillin[a]	122	4.0	5.0	94.3	0	2.4	3.3
Penicillin[a]	122	1.0[b]	2.0[b]	98.4	0.8	0	0.8
Overall accord				96.8	1.5	0.6	1.0

[a] Tested with organisms listed above.
[b] Units of penicillin.

TABLE 21. *Interlaboratory reproducibility of the three susceptibility methods with the phase III challenge strains*

Organism	No. of laboratories in agreement[a]	Agreement (%)		
		MS-2	MTD[b]	K-B[c]
Gram-positive cocci	4	92.4	87.5	86.7
	3	97.3	95.8	96.2
Gram-negative bacilli	4	87.1	83.6	90.1
	3	96.2	95.0	97.4
All organisms (composite)[d]	4	88.9	85.0	88.9
	3	96.6	95.3	97.0

[a] Total of four laboratories.
[b] MTD, Microdilution method.
[c] K-B, Kirby-Bauer standardized disk diffusion method.
[d] Total numbers of drug-organism combinations tested were as follows: MS-2, 6,055; MTD, 6,000; K-B, 6,050.

TABLE 22. *Summary of MS-2 test times*

Organism	Test time (h)	
	Range	Mean
Gram-positive		
S. aureus	3.0-5.8	4.2
S. epidermidis	3.3-6.5	4.8
Enterococci	3.2-5.8	4.0
Other	3.5-5.8	4.6
Gram-negative		
E. coli	2.9-5.3	3.8
P. aeruginosa	2.8-6.4	4.6
Enterobacter spp.	2.8-6.2	4.2
P. mirabilis	3.7-4.7	4.1
Indole-positive *Proteus* spp.[a]	3.5-6.3	4.7
Klebsiella spp.	3.2-5.2	3.9
Other[b]	3.3-6.4	4.5
Composite of all tests		4.2

[a] See Table 3, footnote *b*.
[b] See Table 3, footnote *c*.

observed with penicillin, cephalothin, and kanamycin were predominantly minor and due to the large number of intermediate results by disk diffusion. In the case of penicillin, this may have been the result of interpreting zone diameters for enterococci on the same basis as penicillin and gram-negative bacilli (12 to 21 mm as intermediate). The MS-2 usually recorded these strains (with MICs of 4 to 8 μg/ml) as resistant. The aminoglycoside discrepancies were probably due to the known difficulty in performing disk diffusion tests with these combinations. Recommendations have been made that enterococci not be tested with aminoglycosides by the disk method (6). Phase II agreement between the two reference methods themselves (Table 9) showed low accord for these same enterococci-antimicrobial agent combinations, indicating that estimations of accuracy depend heavily upon which system is used as a reference.

For the gram-positive challenge strains (phase IV testing), initial problems concerned the very major discrepancies observed with penicillin and clindamycin. The majority of penicillin discrepancies occurred with strains of *S. epidermidis*

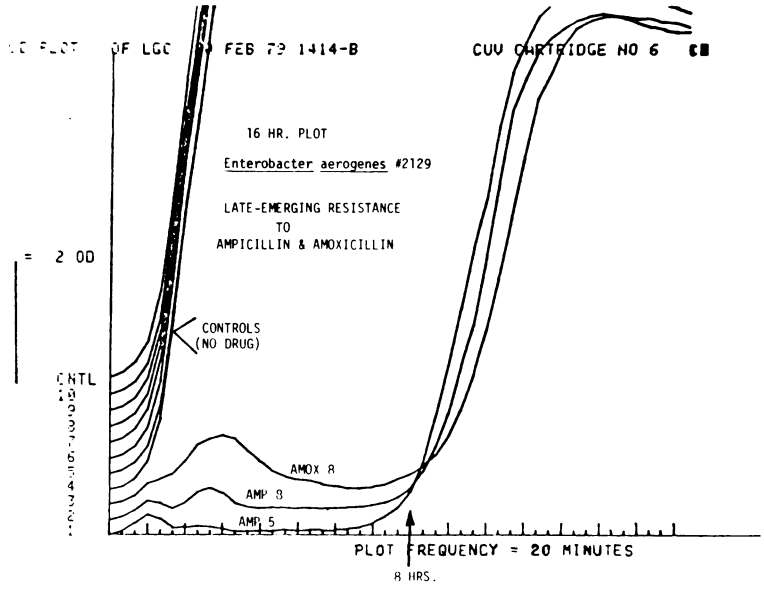

FIG. 3. *MS-2-generated 16-h growth curves for a strain of Enterobacter aerogenes. Curves 1 and 2 show response to ampicillin (Amp) at 5 and 8 μg/ml, respectively; curve 3 is with amoxicillin (Amox) at 8 μg/ml. Note initial growth, subsequent lysis (downward slope), and suppression, with emergence of resistant outgrowth at 7 to 8 h. Other abbreviations are as in the legend to Fig. 2.*

that produced low levels of β-lactamase. One approach to resolving this problem was to reduce the amount of antibiotic substrate, thus requiring less enzyme for the resistance breakthrough. This approach was highly successful (Table 20). Reducing the clindamycin concentration in the MS-2 disk also eliminated the discrepancies previously encountered with enterococci.

It should be reemphasized that diffusion tests with enterococci and aminoglycosides yielded inaccurate results and that these combinations should not be tested routinely in the clinical laboratory (6). Discrepancies in results obtained by different methods when β-lactamase-negative organisms are tested with penicillin were largely due to the definitions of the categories of susceptibility that were used.

Problems encountered with gram-negative bacilli were essentially limited to some strains of *Enterobacter* and the β-lactam antibiotics ampicillin and cephalothin. These discrepancies were for the most part false-susceptible results. Growth curves for such strains in the presence of these drugs showed that within the normal 4- to 6-h test period, growth, lysis, and complete suppression had occurred, and the MS-2 had correctly (for that time period) analyzed the reaction as susceptible. However, subsequent emergent resistant growth could be clearly observed, occurring 8 to 14 h after antibiotic exposure (Fig. 3). Recent studies of some of these strains suggest that this resistant component may represent emergence of low-level mutants present in small numbers in the original susceptible population (3). It is possible that in the disk diffusion test some induction occurs and that the use of Mueller-Hinton agar contributes to the diffusion result. At present, no final solution has been defined for the *Enterobacter* spp.-β-lactam discrepancies, although preliminary studies suggest that using a different cephalosporin and penicillin may substantially decrease this problem.

In the miscellaneous gram-negative organism group designated "other," the great majority of discrepancies occurred with *Serratia* spp. and colistin (false-susceptible results). Of the very major discrepancies seen with colistin, 79% occurred with *Serratia* spp. This problem and this combination is contraindicated for the MS-2 system. When *Serratia* and colistin results are removed from the phase IV data, the very major discrepancies with colistin are reduced from 7.6 to 1.8%, and the overall agreement with colistin is 96.4%.

The microdilution MICs were determined in modified Sensitest broth (Oxoid). Since the disk diffusion test was the prime point of reference, it was felt to be important to use the same medium in the microdilution test as in the MS-2 tests. However, microdilution tests in two laboratories using the modified Sensitest broth and

two lots of Mueller-Hinton (BBL Microbiology Systems and Difco Laboratories) showed that the MICs were essentially the same in both media.

The MICs generated by the MS-2 differ from the conventional MICs in at least two respects. First, they are short-term MICs based upon incubation times of 4 to 6 h, as opposed to 18 to 24 h. Second, MS-2 results are calculated from a single concentration of drug as opposed to conventional testing in which multiple concentrations are used. Although it was difficult to directly compare microdilution MICs (the concentrations tested were a wide range of twofold geometric progressions) with those obtained from the MS-2 (limited-range continual concentrations in an arithmetic progression and threshold values), when based upon the correlation assumptions described earlier, the overall level of agreement for all antibiotic-organism combinations was 95% (Tables 17 and 18).

On the basis of these studies, the MS-2 system can be expected to yield rapid, accurate, and reproducible antimicrobial susceptibility results with most of the nonfastidious, facultative, or aerobic bacterial pathogens isolated from clinical materials. The overall agreement compared with defined susceptibility results (reference antibiogram) for the set of phase IV challenge strains was 96.8% for the gram-positive cocci and 92.4% for gram-negative organisms. The time required to obtain test results in the MS-2 was between 3 and 6 h. The efficacy of this system for testing more fastidious organisms and anaerobic bacteria has not yet been determined.

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