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# Clinical Evaluation of Automated Antibiotic Susceptibility Testing with the MS-2 System

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The MS-2 (Abbott Laboratories) system for automated antimicrobial susceptibility testing was evaluated for both accuracy and general utility in our clinical laboratory. A total of 984 fresh clinical bacterial isolates (745 gram-negative, 239 gram-positive) were tested with the MS-2 system, and results were compared directly with those from a conventional agar disk diffusion method. Discrepancies between the two methods were categorized as very major, major, and minor. For gram-positive isolates, full accord (all discrepancies considered) was 91.6%, and essential accord (minor discrepancies not included) was 96.2%. With gram-negative isolates, full accord was found to be 93.9%, with essential accord of 97.9%. Agreement as a function both of organism group and of antimicrobial agent was determined. Full accord of 90% or more was found for all major organism groups tested, with the exception of enterococci, where discrepant results between the two methods were observed. Mean test time for all isolates tested was 4.3 h. The MS-2 was found to be an accurate and highly automated instrument which required minimal technician time and was readily adaptable to work flow in our clinical laboratory.

The need for rapid, automated instrumentation for performing routine procedures in the clinical microbiology laboratory is an obvious one. Since antibiotic susceptibility testing should guide the physician in the choice of appropriate antimicrobial therapy, it is especially important that accurate susceptibility test results be made available as quickly as possible. Automated systems should also provide the laboratory with increased reliability and uniformity of results, as well as providing greater efficiency and convenience over manual methods. In addition, any such instruction should be readily adaptable to normal work flow in the laboratory and require minimal technical attention and interface time. At present, several mechanized or semiautomated instruments are available for laboratory susceptibility testing applications (3, 5, 6). The purpose of this report is to compare, in a clinical setting, antibiotic susceptibility results obtained from 984 clinical bacterial isolates by using the Abbott MS-2 system with those obtained by a standardized manual disk diffusion method, and to evaluate the general adaptability of the MS-2 system to a clinical microbiology laboratory.

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## MATERIALS AND METHODS

**Organisms.** Organisms used in this study were current bacterial isolates collected from a variety of patient specimens, plus a limited number of stock cultures. Isolates were grown on a variety of primary isolation media appropriate to the type of clinical specimen. For MS-2 susceptibility testing, organisms were obtained directly from the isolation plate by using a sterile cotton swab or inoculating loop, transferred to a 4-ml tube of saline, blended on a Vortex mixer, and adjusted to a visual turbidity equal to an 0.5 McFarland barium sulfate standard. For disk diffusion testing, organisms were transferred from isolation medium to a tube of brain heart infusion broth and processed by a standardized method (1, 2). Organisms were stored on either Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) or blood agar slants.

**Reagents and media.** Primary isolation media used included plates of 5% sheep blood agar, phenylethyl agar, xylose-lysine-deoxycholate agar, colistin-nalidixic acid agar, Hektoen agar, and chocolate agar. Brain heart infusion broth and Mueller-Hinton agar were used for diffusion testing, and a modified Iso-Sensitest broth (Oxoid) was supplied by the manufacturer for use in the MS-2 system. Antimicrobial disks used for agar diffusion were of the standard concentrations recommended for this method (2) and were obtained from regular commercial sources. Concentrations of disks used in the MS-2 (supplied by the manufacturer) are shown in Table 1.

**Susceptibility testing methods. (i) Disk diffusion method.** The standardized disk agar diffusion

method used in this study was that defined by the National Committee for Clinical Laboratory Standards (2).

**(ii) MS-2 method.** The MS-2 is a computerized automated instrument system (Fig. 1) for determining antimicrobial susceptibility of bacterial isolates. Tests are carried out in single-use plastic cartridges in which microbial growth is repeatedly monitored at 5-min intervals by individual electro-optical systems. Susceptibility results are normally obtained within 3 to 5 h and are reported as resistant, susceptible, or intermediate. For those isolates having intermediate susceptibility, a finite minimal inhibitory concentration is automatically computed, and threshold minimal inhibitory concentrations are also produced for both resistant and susceptible isolates.

Results are determined by computer analysis through use of appropriate algorithms, by comparing growth obtained in each antibiotic test chamber (cuvette) with that of an untreated control. The system is composed of (i) a mechanized antimicrobial disk loader/sealer unit, (ii) one or more analysis modules containing the multiple optical systems for monitoring bacterial growth within the cuvette cartridges, and (iii) a control module containing a microprocessor (computer) which controls the system operation, stores and evaluates data from the analysis module, and prints out final results (Fig. 1). To use the MS-2, an appropriate battery of antimicrobial disks was selected, dispensed, and sealed into each cartridge, using the loader/sealer unit. One cartridge was used for each isolate to be tested. Fifteen milliliters of MS-2 broth was then placed into the upper chamber of each cuvette cartridge, and the medium was inoculated with a suspension of the test organism (200 μl if gram negative, 400 μl for gram positives). To prepare the inoculum, several morphologically similar colonies were touched with a sterile loop or cotton swab, transferred to a tube containing 4 ml of physiological saline, and blended in the Vortex mixer to obtain a uniform suspension. The suspension was then visually adjusted to match an 0.5 McFarland barium sulfate standard. After inoculation of the cartridge, the filling port was sealed, and the cartridge was inserted into one position within an analysis module. Each analysis module contains eight operating cartridge positions. Multiple analysis modules can be accommodated by each control module, thus providing increased capacity for simultaneous testing as required. Insertion of the inoculated cartridge into the analysis module initiates the automated analysis cycle, and no further operator attention is required. Cartridges are maintained at a constant 35°C within the analysis module and are subjected to continuous linear impact agitation during the test. At 5-min intervals, each cuvette chamber is automatically read for growth (optical density) by individual light-emitting diodes and matched detectors.

Discrepancies between the results obtained with the MS-2 and by disk diffusion methods were considered to be "very major" if the organism was found susceptible by the MS-2 but resistant by Kirby-Bauer, "major" if the opposite was found, and "minor" if a discrepant intermediate result was obtained by either of the two methods. In the accompanying tables of results, the numbers and types of discrepancies are listed in this order, with the total number of strains tested; i.e., an entry of 0,1,2/77 indicates no very major, one major, and two minor discrepancies observed in testing 77 isolates with a specific antimicrobial agent.

## RESULTS

Results with gram-positive isolates (Table 2) show that full accord was greater than 90% for all antibiotics tested except erythromycin (88.3%), gentamicin (84.5%), and penicillin (81.6%). Essential accord (minor discrepancies disregarded) ranged from a low of 89.5% (penicillin) to 99.6% agreement for cephalothin and chloramphenicol. When examined by organism group, full accord was 90% or more for *Staphylococcus aureus*, *Staphylococcus epidermidis*, and group B streptococci, but enterococci showed a low full accord of 65.4%, resulting from a high incidence of minor discrepancies with penicillin, cephalothin, and kanamycin, major discrepancies with gentamicin, and very major discrepancies with clindamycin. Very major discrepancies occurred with penicillin with both

TABLE 1. *MS-2 antibiotic batteries and disk concentrations*

| Cuvette position no. | Gram-positive organisms | | Gram-negative organisms (non-*Pseudomonas*) | | *Pseudomonas* | |
|---|---|---|---|---|---|---|
| | Drug | Amt (μg) | Drug | Amt (μg) | Drug | Amt (μg) |
| 1 | Cephalothin | 10 | Ampicillin | 5 | Ampicillin | 9 |
| 2 | Clindamycin | 1 | Tobramycin | 4 | Tobramycin | 4 |
| 3 | Erythromycin | 3 | Carbenicillin | 16 | Carbenicillin | 100 |
| 4 | Gentamicin | 4 | Cephalothin | 10 | Cephalothin | 10 |
| 5 | Methicillin | 5 | Chloramphenicol | 9 | Chloramphenicol | 9 |
| 6 | Penicillin | 2 | Gentamicin | 4 | Gentamicin | 4 |
| 7 | Tetracycline | 5 | Kanamycin | 8 | Kanamycin | 8 |
| 8 | Kanamycin | 8 | SXT[a] | 25 | Colistin | 6 |
| 9 | Chloramphenicol | 9 | Tetracycline | 5 | Tetracycline | 5 |
| 10 | Ampicillin | 2.5 | Amoxicillin | 8 | Nitrofurantoin | 30 |

[a] Sulfamethoxazole, 23.75 μg, plus trimethoprim, 1.25 μg.

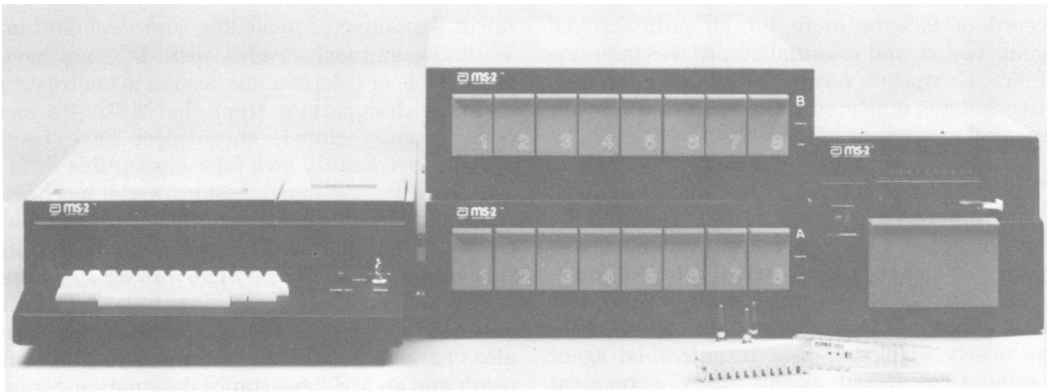

FIG. 1. *The MS-2 System. From left to right are the control module, two analysis modules, and the disk loader/sealer. Several types of cuvette cartridges and adapters are shown in the foreground.*

TABLE 2. *Discrepancies and accord between MS-2 and disk diffusion*[a]

| Antimicrobial agent | Results with isolates: | | | | Accord by antimicrobial agent | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *S. epidermidis* | Enterococci | Other[b] | % Full accord | % Essential accord |
| Ampicillin | NT[c] | NT[c] | 0,0,0/15 | 0,1,0/28 | 97.6 | 97.6 |
| Cephalothin | 0,0,0/119[c] | 1,0,4/77 | 0,0,9/15 | 0,0,0/28 | 94.1 | 99.6 |
| Chloramphenicol | 0,0,1/119 | 0,1,1/77 | 0,0,0/15 | 0,0,0/28 | 98.7 | 99.6 |
| Clindamycin | 0,1,0/119 | 1,1,2/77 | 4,0,0/15 | 2,0,0/28 | 95.4 | 96.2 |
| Erythromycin | 4,1,6/119 | 4,2,5/77 | 1,0,5/15 | 0,0,0/28 | 88.3 | 95.0 |
| Gentamicin | 0,2,7/119 | 1,4,5/77 | 0,9,1/15 | 1,2,5/28 | 84.5 | 92.0 |
| Kanamycin | 0,2,5/119 | 0,1,2/77 | 0,1,9/15 | 0,1,1/28 | 90.8 | 97.9 |
| Methicillin | 0,0,1/119 | 4,1,4/77 | 0,1,0/15 | 0,1,1/28 | 94.6 | 97.1 |
| Penicillin | 9,0,1/119 | 13,1,6/77 | 1,9,11/15 | 0,1,1/28 | 81.6 | 89.5 |
| Tetracycline | 0,1,3/119 | 0,2,0/77 | 0,0,0/15 | 0,0,4/28 | 95.8 | 98.7 |
| % Full accord by organism group | 95.8 | 90.4 | 65.4 | 92.4 | 91.6[d] | 96.2[d] |
| % Essential accord by organism group | 98.1 | 94.7 | 88.7 | 96.7 | | |

[a] Results with 239 gram-positive isolates, shown as number of very major, major, and minor discrepancies/number of isolates tested with the specific drug.

[b] Other gram-positive isolates tested included 26 group B streptococci and 2 *Listeria* spp.

[c] Staphylococci were not tested with ampicillin.

[d] Overall composite accord of all gram-positive isolates.

groups of staphylococci, but were most evident with *S. epidermidis* isolates. All other gram-positive organism/antimicrobial agent combinations showed acceptable levels of agreement. Overall full accord for all gram-positive strains was 94%.

With the gram-negative specimens (Table 3), all organism groups tested showed full accord of 90% or more, and essential agreement was above 96% in each case. Examination of individual organism-antibiotic combinations showed only one area (*Enterobacter*-cephalothin) where combined major and very major discrepancies exceeded 10%. This particular combination showed 10 false-susceptible results of 58 tested, suggesting that some cephalothin-resistant strains of *Enterobacter* were not being defined correctly by the MS-2. Several other specific combinations showed a combined major/very major discrepancy rate of more than 5%, including *Serratia*-sulfamethoxazole-trimethoprim, *Pseudomonas*-carbenicillin, and *Enterobacter*-ampicillin.

Results by individual antibiotic showed full

accord of 92% or more for all antimicrobial agents tested, and essential accord was in excess of 96%. Composite results for all 745 gram-negative isolates indicated full accord of 94%, with essential agreement of 98%.

An overall summary of results is shown in Table 4. Minor discrepancies were the most frequently observed type (4.1%), followed by very majors at 1.4% and majors (1.2%). All categories of discrepancies were observed somewhat more frequently with gram-positive isolates, which will be discussed later. In total, for the nearly 9,000 organism-antimicrobial agent combinations tested in this study, agreement between MS-2 and disk diffusion results was found to be 93.3% full accord and 97.4% essential agreement. Results from the MS-2 were generally obtained within 3 to 5 h after initiation of the test procedure. Actual times to final printout of results for all organism groups tested are shown in Table 5.

## DISCUSSION

As compared to disk diffusion results, accuracy of the MS-2 was found to be 90% or greater for all organism groups tested, with the exception of enterococci. Closer examination of results from this group (Table 2) shows a high incidence of major and minor discrepancies with gentamicin, kanamycin, penicillin, and cephalothin. With the aminoglycosides, disk diffusion gave susceptible or intermediate results in contrast to resistant designations from the MS-2. As has been recently noted, enterococci have been found to frequently give false-susceptible diffusion zones with aminoglycosides, and it has been recommended that such combinations should not be tested by disk diffusion methods (5). In this case it would appear that the MS-2 results are probably correct. Regarding the beta-lactam antibiotics with enterococci, discrepancies were also characterized by an intermediate diffusion result and an MS-2 resistance designation. Since most strains of enterococci are considered to be cephalothin resistant, the MS-2 result may again be more indicative. It is important to note that in any comparison of methodologies, accuracy is a relative term. What one measures is a level of agreement between two methods, each of which may have its own distinct strengths and weaknesses. It seems probable that the low level of accord observed with enterococci in these studies may be due to the specific problems noted with the diffusion assay. Conversely, examination of other gram-positive results (Table 2) indicates a significant level of false-susceptible MS-2 results with penicillin-staphylococci (particularly *S. epidermidis*), with clindamycin-en-

TABLE 3. *Discrepancies and accord between MS-2 and disk diffusion*[a]

| Antimicrobial | Results with isolates: | | | | | | | Accord by antimicrobial agent | |
|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* | *Klebsiella* | *Enterobacter* | *Serratia* | *Proteus* | *Pseudomonas* | Others[b] | % Full accord | % Essential accord |
| Ampicillin | 0,1,6/142 | 0,0,1/46 | 3,0,1/32 | 0,0,1/40 | 0,2,1/69 | 5,0,0/145 | 3,1,1/46 | 94.9 | 97.0 |
| Carbenicillin[c] | 0,1,1/215 | 0,2,6/85 | 1,0,1/58 | 0,0,2/79 | 0,1,1/127 | 4,7,10/135 | 1,2,4/46 | 94.1 | 97.3 |
| Cephalothin | 1,2,8/215 | 0,0,9/85 | 10,0,4/58 | 0,0,1/79 | 4,4,6/127 | 1,0,3/135 | 0,2,2/46 | 92.3 | 96.8 |
| Chloramphenicol | 1,0,2/215 | 0,1,4/85 | 0,0,3/58 | 1,2,7/79 | 5,2,10/127 | 0,1,7/135 | 1,0,7/46 | 92.8 | 98.1 |
| Colistin[d] | | | | | | 1,0,2/135 | | 97.8[d] | 99.3[d] |
| Gentamicin | 0,0,1/215 | 1,0,1/85 | 0,0,2/58 | 2,0,1/79 | 1,1,2/127 | 1,0,7/135 | 0,0,3/46 | 96.9 | 99.2 |
| Kanamycin | 1,9,6/215 | 1,2,4/85 | 0,0,1/58 | 0,1,19/79 | 1,0,2/127 | 0,0,3/135 | 0,0,4/46 | 92.7 | 98.0 |
| Nitrofurantoin[d] | | | | | | 2,1,4/135 | | 94.8[d] | 97.8[d] |
| Sulfamethoxazole/trimethoprim | 1,3,9/215 | 1,1,3/85 | 0,1,2/58 | 5,1,2/79 | 7,4,6/127 | | 0,2,1/46 | 92.0 | 95.7 |
| Tetracycline | 1,1,4/215 | 1,0,2/85 | 0,0,6/58 | 1,2,8/79 | 1,0,1/127 | 1,0,8/135 | 1,0,4/46 | 94.9 | 98.8 |
| Tobramycin | 0,0,6/215 | 0,0,6/85 | 0,0,5/58 | 3,1,8/79 | 0,2,3/127 | 1,0,5/135 | 1,1,2/46 | 94.1 | 98.8 |
| % Full accord by organism group | 96.5 | 93.7 | 91.9 | 89.9 | 93.8 | 94.5 | 89.6 | | |
| % Essential accord by organism group | 98.8 | 98.6 | 97.0 | 97.2 | 96.8 | 98.1 | 96.4 | 93.9[e] | 97.9[e] |

[a] Results with 745 gram-negative isolates.

[b] Other gram-negative isolates tested included 10 *Salmonella*, 9 *Providencia*, 13 *Citrobacter*, 7 *Acinetobacter*, and 7 miscellaneous. Numbers indicate number of very major, major, and minor discrepancies/total number of isolates tested.

[c] 100-μg disk was used for testing *Pseudomonas*; 16-μg disks were used for all other gram-negative isolates.

[d] Colistin and nitrofurantoin were tested only against *Pseudomonas* spp.

[e] Overall composite accord of all gram-negative isolates.

TABLE 4. *Summary of MS-2 susceptibility test results*

| Isolates | No. of isolates tested | Total no. of organism-antibiotic test combinations | No. (%) of MS-2 discrepancies by category | | | | % Accord | |
|---|---|---|---|---|---|---|---|---|
| | | | Very major | Major | Minor | Total | Full | Essential |
| Gram-positive | 239 | 2,190 | 46 (2.1) | 37 (1.7) | 100 (4.6) | 183 (8.4) | 91.6 | 96.2 |
| Gram-negative | 745 | 6,603 | 77 (1.2) | 64 (1.0) | 262 (4.0) | 403 (6.2) | 93.9 | 97.9 |
| Overall composite | 984 | 8,793 | 123 (1.4) | 101 (1.2) | 362 (4.1) | 586 (6.7) | 93.3 | 97.4 |

TABLE 5. *MS-2 test times by organism group*

| Organism group | No. of isolates tested | Range (min) | Mean[a] (h) |
|---|---|---|---|
| *E. coli* | 215 | 114–355 | 3.8 |
| *Pseudomonas* | 135 | 160–440 | 4.8 |
| *Proteus* | 127 | 185–465 | 4.5 |
| *Klebsiella* | 85 | 115–406 | 4.3 |
| *Serratia* | 79 | 160–360 | 4.5 |
| *Enterobacter* | 58 | 190–355 | 4.3 |
| *Citrobacter* | 13 | 175–335 | 4.2 |
| *Salmonella* | 10 | 210–310 | 4.3 |
| *Providencia* | 9 | 220–325 | 4.5 |
| *Acinetobacter* | 7 | 230–375 | 5.1 |
| *Shigella* | 2 | 245–265 | 4.3 |
| *S. aureus* | 119 | 140–380 | 3.9 |
| *S. epidermidis* | 77 | 135–410 | 5.0 |
| Group B streptococci | 26 | 170–340 | 3.7 |
| Group D streptococci | 15 | 150–345 | 3.9 |
| *Listeria* | 2 | 240–290 | 4.5 |

[a] Overall mean was 4.3 h.

terococci, and with erythromycin-staphylococci. These specific problem areas were quite real, but have since been effectively resolved by the manufacturer through use of lower disk concentrations for penicillin and clindamycin and by incorporation of subinhibitory levels of erythromycin inducer for gram-positive testing (4).

Regarding gram-negative isolates, all organism groups showed full accord of at least 90%, with essential accord of 96% or higher (Table 3). Discrepancies were randomly scattered throughout the data matrix except for the combination of *Enterobacter*-cephalothin, where a significant level of false-susceptible results was observed.

Overall accuracy of the MS-2 (Table 4) showed a full accord rate of 93.3% for the nearly 9,000 organism-antibiotic combinations tested, which we regard as highly acceptable performance for a rapid automated system. It should also be noted that there is a finite limit of complete agreement when any reference method is compared to itself, as in reproducibility experiments. For the disk diffusion test, this level has recently been estimated at 92% (6).

Regarding general utility of the system, we found that the MS-2 was easily adaptable to the daily work flow in our clinical laboratory. Since test time for most isolates was about 4 h (see Table 5), both a morning and late afternoon run were possible when needed. These studies utilized an MS-2 system with two analysis modules, giving us the capacity to test at least 32 isolates per day. Additional analysis modules would have provided greater capacity if required.

Since inoculum preparation for the MS-2 involved the same standardization procedure as used for the disk diffusion method, no special training was required, and our personnel adapted quickly to routine use of the instrument. Inoculation of the cuvette cartridge was straightforward, using 200- or 400-μl micropipettes which are readily available. Dispensing of the appropriate antibiotic disks into the MS-2 cartridges using the mechanized loader/sealer was found to be rapid and essentially trouble-free. In those rare instances where a jam occurred, it was readily corrected by pressing the mechanism release button on the rear of the unit. The use of a semiautomatic pipetting device for delivery of the MS-2 medium into the upper growth chamber of the cartridge was found to reduce set-up time, and is strongly recommended.

Upon completion of analysis in the MS-2, results are automatically printed out on thermal roll paper. One must, however, separate the individual reports and make copies, either by duplication or transcription to conventional hospital report forms.

One distinct advantage noted with the MS-2 was the fully automated operation after insertion of the test sample. Once the inoculated cartridges were loaded into the analysis module, no further operator attention was required, and the technologist was free to pursue other duties. During these studies, personnel operating the MS-2 also carried out the disk diffusion assays and their other regular bench assignments while the instrument was in operation.

The MS-2 culture medium as supplied was found to effectively support growth of the great majority of clinical isolates. As indicated by the manufacturer, however, it is not suitable for anaerobes or fastidious microorganisms, e.g., *Haemophilus* spp. or pathogenic *Neisseria*. We

were also unable to obtain uniform growth with limited numbers of group A streptococci and *Streptococcus pneumoniae*, although group B and D streptococci grew very well. Test stops (failure to grow in suspension) were also encountered with some strains of *S. epidermidis*.

In summary, the MS-2 system in our hands gave a highly acceptable level of accuracy while producing rapid (3- to 5-h) susceptibility tests results. Specimen preparation and use of the instrument were learned quickly, and the system was readily adapted into our normal laboratory work patterns and schedule. The ability of this instrument to operate without attention or technical manipulation was notable, and our results indicate that the MS-2 system can be expected to yield rapid and accurate results with those aerobic or facultative bacterial pathogens most frequently isolated from clinical specimens.

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