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Evaluation of a Rapid Inoculum Preparation Method for Agar Disk Diffusion Susceptibility Testing

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A rapid inoculum preparation method for agar disk diffusion susceptibility testing which does not require incubation before inoculation of Mueller-Hinton plates was compared with the National Committee for Clinical Laboratory Standards (NCCLS) method. A total of 326 fresh clinical isolates were tested, and the NCCLS-recommended quality control organisms were included with each test series. Randomly distributed interpretative changes occurred with 27 (0.8%) of the 3,215 test results for the clinical isolates. The quality control organisms were tested on 29 separate days, and results were consistently within tolerance limits. The rapid method was found to be equivalent to the standard NCCLS method and required less time and expense.

Several techniques are used to determine the in vitro susceptibility of bacteria to antimicrobial agents. The agar disk diffusion method described by Bauer et al. (1) and currently recommended with minor modifications by the U.S. Food and Drug Administration (2) and by the National Committee for Clinical Laboratory Standards (NCCLS) (4) is used in most clinical laboratories. In addition to the 16 to 18 h of incubation necessary for the inoculated Mueller-Hinton agar plates, the most time-consuming and logistically problematic step in the method is the preparation of the standardized inoculum which not only requires an incubation period of 2 to 8 h but also an interruption in the performance of the test. To circumvent these problems, some laboratories have modified the inoculum preparation method by placing a sufficient amount of bacterial growth, obtained from primary isolation plates, in a broth medium or in sterile water to equal a 0.5 McFarland barium sulfate turbidity standard. This rapid inoculum preparation method precludes the need for the 2 to 8 h of incubation and permits the completion of the test without interruption. However, there is a paucity of published data regarding its efficacy. We therefore decided to evaluate the rapid method by comparing it with the NCCLS standard method for inoculum preparation (4).

MATERIALS AND METHODS

Test organisms. A total of 326 fresh clinical bacterial isolates were tested. All isolates were identified to the species level by standard methods (3). The number of isolates of each species is shown in Table 1.

Test procedure. The antimicrobial susceptibility test procedure for the rapid inoculum preparation method and NCCLS standard method differed only in the method of inoculum preparation. Inocula for both the rapid method and the standard method were taken from the same primary isolation plates, and both methods were tested simultaneously. The rapid method consisted of suspending a sufficient number of bacteria obtained from well-isolated, morphologically similar colonies in 4 to 5 ml of sterile distilled water to equal a 0.5 McFarland barium sulfate turbidity standard (4). Within 15 min of preparation, this suspension was used to inoculate a Mueller-Hinton agar plate.

Antimicrobial agents. Each bacterial group, *Enterobacteriaceae, Pseudomonas* spp. and other nonfermenters, *Staphylococcus* spp., and enterococci, was tested with the antimicrobial agents listed in Tables 2 to 5.

Quality control. *Staphylococcus aureus* (ATCC

TABLE 1. Organisms tested

Organism	No. of clinical isolates
Escherichia coli	57
Klebsiella spp.	23
Serratia spp.	18
Enterobacter spp.	19
Morganella morganii	7
Proteus spp.	29
Providencia spp.	6
Salmonella spp.	12
Citrobacter spp.	6
Arizona hinshawii	2
Shigella spp.	5
Acinetobacter calcoaceticus	12
Pseudomonas aeruginosa	40
Pseudomonas spp.	7
Staphylococcus aureus	48
Staphylococcus epidermidis	20
Enterococci	15

TABLE 2. Differences in zone diameter between rapid and standard methods for *Enterobacteriaceae*[a]

Drug	No. of tests with following zone difference (mm):					
	Unchanged	1	2	3	4	≥5
Amikacin	43	65	46	28	1	1
Ampicillin	121	34	24	3	1	1
Carbenicillin	73	43	35	16	9	8
Cephalothin	71	51	35	17	8	2
Chloramphenicol	47	63	46	17	7	4
Gentamicin	45	64	42	23	6	4
Nitrofurantoin	59	62	32	22	8	1
Tetracycline	90	40	34	18	2	0
Tobramycin	48	62	47	17	6	4
Trimethoprim-sulfamethoxazole	50	54	42	17	16	5
Total %	35.2	29.2	20.8	9.6	3.4	1.6

[a] Based on 1,840 test results.

TABLE 3. Differences in zone diameter between rapid and standard methods for *Pseudomonas* spp. and other nonfermenters[a]

Drug	No. of tests with following zone difference (mm):					
	Unchanged	1	2	3	4	≥5
Amikacin	18	26	9	3	1	2
Ampicillin	54	3	0	0	2	0
Carbenicillin	30	22	4	2	1	0
Cephalothin	57	1	1	0	0	0
Chloramphenicol	52	3	3	0	1	0
Gentamicin	24	18	10	5	2	0
Nitrofurantoin	59	0	0	0	0	0
Tetracycline	37	9	7	2	4	0
Tobramycin	22	24	7	1	4	1
Trimethoprim-sulfamethoxazole	46	6	4	2	1	0
Total %	67.6	18.9	7.6	2.5	2.7	0.5

[a] Based on 590 test results.

TABLE 4. Differences in zone diameter between rapid and standard methods for *Staphylococcus* spp.[a]

Drug	No. of tests with following zone difference (mm):					
	Unchanged	1	2	3	4	≥5
Cephalothin	12	24	15	10	4	3
Chloramphenicol	18	16	20	9	3	2
Clindamycin	18	19	13	9	7	2
Erythromycin	24	18	14	7	5	0
Gentamicin	8	29	17	9	4	1
Nafcillin	23	27	15	0	1	2
Penicillin	20	20	7	7	10	4
Tetracycline	21	19	7	13	5	3
Trimethoprim-sulfamethoxazole	14	25	10	10	2	2
Vancomycin	21	22	16	6	1	2
Total %	26.4	32.3	19.8	11.9	6.3	3.0

[a] Based on 680 test results.

TABLE 5. Differences in zone diameter between rapid and standard methods for enterococci[a]

Drug	No. of tests with following zone difference (mm):					
	Unchanged	1	2	3	4	≥5
Ampicillin	1	6	7	1	0	0
Chloramphenicol	6	6	2	0	1	0
Erythromycin	1	9	2	2	1	0
Nitrofurantoin	7	6	1	0	1	0
Penicillin	2	8	2	3	0	0
Tetracycline	10	5	0	0	0	0
Trimethoprim-sulfamethoxazole	12	0	2	1	0	0
Total %	37.1	38.0	15.2	6.6	2.8	0

[a] Based on 105 test results.

25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were tested by the rapid method and the NCCLS standard method and were included with each test series.

RESULTS

The differences in zone diameter between the rapid and standard methods for the fresh clinical isolates, based on a total of 3,215 test results, are shown in Tables 2 to 5. Approximately 95% of the zone diameters for the *Enterobacteriaceae*, *Pseudomonas* spp. and other nonfermenters, and enterococci, differed by ≤3 mm. For the *Staphylococcus* spp., approximately 90% of the diameters differed by ≤3 mm, and 96% differed by ≤4 mm. There were no consistent drug-organism combinations which showed differences between the two methods. The rapid method did not demonstrate any trend toward larger or smaller zone diameters when compared with the standard method.

The effect of the rapid method on the interpretative susceptibilities of fresh clinical isolates (that is, whether they were susceptible, intermediate, or resistant) is shown in Table 6. Of 3,215 determinations, 27 (0.8%) changes in interpretative susceptibility occurred; 26 were minor, and 1 was very major. A zone diameter variation of ≤2 mm between the rapid and standard methods was responsible for all of the minor changes. A 6-mm variation for *Morganella morganii* with carbenicillin was responsible for the very major change. The interpretative changes were randomly distributed among the drugs tested. As a group, the *Enterobacteriaceae* showed the lowest percent change (0.6%), and the enterococci showed the highest (3.8%).

Tables 7 to 9 show the mean and range of zone diameters for the quality control organisms, which were tested on 29 separate days. The mean and range for the rapid and standard methods were very similar. The mean zone diameter differed by ≤1 mm when the two methods were compared. The greatest difference in the range between the two methods occurred for *E. coli* ATCC 25922 with cephalothin and tobramycin. In all instances, the accuracy and precision of controls were within acceptable ranges (4, 5).

For *E. coli* ATCC 25922, the individual daily test control zone diameters exceeded the limits (4, 5) for cephalothin and trimethoprim-sulfamethoxazole in both methods. This occurred twice for cephalothin and once for trimethoprim-sulfamethoxazole. The individual daily test control zone diameters were within limits (4, 5) for *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. It should be noted that on two occasions, a zone diameter of 19 mm was obtained for *P. aeruginosa* ATCC 27853 with carbenicillin by the standard method. However, the individual daily test control zone diameters have recently been changed from 20 to 24 mm (4) to 18 to 24 mm (5).

TABLE 6. Effect of rapid method on interpretative susceptibilities of fresh clinical isolates

Organism	No. of tests	No. of tests with following interpretation[a]:						
		S→I	S→R	I→S	I→R	R→S	R→I	%
Enterobacteriaceae	1,840	1	0	4	2	1	4	0.6
Pseudomonas spp. and other nonfermenters	590	1	0	2	1	0	2	1.0
Staphylococcus spp.	680	0	0	0	0	0	5	0.7
Enterococci	105	2	0	0	1	0	1	3.8

[a] The standard method is listed first. S, susceptible; I, intermediate; R, resistant.

TABLE 7. Mean and range of zone diameters for *E. coli* ATCC 25922[a]

Drug	Zone diam (mm) for following method:			
	Rapid		Standard	
	Mean	Range	Mean	Range
Amikacin	24.8	23–26	24.4	22–26
Ampicillin	19.5	18–20	18.9	18–20
Carbenicillin	27.7	24–29	26.7	24–29
Cephalothin	20.2	20–23	20.3	18–24
Chloramphenicol	25.0	23–27	24.7	22–27
Gentamicin	24.0	22–26	24.7	22–26
Nitrofurantoin	23.2	20–25	22.3	20–25
Tetracycline	23.7	20–25	22.7	19–25
Tobramycin	24.6	18–26	24.0	20–26
Trimethoprim-sulfamethoxazole	27.4	23–30	26.4	23–30

[a] Tested on 29 separate days.

TABLE 9. Mean and range zone diameters for *S. aureus* ATCC 25923[a]

Drug	Zone diam (mm) for following method:			
	Rapid		Standard	
	Mean	Range	Mean	Range
Cephalothin	34.8	28–37	35.0	28–37
Chloramphenicol	24.4	21–26	24.5	21–26
Clindamycin	27.8	23–29	28.0	24–29
Erythromycin	26.2	24–30	27.1	23–30
Gentamicin	24.1	21–27	24.6	22–27
Nafcillin	18.9	17–22	18.4	17–21
Penicillin	33.9	31–37	34.0	30–37
Tetracycline	26.1	23–28	26.4	23–28
Trimethoprim-sulfamethoxazole	30.4	26–31	30.6	26–32
Vancomycin	17.8	16–19	18.1	16–19

[a] Tested on 29 separate days.

DISCUSSION

Although the rapid inoculum preparation method for agar disk diffusion susceptibility testing is used by many laboratories, there is a paucity of published data regarding its efficacy. These results demonstrated that the rapid method is equivalent to the NCCLS standard method (4) for inoculum preparation. The distinct advantage of the rapid method over the standard method is that the former obviates the 2 to 8 h of incubation and the attendant interruption in the performance of the susceptibility test. Additionally, the rapid method is less expensive because the inoculum is prepared with water instead of a broth medium.

From the standpoint of differences in zone diameter between the rapid and standard methods, approximately 95% of the test results differed by ≤3 mm for all of the bacterial groups except *Staphylococcus* spp. For *Staphylococcus* spp., approximately 90% of the test results differed by ≤3 mm. A possible explanation is that the larger zone diameters obtained for the staphylococci were more difficult to reproduce.

TABLE 8. Mean and range of zone diameters for *P. aeruginosa* ATCC 27853[a]

Drug	Zone diam (mm) for following method:			
	Rapid		Standard	
	Mean	Range	Mean	Range
Amikacin	21.2	20–25	20.9	19–26
Carbenicillin	20.9	20–23	20.7	19–23
Gentamicin	18.4	18–21	18.3	17–21
Tobramycin	22.9	21–25	22.3	21–25

[a] Tested on 29 separate days.

A statistical analysis of the data was not performed, but in the vast majority of cases, there were no clinical differences between the rapid and standard methods.

The use of the rapid inoculum preparation method for dilution susceptibility testing has not been systematically evaluated. However, based upon limited experience in our laboratories, the rapid method is equivalent to the standard method for both macro and micro broth dilution procedures.

In our opinion, the rapid method can be routinely used for disk diffusion susceptibility testing in the clinical microbiology laboratory where time is of the essence.

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