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Sensititre Autoreader for Same-Day Breakpoint Broth Microdilution Susceptibility Testing of Members of the Family *Enterobacteriaceae*

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The Sensititre Autoreader system is an instrument-assisted broth microdilution susceptibility test procedure based on the detection of fluorogenic growth substrate metabolism by test bacteria with different concentrations of antimicrobial agents. In the current investigation, this system was assessed as a means for predicting the in vitro activity of 17 antimicrobial agents versus numerous species of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* by using a breakpoint broth microdilution test format. Same-day and overnight determinations of susceptibility were made with the Sensititre Autoreader system, and in both cases, the results were compared with those obtained with a manual overnight breakpoint broth microdilution susceptibility test. Among a total of 6,086 organism-antimicrobial agent comparisons with *Enterobacteriaceae*, concordance was noted between the results of the same-day Autoreader system and the manual overnight test in 94.4% of cases. The same-day Autoreader results with members of the *Enterobacteriaceae* other than *Proteus* spp. were determined after 4 h of incubation; with *Proteus* spp. the same-day Autoreader results were determined after 5 h of incubation. When the *Enterobacteriaceae* Autoreader results were determined after 18 h of incubation, concordance was noted in 97.2% of comparisons. Among a total of 1,377 organism-antimicrobial agent comparisons with *P. aeruginosa* after 18 h of incubation, agreement of results from the manual overnight test and the Autoreader system was achieved in 92.2% of cases.

Breakpoint susceptibility testing refers to the in vitro susceptibility test procedures which measure the activity of one or two discrete concentrations of antimicrobial agents by either an agar or broth dilution technique. The concentration(s) of antimicrobial agents tested are those that define the usual susceptibility categories: susceptible, intermediate (or moderately susceptible), and resistant (3). Results are conveyed in the form of susceptibility categories or MIC ranges. The principal advantages of breakpoint susceptibility testing are low cost, when performed by using a broth microdilution format, and interpretive ease (G. V. Doern, Clin. Microbiol. Newsl., in press). A recent clinical laboratory evaluation of one commercially available breakpoint broth microdilution test system (Sensititre Breakpoint system; Sensititre Inc., North Andover, Mass.) revealed that the results obtained with this system were at least as accurate as those obtained with the Bauer-Kirby disk diffusion susceptibility test (1).

Recently, an instrument-assisted broth microdilution susceptibility test system, the Sensititre Autoreader system, became commercially available. This system is based on the use of fluorogenic growth substrates to detect bacterial metabolism and growth. Briefly, the broth used in microdilution susceptibility tests is supplemented with fluorogenic growth substrates. In their native state, the substrates do not fluoresce. In the absence of antimicrobial inhibition, the fluorogenic substrates are metabolized by bacteria in a manner that causes them to fluoresce. The amount of fluorescence is roughly proportional to the amount of bacterial growth. With the Sensititre Autoreader system, fluorescence in the wells of microdilution susceptibility test plates is detected with a multichannel fluorimeter, the Sensititre Autoreader. The results of Autoreader determinations of MICs after overnight incubation of microdilution test plates have been shown to compare favorably with the results of manual overnight tests (2). Because the metabolism of fluorogenic substrates, with the resultant production of fluorescent signals, is a more sensitive indicator of bacterial growth than measurements of turbidity are, it is possible that this system could be used to perform same-day susceptibility testing. Indeed, in a recent evaluation of a same-day microdilution MIC procedure, concordance of 92% was achieved between results obtained with the Autoreader and an overnight manual test (J. L. Staneck, S. D. Allen, and R. C. Tilton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C89, p. 314). It was the intent of the present investigation to examine the utility of the Sensititre Autoreader system as a means for performing same-day susceptibility tests of members of the family *Enterobacteriaceae* with breakpoint broth microdilution test plates. In addition, the results of overnight breakpoint broth microdilution susceptibility tests of *Enterobacteriaceae* and *Pseudomonas aeruginosa* were assessed by using the Sensititre Autoreader system.

MATERIALS AND METHODS

Organisms. A total of 358 different clinical isolates of members of the family *Enterobacteriaceae* and 81 different strains of *Pseudomonas aeruginosa* were examined in this study (Table 1). Among the *Enterobacteriaceae*, 215 isolates were characterized at the University of Massachusetts Medical Center (UMMC), and 143 were characterized at the University of Cincinnati Medical Center (UCMC). Forty-eight strains of *P. aeruginosa* were tested at UMMC, and thirty-three were tested at UCMC. The inocula for susceptibility tests were prepared from primary specimen culture plates which had been incubated for 18 to 24 h at 35°C.

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TABLE 1. Organisms characterized by using the Sensititre Breakpoint Autoreader system

Organism	No.[a] characterized at:	
	UMMC	UCMC
Escherichia coli	63 (25)	54
Klebsiella pneumoniae	41 (20)	13
Klebsiella oxytoca	8 (5)	5
Enterobacter cloacae	17 (10)	14
Enterobacter aerogenes	8 (5)	6
Enterobacter agglomerans	6 (5)	
Enterobacter gergoviae	1	
Serratia marcescens	12 (10)	10
Serratia liquefaciens	1	
Citrobacter freundii	7 (5)	7
Citrobacter diversus	3	2
Proteus mirabilis	21	11
Proteus vulgaris	3	6
Morganella morganii	8 (5)	7
Providencia stuartii	3	3
Providencia rettgeri	2	2
Hafnia alvei	1	2
Salmonella species	5 (5)	1
Shigella species	5 (5)	
Pseudomonas aeruginosa	48	33

[a] Number in parentheses indicates those strains examined with alternate procedures (Table 3).

Susceptibility test plates. Dehydrated, commercially available breakpoint microdilution susceptibility test plates of a single lot number were used throughout the study (Urinary/Gram Negative Plate, APB3A; Sensititre). These plates contained antimicrobial agents at the following concentrations (in micrograms per milliliter): sulfamethoxazole, 256; naladixic acid, 16; nitrofurantoin, 64; trimethoprim-sulfamethoxazole, 2/38; tetracycline, 8; gentamicin, 4 and 8; amikacin, 16 and 32; tobramycin, 4 and 8; moxalactam, 8 and 32; cephalothin, 8 and 16; cefoxitin, 8 and 16; cefamandole, 8 and 16; cefotaxime, 8 and 32; ampicillin, 1 and 16; carbenicillin, 32 and 128; piperacillin, 16 and 64; and chloramphenicol, 8 and 16. Plates were stored at room temperature before use.

Overnight susceptibility studies. Filter paper strips impregnated with fluorogenic substrates (18-h fluorogenic substrate strips; Sensititre) were placed in screw-cap glass test tubes (16 by 125 mm) containing 10 ml of cation-supplemented Mueller-Hinton broth (GIBCO Diagnostics, Madison, Wis.). Tubes were incubated at 35°C for 1 h, while the fluorogenic substrates eluted from the strips into the broth. An initial suspension of the test organism equivalent to a 0.5 McFarland standard was prepared in sterile distilled water. When testing *P. aeruginosa* and members of the *Enterobacteriaceae* other than *Proteus* spp., 0.01 ml of this suspension was transferred with a disposable plastic calibrated loop into the 10 ml of prewarmed cation-supplemented Mueller-Hinton broth containing fluorogenic substrates. This suspension contained ca. 10^5 CFU/ml. When testing *Proteus* spp., a 0.001-ml inoculum was used, resulting in a suspension containing ca. 10^4 CFU/ml. After the desired inocula had been prepared, a plastic dosing head was attached to the tubes, and breakpoint microdilution susceptibility test plates were inoculated with an automatic inoculating device (Sensititre) which delivered 50 μl to each well. The plates were sealed with a Mylar film and incubated for 18 h at 35°C.

After incubation for 18 h, the plates were manually inspected and examined with the Sensititre Autoreader. The Autoreader consisted of a reader module driven by a DEC 350 professional computer. Plate reading was accomplished by manually placing a microdilution tray into the carriage of the reading module and initiating a command function via the computer keyboard. The total reading sequence required approximately 45 s per tray. With respect to manual inspection, growth was defined as the presence of turbidity or a conspicuous button of cells on the bottom of wells. With those antimicrobial agents present in two concentrations, an organism was considered resistant if growth was observed in both concentrations of the antimicrobial agent. Growth only in the higher concentration was defined as intermediate. The absence of growth in either concentration was considered susceptible. With those antimicrobial agents present in a single concentration, the presence of growth was interpreted as resistant and no growth was defined as susceptible.

Same-day susceptibility studies. The inocula for same-day susceptibility studies were prepared from the same initial 0.5 McFarland suspension of the test organism used for overnight tests. For same-day tests with members of the *Enterobacteriaceae* other than *Proteus* spp., 0.2 ml of the 0.5 MacFarland suspension was transferred with a plastic 1.0-ml graduated pipette into 10 ml of a specially formulated broth (Rapid Broth; GIBCO) containing fluorogenic substrates. This suspension contained ca. 2×10^6 CFU/ml. When testing *Proteus* spp., 0.01 ml of the initial suspension was transferred into the Rapid Broth with a disposable plastic calibrated loop. The resultant concentration of organisms was ca. 10^5 CFU/ml. As with the overnight tests, fluorogenic substrates (Sensititre) were added 1 h before inoculation. Tubes were incubated at 35°C. The strips used for same-day tests were, however, different from those employed with overnight tests in that they contained different fluorogenic growth substrates at higher concentrations. Plates were incubated at 35°C for either 4 h (*Enterobacteriaceae* other than *Proteus* spp.) or 5 h (*Proteus* spp.) at 35°C, and results were ascertained with the Sensititre Autoreader. Strains of *P. aeruginosa* were not examined with the same-day susceptibility test procedure.

Data analysis. The results of overnight manual tests were considered definitive and were used to assess both the same-day and overnight Autoreader results. Discrepancies for individual organism-antimicrobial agent comparisons were defined as follows: very major error, a resistant result with the overnight manual procedure but a susceptible result with the Autoreader procedure; major error, a susceptible result with the overnight manual test but a resistant result with the Autoreader test; and minor error, all other discrepancies.

RESULTS

The results of same-day and overnight Autoreader determinations of *Enterobacteriaceae* are shown in Table 2. In all cases, Autoreader results were compared with the results of manual susceptibility tests which had been incubated for 18 h. A total of 6,086 different organism-antimicrobial agent comparisons were made with the *Enterobacteriaceae*. A total of 84 very major errors (1.4%), 40 major errors (0.7%), and 216 minor errors (3.5%) were noted with the same-day Autoreader test. The overall discrepancy rate was thus 5.6%. In contrast, when Autoreader results were determined after 18 h of incubation, approximately one-half as many errors were observed, i.e., 49 very major errors (0.8%), 25 major errors (0.4%), and 98 minor errors (1.6%). The overall discrepancy rate with the overnight Autoreader test was thus 2.8%.

TABLE 2. Comparison of Sensititre Autoreader results with those obtained by an overnight manual procedure[a]

Antimicrobial agent	No. (%) of discrepancies[b] after incubation at 35°C for:					
	4 h			18 h		
	VM	M	Mi	VM	M	Mi
Sulfamethoxazole	6 (1.7)	10 (2.8)	9 (2.5)	23 (6.4)	12 (3.4)	0
Nalidixic acid	0	1 (0.3)	2 (0.6)	1 (0.3)	0	0
Nitrofurantoin	12 (3.4)	2 (0.6)	5 (1.4)	11 (3.1)	1 (0.3)	0
Trimethoprim-sulfamethoxazole	2 (0.6)	2 (0.6)	6 (1.7)	1 (0.3)	4 (1.1)	3 (0.8)
Tetracycline	12 (3.4)	0	6 (1.4)	6 (1.7)	0	0
Gentamicin	4 (1.1)	0	4 (1.1)	0	0	0
Amikacin	0	4 (1.1)	2 (0.6)	0	0	0
Tobramycin	1 (0.3)	1 (0.3)	7 (2.0)	1 (0.3)	0	4 (1.1)
Moxalactam	0	0	6 (1.4)	0	0	4 (1.1)
Cephalothin	12 (3.4)	7 (2.0)	33 (9.2)	1 (0.3)	2 (0.6)	14 (3.9)
Cefoxitin	1 (0.3)	3 (0.8)	12 (3.4)	1 (0.3)	3 (0.8)	8 (2.2)
Cefamandole	13 (3.6)	1 (0.3)	11 (3.1)	0	3 (0.8)	10 (2.8)
Cefotaxime	2 (0.6)	2 (0.6)	6 (1.4)	0	0	2 (0.6)
Ampicillin	2 (0.6)	3 (0.8)	45 (12.7)	0	0	16 (4.5)
Carbenicillin	1 (0.3)	3 (0.8)	17 (4.7)	1 (0.3)	0	13 (3.6)
Piperacillin	10 (2.8)	0	12 (3.4)	1 (0.3)	0	6 (1.4)
Chloramphenicol	6 (1.7)	1 (0.3)	33 (9.2)	2 (0.6)	0	18 (5.0)

[a] A total of 358 different strains of *Enterobacteriaceae* were examined. The plates examined by the Autoreader were incubated under various conditions. Autoreader results with the *Enterobacteriaceae* other than *Proteus* spp. were determined after 4 h of incubation. Autoreader results with *Proteus* spp. were determined after 5 h of incubation.

[b] VM, Very major errors; M, major errors; Mi, minor errors.

The overall discrepancy rates obtained after both same-day and overnight incubation with *Enterobacteriaceae* characterized at the UMMC versus those examined at the UCMC were similar; 4.2 versus 7.6% with the same-day test and 2.4 versus 3.5% with the overnight test, respectively. Furthermore, the relative percentage of very major, major, and minor errors were similar at both medical centers. Among the different genera and species of *Enterobacteriaceae* examined in this study, no single organism or organism group accounted for a disproportionate number of discrepancies.

Selected antimicrobial agents, however, were associated with disproportionately large numbers of discrepancies with the *Enterobacteriaceae* (Table 2). For instance, with the same-day Autoreader test, five antimicrobial agents (nitrofurantoin, tetracycline, cephalothin, cefamandole, and piperacillin) accounted for 59 of the 84 (70.2%) very major errors. Two antimicrobial agents (sulfamethoxazole and cephalothin) were responsible for 17 of the 40 (42.5%) major errors, and three antimicrobial agents (cephalothin, ampicillin, and chloramphenicol) accounted for 111 of the 215 (51.6%) minor errors. After 18 h of incubation, sulfamethoxazole and nitrofurantoin were responsible for 34 of the 49 (67.3%) very major errors with the Autoreader. Sulfamethoxazole alone accounted for 12 of the 25 (48%) major errors. Among the 98 minor errors noted after incubation for 18 h

TABLE 3. Evaluation of same-day Sensititre Autoreader results with various incubation conditions[a]

Antimicrobial agent	No. of discrepancies[b] at incubation for:								
	4 h at 35°C			5 h at 35°C			4 h at 37°C		
	VM	M	Mi	VM	M	Mi	VM	M	Mi
Sulfamethoxazole	18	13	0	1	1	4	1	1	2
Nalidixic acid	0	15	0	0	0	0	1	1	0
Nitrofurantoin	15	0	0	9	1	1	8	0	1
Trimethoprim-sulfamethoxazole	1	12	0	0	2	2	0	1	3
Tetracycline	11	12	0	0	1	1	1	1	1
Gentamicin	1	5	4	2	0	1	3	0	2
Amikacin	0	28	1	2	3	0	3	1	0
Tobramycin	1	5	5	0	1	2	1	2	3
Moxalactam	0	13	1	0	1	1	0	1	0
Cephalothin	1	20	6	3	2	9	3	2	11
Cefoxitin	0	22	8	0	1	6	1	1	6
Cefamandole	0	14	8	4	0	6	3	1	9
Cefotaxime	0	13	7	0	0	1	0	1	3
Ampicillin	0	5	23	0	1	7	0	1	8
Carbenicillin	0	21	13	2	0	5	1	1	7
Piperacillin	3	7	7	0	0	2	0	1	3
Chloramphenicol	1	44	11	0	1	7	0	0	10

[a] Inocula were prepared from nonprewarmed broth.

[b] Number also equals percent, because a total of 100 different strains of *Enterobacteriaceae* were examined. VM, Very major errors; M, major errors; Mi, minor errors.

with the Autoreader, 79 (80.6%) were due to six antimicrobial agents (cephalothin, cefoxitin, cefamandole, ampicillin, carbenicillin, and chloramphenicol).

The results of the same-day *Enterobacteriaceae* Autoreader susceptibility tests were clearly influenced by the temperature and time of incubation of breakpoint plates and by the temperature of the broth used to inoculate plates (Table 3). These variables were assessed at the UMMC on 100 isolates of *Enterobacteriaceae* selected from the 215 UMMC strains examined in the studies discussed previously (Table 1). In all cases, same-day Autoreader results were compared with the results obtained by visual inspection of test plates after 18 h of incubation at 35°C.

When same-day Autoreader results were determined after incubation at 35°C with plates that had been inoculated with inocula prepared in broth at room temperature, a total of 395 discrepancies was noted among 1,700 organism-antimicrobial agent comparisons. The overall rate of discrepancies (23.3%) observed under these conditions was significantly higher than the 5.6% discrepancy rate obtained from plates with inocula prepared in broth prewarmed to 35°C under identical incubation conditions (Table 2). The deleterious effect of using inocula prepared in broth at room temperature was eliminated, however, when susceptibility plates were incubated at 35°C for 1 h longer before examination with the Autoreader (i.e., 5-h incubation period) or by increasing the incubation temperature to 37°C while maintaining the time of incubation at 4 h (overall discrepancy rates, 4.9 and 5.8%, respectively).

Autoreader results with *P. aeruginosa* were assessed only after incubation of susceptibility test plates at 35°C for 18 h. Among a total of 1,377 organism-antimicrobial agent comparisons performed under these conditions, discrepancies were noted in 107 cases (7.8%). Twenty-five discrepancies were very major errors, one discrepancy was due to a major error, and the remaining eighty-one discrepancies were minor errors. Antimicrobial agents yielding disproportionately large numbers of discrepancies included tetracycline (32% of the very major errors) and trimethoprim-sulfamethoxazole, gentamicin, moxalactam, cefotaxime, and carbenicillin (collectively, 82.7% of minor errors).

DISCUSSION

Through the use of fluorogenic growth substrates, the Sensititre Autoreader system makes possible nonturbidimetric determination of in vitro antimicrobial activity. In the current investigation, the Sensititre Autoreader system was assessed as a means of performing same-day breakpoint broth microdilution susceptibility tests with a variety of different species of *Enterobacteriaceae*. In addition, the utility of this system was examined after incubation of *Enterobacteriaceae* and *Pseudomonas aeruginosa* for 18 h. The results of overnight Autoreader susceptibility tests determined after 18 h of incubation revealed that the in vitro activity of 17 different antimicrobial agents could be accurately predicted with both the *Enterobacteriaceae* and *P. aeruginosa*. Among a total of 6,086 organism-antimicrobial agent comparisons with *Enterobacteriaceae* after 18 h of incubation, concordance was achieved between the results of Autoreader tests and visually inspected susceptibility test plates in 97.2% of cases. The overall concordance with *P. aeruginosa* at 18 h (1,377 organism-antimicrobial agent comparisons) was 92.2%.

Autoreader results were also determined after 4 h of incubation of susceptibility test plates with *Enterobacteriaceae* other than *Proteus* spp. and after 5 h of incubation with *Proteus* spp. As with the overnight Autoreader results, the results of same-day Autoreader tests were compared with the results obtained by visual inspection of susceptibility test plates after 18 h of incubation. Concordance was achieved between the results of the same-day Autoreader test and overnight manual procedure in 94.4% of 6,086 organism-antimicrobial agent comparisons. The use of visually inspected susceptibility test plates after 18 h of incubation to assess the correctness of Autoreader results after either same-day or overnight incubation was considered justified on the basis of a previous study (1). In this study of the Sensititre Breakpoint system (1), the results of microdilution susceptibility tests determined manually after overnight incubation were shown to be at least as accurate as the results of a standardized disk diffusion procedure.

As stated previously, all of the same-day *Enterobacteriaceae* results were determined after plates had been incubated for 4 h, except for plates of *Proteus* spp. Same-day results with *Proteus* spp. were determined after 5 h of incubation. This was a result of the necessity of testing *Proteus* spp. at a 20-fold-lower inoculum than that used with the other *Enterobacteriaceae* (i.e., ca. 1×10^5 versus 2×10^6 CFU/ml). The use of the higher inoculum with *Proteus* spp. results in a significant early conversion of fluorogenic substrates, even in the presence of inhibitory concentrations of antimicrobial agents (J. Staneck, unpublished observation). This background substrate conversion, which is probably due to excessive endogeneous levels of enzymes active against the fluorogenic substrates, leads to false-resistant results with the Autoreader. Use of the lower inoculum eliminates this problem but does necessitate a 1-h-longer incubation period to achieve accurate measurements of inhibition. Same-day Autoreader results were not determined with *P. aeruginosa*. Preliminary observations suggested that it was not possible to perform same-day susceptibility tests with *P. aeruginosa* with the Sensititre Autoreader system because of inadequate fluorogenic substrate metabolism by this organism.

The results of this investigation also underscore the importance of using inocula prepared in prewarmed broth to obtain accurate results with the Autoreader after 4 h of incubation of plates at 35°C. The overall discrepancy rate of results obtained with nonprewarmed broth (i.e., 23.2%) was approximately four times higher than the error rate obtained when inocula were prepared in broth prewarmed to 35°C (i.e., 5.6%). These results suggest that it is important that organisms be growing actively when Autoreader results are determined after incubation periods as short as 4 h. This hypothesis is supported by two other observations made in this study. By increasing the temperature at which susceptibility test plates were incubated from 35 to 37°C, the detrimental effect of using inocula prepared in nonprewarmed broth was obviated. Similarly, when the length of incubation of test plates at 35°C was increased from 4 to 5 h, inocula prepared in nonprewarmed broth were satisfactory.

It seems apparent from these results that same-day Autoreader results are very much dependent on sufficient growth of the test organism before instrument determination of antimicrobial agent activity. This is probably related to the manner in which test results are achieved with the Sensititre Autoreader system. The amount of fluorogenic substrate conversion in a no-growth control well is subtracted from the amount of substrate conversion in a growth control well, thus establishing a threshold value to which readings in wells containing various concentrations of anti-

microbial agents are compared. If the amount of substrate conversion in an antimicrobial agent-containing well exceeds an empirically derived percentage of the threshold value, the organism is judged not to have been inhibited by that concentration of antimicrobial agent. This would be the case when the MIC for the organism is higher than the concentration of antimicrobial agent in the well. Conversely, if the amount of substrate conversion in the antimicrobial agent-containing well is less than an empirically derived percentage of the threshold value, the organism is judged to have been inhibited. This would apply to organisms for which MICs are equal to or less than the concentration of the antimicrobial agent in the well. Clearly, the higher the threshold value, the more accurate the test results. Acceptably high threshold values can be achieved only with sufficient growth of the test organism. The results of the current investigation suggest that with the Sensititre Autoreader system, sufficient growth of *Enterobacteriaceae* can be achieved after 4 h only when growth conditions are optimized, i.e., when prewarmed inoculating broth is used or when the incubation of plates is at 37°C rather than 35°C. If the incubation temperature of the test plates is maintained at 35°C and nonprewarmed inoculating broth is used, accurate readings can be achieved only after 5-h incubation periods.

One issue not addressed in this study is the lack of a convenient quality control procedure to ensure that the concentrations of antimicrobial agents in breakpoint susceptibility test plates are present in precisely the desired amounts. The use of quality control organisms with known susceptibility patterns is inadequate since discrete concentrations of antimicrobial agents are tested. For instance, assume that two concentrations of gentamicin, 4.0 and 8.0 μg/ml, are used for breakpoint susceptibility testing of gram-negative bacilli. A strain of *E. coli* (ATCC 25922) is used for quality control of the breakpoint plates. For this strain, the gentamicin MIC is 0.25 to 1.0 μg/ml, and therefore the strain should always yield a susceptible result with the breakpoint plates since it would be inhibited by both concentrations of gentamicin. A susceptible quality control test result, however, does not prove that the concentration of gentamicin in the breakpoint plates is exactly 4.0 or 8.0 μg/ml, which is the requirement of an adequate quality control test. A susceptible result with this strain of *E. coli* only proves that concentrations of gentamicin greater than 1.0 μg/ml are present in the breakpoint plates.

Strictly speaking, an adequate quality control procedure of a breakpoint susceptibility system would necessitate assaying the precise concentrations of each antimicrobial agent present in test plates at some interval. Such a procedure, at least one that could be conveniently applied in most clinical microbiology laboratories, does not now exist. We have initiated studies, however, aimed at developing a suitable quality control procedure for breakpoint plates.

In conclusion, the results of this study demonstrated that the Sensititre Autoreader system accurately predicted the in vitro activity of a variety of different antimicrobial agents versus numerous species of *Enterobacteriaceae* by a same-day breakpoint broth microdilution test system. After overnight incubation, satisfactory susceptibility results were obtained with both the *Enterobacteriaceae* and *P. aeruginosa*.

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