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Comparison of Sensititre Dried Microtitration Trays with a Standard Agar Method for Determination of Minimum Inhibitory Concentrations of Antimicrobial Agents

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A total of 222 clinical isolates were used to test the accuracy of Sensititre dried microtitration trays for determining minimum inhibitory concentrations (MICs) of antimicrobial agents. In comparison with an agar dilution technique, 89.5% of all the pairs of results were within one doubling dilution. The 2,420 pairs of MIC results with finite values gave a corresponding figure of 86.8%. Exclusion of sulfisoxazole results, which demonstrated a significant interlaboratory variation in accuracy, raised this value to 89.1%. Very good differentiation of β-lactamase-producing strains of *Staphylococcus aureus* (24 of 24 giving an MIC ≥ 0.25 μg/ml) and *Haemophilus influenzae* (3 of 3 giving an MIC ≥ 32 μg/ml) was obtained with the Sensititre system. This method also clearly distinguished erythromycin-resistant *S. aureus* strains (7 of 7 giving an MIC > 32 μg/ml) from the susceptible strains (26 of 28 giving an MIC ≤ 0.5 μg/ml plus 1 strain at 1.0 μg/ml and 1 at 2.0 μg/ml). Sensititre offers an accurate and convenient method of determining MICs comparable to those obtained with the agar dilution procedure, with the advantage of an extended shelf life when stored at room temperature.

The Kirby-Bauer disk diffusion method is widely used for antimicrobial susceptibility tests, but there are limitations to this method, mainly because of the sensitivity of the test to changes in operator technique and also in the subsequent interpretation of zone diameters. In recent years there has been a move towards a more quantitative method, namely, the measurement of an antimicrobial agent's minimum inhibitory concentration (MIC). To date this type of susceptibility testing has relied on freshly prepared dilutions of antimicrobial agents in nutrient media or on frozen microtitration trays, either made in the hospital's laboratory (9) or, more recently, brought in from an outside supplier (3). The frozen systems have to be stored at −20°C to retain the potencies of the antimicrobial drugs, and at this temperature the trays have a relatively limited storage life. A microtitration system, Sensititre, is now commercially available; it consists of plates containing dried antibiotic which are stable for 12 months at ambient temperature (data are on file with the manufacturer).

A previous study has demonstrated the good performance of Sensititre when compared with another broth microdilution procedure, and also the high reproducibility of results obtained in several laboratories (6). This report compares Sensititre with the agar dilution procedure for MIC determinations. Particular emphasis was put on selecting organisms known to be resistant to antimicrobial agents.

MATERIALS AND METHODS

Sensititre plates were supplied by Seward Laboratory, UAC House, Blackfriars Road, London (available in the U.S. from GIBCO Diagnostics, Madison, Wis.) and were kept at room temperature before use.

The drugs used were obtained directly from the manufacturers, together with accurate potency values. These were stored at 4°C over silica desiccant before use.

Bacterial strains. The 222 organisms used in this study were all recent clinical isolates. They were checked for purity and identification, then stored either under liquid nitrogen or on agar slopes at room temperature. Immediately before use, the organisms were rechecked for purity and subcultured into nutrient broth. The isolates included 35 of *Staphylococcus aureus*, 13 of coagulase-negative staphylococci, 15 of *Streptococcus faecalis* (Lancefield group D), 8 of *Micrococcus* spp., 39 of *Escherichia coli*, 38 of *Klebsiella* spp., 29 of *Proteus* spp., 6 of *Haemophilus influenzae*, 16 of *Pseudomonas aeruginosa*, 6 of *Enterobacter* spp., 1 of *Pasteurella/Moraxella* sp., 5 of *Serratia* spp., 2 of *Hafnia* spp., and 9 of *Citrobacter* spp. By design, a high proportion of the isolates showed resistance to relevant antimicrobial agents, e.g., three of the six *H. influenzae* isolates produced β-lactamase.

A separate study was performed to examine the performance of Sensititre when testing *Pseudomonas* species against aminoglycoside antibiotics. A further 20 *P. aeruginosa*, 3 *Pseudomonas fluorescens*, 3 *Pseudomonas maltophilia*, and 3 *Pseudomonas* spp. isolates were used in tests with gentamicin, amikacin, tobramycin, and kanamycin. These strains were collected from a variety of sources within the U.K. and were all reported to be resistant to at least one aminoglycoside by the disk diffusion test.

Inoculum preparation. An important factor in the determination of MICs is the inoculum size used, which can easily affect the final endpoint observed. The original International Collaborative Study report of Ericsson and Sherris (4) recommended inocula sizes of 10^4 colony-forming units (CFU) per spot for the agar method and 10^5 to 10^6 CFU/ml for the broth dilution technique. However, no sulfonamides were included in this study, and previous experience has shown the need for a light surface inoculum when the agar technique is used to determine MICs for sulfonamides (7).

The possibility that a surface inoculum suitable for sulfonamides and most other antimicrobial drugs (7, 8, 13) would not be heavy enough to detect extracellular β-lactamase production (1, 7) was disposed of by the separate use of a β-lactamase indicator (10). A light surface inoculum of 10^3 CFU was used for the agar method, together with the chromogenic cephalosporin for detecting β-lactamase-producing organisms. Sensititre was used with an inoculum of 10^5 CFU/ml according to the manufacturer's instructions. The six *H. influenzae* strains were also tested at 10^4 CFU/ml, as explained below.

For the Sensititre method the overnight culture, at approximately 10^9 CFU/ml, was diluted 1:10,000 into Mueller-Hinton broth (pH 7.2 ± 0.1) (GIBCO Diagnostics) to yield a final inoculum density of 10^5 CFU/ml. With the six *H. influenzae* strains, two dilutions were used to give inocula of 10^4 and 10^5 CFU/ml. An inoculum of 10^4 CFU/ml has been recommended by Barry (2) for testing the susceptibility of *H. influenzae* with microdilution procedures. Schaedler's broth (BBL Microbiology Systems) plus 5% Fildes peptic digest (Difco) was used with this organism.

For the agar incorporation technique the overnight culture was diluted 1:1,000 with Mueller-Hinton broth to give a 10^6-CFU/ml suspension, i.e., 10^3 CFU per inoculum on the surface. As in the Sensititre method, Schaedler's broth (BBL) plus 5% Fildes peptic digest (Difco) was used for culture of the six *H. influenzae* strains. Previous hospital experience with agar dilution systems had demonstrated that the differentiation of β-lactamase-positive *H. influenzae* strains from negative strains was easier if a heavy inoculum was used. Therefore, overnight cultures of these organisms were diluted 1:100 and 1:10, giving final suspensions of 10^7 and 10^8 CFU/ml, i.e., 10^4 and 10^5 CFU per 1-μl spot on the agar plate.

Broth MICs. Broth MICs were determined in Sensititre microtitration plates, which contain an array of 8 by 12 wells with a series of eight doubling dilutions of either 10 or 11 drugs, depending on the plate type. These antimicrobial agents, when dried into the wells, are stable at room temperature for up to 12 months from the date of manufacture. The three types of plate used in this study were as shown in Table 1.

Strains of *S. aureus*, coagulase-negative staphylococci, *Micrococcus* spp., and *S. faecalis* were tested using APO1 plates. The six *H. influenzae* strains at the two inoculum levels were tested against APO2 plates and also against benzylpenicillin. The nine other gram-negative genera were tested on both APO2 and APO3 plates.

The Sensititre wells were inoculated with the bacterial suspension prepared as described above, using an eight-channel micropipettor delivering eight 50-μl samples (Finnpipette, obtained in the U.K. from Jencons Scientific Limited, Hemel Hempstead, U.K.). The plates were sealed with the transparent tape provided with the Sensititre plates and then incubated aerobically for 18 h at 37°C.

Agar MICs. The agar incorporation plates were prepared immediately before use. With the exception of the tests involving the six *H. influenzae* strains, the drugs were incorporated into Mueller-Hinton agar (the same batch of broth, plus 1.5% agar [Difco], was used for the Sensititre MIC technique) in amounts that covered the same concentration ranges as were used in the Sensititre plates. Schaedler's broth (BBL) with 5% Fildes peptic digest (Difco) and 1.5% agar (Difco) was used in tests of the *H. influenzae* strains. When dry, the agar plates were inoculated using a replica-plating device (Denley-Tech, Denley Instruments, Billingshurst, U.K.), which transferred 1 μl of each inoculum to the agar surface. After drying of the liquid, the agar plates were incubated aerobically at 37°C for 18 h.

Determination of MICs. The MICs on the Sensititre plates were read in a special viewer which projects the plate image against a dark background with a superimposed grid pattern (obtained from Seward Laboratory, UAC House, London, U.K.). This device simplifies reading and recording of the results. Except for sulfisoxazole, the MIC was defined as the lowest concentration of drug inhibiting the development of a button in the base of the well. With sulfisoxazole the endpoints were not clear-cut; hazy growth was often visible at higher drug concentrations than those which blocked button formation. This hazy appearance was ignored, and the MIC was taken as the concentration of sulfisoxazole at which the button of growth in the bottom of the wells was no longer visible. This difficulty with sulfisoxazole was also encountered with agar plates. Again the MIC was taken as the drug concentration that effected the greatest diminution of growth. Other agar MICs were read as the lowest drug concentrations causing an absence of growth.

β-Lactamase determination. β-Lactamase production by strains of *S. aureus* and *H. influenzae* was monitored with the chromogenic cephalosporin (nitrocefin) procedure described by O'Callaghan et al. (10).

RESULTS

Analysis of results. Because of the large quantity of data generated in this study the individual comparisons between the Sensititre and agar MICs are not presented here. The results of clinically more important organism-

TABLE 1. *Sensititre plate formats as used in this study*

Plate type	Column	Drug	Concn range (μg/ml)
APO1 (gram-positive isolates)	1	Benzylpenicillin	0.06–8
	2	Methicillin	0.12–16
	3	Ampicillin	0.12–16
	4	Cephalothin	0.5–64
	5	Gentamicin	0.12–16
	6	Kanamycin	0.5–64
	7	Erythromycin	0.25–32
	8	Chloramphenicol	0.25–32
	9	Clindamycin	0.12–16
	10	Tetracycline	0.12–16
	11	Vancomycin	0.25–32
APO2 (gram-negative isolates)	1	Ampicillin	0.25–32
	2	Carbenicillin	4–512
	3	Cephalothin	1–128
	4	Amikacin	0.25–32
	5	Gentamicin	0.12–16
	6	Kanamycin	0.5–64
	7	Tobramycin	0.12–16
	8	Chloramphenicol	0.5–64
	9	Sulfisoxazole[a]	0.5–64
	10	Tetracycline	0.25–32
APO3 (urinary tract isolates)	1	Trimethoprim-sulfamethoxazole	0.25/4.75–32/608
	2	Sulfisoxazole[a]	2–256
	3	Nalidixic acid	1–128
	4	Nitrofurantoin	2–256
	5	Ampicillin	1–128
	6	Carbenicillin	4–512
	7	Cephalothin	1–128
	8	Gentamicin	0.5–64
	9	Kanamycin	2–256
	10	Tetracycline	0.5–64

[a] Sulfisoxazole is not currently included on APO2 and APO3 plates.

antimicrobial agent combinations are given in Tables 2 to 6. The overall results are summarized in Tables 8 to 10, where the data are analyzed both by organism and by antimicrobial agent. These latter analyses present the results in terms of doubling dilution differences for pairs of Sensititre and agar MIC results.

Because the overall range of agar MICs was often greater than the antimicrobial concentration range on a single Sensititre plate, it was necessary to make various interpretations of results falling outside the actual concentration range in order to analyze all the data. These interpretations are summarized below, with an example of an agar dilution MIC, followed by the Sensititre MIC and the ratio taken in such a case: (i) 128 μg/ml:>32 μg/ml, ratio 1; (ii) >128 μg/ml:>32 μg/ml, ratio 1; (iii) >128 μg/ml:32 μg/ml, ratio 8 (since the agar value was greater than 128 μg/ml it was taken as 256 μg/ml); (iv) >128 μg/ml:128 μg/ml, ratio 2.

To ensure that this approach did not bias results, data were also analyzed, excluding any results where one or both MICs were either ">" or "≤".

Gram-positive organisms and APO1 plates. Table 2 shows the MICs of benzylpenicillin for *S. aureus* strains. Using the criterion of an MIC ≥ 0.25 μg/ml for penicillin resistance (2), there was a good correlation between β-lactamase production, as shown by the use of the chromogenic cephalosporin, and the MIC value by the Sensititre system.

By the agar dilution method one β-lactamase-positive strain required an MIC of 0.06 μg/ml, and another strain required an MIC of 0.125 μg/ml. Generally the MICs of β-lactamase-positive strains obtained with the Sensititre method were higher than with the agar dilution system, probably because of the lower inoculum used in the agar method. Eight of the β-lactamase-negative strains showed very low MICs (≤0.06 μg/ml)

TABLE 2. *Comparative MICs (μg/ml) for S. aureus strains tested against benzylpenicillin*

β-Lactamase	Agar dilution MIC	Sensititre MIC (APO1)
Positive	1	8
	1	8
	>8	>8
	0.25	0.25
	0.25	8
	0.25	0.5
	>8	>8
	>8	>8
	4	>8
	4	8
	0.06	0.25
	4	>8
	0.5	1
	4	>8
	0.5	0.5
	4	8
	0.25	2
	0.25	2
	0.125	0.5
	0.25	1
	0.5	1
	0.25	1
	0.5	2.0
Negative	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	≤0.06	≤0.06
	0.25	0.25
	0.5	1.0
	0.5	1.0

with both systems; however, three strains, β-lactamase negative by the chromogenic cephalosporin test, had MIC values ≥0.25 μg/ml by both techniques. There is no obvious explanation for this observation. The overall differentiation of β-lactamase-positive from β-lactamase-negative strains with the Sensititre method was satisfactory, there being no "false-negatives," i.e., β-lactamase-positive strains showing MICs of ≤0.125 μg/ml.

MICs measured for methicillin-resistant *S. aureus* by the two methods were in good agreement (Table 3). All strains of *S. aureus* previously shown to be erythromycin resistant by conventional disk diffusion methods (15-μg erythromycin disk giving a zone diameter < 15 mm) gave MICs of >32 μg/ml by the Sensititre procedure and from 2 to >32 μg/ml by the agar method (Table 3). All erythromycin strains susceptible by disk diffusion gave Sensititre MICs of ≤2.0 μg/ml (26 of 28 at ≤0.5 μg/ml, 1 at 1.0 μg/ml, and 1 at 2.0 μg/ml).

With one exception involving the MIC of penicillin for a strain of *S. faecalis*, where there was a three-doubling-dilution difference between the two methods, there were no discrepancies with other gram-positive organism-antibiotic combinations.

Gram-negative organisms and APO2 and APO3 plates. The MICs of ampicillin for three β-lactamase-positive and three β-lactamase-negative strains of *H. influenzae* are shown in Table 4. Sensititre gave better differentiation between ampicillin-resistant and -susceptible strains, but only when the 10^5-CFU/ml inoculum was used. With the lower inoculum density, there was no clear differentiation between β-lactamase-positive and -negative strains with either method.

The results of aminoglycoside testing with *Pseudomonas* strains are presented in Table 5, where they are analyzed both as those pairs of

TABLE 3. *Comparative MICs (μg/ml) for S. aureus strains resistant to either erythromycin or methicillin*

Strain resistant to:	MICs (μg/ml)	
	Agar dilution	Sensititre (APO1)
Erythromycin	>32	>32
	4	>32
	4	>32
	2	>32
	4	>32
	>32	>32
	>32	>32
Methicillin	8	16
	8	16
	8	8

TABLE 4. *Inoculum effect on ampicillin MICs against H. influenzae*

β-Lactamase	Ampicillin (μg/ml)			
	10^{4a}		10^{5a}	
	A	S	A	S
Positive	4	>32	4	>32
	2	4	16	>32
	2	4	16	32
Negative	<0.25	0.5	0.25	0.5
	1	0.5	1	1
	2	4	2	4

[a] 10^4 and 10^5 refer to either CFU per milliliter in the Sensititre (S) broth inoculum (using APO2 plates for ampicillin determinations) or to CFU transferred to the agar surface for the agar (A) MIC method.

MICs having two "finite" results, as defined earlier, and also in toto. The results were further subdivided by aminoglycoside and then again as to whether or not they indicated the organism was resistant or susceptible. This latter division was made by assigning breakpoint values to each aminoglycoside. When both of the MICs for a given organism were equal to or less than the breakpoint value, the organism was classified as susceptible. If one or both MICs were greater than the breakpoint, the organism was classified as resistant to the aminoglycoside. The breakpoint values chosen were as follows: gentamicin and tobramycin, 4.0 μg/ml; amikacin and kanamycin, 8.0 μg/ml. In this analysis the organism-antibiotic MIC pairs were treated individually, i.e., a particular organism may have given resistant values with one aminoglycoside and susceptible values with the next. Table 5 shows that when all the results were considered, the modal ratio was consistently 1, but with a tendency for the agar results to be higher than the Sensititre values, particularly for amikacin and gentamicin. This tendency was more marked when only those pairs of results with finite values were considered, especially for strains resistant to gentamicin, where the modal ratio was 2. Kanamycin and tobramycin did not demonstrate this phenomenon. This distinction between broth and agar MICs for aminoglycoside antibiotics has been demonstrated previously (12). To test whether this tendency toward higher agar MIC values could lead to a misinterpretation of the values, the results were examined according to the breakpoint values given earlier for classifying an organism as susceptible or resistant.

The results where there were discrepancies, i.e., susceptible by one method and resistant by the other, are shown in Table 6. For disk diffusion testing of *Pseudomonas* spp. against aminoglycosides it is normal to include an intermediate category in the results. It would probably be more correct to consider the results occurring at these breakpoint values in this intermediate category of susceptibility and reanalyze the results for major discrepancies, i.e., where the result was fully susceptible by one method and fully resistant by the other. There were only two

TABLE 6. *Pseudomonas species and aminoglycosides: discrepant results*

Aminoglycoside (breakpoint MIC in μg/ml)	Agar MIC (μg/ml)	Sensititre MIC (μg/ml)
Amikacin (≤8)	16	8
	16	8
Gentamicin (≤4)	8	2
	8	4
	8	4
	8	4
	16	4
Kanamycin (≤8)	16	8
Tobramycin (≤4)	4	8
	8	2
	8	4

TABLE 5. *Comparative MIC ratios for Pseudomonas strains and aminoglycosides*[a]

Results	Drug	Susceptibility	No. of strains having agar MIC/Sensititre MIC ratio of:					% of results with ratio of 2, 1, or 0.5
			4	2	1	0.5	0.25	
Total	Amikacin	R		5	**13**	1		100.0
		S	2	9	**12**	3		80.7
	Gentamicin	R	6	7	**15**	1		79.3
		S	3	4	**9**			81.25
	Kanamycin	R	1	3	**28**	5	1	94.7
		S		1	**4**	2		100.0
	Tobramycin	R	1	3	**9**	2		93.3
		S	3	4	**16**	6	1	86.7
Finite	Amikacin	R		4	**5**			100.0
		S	2	9	**12**	3		80.7
	Gentamicin	R	6	**8**	2			62.5
		S	3	4	**7**			78.5
	Kanamycin	R	1	3	**5**	5		92.8
		S		1	**2**			100.0
	Tobramycin	R	1	**3**	**3**	1		87.5
		S	3	4	**15**	6	1	86.2

[a] A total of 45 isolates were examined: 36 *P. aeruginosa*, 3 *P. fluorescens*, 3 *P. maltophilia*, and 3 *Pseudomonas* spp. Modal values are in boldface type. R, Resistant; S, susceptible.

such cases with two different organisms, one with gentamicin and the other with tobramycin (Table 6). Thus, of the 132 pairs of aminoglycoside-*Pseudomonas* spp. results, only 2 can be considered as major discrepancies, both classifying an organism susceptible by Sensititre and resistant by the agar method.

The sulfisoxazole MIC results in Table 7 are divided according to the laboratory from which they were obtained. Although apparently identical methods were used, the results of one laboratory showed a markedly skewed distribution towards obtaining higher MICs by the agar dilution than by Sensititre, whereas at the second laboratory good agreement was found between the two methods. There is no ready explanation for this observation. Co-trimoxazole, a mixture of trimethoprim plus sulfamethoxazole, did not show this discrepancy at either laboratory.

Overall distribution of results. The overall results were analyzed by organism type (Table 8) and by antimicrobial agent (Table 9 and 10). The two methods gave at least 80% agreement within one doubling dilution, with all organisms except *Hafnia* spp., when the APO2 plates were used. Since only two species of *Hafnia* were tested, the low value of 70% agreement may be due to sampling error; this is supported by the

TABLE 7. *Distribution of sulfisoxazole results by laboratory*[a]

Laboratory	No. of isolates showing agar MIC/Sensititre MIC ratio of:					
	≥8	4	2	1	0.5	0.25
1	23	**40**	32	14	6	
2		12	19	**30**	18	6
Overall (% distribution)	23 (11.5)	**52** (26.0)	51 (25.5)	44 (22.0)	24 (12.0)	6 (3.0)

[a] The modal values are in boldface type; only finite results are included in this table.

TABLE 8. *Analysis of all results*

Isolate	Plate type	No. of tests[a]	% Distribution of paired MICs		
			Absolute agreement	±1 doubling dilution	±2 doubling dilutions
S. aureus	APO1	385	49.1	90.1	96.4
Staphylococcus albus	APO1	165	67.2	95.7	100
Micrococcus spp.	APO1	66	66.7	96.9	100
S. faecalis	APO1	165	49.7	96.9	99.4
E. coli	APO2	390	50.2	87.2	97.1
Klebsiella spp.	APO2	380	60.5	91.8	99.7
Proteus spp.	APO2	290	38.9	81.1	98.6
Citrobacter spp.	APO2	90	53.3	86.1	100
Enterobacter spp.	APO2	60	70	98.3	100
P. aeruginosa	APO2	160	51.2	80.6	99.3
Serratia spp.	APO2	50	62	94	100
Hafnia app.	APO2	20	45	70	100
Pasteurella/Moraxella spp.	APO2	10	80	100	100
H. influenzae, 10^5 inoculum	APO2[b]	66	40.9	80.2	95.5
H. influenzae, 10^4 inoculum	APO2[b]	66	31.8	83.3	97.0
E. coli	APO3	390	62.5	93.6	100
P. aeruginosa	APO3	160	50.6	86.2	99.3
Klebsiella spp.	APO3	380	62.4	92.1	99.5
Proteus spp.	APO3	290	47.2	84.1	97.6
Citrobacter spp.	APO3	90	58.9	83.3	98.9
Enterobacter spp.	APO3	60	70.0	98.3	100
Pasteurella/Moraxella spp.	APO3	10	70.0	100	100
Serratia spp.	APO3	50	62.0	88.0	96.0
Hafnia spp.	APO3	20	60	85	100

[a] Total, 3,813.
[b] Plus benzylpenicillin.

85% agreement when the same two species were tested with APO3 plates. Other results are in good agreement especially in view of the different methods used for the MIC determinations.

An analysis of all the pairs of MICs by antimicrobial agent regardless of whether or not finite MICs were obtained is presented in Table 9. To ensure that the analysis was not unduly biased by this treatment the data were reanalyzed, including now only those results giving finite values, i.e., no "<" or ">" values. As shown in Table 10, this analysis did not significantly alter the distribution of results. In both analyses the sulfisoxazole MICs showed the most skewed distribution, as discussed above. In Table 10 the benzylpenicillin results were also biased towards low agar MICs, but, as shown in Table 2, the Sensititre results more clearly distinguished between β-lactamase-positive and -negative organisms. The results are highly acceptable, if those of benzylpenicillin and sulfisoxazole are excluded, with a modal ratio of 1 for 14 of the remaining 16 antimicrobial agents. Vancomycin and cephalothin had modal ratios of 0.5; vancomycin showed a tendency to give lower agar dilution MICs in both laboratories, whereas the cephalothin MICs were skewed in this direction only in the results from one laboratory. When all the MIC values were included 89.5% of the results showed a ratio of agar MIC to Sensititre MIC of 2, 1, or 0.5; when only those results having finite values were used, 86.6% of the paired MICs were within one doubling dilution of each other. Exclusion of the sulfisoxazole results gives corresponding values of 91.2% and 89.1% for the total and finite value analyses.

DISCUSSION

In this study with 222 clinical isolates we have demonstrated good overall agreement between two different methods of MIC determination. Sensititre, a microtitration broth system available as dried trays, gave accurate results comparable to those obtained with an agar dilution method. These trays, stable for extended periods at room temperature, should enable laboratories to obtain accurate MICs for a wide range of antimicrobial agents.

When testing organisms synthesizing extracellular β-lactamase enzymes, the MICs for β-lac-

TABLE 9. *Distribution of results by drug: all results*

Drug	No. of tests	No. of isolates with agar MIC/Sensititre MIC ratio of[a]:							% of results with ratio of 2, 1, or 0.5
		>8	4	2	1	0.5	0.25	≤0.125	
Penicillin	83	1	0	6	**31**	29	11	5	79.5
Methicillin	71	0	0	11	**45**	13	2	0	97.2
Ampicillin	373	0	6	44	**216**	79	23	5	90.9
Cephalothin	373	3	13	31	**181**	112	30	3	86.9
Gentamicin	373	0	17	68	**167**	105	15	1	91.2
Kanamycin	373	0	4	46	**186**	111	25	1	92.0
Erythromycin	71	0	0	2	**40**	20	4	5	87.3
Chloramphenicol	228	1	7	39	**112**	61	6	2	93.0
Clindamycin	71	0	0	4	**53**	13	0	1	98.6
Tetracycline	373	1	11	47	**194**	112	8	0	94.6
Vancomycin	71	0	0	3	28	**38**	2	0	97.2
Carbenicillin	302	0	1	16	**226**	40	18	1	93.4
Amikacin	157	0	2	23	**71**	48	12	1	90.4
Tobramycin	157	0	6	12	**66**	49	22	2	80.9
Sulfisoxazole	302	23	52	61	**121**	30	13	2	70.2
Cotrimoxazole	145	0	4	12	**126**	2	1	0	96.6
Nalidixic acid	145	3	7	18	**77**	37	3	0	91.0
Nitrofurantoin	145	1	2	17	**75**	41	7	2	91.7
Percentages of overall results		0.8%	3.4%	12.1%[b]	52.8%[b]	24.6%[b]	5.3%	0.8%	89.5
Percentages of results without sulfisoxazole data		0.3%	2.3%	11.4%[c]	53.9%[c]	25.9%[c]	5.4%	0.8%	91.2

[a] The modal value within the distribution is shown in boldface type.
[b] Total, 89.5%.
[c] Total, 91.2%.

TABLE 10. *Distribution of results by drug: finite pairs of MICs*

Drug	No. of tests	No. of isolates with agar MIC/Sensititre MIC ratio of[a]:							% of results with ratio of 2, 1 or 0.5
		≥8	4	2	1	0.5	0.25	≤0.125	
Penicillin	48	0	0	4	11	**22**	6	5	77.1
Methicillin	58	0	0	11	**35**	10	2	0	96.5
Ampicillin	190	0	6	28	**72**	60	21	3	84.2
Cephalothin	226	2	12	20	67	**100**	23	2	82.7
Gentamicin	251	0	12	55	**109**	66	8	1	91.6
Kanamycin	225	0	4	36	**97**	69	19	0	89.8
Erythromycin	16	0	0	2	**8**	4	2	0	87.5
Chloramphenicol	192	1	5	27	**95**	60	4	0	94.8
Clindamycin	13	0	0	3	**5**	**5**	0	0	100.0
Tetracycline	272	1	11	45	**121**	86	8	0	92.6
Vancomycin	67	0	0	3	27	**37**	0	0	100.00
Carbenicillin	106	0	1	12	**51**	26	14	1	83.9
Amikacin	150	0	2	23	**70**	44	11	0	91.3
Tobramycin	141	0	6	10	**61**	42	21	1	80.1
Sulfisoxazole	200	23	**52**	51	44	24	6	0	59.5
Cotrimoxazole	33	0	4	6	**22**	1	0	0	87.9
Nalidixic acid	111	0	7	13	**62**	23	3	0	88.3
Nitrofurantoin	121	1	1	16	**60**	38	5	0	94.2
Percentages of overall results		1.2%	5.1%	15.1%[b]	42.1%[b]	29.6%[b]	6.4%	0.5%	86.8
Percentages of results without sulfisoxazole data		0.2%	3.2%	14.1%[c]	43.8%[c]	31.2%[c]	6.9%	0.6%	89.1

[a] The modal value within the distribution is shown in boldface type.
[b] Total, 86.8%.
[c] Total, 89.1%.

tamase-susceptible antibiotics were generally lower with the agar method than with Sensititre. The use of a 10^3-CFU inoculum contributed to this difference, but when Ericsson and Sherris also tested a β-lactamase-producing *Staphylococcus epidermidis* against benzylpenicillin they obtained a lower agar MIC than by a broth method even though a 10^4-CFU surface inoculum had been used (4).

When the Ericsson and Sherris inoculum level of 10^4 CFU was used with *H. influenzae* strains and the agar method, there was poor differentiation between β-lactamase-positive and -negative strains, as was also the case for the 10^4-CFU/ml inoculum with Sensititre. With both methods it was only when higher inoculum levels were used that there was good differentiation between the strains.

All the β-lactamase-positive *S. aureus* strains had Sensititre MICs against benzylpenicillin of ≥0.25 μg/ml, and those for 18 of 23 of the strains were at least 1.0 μg/ml. There is no explanation for the three β-lactamase-negative strains with MICs ≥0.25 μg/ml by both methods.

Determination of the methicillin MICs against *S. aureus* strains, where any inoculum effect is much less significant, gave very similar values by both methods, which also clearly differentiated between methicillin-susceptible and -resistant strains.

Erythromycin-resistant *S. aureus* strains had higher MICs in the Sensititre system than with the agar method. Ericsson and Sherris (4) gave data for one *S. aureus* strain, erythromycin resistant by the disk diffusion test zone size, which showed showing geometric mean MICs of 10 μg/ml by the agar method and 36 μg/ml for the broth macrodilution method.

The MICs of aminoglycosides for *Pseudomonas* tended to be higher by the agar method, although the modal ratio of agar MIC to Sensititre MIC in nearly all cases was still 1. The higher agar MICs were probably due to raised Ca^{2+} and Mg^{2+} concentrations, which are known to antagonize aminoglycoside activity against *Pseudomonas* spp. (J.B. Cassals, Proc. 9th. Int. Cong. Chemother., abstr. no. M-521, 1975). However, examination of the results demonstrates that only 2 of the 132 *Pseudomonas*-aminoglycoside tests gave major discrepancies in which

one method indicated susceptibility and the other indicated resistance.

The main difficulty encountered in this study was determining sulfisoxazole MICs. Since discrepant results were only obtained in one laboratory, the reason probably lies not in the Sensititre system but in the extreme sensitivity of sulfisoxazole MIC determinations to variations in laboratory technique.

A previous study comparing commercially prepared frozen microdilution plates with a broth macrodilution system gave agreement within one doubling dilution of 85% (3). We regard the overall correlation between Sensititre and the agar MIC technique as highly acceptable: 86.8% of finite pairs of MIC results agree within one doubling dilution (89.1% with exclusion of the sulfisoxazole results).

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