Journal of Clinical Pathology, 1978, **31**, 536-538

An assessment of the Sensititre method for determining minimum inhibitory and bactericidal concentrations

ANGELA H. PYKETT

From the Public Health Laboratory, Bridle Path, York Road, Leeds LS15 7TR, UK

SUMMARY Minimum inhibitory and bactericidal concentrations of various antibiotics were determined for 90 strains of five organisms by Sensititre and compared with those obtained by conventional methods. Results by both methods correlated well. The advantages and limitations of Sensititre are discussed.

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) by conventional tube or plate dilution methods is a time-consuming and technically demanding procedure, involving weighing out and making up serial dilutions of the antibiotics accurately and aseptically.

A new product, Sensititre, marketed by Seward Laboratory, is essentially a miniaturised method of determining MICs in which the work of preparing antibiotic dilutions has already been done by the manufacturer, thus saving technician time and equipment.

The present investigation was undertaken to compare the results obtained using Sensititre with those by conventional tube or plate dilution methods.

Material and methods

Thirty strains of *Staphylococcus pyogenes*, 10 each of *Proteus mirabilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and 30 streptococci, all recently isolated from clinical material, were used.

Sensititre consists of a microtitre plate with doubling dilutions of antibiotics dried into the wells. Three types of plates are available. For staphylococci the antibiotics are benzypenicillin, cloxacillin, fusidic acid, and gentamicin; for Gram-negative bacilli, ampicillin, carbenicillin, cephaloridine, and gentamicin; for streptococci, benzylpenicillin, ampicillin, cephaloridine, and erythromycin. A duplicate set of antibiotics is provided in each plate for a standard control organism. Ten dilutions of each antibiotic and two wells without antibiotic (to serve as negative and positive controls) are accommodated on each row of a plate.

Sensititre plates were set up as follows. The test organism and control were grown overnight in Oxoid Isosensitest broth and the opacity was adjusted to 10^9 organisms per ml using Brown's tubes. The growth was further diluted to give an inoculum of 10^6 per ml, as recommended by the manufacturer; 0·05 ml of inoculum was then delivered into the appropriate wells using the disposable pipettes provided with the kit and broth alone to one well in each row to serve as a negative control.

The plates were sealed with adhesive plastic and incubated at 37°C overnight. After incubation the plates were read from below using the Sensititre viewer, and the MIC was recorded as the last well showing no button of growth. MBCs were then determined by subculture to a nutrient agar for the staphylococci and Gram-negative bacilli and to blood agar for the streptococci using the Sensititre multi-inoculator.

MICs and MBCs for all the strains of staphylococci and Gram-negative bacilli were determined by a standard tube dilution method. Antibiotic dilutions were made from the pure powder in sterile demineralised water. Final dilutions were made in Oxoid Isosensitest broth in 1 ml volumes, and 0·05 ml of an inoculum of 10^6 per ml was used, as recommended by Waterworth (1973a). After incubation overnight at 37°C, tubes showing no growth were subcultured to a nutrient agar for the MBC determinations. MICs for the streptococci were determined by a plate method. The antibiotic dilu-

Received for publication 17 October 1977

tions were prepared in Oxoid Isosensitest agar, and 0·02 ml of an inoculum of 10^6 per ml was used.

Control organisms used throughout were the Oxford staphylococcus for the staphylococcal and streptococcal estimations and *Escherichia coli*, NCTC 10418, for the Gram-negative bacilli. Sensitivity was also determined for all the organisms by the disc method using Stokes (1968) technique.

Results

Nearly 30% of the values for the Gram-negative bacilli and streptococci and 10% of those for the staphylococci fell outside the range of concentrations of antibiotics in the Sensititre plates and were not included in the results. The Gram-negative bacilli and staphylococci were too resistant and the streptococci too sensitive for endpoints to be obtained. This finding applied in particular to ampicillin and cephaloridine with the Gram-negative bacilli, to benzylpenicillin with the staphylococci, and to benzylpenicillin and cephaloridine with the streptococci.

The remaining values were used for analysis. Tables 1 and 2 show how the results by Sensititre compare in terms of dilution difference from results by the conventional methods. Regression lines were drawn for each organism and for each antibiotic (Figs 1 and 2).

For the staphylococci, 83% of the MICs and 77% of the MBCs showed no more than one dilution difference by the two methods, and 96% of the results were no more than two dilutions different.

Table 1 *Overall results by Sensititre in terms of number of dilutions difference from results by conventional methods*

	Dilution difference								
	−4	*−3*	*−2*	*−1*	*0*	*+1*	*+2*	*+3*	*+4*
Total no. of tests	1	3	31	69	132	132	29	4	1
% of total	0·3	0·8	7·7	17·2	32·8	32·8	7·2	1·0	0·2

Table 2 *Percentages of Sensititre results differing from results by conventional methods in terms of dilution difference for each organism and antibiotic*

	Dilution difference								
	−4	*−3*	*−2*	*−1*	*0*	*+1*	*+2*	*+3*	*+4*
MICs:									
Staphylococci			5	19	31	33	9	2	1
Gram-negative bacilli		1	2	16	35	40	5	1	
Streptococci	3		12	20	20	31	14		
Benzylpenicillin				11	33	11	28	11	6
Cloxacillin				6	34	47	13		
Fusidic acid			17	50	30	3			
Gentamicin			2	22	35	39	2		
Ampicillin			13	0	50	37			
Carbenicillin			5	9	18	40	28		
Cephaloridine					18	75	7		
Erythromycin			8	14	50	14	14		
MBCs:									
Staphylococci		2	13	17	29	31	6	2	
Gram-negative bacilli			9	14	41	31	5		
Benzylpenicillin				13	40	7	27	13	
Cloxacillin				14	36	43	7		
Fusidic acid		11	50	23	10	6			
Gentamicin			8	17	41	32	2		
Ampicillin			17	13	44	17	9		
Carbenicillin				10	24	52	14		
Cephaloridine	6		6	24	59	6			

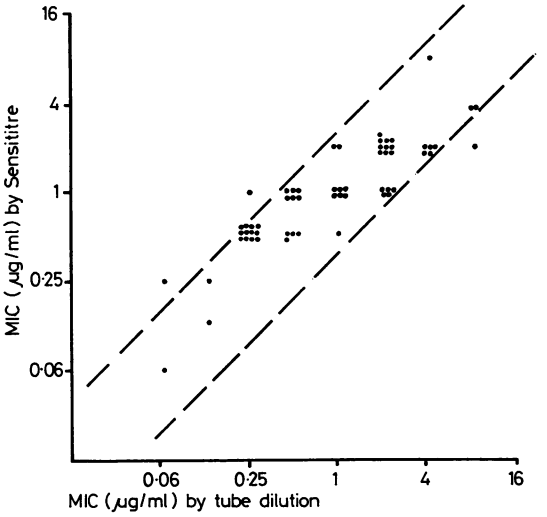

Fig. 1 *Regression line for gentamicin*

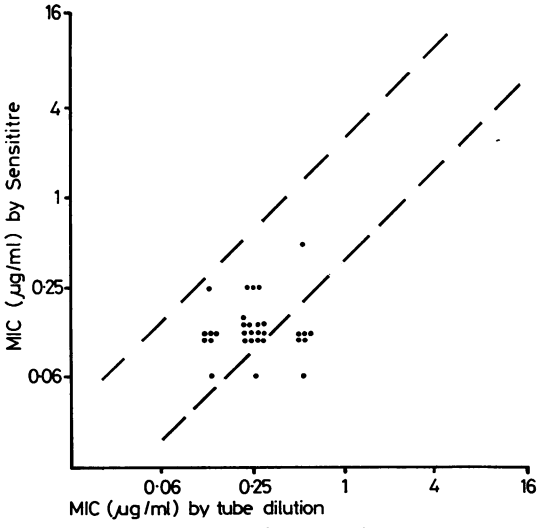

Fig. 2 *Regression line for fusidic acid.*

For the Gram-negative bacilli, 91% of the MICs and 86% of the MBCs were within one dilution different and 98% of the MICs and all the MBCs were not more than two dilutions different. For the streptococci, the correlation was less good, 71% and 97% of the MICs being respectively not more than one and two dilutions different.

For all antibiotics except fusidic acid, a greater percentage of Sensititre results were higher than with the conventional method. The best correlation was for gentamicin, 96% of MICs and 90% of MBCs being within one dilution difference and none of the results being more than two dilutions different. The poorest correlation appeared to be with benzylpenicillin and staphylococci. Only 55% of MICs and 60% of MBCs were within one dilution difference and 45% of the MICs by Sensititre were two to four dilutions higher than the tube dilution results.

However, in 11 strains of staphylococci established as resistant to benzylpenicillin on testing by the disc method as described by Stokes (1968) and applying the criteria laid down by Waterworth (1973b), the MIC established by the Sensititre method correlated well in nine out of these 11 strains. The tube dilution MICs, carried out as advised by Waterworth (1973a), on the other hand, gave very poor correlation with the disc method, with only two out of 11 in agreement. The tube dilution method cannot be used as a valid basis of comparison, probably because of the relatively smaller inoculum used in the tube dilution method giving rise to anomalous results with these penicillinase-producing strains.

Although two of the strains of staphylococci appeared to be resistant to methicillin on disc testing at 30°C, neither Sensititre nor the tube dilution method showed clear evidence of resistance to cloxacillin at 37°C. There were no major discrepancies between the results by all three methods for the Gram-negative bacilli and the streptococci.

Discussion

The results by Sensititre correlated well on the whole with those obtained by conventional methods. The higher results by Sensititre for staphylococci and benzylpenicillin were probably more valid than the results by the tube dilution method. The problem of determining sensitivity to cloxacillin remains to be resolved. There is obviously no provision with Sensititre for incubation at 30°C (Hewitt *et al.*, 1969) or for testing in the presence of 5% salt (Barber, 1964).

Particular difficulty was encountered with alpha-haemolytic streptococci. Some strains could not be persuaded to grow in the Sensititre plate although they grew well in Isosensitest broth overnight. The initial inoculum in the microtitre plate was probably too small to initiate growth.

Difficulty was also occasionally encountered in reading the endpoint with Sensititre. Instead of a sharp cut-off, the buttons of growth sometimes showed a gradual diminution in size around the endpoint. The manufacturers state that 'minute' buttons should be ignored as being due to slightly more resistant variants within a strain. In practice, it was difficult to decide how much smaller than the control a button should be before it could be classed as 'minute'. In contrast, the tube and plate dilution methods consistently gave clear-cut endpoints.

The advantage of Sensititre is, of course, that it takes only a few minutes to set up a plate once the inocula have been prepared compared with the much more time-consuming conventional methods. It requires very little technical expertise and should avoid errors in making up antibiotic dilutions. It can also be adapted for use with anaerobic organisms, the multi-inoculator being used to perforate the sealing plastic before incubation in an anaerobic atmosphere.

The major disadvantage of Sensititre is that the range of antibiotics provided for a particular organism is limited. Plates with a much wider range of antibiotics are being developed by Seward Laboratory. The two control wells for each row seemed superfluous, and it would probably be more useful to have a wider range of dilutions for each antibiotic.

I acknowledge the help and advice of Dr G. L. Gibson in preparing this paper.

References

Barber, M. (1964). Naturally occurring methicillin-resistant staphylococci. *Journal of General Microbiology*, **35**, 183-190.

Hewitt, J. H., Coe, A. W., and Parker, M. T. (1969). The detection of methicillin resistance in *Staphylococcus aureus*. *Journal of Medical Microbiology*, **2**, 443-455.

Stokes, E. J. (1968). *Clinical Bacteriology*, 3rd edition, pp. 217-221. Arnold, London.

Waterworth, P. M. (1973a). In *Antibiotic and Chemotherapy*, 4th edition, edited by L. P. Garrod, H. P., Lambert, and F. O'Grady, pp. 508-510. Churchill Livingstone, Edinburgh and London.

Waterworth, P. M. (1973b). In *Antibiotic and Chemotherapy*, 4th edition, edited by L. P. Garrod, H. P. Lambert, and F. O'Grady, pp. 496-500. Churchill Livingstone, Edinburgh and London.

Requests for reprints to: Dr A. H. Pykett, Senior Registrar, Public Health Laboratory, Bridle Path, York Road, Leeds LS15 7TR, UK.