ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1979, p. 255-261
0066-4804/79/09-0255/07$02.00/0

Vol. 16, No. 3

Australian Evaluation of Autobac I with Suggested Interpretive and Technical Modifications

GLENN R. FUNNELL* AND MICHAEL D. G. GUINNESS

Department of Microbiology, Repatriation General Hospital, Concord, NSW, Australia 2139

Received for publication 14 June 1979

Autobac I, a recently introduced semiautomated method for rapid antibiotic susceptibility testing, has been evaluated by comparison with the calibrated dichotomous sensitivity disk diffusion technique, which is routinely used in many Australian hospitals. Only the most common clinical isolates, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp., and *Proteus mirabilis*, were included in this evaluation, and an overall interpretive agreement of 93% was obtained. However, an unusually high rate of discrepancy was noted in several organism-antibiotic combinations, in particular *E. coli* and *P. mirabilis* with ampicillin, *S. aureus* with penicillin, and methicillin-resistant *S. aureus* with methicillin, erythromycin, and clindamycin. The discrepancies associated with ampicillin have been reduced from 29 and 24% for *E. coli* and *P. mirabilis*, respectively, to less than 5% after the utilization of commercial 10-μg diffusion disks, in preference to the lower antibiotic content disks supplied by the Autobac manufacturer. Furthermore, modifications in the interpretive procedure have eliminated discrepancies associated with *S. aureus* and penicillin.

The manufacturers of Autobac I, Pfizer Diagnostics Inc., claim that their product offers high interpretive agreement (average, 90%; range, 80 to 100%) with the results of the Bauer-Kirby agar diffusion method. These figurés were confirmed in a collaborative study performed at several United States hospitals (7). Since the Autobac in the Division of Microbiology, Repatriation General Hospital, Concord, was the first to be installed in Australia, a similar evaluation was undertaken to compare Autobac I susceptibility results with those obtained by the calibrated dichotomous sensitivity (CDS) disk diffusion technique. Organism-antibiotic combinations which showed poor correlation were studied in greater detail.

MATERIALS AND METHODS

Organisms. Clinical isolates of *S. aureus*, *E. coli*, *Klebsiella* sp., and *P. mirabilis* were used. The staphylococci were differentiated into three groups according to their susceptibility to methicillin as determined by the disk diffusion technique, and to their ability to produce penicillinase, this being determined by direct observation of the inhibitory zone surrounding the penicillin disk. A sharp well-defined border or large, discreet colonies at the zone edge indicated penicillinase production (1, 5, 8). The validity of this technique has been confirmed in our laboratory by comparative studies with iodometric and acidometric methods.

Susceptibility test methods. All antibiotic susceptibility tests were performed on fresh overnight cultures grown on Columbia agar supplemented with 5% defibrinated horse blood.

The Autobac I testing was performed according to the manufacturer's instructions except where indicated. Commercially prepared ampicillin diffusion disks (Oxoid Ltd.; 10 μg) were used in the Autobac I system during the investigation of discrepancies associated with ampicillin.

The CDS agar disk diffusion technique as described by Bell (1) was followed. Oxoid Sensitest agar CM409 and Oxoid diffusion disks were used. This diffusion technique has several features which differ from the Bauer-Kirby single disk method. The agar plates are inoculated by the flood plate method by using a bacterial suspension of 10^6 to 10^7 organisms per ml, while zone sizes are measured as the annular radius rather than as the zone diameter. Antibiotic disk masses were selected so that a single interpretive criterion for all organism-antibiotic combinations tested could be used. An organism is considered susceptible to an antibiotic if the annular radius is $\geq$6 mm, whereas it is interpreted as resistant if the annular radius is $<$6 mm. There is no interpretation of intermediate susceptibility in the CDS system. Similar to the Bauer-Kirby technique, this system was calibrated with the International Collaborative Study (ICS) reference method for quantitative sensitivity testing using the universally accepted inoculum of 10^4 organisms. The validity of this calibration has since been confirmed in our laboratory. Cephalothin sensitivity testing was included in the present evaluation even though there is lack of calibration with the ICS method (1). The ampicillin disk mass used was 10 μg rather than 25 μg as specified by Bell. This modification does not affect the correlation between disk diffusion and quantitative sensitivity results.

The results from the CDS and Autobac I procedures were compared, and any discrepancies were catego-

rized as minor, major, or very major as defined by Thornsberry et al. in the original collaborative evaluation (7).

Minimum inhibitory concentrations (MIC) were determined by using the ICS agar dilution method (3) on those organism-antibiotic combinations demonstrating discrepant results in the CDS-Autobac I evaluation—specifically *E. coli* and *P. mirabilis* with ampicillin and *S. aureus* with methicillin, erythromycin, and clindamycin.

RESULTS

The correlations in the antibiotic susceptibility results obtained in comparative trials using Autobac I and the CDS disk diffusion technique are demonstrated in Table 1.

Whereas the majority of organism-antibiotic combinations showed good correlation (>90%) there were several which were unsatisfactory, including *S. aureus* and penicillin, methicillin-resistant *S. aureus* and methicillin, erythromycin, and clindamycin, *E. coli* and ampicillin, and also *P. mirabilis* and ampicillin. Further investigations were performed on these combinations.

***S. aureus*-penicillin.** A total of 152 isolates of *S. aureus*, consisting of 42 non-penicillinase producers and 110 penicillinase producers, were studied.

Although the manufacturers have claimed that *S. aureus* should have a light-scattering index (LSI) greater than 0.90 before it is interpreted as susceptible to penicillin, 36% of the non-penicillinase producers tested had an LSI in the range 0.6 to 0.9. Concurrently, 6% of the penicillinase-producing strains had an LSI within the same range, thereby making it impossible to accurately interpret such a result. (Table 2)

This problem was resolved by simply reincubating the cuvette if the LSI for penicillin was between 0.6 and 0.9, thus allowing the eluted penicillin further time to inhibit cell wall production. An increase in the LSI after reincubation was consistently associated with a penicillin-susceptible result by the disk diffusion method. Similarly, a reduction in the LSI was associated with penicillin resistance.

This interpretive modification enabled every member of this group of staphylococci to be correctly classified as susceptible to penicillin even though the LSI might never have reached 0.90. It effectively eliminated discrepancies involving non-penicillinase-producing *S. aureus*.

Enterobacteriaceae-ampicillin. The expected bipolar distribution of LSI was not obtained when the ampicillin susceptibility of 94 strains of *E. coli* was examined. Instead, the results were widely scattered (Fig. 1).

Only 60% of the *E. coli* susceptible to ampicillin by the disk diffusion technique were similarly interpreted by Autobac I. The remainder were interpreted as either intermediate or resistant.

This problem was further emphasized in a result precision trial. Seven strains of *E. coli* previously demonstrated to be susceptible to ampicillin by both disk diffusion and MIC testing were tested in replicate by Autobac I. Only the laboratory control *E. coli* (MIC, 2 μg/ml) demonstrated consistent susceptibility to ampicillin (Table 3). The other six strains (MIC, 4 to 8 μg/ml) all had an average LSI of <0.60, and in each case the degree of variation was completely unacceptable. Neither the individual LSI nor

TABLE 2. *LSI for S. aureus-penicillin*[a]

Strain	% Organisms within LSI range		
	<0.60	0.60–0.90	>0.90
Non-penicillinase producing (n = 42)		36	64
Penicillinase producing (n = 110)	89	6	5

[a] n = Number of isolates tested.

TABLE 1. *Correlation of antibiotic susceptibility results (%)*[a]

S. aureus[b]	Penicillin	Methicillin	Erythromycin	Clindamycin	Kanamycin
Non-penicillinase producers (n = 42)	64	100	100	100	100
Penicillinase producers (n = 110)	95	100	92	99	97
Methicillin resistant (n = 39)	100	74	77	36	100
Enterobacteriaceae[c]	**Ampicillin**	**Cephalothin**	**Kanamycin**	**Carbenicillin**	**Chloramphenicol**
E. coli (n = 94)	71	86	95	100	91
Klebsiella sp. (n = 84)	99	87	94	95	95
P. mirabilis (n = 37)	76	84	100	97	92

[a] n = Number of isolates tested.
[b] Cephalothin, gentamicin, and vancomycin had a correlation of 100% for each strain of *S. aureus* tested.
[c] Gentamicin and tobramycin had a correlation of 100% for each strain of enterobacteriaceae tested.

the resultant sensitivity interpretations were reproducible.

The ampicillin elution disk used in this trial had a disk mass (antibiotic content) of 3.6 μg. Since this was eluted in 1.5 ml of eugonic broth, the final concentration in the cuvette was only 2.4 μg/ml, which would seem too low to test organisms with an MIC of 4 to 8 μg/ml. Subsequently, Pfizer introduced a new ampicillin disk with a mass of 4.5 μg. This produced an intracuvette concentration of 3.0 μg/ml, which proved to be similarly insufficient. Scattering similar to that depicted in Fig. 1 was obtained, whereas the results of the precision trial demonstrated an identical trend to that achieved with the 3.6-μg disk.

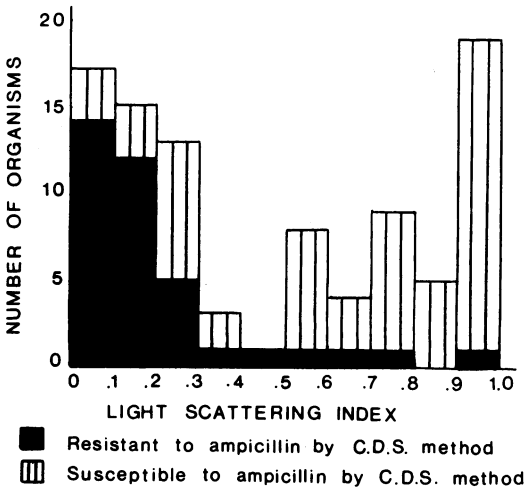

FIG. 1. *LSI for E. coli-ampicillin (3.6 μg). Resistant (■) or susceptible (▥) to ampicillin by CDS method.*

A third ampicillin disk, an Oxoid 10-μg diffusion disk, was tested in the Autobac I system. In the result precision trial, discrepancies from an interpretation of susceptibility were nil (Table 4). The results were reliable and reproducible, and each of the seven organisms had an average LSI greater than 0.90. Furthermore, in a comparative trial in which 94 clinical isolates of *E. coli* were tested by disk diffusion and Autobac I methodology, a definite bipolar distribution was obtained (Fig. 2). There were no major discrepancies, although very major discrepancies occurred in 5% of the organisms tested. This did not pose a serious problem because these organisms were invariably resistant to carbenicillin, which in the case of *E. coli* is an indication of concurrent ampicillin resistance. The carbenicillin result therefore served as an internal control in the interpretation of the ampicillin 10-μg result.

Use of 10-μg ampicillin disks similarly reduced the incidence of minor and major discrepancies for *P. mirabilis* from 24 to 3%. The LSI of seven ampicillin-resistant strains which were included in this survey group were not affected by the higher-content disks.

A similar trial involving 84 isolates of *Klebsiella* sp. was also performed, but with less satis-

TABLE 3. *Result precision trial: ampicillin (3.6 μg)*

E. coli strain	MIC (μg/ml)	Discrepancies[a]	LSI range	Avg LSI
E-59	8	0, 11, 8/22	0.12–0.99	0.50
E-60	8	0, 20, 2/22	0.15–0.57	0.34
E-61	8	0, 11, 0/12	0.12–0.69	0.32
E-62	4	0, 4, 4/12	0.28–0.93	0.53
E-64	4	0, 11, 5/22	0.14–0.77	0.47
E-65	4	0, 14, 2/18	0.14–0.73	0.35
ATCC 25922	2	0, 0, 0/20	0.95–1.00	1.00

[a] Numbers of very major, major, and minor discrepancies/total number of strains tested.

TABLE 4. *Result precision trial: ampicillin (10 μg)*

E. coli strain	MIC (μg/ml)	Discrepancies[a]	LSI range	Avg LSI
E-59	8	0, 0, 0/12	0.80–1.00	0.94
E-60	8	0, 0, 0/18	0.87–1.00	0.99
E-61	8	0, 0, 0/18	0.78–1.00	0.95
E-62	4	0, 0, 0/18	0.94–1.00	1.00
E-64	4	0, 0, 0/18	0.91–1.00	0.99
E-65	4	0, 0, 0/18	0.82–0.99	0.91
ATCC 25922	2	0, 0, 0/18	0.98–1.00	1.00

[a] Numbers of very major, major, and minor discrepancies/total number of strains tested.

factory results. The 10-μg disk initiated a generalized shift in the LSI toward 1.00, and, hence, many *Klebsiella* that were resistant to ampicillin were interpreted as susceptible by Autobac I. These very major discrepancies occurred in 11% of the organisms tested, compared with only 1% when the low-content disks were used.

MIC correlation. MIC data were correlated with susceptibility results obtained by both the CDS disk diffusion technique and Autobac I on those organism-antibiotic combinations which demonstrated a high discrepancy rate in the initial evaluation. A comparison of CDS and MIC results revealed an overall agreement of 99% (Table 5), whereas the same comparison between Autobac I and MIC results yielded only a 78% correlation (Table 6). The individual discrepancy rates for each of the organism-antibiotic combinations tested in this comparative study were similar to those observed in the initial CDS-Autobac I evaluation, thereby confirming those figures. It is also apparent that the use of 10-μg ampicillin disks in preference to the lower-content disks reduced the discrepancy rate associated with *E. coli* and *P. mirabilis* from 28 to 1% for the 95 strains tested by both Autobac I and the agar dilution method, verifying the findings presented in Fig. 1 and 2.

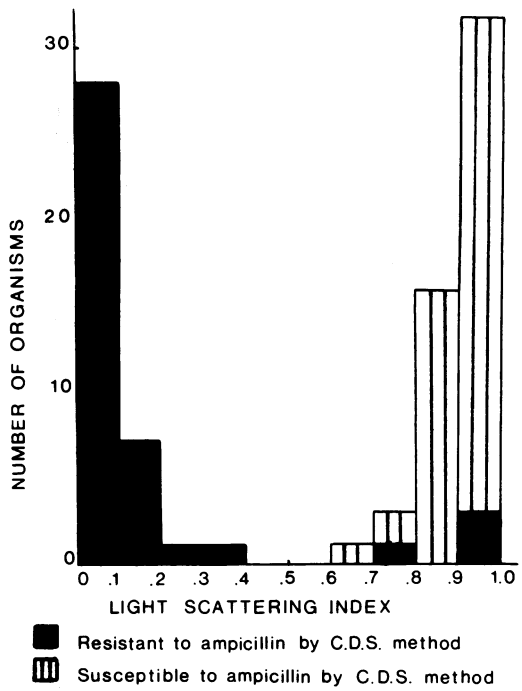

FIG. 2. *LSI for E. coli-ampicillin (10 μg). Resistant (■) or susceptible (▥) to ampicillin by CDS method.*

DISCUSSION

The comparative study between Autobac I and CDS disk diffusion susceptibility methods was limited to clinical isolates of *S. aureus*, *E. coli*, *P. mirabilis*, and *Klebsiella* sp. and involved a total of 3,054 tests. Other genera were not included in this evaluation because of the low number of strains isolated. Overall, the correlation between the two procedures was 93%, the results from the majority of organism-antibiotic combinations testing being in complete agreement. Nevertheless, several significant inconsistencies were demonstrated.

Discrepancies occurred in 20 of the 186 *S. aureus* tested against penicillin, the majority of which were in the non-penicillinase-producing group. Thornsberry et al. obtained similar results in their evaluation (7) in which discrepancies occurred in 21 of the 280 staphylococci tested. Unlike the present investigation, that of Thornsberry did not differentiate between penicillinase-producing and non-penicillinase-producing strains, and hence the high percentage of major discrepancies in the latter group was not apparent.

In our evaluation it has been shown that these discrepancies can be avoided if the cuvettes are incubated longer than the suggested 3 h because

TABLE 5. *Comparative study between the CDS disk diffusion and the ICS agar dilution test results*

Organism	Antimicrobial agent	Discrepancies[a]	% Agreement
E. coli	Ampicillin	0, 2, 0/64	97
P. mirabilis	Ampicillin	0, 1, 0/31	97
Non-penicillinase-producing *S. aureus*	Methicillin	0, 0, 0/15	100
	Erythromycin	0, 0, 0/15	100
	Clindamycin	0, 0, 0/15	100
Penicillinase-producing *S. aureus*	Methicillin	0, 0, 0/29	100
	Erythromycin	0, 1, 0/29	97
	Clindamycin	0, 0, 0/29	100
Methicillin-resistant *S. aureus*	Methicillin	0, 0, 0/38	100
	Erythromycin	0, 0, 0/38	100
	Clindamycin	0, 0, 0/38	100

[a] Numbers of very major, major, and minor discrepancies/total number of strains tested.

TABLE 6. *Comparative study between Autobac I and ICS agar dilution test results*

Organism	Antimicrobial agent	Discrepancies[a]	% Agreement
E. coli	Ampicillin (4.5 μg)	0, 14, 4/64	72
	Ampicillin (10 μg)	0, 0, 0/64	100
P. mirabilis	Ampicillin (4.5 μg)	0, 4, 5/31	71
	Ampicillin (10 μg)	0, 1, 0/31	97
Non-penicillinase-producing *S. aureus*	Methicillin	0, 0, 0/15	100
	Erythromycin	1, 0, 0/15	94
	Clindamycin	0, 0, 0/15	100
Penicillinase-producing *S. aureus*	Methicillin	0, 0, 0/29	100
	Erythromycin	3, 0, 0/29	90
	Clindamycin	1, 0, 0/29	97
Methicillin-resistant *S. aureus*	Methicillin	4, 0, 6/38	74
	Erythromycin	8, 0, 1/38	76
	Clindamycin	20, 0, 4/38	37

[a] Numbers of very major, major, and minor discrepancies/total number of strains tested.

this allows the penicillin more time to inhibit cell wall synthesis. Accordingly, in our laboratory the criteria for determining staphylococcal susceptibility to penicillin varies from the manufacturer's instructions. When the initial LSI reading is in the range of 0.6 to 0.9, instead of interpreting the result as resistant, the cuvette is reincubated for 30 min. If after this time the LSI has increased, further lysis of the bacteria has occurred and an interpretation of susceptibility is made. Similarly, if the LSI decreases, the result is interpreted as resistant. This modification has eliminated discrepancies associated with non-penicillinase-producing *S. aureus*.

The problem that might confront Autobac I susceptibility testing by methicillin-resistant strains of *S. aureus* was first suggested in the original collaborative report (7) and has since been demonstrated in a study by Cleary and Maurer (2). The findings reported in the present paper confirm the high discrepancy rate associated with these organisms, but the actual figures differ markedly from those of the previous study. There was a 74% agreement between the CDS and Autobac I techniques for the susceptibility testing of methicillin, whereas the equivalent figure obtained by Cleary and Maurer was only 21% (2). Similarly, for erythromycin these values were 77 and 98%, whereas for clindamycin the comparative figures were 36 and 79%, respectively. Subsequent MIC determinations have confirmed the validity of the CDS disk diffusion results because there was a 100% result correlation between the two testing procedures. The reasons for these discrepancies are therefore uncertain, although there is little doubt that different hospitals harbor different strains of methicillin-resistant staphylococci.

It has been our experience that staphylococci which are susceptible to methicillin by agar disk diffusion invariably have an LSI of >0.90, whereas those which are resistant have an LSI of <0.70. Consequently whenever the latter occurs, and if the organism is also resistant to kanamycin, a methicillin-resistant strain is immediately suspected. Furthermore, MIC testing has shown that all methicillin-resistant staphylococci isolated at Repatriation General Hospital, Concord, are also resistant to erythromycin. Hence, if the isolate is interpeted as methicillin resistant, it is automatically resistant to erythromycin, irrespective of the Autobac I interpretation. The high incidence of very major and minor discrepancies associated with these two antibiotics therefore is not as serious a problem as it may first appear. Unlike erythromycin, MIC determinations of clindamycin have demonstrated that 25% of the methicillin-resistant staphylococcal isolates are susceptible to this antibiotic. Because 64% of these isolates are falsely interpreted by Autobac I as being susceptible to clindamycin and because this antibiotic may be an important agent in the treatment of methicillin-resistant *S. aureus* infections, we have found no alternative but to repeat any methicillin-resistant, clindamycin-susceptible Autobac I result by the disk diffusion technique. Likewise, if there is any doubt regarding an organism's susceptibility to methicillin, a confirmatory disk test is performed.

It is apparent from the *E. coli*-ampicillin evaluation that an Autobac I "resistant" result is not a reliable interpretation if 3.6-μg or 4.5-μg elution disks are used because organisms with an MIC of 4 to 8 μg/ml, which are considered sensitive by the ICS agar dilution method, give non-reproducible results with an LSI often in the resistant range. Minor or major discrepancies have been demonstrated in 26% of *E. coli* tested with the 3.6-μg disk. This figure altered marginally to 28% when the 4.5-μg disks were evaluated. Similar discrepancies were noted in 24% of *P. mirabilis* tested.

These results confirm the findings of Mogyo-

ros et al. (4), who reported that minor or major discrepancies occurred in 14% of 400 gram-negative bacilli tested against ampicillin, although they did not indicate which organisms had the highest discrepancy rates.

Likewise, Stubbs and Wicher ran an extensive trial which demonstrated that 3.6-μg ampicillin elution disks gave highly discrepant results when *E. coli*, *Citrobacter*, indole-positive *Proteus*, and *Providencia* species were tested (6). Unfortunately, these workers made no attempt to differentiate the discrepancies involving ampicillin into categories similar to those implemented by the collaborative study and used in the present study. It is therefore difficult to compare their findings with those presented in this paper, although it is apparent that ampicillin susceptibility testing by Autobac I has been proven to be a major cause for concern in all of the reported clinical evaluations performed subsequent to the original collaborative study of Thornsberry et al. (7).

The report of the collaborative study noted that discrepancies between the Autobac I and Bauer-Kirby techniques occurred in only 7% of the cases, most of these being minor. The disks used in this evaluation were designated 3.6 μg, but they were actually assayed at 6.7 μg. It is probable that the increased disk mass was responsible for the higher agreement noted by these workers, a supposition which is supported by the findings presented in this paper.

This problem has been resolved by us, at least partially, by use of 10-μg Oxoid diffusion disks in the Autobac I system, which has eliminated the discrepancies described previously, suggesting that the low disk mass was responsible for the high discrepancy rate in our initial evaluation. Nevertheless, it is possible that local bacterial strains and, perhaps even more importantly, loss of activity of the antibiotic during shipping, were other factors involved. Routine quality control checks of the elution disks using control organisms of known MIC did not indicate that the latter was a significant problem.

The occurrence of very major discrepancies in 5% of the *E. coli* evaluated is one of several problems associated with the use of 10-μg ampicillin disks. An uncertainty regarding the extent of antibiotic elution from the diffusion disk plus disk mass quality control regulations which are less rigid than those imposed by the Food and Drug Administration on disks sold or manufactured in the United States may be factors which could explain these discrepancies, although it is most probable that a disk mass of 10 μg is simply excessive. The ideal disk mass for testing *E. coli* and *P. mirabilis* should lie between 6.7 and 10 μg; the actual value will have to be determined by further intensive experimentation.

Furthermore, certain genera of the enterobacteriaciae perform less satisfactorily with the 10-μg disk than they do with the lower antibiotic content disks. For instance the correlation between CDS and Autobac I methods for *Klebsiella* sp. decreased from 99% for the 3.6-μg and 4.5-μg disks to 89% for the 10-μg disk, a decrease due entirely to the occurrence of very major discrepancies. Likewise, the 3.6-μg ampicillin disk used by Thornsberry et al. (assay mass, 6.7 μg) also initiated a trend towards intermediate and susceptible interpretations instead of the anticipated resistant result with genera such as *Klebsiella* and *Enterobacter*, confirming that the larger disk mass is not satisfactory under all circumstances.

At Repatriation General Hospital, Concord, two ampicillin disk masses are utilized in the determination of antibiograms of gram-negative bacilli: 4.5 μg and 10 μg. The result ultimately reported is dependent upon organism identification. At the present time the 10-μg disk is only used in conjunction with *E. coli* and *P. mirabilis*.

Owing to its rapidity, standardization of methodology, and high overall correlation (93%) with the CDS disk diffusion technique, Autobac I is now used in our laboratory for routine antibiotic susceptibility testing. Nevertheless, Autobac I results are not reported without due consideration by the technologist involved, since a significant proportion of discrepant results has been demonstrated in certain organism-antibiotic combinations. Indeed, the technique modifications outlined, which have greatly reduced the number of discrepancies in several of these combinations, are dependent upon operator awareness.

LITERATURE CITED

1. **Bell, S. M.** 1975. The C.D.S. disc method of antibiotic sensitivity testing. Pathology **7 (Suppl.)**:1–48.
2. **Cleary, T. J., and D. Maurer.** 1978. Methicillin-resistant *Staphylococcus aureus* susceptibility testing by an automated system, Autobac I. Antimicrob. Agents Chemother. **13**:837–841.
3. **Ericsson, H., and J. C. Sherris.** 1971. Antibiotic susceptibility testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Suppl. **217**:1–90.
4. **Mogyoros, M., J. R. Morgan, and J. A. Smith.** 1977. Evaluation of the Autobac I susceptibility testing system in a clinical diagnostic laboratory. Antimicrob. Agents Chemother. **11**:750–752.
5. **Stokes, J. E., and P. M. Waterworth.** 1972. Antibiotic sensitivity tests by diffusion methods. Association of Clinical Pathologists: Broadsheet 55 (revised).
6. **Stubbs, K. G., and K. Wicher.** 1977. Laboratory evalu-

ation of an automated antimicrobial susceptibility system. Am. J. Clin. Pathol. **68**:769–777.

7. **Thornsberry, C., T. L. Gavan, J. C. Sherris, A. Balows, J. M. Matsen, L. D. Sabath, F. Schoenknecht, L. D. Thrupp, and J. A. Washington II.** 1975. Laboratory evaluation of a rapid, automated susceptibility testing system: report of a collaborative study. Antimicrob. Agents Chemother. **7**:466–480.
8. **Waterworth, P. M.** 1973. Laboratory control—common sources of error, p. 500. *In* L. P. Garrod, H. P. Lambert, and F. O'Grady (ed.), Antibiotic and chemotherapy, 4th ed. Livingstone, Edinburgh.