Dependability of sensitivity tests in primary culture

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SYNOPSIS Primary sensitivity tests were done on 90 specimens of infected urine, and the results were compared with those of secondary tests on pure cultures done by three diffusion methods. There was good correlation between the four methods. In a second study, the reliability of primary tests prepared in the clinical laboratory on specimens of pus was assessed, and the frequency with which a definitive result was obtained with different types of specimen was determined. Recommendations are made for the economic use of these tests.

Direct (or 'primary') sensitivity tests, in which discs are applied to plates inoculated from the specimen, are widely used in Great Britain, but their reliability is often questioned by workers in other countries. The advantages claimed for these tests are, first, speed, as the result may be available on the following day; second, the identification of small numbers of resistant organisms in a predominantly sensitive population; and, third, their value as selective media in mixed cultures. Nevertheless, it has to be admitted that little attempt has been made to prove their validity. This paper describes two studies in which the reliability of primary sensitivity tests and their value in relation to different types of specimen are assessed.

The first study concerns only specimens of urine. In this, the results of primary sensitivity tests were compared with those of tests on pure cultures done by the same method, by the Kirby-Bauer method, and by the method recommended by the International Collaborative Study (ICS). The second investigation was concerned with specimens other than urine. In this, primary sensitivity tests prepared in the clinical laboratory were collected daily. The results from these were compared with those of the same tests repeated on pure cultures. They were then related to the source of the specimen, and the frequency with which a definitive result was obtained was determined.

Methods and Material

PRIMARY TESTS

Primary tests were done by the Stokes (1975)

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modification of the method in which the zone given by the unknown organism is compared to that given by a control organism of known sensitivity. In this, an 85 mm plate of suitable medium is divided horizontally into three; the control is seeded onto the top and bottom areas, and the specimen or culture onto the centre band, leaving 2-3 mm between them. Two discs are placed on each side of the specimen so that the zones of inhibition are produced half in the control and half in the unknown, thus making direct comparison easy (fig 1). Zone sizes are interpreted as follows:

Zone radius (from the edge of the zone to the disc) larger, the same, or not > 3 mm smaller than the control—sensitive.

Fig 1 Primary sensitivity test on a specimen of urine.

Radius of at least 3 mm but > 3 mm smaller than the control—moderate

Radius of < 3 mm—resistant

Escherichia coli (NCTC 10418) was used as control for specimens of urine and the Oxford *Staphylococcus aureus* (NCTC 6571) for the remainder. These were applied to the plates with swabs which had been impregnated in bulk with dilute broth culture (Felmingham and Stokes, 1972).

When the specimen was on a swab, this was spread evenly over the centre area of the plate. When it was urine, a 4 mm loopful was applied to the centre of the plate and then spread evenly with a dry sterile swab. The discs were applied at once and the cultures incubated without prediffusion.

SECONDARY TESTS

Secondary tests were done by the same method, applying a 2 mm loop (held vertically) of broth culture to the centre band, and spreading this evenly with a dry sterile swab. This method will be referred to as the comparison with a control (CC).

In the urine study, 100 mm square plates were used for both these methods, with three discs on both sides of the test organism.

The Kirby-Bauer (KB) method was done following the description of the method by Anderson (1970), and zone diameters were translated into sensitive, intermediate or resistant, according to the table in that paper.

The ICS method was done following the directions of Ericsson and Sherris (1971). Zone diameters were measured with calipers which were applied directly to charts kindly supplied by Dr H. Ericsson. These charts automatically translate zones into four categories: 1—sensitive; 2—fairly sensitive; 3—slightly sensitive, 'infection in organs where the drug may be concentrated, eg, urine'; and 4—resistant.

Oxoid Diagnostic Sensitivity test agar was used for the primary tests and the CC method and Oxoid Mueller-Hinton agar for the KB and ICS methods. Five per cent of lysed horse blood was added to all media.

All specimens were from the routine work of the laboratory. In the urine study the primary tests were performed by one of us (M del P) on specimens already shown to be infected, which had been stored at 4°C since receipt. The discs used are given in table I; these drugs were not chosen on merit but because discs of appropriate content were readily available to us. This study was concerned only with the technical aspects of sensitivity testing and not with the clinical significance of the organisms isolated.

In the second study, the primary tests were those

Drug (μ g)	Primary CC	KB	ICS
Ampicillin	25	10	10
Cephaloridine	25	30	30
Gentamicin	10	10	30
Kanamycin	30	30	30
Tetracycline	30	30	30
Nalidixic acid	30	30	30

Table I Drug content of the discs used in the four methods

prepared in the laboratory. Four drugs were always tested; these varied according to the specimen, but in the majority were penicillin, tetracycline, erythromycin, and clindamycin. When thought appropriate, ampicillin, kanamycin, chloramphenicol or gentamicin sometimes replaced one or more of these.

Results

PRIMARY SENSITIVITY TESTS ON INFECTED URINE

Primary sensitivity tests were performed on 90 specimens of urine: 73 yielded a pure growth of a single organism, but in six the inoculum was too light for the satisfactory interpretation of the test, and in one it was too heavy. Seventeen specimens contained two organisms. *Proteus mirabilis* was present in 13, and while it was always possible to read the sensitivity of this, that of the second organism was masked. In three of the remaining four, the sensitivity of both organisms was readily apparent, and in the last, the second organism was present in very small numbers.

Secondary sensitivity tests were done by the CC, KB, and ICS methods on the 86 organisms which had satisfactory primary tests. These comprised mainly *E. coli* and *P. mirabilis* with small numbers of *Enterobacter-Klebsiella*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa*; all were tested against the drugs shown in table I.

The results are summarized in table II. It is clear that the results obtained by primary tests do not differ significantly from those obtained in tests done by the same method on pure cultures, though there might appear to be some discrepancy between the

Method	Sensitive ICS 1	Moderate Intermediate ICS 2	ICS 3	Resistant ICS 4
Primary	418	20		78
CC	418	17		75
KB	392	37		87
ICS	237	167	25	87

Table II Comparison of four methods of sensitivity testing: Results of tests with six drugs on 86 urinary pathogens

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results obtained by the three methods on pure cultures. However, this can largely be accounted for by the different approach in these methods as to what constitutes 'sensitive' in urinary tract infection. In the CC method allowance is made for the high concentration of many drugs in the urine by using high-content discs and a more resistant control organism. The ICS method includes a special category, 3, for urinary infections, so presumably all strains falling into categories 1, 2 or 3 can be classed as sensitive. On the other hand, the KB method makes no allowance for the site of the infection.

The great majority of these differences arise in three drugs. The only significant difference in the number of strains found resistant occurred with ampicillin (table III), probably because this is the one drug for which the CC method uses a higher disc content (25 µg) than the KB and ICS methods (10 µg). With cephaloridine (table IV), when all the methods use the same disc (30 µg), the resistance rates are similar. The ICS method presumably has lower break-points for gentamicin (table V), but if groups 1, 2, and 3 are taken to indicate sensitivity, there is good correlation between the methods.

It is not the purpose of this paper to discuss the relative merits of these three methods, but these results are emphasized to prove not only that primary

sensitivity tests give reliable results, but also that there is good correlation between the CC method and the KB and ICS methods. In the second study only the first-named was used.

PRIMARY SENSITIVITY TESTS ON PUS

All primary tests on specimens of pus, exudates or washings which gave any growth, whether or not this was ultimately considered significant, were collected from the clinical laboratory daily. The suitability of the inoculum was assessed, taking the optimal as dense but not confluent growth. Zone sizes were measured for as many organisms as possible, including those in which the inoculum was, by the strict standards used, either too light or too heavy. Pure cultures were prepared in broth and the same tests were repeated by the CC method.

Tests were repeated on the first 250 organisms for which it had been possible to measure the zones in the primary test. They included 19 in which the original inoculum had been classed as unsatisfactory and 91 from mixed cultures, not all of which were considered to be clinically significant. Of these 1000 tests, 983 gave the same result when retested. There were 10 instances of false resistance due to the presence of a second strain, and five in which a sensitive organism (usually *Str. pyogenes*), when present with a beta-lactamase-producing *Staph. aureus*, appeared moderately resistant to penicillin or ampicillin.

In the latter it can be argued that the primary test is highly significant, as such infections do not always respond to treatment with penicillin. One strain of *Str. faecalis* appeared resistant to tetracycline originally and gave a moderate zone on re-testing, and one group B streptococcus in a mixed culture gave no zone with chloramphenicol in the primary test but appeared fully sensitive on re-testing. This too may have been due to the enzymic destruction of the drug.

In all, 500 primary sensitivity plates were examined and the results were assessed in relation to the laboratory's report. A final report was possible the day following the receipt of the specimen in 200 (40%), and in a further 30 (6%) a report was possible on the predominating organism, but a second organism, present in only small numbers, required re-testing. In 74 (14.8%) the inoculum was classed as unsatisfactory, but 30 of these could in fact be read (the results were confirmed). In 13 (2.6%) the inoculum was satisfactory, but further drugs required testing. The growth was too mixed in 72 (14.4%), including *Proteus* in 30. *Ps. aeruginosa* was present in 36 (7.2%), and it is our practice to test this organism separately with a sensitive strain of the same species as the control. Finally, there were 75

Method	Sensitive ICS 1	Moderate Intermediate ICS 2	ICS 3	Resistant ICS 4
Primary	60	7		19
CC	60	8		18
KB	59	2		25
ICS	1	60	1	24

Table III Results of ampicillin sensitivity tests on 86 organisms done by four methods

Method	Sensitive ICS 1	Moderate Intermediate ICS 2	ICS 3	Resistant ICS 4
Primary	73	3		10
CC	71	7		8
KB	66	10		10
ICS	11	62	4	9

Table IV Results of cephaloridine sensitivity tests on 86 organisms done by four methods

Method	Sensitive ICS 1	Moderate Intermediate ICS 2	ICS 3	Resistant ICS 4
Primary	84	2		
CC	85	1		
KB	86			
ICS	36	43	7	

Table V Results of gentamicin sensitivity tests on 86 organisms done by four methods

cultures (15%) in which the growth was not considered to be of clinical significance.

These results were then classified according to the source of the specimen and are given in table VI. It is clear that primary tests are of little value for specimens from ulcers or ears; both usually yielded a heavy mixed growth, frequently including *Proteus* or *Ps. aeruginosa*.

Source of Specimen	Total No. examined	Report Possible	
		No.	%
Casualty department	138	87	63.0
Wards and outpatient departments	246	95	39.0
Genitourinary	36	7	19.4
Ears	49	7	14.3
Ulcers	31	4	12.9

Table VI Primary sensitivity tests on which a final report was possible related to the source of the specimen

In more than half the genitourinary specimens, which were mainly high vaginal swabs, the growth was not considered to be significant. In the remaining specimens the success rate was much higher in those received from the casualty department, than in those from the wards and outpatient departments; mixed infections were much more frequent in the latter, and Gram-negative bacilli were present in 40%. There was also a high proportion (20%) of cultures on which growth was scanty, often from patients already receiving treatment.

Discussion

It is clear from both these studies that primary sensitivity tests give reproducible results. The objection most commonly raised against such tests is the difficulty of controlling the inoculum, and we fully accept the importance of this. The Stokes modification of the CC method has two advantages

Fig 2 (a) Primary test made from a swab bearing pus. The inoculum is lighter than that of the control but the result is clear. (b) Secondary test on the same organism. The plate was inoculated by applying a small loop of an overnight broth culture and spreading this with a dry swab.

Fig 3 Primary sensitivity tests on urine done by: (a) the conventional four-disc Stokes method, and (b) using a rotary plater and six discs.

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in primary tests. First, because the results are interpreted by the direct comparison of the zone given by the unknown with that of the control, it is possible to make some allowance when the inocula of the two organisms are not identical (see fig 2). Secondly, the method of inoculation used contributes greatly to the success. If a small loopful of a broth culture is applied to a plate and spread with a dry swab, it seems that the bulk of the organisms adhere to the cotton wool, and the inoculum left on the plate almost invariably yields dense but not confluent growth which is evenly distributed (see fig 2b). This presumably accounts for the frequently successful inoculation of primary tests with swabs bearing pus.

The other objection often raised is that these tests are a waste of time and materials because so many have to be repeated. The overall success rate in the study of primary sensitivity tests on 'pus' (40%) was much lower than anticipated, and clearly these tests should be done much more selectively. While a primary sensitivity test on a high vaginal swab from puerperal fever or septic abortion may be life-saving, in general the test should not be done on specimens from sites with an indigenous flora. Similarly, these tests are seldom worth while on specimens from sites where heavy contamination is likely, such as ears, chronic ulcers, bed-sores or lesions communicating with the large bowel. The low success rate with other specimens from the wards reflects the problems of present-day chemotherapy, in contrast to the much better results with those from the casualty department, where the majority of specimens were from acute, untreated infection mainly due to Gram-positive cocci. Better results would be achieved if specimens from patients receiving antibacterial treatment were omitted and the spectrum of the drugs tested were broadened.

Primary tests on urine, when a large loopful is applied to the plate and spread with a dry swab, are highly successful, and in the study reported here 92% of the primary cultures yielded dense, but not confluent, well distributed growth. The use of a rotary plater, enabling the control to be seeded in a band round the outer part of the plate and the specimen in the centre (Pearson and Whitehead, 1974), has made it possible to test six drugs on an 85 mm plate (fig 3) with the result that further tests are seldom required, and the majority of specimens can be reported on the day following their receipt.

This technique has been further simplified by the introduction of a new Mastring designed for use with this method, which enables the six discs to be applied with one movement (fig 4). This method of inoculation can also be used very successfully for inoculating whole plates if this is preferred (fig 5).

The difficulty of selecting the relatively small

Fig 4 Primary sensitivity test on urine using the Mastring-S designed for this method.

Fig 5 Whole plate inoculated with a rotary plater. The inoculum (a small loopful of broth culture) was first streaked across the plate once with a dry swab, and the swab was then drawn slowly outwards from the centre as the plate revolved.

number of infected specimens from the very large number of urines received in most laboratories remains. A recent survey of the results in this laboratory revealed that, although primary sensitivity tests were done only on specimens shown to

contain at least moderate numbers of leucocytes, nearly 60% of these yielded no significant growth. If economy of effort and materials is to be achieved, it would seem that these tests should be done only when bacteria have been demonstrated either microscopically or by screening culture.

Conclusions

1 Sensitivity tests inoculated with the original specimen give reproducible results.

2 Such tests should be done only on specimens from sites normally sterile.

3 Primary tests on infected urine are highly successful, and 92% of those tested in this study yielded a satisfactory inoculum. If economy of effort is to be achieved, it is essential that these tests be confined to specimens shown to contain bacteria; sensitivity tests should not be reported unless the organism is considered to be clinically significant.

4 Primary tests should not be done on specimens from areas where heavy contamination is likely, eg, bed sores, ulcers, and lesions connecting with the large bowel.

5 Primary tests are very successful in acute untreated infection. Results with some specimens from

inpatients could be improved by broadening the spectrum of the drugs tested.

6 If these suggestions are followed, the advantages of rapid reporting on the majority of specimens will more than compensate for the need to repeat the tests in a minority.

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