Antibiotic sensitivity testing: a survey undertaken in September 1970 in the United Kingdom

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Purpose of Survey

A national survey was carried out in the United Kingdom in 1965 in order to identify the range of antibiotics and chemotherapeutic substances used in hospital laboratories for establishing the sensitivity of microorganisms from urinary infections, staphylococcal infections, and other infections. The results of this survey (which were published in booklet form by Oxoid Limited) revealed that whilst antibiotics were equally favoured in laboratories of all hospitals, large differences were evident in the choice of many other drugs.

A further survey was undertaken in September 1970 in order to identify any major changes in the choice of drugs for sensitivity testing since the last survey and also to obtain details of techniques currently employed. The results confirm some changes in the choice of drug and show very wide differences in technique.

A questionnaire was sent to most of the hospitals in the United Kingdom having laboratory facilities with an invitation to participate in the survey. In order to see if there were any major differences in the choice of drugs with hospitals of different size and status, they were divided into three groups as follows: teaching hospitals, large non-teaching hospitals, and other hospitals.

Teaching hospitals are those classified as such in the 1970 *Hospital Year Book*. Large non-teaching hospitals are those where the number of beds exceeds 500.

Response Rate

A small number of laboratories did not complete the questionnaire because they omitted to answer one or more of the questions. It is for this reason that the figures in some of the tables and also in some sections of the text do not total 330—the number of hospitals responding. The response rate is shown in Table I.

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<i>Hospital</i>	<i>Total No. of Hospitals</i>	<i>No. of Hospitals Responding</i>	<i>Percentage Response Rate</i>
All	645	330	51
Teaching	64	35	55
Large non-teaching	194	116	60
Other	387	179	45

Table I *Response rate*

There were not many significant differences between practices in these three groups, and in the absence of such differences, results are given only for all hospitals.

Techniques and Test Materials

It has often been stated that the validity of results in a sensitivity test is dependent on a number of factors, such as the type of growth medium, the type of inoculum, method of spreading the inoculum, drug content of discs, use of controls, and the interpretation of results. In this survey, each of these factors was studied in order to ascertain the extent of their acceptance in the performance of sensitivity tests.

SENSITIVITY TEST MEDIUM

It is clear from Table II that there are differences of opinion among laboratories as to (1) the need to employ media specially formulated for sensitivity testing; (2) the need to employ some form of blood enrichment in the medium; (3) the best type of enrichment; and (4) the need to use plates containing a standard depth of medium.

Two hundred and sixty-two (79%) of all hospitals employ a special medium for sensitivity testing. Of these, 89% use diagnostic sensitivity test agar, and 3% indicated a preference for salt agar when testing the sensitivity of staphylococci to methicillin.

One third approximately of all hospitals use media enriched with whole blood only, another third use media enriched with lysed blood only, and a quarter consider enrichment to be unnecessary. A small

Sensitivity Test Medium	Hospital			
	Teaching	Large Non-teaching	Other	All
Laboratories using a test medium specially formulated for sensitivity testing	27 (77 %)	100 (86 %)	135 (75 %)	262 (79 %)
Laboratories using media enriched with whole blood only				118 (36 %)
Laboratories using media enriched with lysed blood only				101 (31 %)
Laboratories using media enriched with lysed blood and also media enriched with whole blood				31 (9 %)
Laboratories using non-enriched media only				80 (24 %)
Laboratories metering the volume of medium so as to provide a standard depth of medium in the plate	16 (46 %)	34 (30 %)	43 (24 %)	93 (28 %)
Laboratories considering depth of medium in the plate to be important	27 (77 %)	94 (82 %)	141 (79 %)	262 (80 %)

Table II Use of sensitivity test media

number of the hospitals using enriched media stated that non-enriched plates were employed when blood was considered to be unnecessary for growth of the test organism. There is no significant difference between the three 'groups' of hospitals in the use/choice of enrichment for the sensitivity test medium.

Of the 31 hospitals (9%) which use media with lysed blood and also media with whole blood, many stated that the former are employed when testing for sensitivity to sulphonamides. One laboratory uses a medium containing both whole and lysed blood with a view to demonstrating haemolysis, and also to provide more clear-cut results when testing for sensitivity to sulphonamides.

It is interesting to note from Table II that 262 (80%) of all hospitals consider the depth of the medium in the plate to be important, whereas only 67 (28%) actually meter the volume so as to provide a standard depth. It should, however, be pointed out that some laboratories, although not metering the volume, attempt to achieve a standard depth when pouring the plates.

Four hospitals stated that depth of medium was unimportant if 'within reasonable limits', and another felt that provided it is enough to support good growth (3-4 mm) and that each disc is controlled, depth of medium is unimportant.

Inoculum

CHOICE OF INOCULUM

Tests employing pure cultures were done by 176 (53%) of all hospitals: 55 (17%) prefer the direct plating method in which the specimen is used as the inoculum, while 98 (30%) use either direct plating or a subculture according to the nature of the specimen.

In order to clarify the reasons for the above mentioned preferences, the authors telephoned a selection of hospitals and found the following:

1 Those laboratories which normally employ only direct plating of the specimen do so in the belief that the speedy result so obtained is of paramount importance.

2 In the laboratories where only subcultures are used, it was held either that it was essential to use pure cultures of the test organism, or that the primary plating method does not permit control of the size of the inoculum, or both.

3 Laboratories using both direct plating and subcultures have indicated a variety of approaches some of which are: direct plating of urines and subcultures for other specimens; direct plating of urines when microscopy of specimen shows evidence of infections; and direct plating of all specimens except urines.

All of the laboratories with which a personal approach was made concerning the use of direct plating stated that subcultures were undertaken whenever the result with primary plates was in doubt.

SIZE OF THE INOCULUM

The importance of controlling the size of the inoculum has been stressed by many workers. Nevertheless, 40% of teaching hospitals, 27% of large non-teaching hospitals, and 38% of other hospitals replied to the questionnaire that they did not attempt to control the size of the inoculum.

SPREADING THE INOCULUM

The survey confirms that a wide variety of methods (singly and in combination) are used for spreading the inoculum on the plate. It will be seen from Table III that more than one method of spreading is used by 21% of all laboratories. Personal communication with a random selection of these laboratories confirmed that their choice of method is determined by the nature of the specimen. In general, the swab is used when the specimen arrives on a swab and a loop when the specimen is urine or pus.

Means of Spreading	Hospital			
	Teaching	Large Non-teaching	Other	All
Loop only	14 (40%)	48 (42%)	87 (50%)	149 (46%)
Glass rod only	2 (6%)	5 (4%)	5 (2%)	11 (3%)
Swab only	4 (11%)	21 (18%)	21 (12%)	46 (14%)
Flooding only	7 (20%)	17 (15%)	27 (16%)	51 (16%)
Loop and swab	4 (11%)	8 (7%)	16 (9%)	28 (9%)
Loop and flooding	—	9 (8%)	10 (6%)	19 (6%)

Table III Spreading the inoculum¹

¹In addition, six laboratories used both swab and flooding, three both loop and rod, one rod and swab, and 10 used other combinations including three which make a poured plate by mixing the inoculum with molten agar.

It is interesting to note that 46% of all laboratories favour 'loop only' as the means of spreading the plate.

RESULTING GROWTH

The survey revealed little evidence of any correlation between the type of inoculum, method of spreading, and the resulting growth. For example, major differences in growth density have been identified between laboratories which appear to be using identical methods for inoculating and spreading the plate. Fifty-nine percent of all hospitals usually obtain a dense but not confluent growth, 27% usually a confluent growth, 8% usually a variation in growth density, and 6% well separated colonies. Table IV shows the range of growth densities obtained in the various hospital groups.

Resulting Growth	Hospital			
	Teaching	Large Non-teaching	Other	All
Confluent (a)	17 (48%)	20 (17%)	53 (30%)	90 (27%)
Dense but not confluent (b)	16 (46%)	81 (70%)	98 (55%)	195 (59%)
Well separated colonies (c)	—	5 (4%)	14 (8%)	19 (6%)

Table IV Resulting growth¹

¹In addition, 12 laboratories described their growth as ranging between (b) and (c) and eight as either (a) or (b) and four as ranging between (a) and (c).

Controls

The survey revealed that 151 (46%) of all hospitals control their tests with one or more standard organisms. Of these, 58 (32%) stated that a control is provided for each test. Of the 151 laboratories using controls, 63% employ a standard strain of *Staph. aureus* (Oxford) or *Ps. aeruginosa* or *E. coli*, depending on the type of test organism or source of specimen. Thirty-seven per cent use a *Staph. aureus* (Oxford) only.

The value of the control procedure operated by some laboratories is somewhat questionable. In some departments, for example, it appears to be the practice to put up a control plate with each new batch of discs only, whereas others check each new batch of medium only. There are examples of controls consisting of an occasional testing of a *Staph. aureus* (Oxford).

Disc Content

Table V shows large differences in choice of disc content with eight antibiotics in 'front line' testing. Similar differences occur with many other antibiotics. (A table giving details of the entire range of drug levels used for both 'first' and 'second' line testing is available on application to the authors.)

The survey showed that the drugs which have a high ranking in 'frequency of use' are very often associated with a wider range of disc levels. In the case of routine 'front line' testing of staphylococci with penicillin, nine different drug levels are used in the range 0.5 units to 15 units. With tetracycline, eight different levels are used in the range 5 µg to 125 µg. It is probable that the exceptionally high disc levels, such as tetracycline at 200 µg, are used only when testing the sensitivity of organisms from superficial infections to which the antibiotic is to be applied locally.

In 255 (78%) of laboratories in all hospitals it is the practice to use a higher drug content in discs for testing organisms from urinary infections than in discs for staphylococcal infections.

There are no internationally agreed levels of antibiotics in sensitivity testing at the present time. When asked if they felt international standards were desirable, 288 hospitals replied Yes and 30 replied No.

Range of Drugs

A very wide range of antibiotics is used in both 'front-line' and 'second-line' testing procedures for

Antibiotic	First-line Testing Urinary Infections	Staphylococcal Infections	Other Infections
Ampicillin	Six different disc drug contents used Range = 2-50 µg Dominant content 25 µg	Six different disc drug contents used Range = 1.5-25 µg Dominant content 2 µg	Eight different disc drug contents used Range = 1.5-200 µg Dominant content 2 µg
Cephaloridine	Four different disc drug contents used Range = 5-25 µg Dominant content 25 µg	Five different disc drug contents used Range = 5-25 µg Dominant content 25 µg	Five different disc drug contents used Range = 5-25 µg Dominant content 5 µg
Erythromycin	Four different disc drug contents used Range = 5-50 µg Dominant content 10 µg	Six different disc drug contents used Range = 2-50 µg Dominant content 10 µg	Four different disc drug contents used Range = 2-50 µg Dominant content 10 µg
Kanamycin	Four different disc drug contents used Range = 5-30 µg Dominant content 30 µg	Four different disc drug contents used Range = 5-50 µg Dominant content 30/50 µg	Four different disc drug contents used Range = 5-50 µg Dominant content 30 µg
Nalidixic acid	Four different disc drug contents used Range = 25-200 µg Dominant content 30 µg	Three different disc drug contents used Range = 2-50 µg Dominant content 30 µg	Two different disc drug contents used Range = 30-50 µg Dominant content 30 µg
Nitrofurantoin	Five different disc drug contents used Range = 20-500 µg Dominant content 200 µg	One disc drug content used 200 µg	One disc drug content used 200 µg
Penicillin	Eight different disc drug contents used Range = 1-20 units Dominant content 1.5 units	Nine different disc drug contents used Range = 0.5-15 units Dominant content 1.5 units	Seven different disc drug contents used Range = 0.5-5 units Dominant content 1.5 units
Tetracycline	Six different disc drug contents used Range = 10-125 µg Dominant content 50 µg	Eight different disc drug contents used Range = 5-125 µg Dominant content 10 µg	Nine different disc drug contents used Range = 5-200 µg Dominant content 10 µg

Table V Disc drug content (all hospitals)

organisms from urinary infections, staphylococcal infections, and other infections alike. Forty-three different drugs are used in 'first-line' testing of urinary infections and 42 in the case of staphylococcal infections. Some of them have a very high ranking by frequency of use, whereas others are employed by just a few laboratories. There are no significant differences in the choice of drugs between the three groups of hospitals. Tables VI, VII, and VIII cover urinary infections, staphylococcal in-

fections, and other infections respectively. In the first column of each table antibiotics are ranked by frequency of use in routine 'first-line' testing by all hospitals. The number in each column represents the number of laboratories using the antibiotic for each particular purpose, eg, in the case of urinary infections (Table VI) ampicillin is used by 317 hospitals for 'first-line' testing. It will also be seen that nine hospitals use this antibiotic for 'second-line' testing.

Rank	Antibiotic	First Line Testing	Second Line Testing	Comment
1	Ampicillin (5) ¹	317	9	
2	Nitrofurantoin (1)	295	11	Also six and one tested furaltadone or furazolidone
3	Nalidixic acid (7)	275	39	
4	Tetracyclines (2)	271	26	Comprising tetracycline 248 and 25, oxytetracycline 17 and 0, chlortetracycline 6 and 1
5	Sulphonamides (3)	270	12	
6	Polymyxins (8)	208	142	Comprising colistin methane sulphonate 180 and 103, polymyxin B 13 and 36, thiosporin 5 and 3

Rank	Antibiotic	First Line Testing	Second Line Testing	Comment
7	Trimethoprim/ sulphamethoxazole (-)	185	94	Including 19 and 12 testing trimethoprim singly
8	Streptomycin (4)	131	31	
9	Cephaloridine (12)	83	95	
10	Kanamycin (9)	83	122	
11	Chloramphenicol (6)	70	49	
12	Gentamicin (-)	57	186	
13	Penicillin (10)	30	9	
14	Carbenicillin (-)	23	208	
15	Cephalexin (-)	20	33	
16	Erythromycin (11)	17	7	
17	Cloxacillin (15)	11	11	
18	Novobiocin (13)	7	8	
19	Framycetin (19)	5	10	
20	Cephalothin (-)	4	6	
21	Methicillin (20)	3	8	

Table VI Antibiotics ranked by frequency of use in all hospitals in urinary infections.

¹The ranking in the 1965 survey is shown in brackets.

²The following agents were tested by fewer than three (1%) laboratories in 'first-line testing'. Lincomycin, fusidic acid, nystatin, phenethicillin, rifamides, propicillin, methacycline, doxycycline, paromomycin, albamycin, clindamycin, mandelamine, dicloxacillin, oleandomycin, ristocetin, spiramycin, vancomycin, pristinamycin.

Rank	Antibiotic	First Line Testing	Second Line Testing	Comment
1	Penicillin (2) ^a	298	8	
2	Tetracycline (1)	298	8	Comprising tetracycline 275 and 6, oxytetracycline 15 and 1, chlortetracycline 8 and 1
3	Erythromycin (3)	224	51	
4	Ampicillin (7)	197	25	
5	Cloxacillin (6)	162	56	
6	Streptomycin (4)	162	25	
7	Chloramphenicol (5)	109	59	
8	Fusidic acid (11)	101	158	
9	Cephaloridine (13)	92	75	
10	Methicillin (10)	84	61	
11	Lincomycin (14)	84	106	Also 6 and 30 tested clindamycin
12	Sulphonamides (8)	83	13	
13	Neomycin (12)	71	54	
14	Trimethoprim/sulphamethoxazole (-)	78	63	Including 7 and 11 testing trimethoprim singly
15	Novobiocin (9)	46	52	
16	Gentamicin (-)	23	77	
17	Polymyxins (17)	13	19	Comprising colistin methane sulphonate 9 and 14, polymyxin B 3 and 3, thiosporin 1 and 2
18	Kanamycin (16)	12	37	
19	Cephalexin (-)	11	31	
20	Nalidixic acid (27)	10	—	
21	Framycetin (15)	8	23	
22	Nitrofurantoin (20)	8	2	Also 3 and 3 tested furaltadone or furazolidone

Table VII Antibiotics ranked by frequency of use in all hospitals in staphylococcal infections¹

^aSome laboratories also tested the following: paromomycin, lymecycline, methacycline, spiramycin, doxycycline, propicillin, albamycin, demethylchlortetracycline, clindamycin, oleandomycin, carbenicillin, cephalothin, phenethicillin, rifamides, nystatin, vancomycin, bacitracin.

^bRanking in the 1965 survey (Tables VII and VIII)

Number of Drugs

It will be seen from Table IX that the number of different drugs employed in 'first-line' testing by all hospitals varies between one and 17. The majority use either six or eight, and it is felt that the choice of this number may stem from the fact that multitype discs which contain either six or eight drugs are used by 61% of all hospitals.

Changes in Choice of Drug

The survey revealed that 150 laboratories change their range of drugs every two years approximately, 130 every year approximately, and 20 every six months approximately. Eight laboratories stated that they 'review' rather than 'change' their range, and two stated that changes were made 'as and when new drugs become available'.

Rank	Antibiotic	First Line Testing	Second Line Testing	Comment
1	Ampicillin (5)	264	19	
2	Tetracycline (1)	262	12	Comprising tetracycline 240 and 9, oxytetracycline 16 and 2, chlortetracycline 6 and 1
3	Streptomycin (3)	180	27	
4	Penicillin (2)	175	8	
5	Chloramphenicol (4)	125	83	
6	Sulphonamides (7)	126	18	
7	Polymyxins (8)	120	152	Comprising colistin methane sulphonate 94 and 103, polymyxin B 24 and 43, thiosporin 2 and 6 Including 10 and 9 testing trimethoprim singly
8	Trimethoprim/sulphamethoxazole (-)	120	82	
9	Cephaloridine (13)	99	73	
10	Gentamicin (-)	83	138	
11	Neomycin (9)	80	69	
12	Cloxacillin (11)	73	17	—
13	Kanamycin (12)	65	99	
14	Carbenicillin (-)	47	151	
15	Framycetin (15)	24	39	
16	Nalidixic acid (19)	21	7	
17	Nystatin (14)	21	34	
18	Novobiocin (10)	18	15	
19	Cephalexin (-)	17	37	
20	Fusidic acid (21)	17	33	
21	Nitrofurantoin (16)	17	17	Also 4 and 4 tested furaltadone or furazolidone
22	Lincomycin (24)	16	26	

Table VIII Antibiotics ranked by frequency of use in all hospitals in other infections¹

¹Some laboratories also tested the following: demethylchlortetracycline, oleandomycin, doxycycline, lymecycline, spiramycin, vancomycin, methacycline, propicillin, cycloserine, penotran, albamycin, mandelamine, methicillin, bacitracin, paramomycin, cephalothin, clindamycin, rifamides, phenethicillin.

Urinary Infections		Staphylococcal Infections		Other Infections	
No. of Drugs	Hospitals Using this Number	No. of Drugs	Hospitals Using this Number	No. of Drugs	Hospitals Using this Number
1	—	1	1	1	1
2	1	2	1	2	4
3	6	3	8	3	6
4	23	4	26	4	25
5	18	5	30	5	21
6	48	6	57	6	59
7	36	7	32	7	27
8	129	8	111	8	94
9	33	9	28	9	21
10	12	10	10	10	10
11	6	11	1	11	9
12	1	12	3	12	8
13	2	13	2	13	5
14	3	14	1	14	2
15	1	15	1	15	2
16	1	16	2	16	2
17	2	17	—	17	1

Table IX Number of drugs used in first-line testing (all hospitals)

The decision to change the range is made by the pathologist or bacteriologist in almost half of all hospitals. The survey shows that an antibiotics committee influences change of drug in 27% of all hospitals.

Choice of Discs

The various methods for sensitivity testing employed by different hospitals involves the use of different types of sensitivity discs. In all hospitals routine first-line testing, 62% use mainly multitipped devices, 20% use mainly single discs, and 15% use mainly ring devices with 1% using antibiotic tablets. Two per cent of all hospitals were found to be using the ditch plate method.

Interpretation

One hundred and twenty-five (38%) of all hospitals grade their reports according to the size of zone of inhibition for any given antibiotic. Two gradings are employed by 62 (50%) of these hospitals, three gradings by 62 (50%), and four gradings by one laboratory. However, only 100 make any form of a comparison with a standard organism. Of these, 46 actually measure the zone (six by regression line), and 54 stated that a visual comparison (no measurement) is made with a standard organism.

It has often been stated that zones of inhibition measuring less than 2 mm radially from the edge of the disc should be ignored. The survey shows that 47 (15%) of all hospitals answering this question do not ignore zones of less than 2 mm.

Reports

Two hundred and eighty-four (87%) of pathologists at all hospitals report to the clinician the results of

all the antibiotics employed in routine first-line testing procedures. The figure for second-line testing procedures is 235 (72%).

It would appear that 71 (22%) of pathologists at all hospitals limit their reports to the clinician to those antibiotics they believe to be safest when an organism is found to be sensitive to a number of antibiotics.

Discussion

The survey has revealed differences in choice of test materials and techniques employed by hospital laboratories in the United Kingdom. The methods used by most laboratories will no doubt enable the clinician to be provided with reliable information on the sensitivity of bacteria to antibiotics. There are, however, laboratories which employ a method involving discs with a low drug content and a heavy inoculum. There are others which use the reverse, discs with a high drug content and a light inoculum. With some drugs, this difference may result in an organism being reported as resistant by one laboratory, fully sensitive by the other.

It may be felt that differences of this type, and others too, are sufficiently great to give cause for concern and that the adoption of a standard procedure for the performance of sensitivity tests is desirable in order to ensure that all hospitals employ a method which will give meaningful and reproducible results.

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