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Antimicrobial susceptibility testing of *Neisseria gonorrhoeae*: a trial organised as part of the United Kingdom national external quality assessment scheme for microbiology

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SUMMARY Six strains of *Neisseria gonorrhoeae* were distributed to 411 United Kingdom laboratories who were asked to test the susceptibility of the strains to penicillin, cefuroxime, tetracycline and spectinomycin and to test for production of β-lactamase. Details of methods used were requested by means of a questionnaire. The number of reports recording sensitive strains as resistant was 5% for penicillin, 0·7% for cefuroxime, 3% for tetracycline and 4% for spectinomycin. The number of reports recording resistant strains as sensitive was 7% for penicillin (0·2% with β-lactamase producing strains, 20% with non-β-lactamase producing strains), 96% for cefuroxime, 76% for tetracycline and 8% for spectinomycin. There was an association between greater error rates and the use of high content discs for testing tetracycline, the use of low content discs for testing spectinomycin, failure to dilute the inoculum, and use of acidimetric methods rather than methods that use a chromogenic cephalosporin for detecting β-lactamase.

The United Kingdom national external quality assessment scheme for microbiology (UKNEQAS) has been described previously.[1 2] As part of the general bacteriology section of the scheme, participants were asked to perform antimicrobial susceptibility tests on strains of established susceptibility. Results of tests on strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* showed high error rates with some combinations of strain and antimicrobial agent, as well as differences in interlaboratory error rates, and association between certain methods or practices and error rates.[3 4] Results with *Haemophilus influenzae*[5 6] suggested that more fastidious organisms presented even greater problems in susceptibility testing. Increased resistance of *Neisseria gonorrhoeae* to antimicrobial agents is a cause of concern[7] and accurate laboratory results for susceptibility tests with this species are a prerequisite for gathering epidemiological information, correlation of degrees of susceptibility with results of treatment, and investigation of treatment failures. A trial was therefore organised in September 1986 to investigate the standard of performance of susceptibility testing of *N gonorrhoeae* and factors affecting the results in a large number of laboratories in the United Kingdom.

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Material and methods

PARTICIPATING LABORATORIES

All laboratories enrolled in the general bacteriology section of UKNEQAS participated in the trial, but only the results of United Kingdom laboratories were included in this analysis. The type and geographical distribution of the laboratories have been described previously.[1]

ORGANISMS

Six strains of *N gonorrhoeae* were distributed as freeze-dried cultures. Before despatch the susceptibility of the strains to antimicrobial agents was determined in the Division of Microbiological Reagents and Quality Control (DMRQC) and the Clinical Microbiology and Public Health Laboratory, Cambridge. Minimum inhibitory concentrations were determined by an agar dilution method based on that of Ericsson and Sherris,[8] but the medium used was Oxoid Isosensitest agar

supplemented with 5% chocolated horse blood. Production of β-lactamase was examined by both an acidimetric paper strip method (Mast Laboratories) and a chromogenic cephalosporin paper "stick" method (Oxoid).

REPORT FORMS AND QUESTIONNAIRE

A report form was provided for each strain. Four antimicrobial agents were listed and participants were requested to test any that they would normally test and to report their results as sensitive or resistant. Results for β-lactamase production were also requested if normally tested. A questionnaire covering details of antimicrobial susceptibility testing methods was sent with the cultures to all participants.

ASSESSMENT OF RESULTS

Antimicrobial agents

For each combination of strain and antimicrobial agent, a correct result of sensitive or resistant was designated on the basis of minimum inhibitory concentrations and the production of β-lactamase as determined by the reference laboratories (table 1). β-lactamase producing strains were regarded as resistant to penicillin regardless of the minimum inhibitory concentration. The results from each laboratory were assessed at DMRQC. A result recorded as sensitive or resistant was regarded as correct if it was the same as the designated correct result, and as incorrect if different from the designated result. Results recorded as "intermediate" were not assessed.

β-lactamase production

Participants' results recorded as positive or negative were regarded as correct if they were the same as the reference laboratories' results, and as incorrect if different.

MEASUREMENT OF ASSOCIATION BETWEEN METHODS AND RESULTS

The association between methods and results was tested by the χ^2 test to compare the ratios of correct:

Table 1 *Results of susceptibility tests as determined by reference laboratories and reported by participating laboratories*

Strain of N gonorrhoeae and antimicrobial agents	*Reference laboratory results*		*Designated correct result*	*No of laboratories reporting strains as:*					*% correct*
	Modal MICs (mg/l)	*β-lactamase production*		*Sensitive*	*Intermediate*	*Resistant*	*Positive*	*Negative*	
MQCL 1312:									
Penicillin	0·03		Sensitive	269	14	27			87
Cefuroxime	<0·01		Sensitive	272	0	0			100
Tetracycline	0·25		Sensitive	291	0	6			98
Spectinomycin	8		Sensitive	231	1	5			97
β-lactamase		Negative	Negative				4	289	99
MQCL 1313:									
Penicillin	4		Resistant	0	0	318			100
Cefuroxime	0·1		Sensitive	276	0	4			99
Tetracycline	8		Resistant	213	8	82			27
Spectinomycin	16		Sensitive	226	2	14			93
β-lactamase		Positive	Positive				302	6	98
MQCL 1314:									
Penicillin	2		Resistant	61	7	244			78
Cefuroxime	1		Resistant	264	1	10			4
Tetracycline	8		Resistant	242	7	50			17
Spectinomycin	8		Sensitive	227	1	10			95
β-lactamase		Negative	Negative				8	292	97
MQCL 1315:									
Penicillin	<0·01		Sensitive	292	1	5			98
Cefuroxime	<0·01		Sensitive	260	0	1			99
Tetracycline	0·25		Sensitive	284	0	1			99
Spectinomycin	16		Sensitive	220	0	10			96
β-lactamase		Negative	Negative				5	277	98
MQCL 1316:									
Penicillin	2		Resistant	1	0	311			99
Cefuroxime	0·01		Sensitive	270	0	3			99
Tetracycline	1		Sensitive	284	2	10			96
Spectinomycin	16		Sensitive	223	3	14			93
β-lactamase		Positive	Positive				298	3	99
MQCL 1317:									
Penicillin	0·25		Not designated	191	28	88			
Cefuroxime	0·06		Sensitive	265	2	2			99
Tetracycline	1		Sensitive	265	6	20			91
Spectinomycin	>128		Resistant	19	0	217			92
β-lactamase		Negative	Negative				5	291	98

incorrect results achieved by laboratories using different methods. Unless otherwise stated, the numbers of correct and incorrect results were the combined totals from all strains with all antimicrobial agents. To avoid distortions due to small numbers of laboratories using a particular method, association between methods and error rates was tested only when methods were used by a minimum of 20 laboratories. Results achieved with methods used by less than 20 laboratories, or when methods used were not unequivocally stated, have generally not been included in text or tables. The exclusion of these results causes some apparent inconsistency where the sum of laboratories using specific techniques is less than the total using the general method. Thus for example, although 232 laboratories stated that they used control organisms, only two species were used by 20 or more laboratories, the Oxford strain of *Staphylococcus* by 160, and *N gonorrhoeae* by 51; the apparent shortfall of 21 laboratories comprised those using other species, more than one species, or not supplying the required information.

Results

Results of susceptibility tests on at least one strain were received from 320 laboratories and 314 returned the questionnaire on methods.

Table 1 shows the results and error rates of participants for the six strains. The overall error rate for all combinations of strains and antimicrobial agents was 11%. Table 2 shows the numbers of laboratories achieving various percentages of the possible number of correct results. Standards of performance in testing these strains varied considerably, with 88% of laboratories achieving only 90% or less of correct results.

A disc method was used by 282 (90%) laboratories, a minimum inhibitory concentration (MIC) method by two (0·6%), and a breakpoint method by two (0·6%). Combinations of more than one method were used by 27 laboratories: 15 (55%) used disc + MIC methods; 9 (3%) used disc + breakpoint methods; two (0·6%) used breakpoint + MIC methods; and one (0·3%) used disc + breakpoint + MIC methods.

Table 2 *No (%) of laboratories achieving various percentages of their total possible scores*

Percentage of total possible score achieved	*No (%) of laboratories*
51–55	1 (0·3)
56–60	2 (0·6)
61–65	1 (0·3)
66–70	6 (1·9)
71–75	16 (5·0)
76–80	28 (8·8)
81–85	102 (31·8)
86–90	124 (38·8)
91–95	33 (10·3)
96–100	7 (2·2)

ASSOCIATION BETWEEN METHODS AND RESULTS

The number of laboratories using different methods and the number of correct and incorrect results obtained are shown in table 3. Significant association between methods used and error rates was found only with the following.

Inoculum

Laboratories emulsifying growth in fluid made proportionally fewer errors than those inoculating directly from the colony ($\chi^2 = 4{\cdot}53$, $p < 0{\cdot}05$).

β-lactamase tests

Laboratories using a chromogenic cephalosporin made proportionally fewer errors in testing for β-lactamase than those using an acidimetric disc or strip method ($\chi^2 = 6{\cdot}89$, $p < 0{\cdot}01$).

Disc content

Laboratories using low content (2, 5, or 10 μg) tetracycline discs made proportionally fewer errors than those using high content (25, 30, or 50 μg) discs ($\chi^2 = 4{\cdot}39$, $p < 0{\cdot}05$). Laboratories using 10 or 16 μg spectinomycin discs made proportionally more errors than those using 25 or 30 μg discs ($\chi^2 = 13{\cdot}92$, $p < 0{\cdot}001$) and those using 100 μg discs ($\chi^2 = 17{\cdot}84$, $p < 0{\cdot}001$).

Discussion

Participants had little difficulty in recognising that strains were sensitive to antibiotics, and for these an average of 97% correct results were reported. Detection of penicillin resistance in β-lactamase producing strains was also achieved without difficulty by almost 100% of participants. Detection of resistance to penicillin not mediated by β-lactamase, however, proved more difficult. Although there is no international agreement on breakpoints for the assessment of susceptibility to penicillin, a three category scheme is commonly used with MICs of $<0{\cdot}1$ mg/l indicating that a strain is sensitive; 0·1–0·5 mg/l indicating moderate resistance; and ≥ 1 mg/l indicating resistance.[9] Strain 1134, with an MIC of 2 mg/l, a value that would be widely regarded as indicating resistance, was reported as sensitive by 20% of laboratories. In a three category system using the aforementioned breakpoints, strain 1317 (MIC 0·25 mg/l) would be regarded as moderately resistant. Assessment of results for this strain is difficult as different conventions used for reporting in various laboratories could result in reports of "sensitive", "moderately resistant" (or

Table 3 *Distribution of incorrect results according to methods used*

Method detail	*No of laboratories*	*No of results:*		*Ratio of right:wrong*
		Right	*Wrong*	
Medium				
DST (Oxoid)	104	1730	299	6
Isosensitest (Oxoid)	61	1044	165	6
General purpose (non-susceptibility testing)	78	1321	229	6
Additives to medium				
Lysed blood	88	1460	250	6
Chocolated blood	146	2418	418	6
Incubation atmosphere				
Candle jar	39	622	109	6
CO_2 incubator	241	4069	697	6
CO_2 sachet	21	309	54	6
Inoculum				
Direct from colony	126	2020	379	5
Emulsified in fluid	182	3076	493	6
Standardisation of inoculum				
Inoculum standardised	213	3569	594	6
Inoculum not standardised	98	1587	286	6
Application of inoculum				
By loop	52	776	147	5
By swab	150	2557	420	6
By loop followed by swab	86	1437	237	6
Control strains used				
Neisseria gonorrhoeae	51	914	140	7
Oxford *Staphylococcus aureus*	160	2569	449	6
Control not used	81	1335	242	6
Use of controls				
On same plate as test strain	159	2653	437	6
On separate plate from test strain	68	1125	193	6
Frequency of use of controls				
Used each time of testing	198	3311	548	6
Used less frequently	29	462	82	6
Measurement of zone sizes				
Zone size never measured	106	1724	296	6
Zone size always measured	38	652	112	6
Zone size measured only if test < control	117	1977	334	6
Interpretation of results				
Visual comparison of test zone with control zone	126	2132	354	6
Measured comparison of test zone with control zone	57	952	167	6
Visual assessment of test zone without comparison with control zone	71	1125	205	5
Strictly defined cut-off point based on measurement of zone size without reference to control zone	21	345	57	6
Type of β-lactamase test (β-lactamase results only analysed)				
Chromogenic cephalosporin	142	794	5	158
Acidimetric disc or strip	129	715	16	45
Manufacturer of discs				
Oxoid	97	1555	280	6
Mast	142	2365	415	6
Content of penicillin discs (penicillin results only analysed)				
1, 1·5, 2 units	170	741	48	15
More than 1 disc content	118	540	28	19
Content of cefuroxime discs				
Insufficient laboratories used discs other than 30 μg to allow comparison to be made				
Content of tetracycline discs (tetracycline results only analysed)				
2, 5, 10 μg	241	996	369	3
25, 30, 50 μg	46	169	85	2
Content of spectinomycin discs (spectinomycin results only analysed)				
10, 16 μg	49	241	28	9
25, 30 μg	119	656	28	23
100 μg	60	336	8	42

"intermediate"), or "resistant". Laboratories in which any significant decrease in sensitivity is regarded as indicating resistance would report such strains as resistant; those in which moderately resistant strains are distinguished from resistant strains would report them as moderately sensitive or intermediate; while in some laboratories infections caused by such strains would be regarded as being likely to respond to the high doses of penicillin often used as standard treatment, the strains therefore being reported as sensitive. Differences both in breakpoints used and in therapeutic regimens make it difficult to decide at which MIC failures in treatment become more likely. Failures in treatment, however, do occur with strains classified by local criteria as moderately resistant, even when high doses of penicillin are used.[10-14] It is clearly important in this context that clinicians and staff of local laboratories agree on the meaning of susceptibility results reported.

Cephalosporins may be used for treating infections with β-lactamase producing strains, and an unpublished UKNEQAS survey of antibiotics tested by United Kingdom laboratories in 1985 showed that 13% were testing the susceptibility of *N gonorrhoeae* to cefuroxime. Susceptibility to cefuroxime and penicillin is positively correlated in non-β-lactamase producing strains.[13 15] This was the case with strain 1314, a non-β-lactamase producer, resistant to penicillin (MIC 2 mg/l) with an MIC of 1 mg/l for cefuroxime. Only 4% of laboratories reported this strain as resistant to cefuroxime. Little information on the correlation between MIC and treatment failure has been published, but such failures have been recorded with four strains having MICs of 0·5 or 1 mg/l.[16 17] Reports from the United States of America record 86% of strains with MICs of less than 0·5 mg/l between 1983 and 1984,[18] indicating that this strain was more resistant than is normal for the species.

Tetracycline is another alternative to penicillin, both in cases of resistance to penicillin or of penicillin allergy. Strains 1313 and 1314 were designated as resistant to tetracycline on the basis of MICs of 8 mg/l. Only 27% and 17% of participants, respectively, reported these strains as resistant. Treatment failures with tetracycline may, however, be expected to increase for infections with strains having MICs of 2 mg/l or more.[14 19]

Strain 1317 was designated as resistant to spectinomycin on the basis of an MIC of > 128 mg/l. Of all participants, 92% reported resistance to spectinomycin. Strains with MICs greater than 32 mg/l are considered to be resistant to spectinomycin,[20] and treatment failures have occurred[10 21] with strains having increased MICs. Even so, there does not seem to be a clear cut relation between MIC and treatment failures with this antibiotic.[10] Resistant strains are not common and are currently not an important clinical problem.

Few associations were found between methods and results, in contrast to the findings of previous surveys,[3 4 6] in which the type of media used, standardisation of inoculum, antibiotic content of discs and use of suitable controls, all affected the results obtained. As in these previous studies, with some antibiotics there was an association between error rates and the antibiotic content of the discs used. With tetracycline, laboratories using low content (2, 5, or 10 μg) discs made proportionally fewer errors than those using higher content (25, 30, or 100 μg) discs. This was not unexpected in view of the slow growth of the organisms and the large zones produced with the high content discs. In contrast, laboratories using low content (10 or 16 μg spectinomycin) discs made proportionally more errors with both spectinomycin resistant and sensitive strains than did those using higher content (25, 30, or 100 μg) discs, presumably because of the low degree of activity of this antibiotic against gonococci. Low content cefuroxime discs were used in too few laboratories (96% used 30 μg discs) to enable results obtained with high and low content discs to be compared. Even so, 30 μg would seem an extremely high disc content for use with this species in view of its sensitivity to cefuroxime.[18]

Methods for detecting β-lactamase that are based on chromogenic cephalosporins gave slightly better results than acidimetric methods. This was suggested in a previous trial with *H influenzae*[6] but the difference was not then significant. These results with *H influenzae* and *N gonorrhoeae* contrast with studies in individual laboratories[22 23] which have shown one type of acidimetric test (Intralactam, Mast Laboratories Ltd) to be as reliable as chromogenic cephalosporin tests. Some versions of the acidimetric test may be less reliable than others, but this was not investigated in the current trial.

The lower error rates for laboratories preparing inocula by emulsifying growth in fluid compared with those using growth direct from the colony is probably due to a reduced density of inoculum with the former. This has also been found in previous trials with other organisms.[3 4 6]

Results of this survey give rise for concern, as although the most important cause of treatment failures, resistance to penicillin mediated by β-lactamase, was readily recognised, other possible causes were recognised by far fewer laboratories. Treatment of gonorrhoea is more standardised and more empirical than therapeutic regimens for other infectious diseases. It is common practice not to test routinely isolates of gonococci for susceptibility other than for β-lactamase production and to reserve further testing for strains isolated from cases of treatment

failure. With the apparent difficulties experienced in detecting resistance, a policy of referral to reference laboratories may be a sensible alternative to infrequent testing in clinical microbiology laboratories.

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