J Clin Pathol 1988;**41**:384–387

Antimicrobial susceptibility testing of *Streptococcus pneumoniae:* quality assessment results

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SUMMARY Six strains of *Streptococcus pneumoniae* were distributed to 405 United Kingdom laboratories who were asked to test the susceptibility of the strains to penicillin, tetracycline, chloramphenicol and erythromycin and to provide details of methodology to test the standards of susceptibility testing. High error rates were seen only in failure to detect moderate resistance to penicillin (12%) and resistance to chloramphenicol (16%). Increased error rates were associated with several methods or practices. These included the use of certain culture media; failure to standardise the inoculum; inoculation by loop rather than by swab; failure to use control organisms; failure to measure zone sizes; the use of discs containing a high content of penicillin to test susceptibility to penicillin, and the use of high content discs for testing erythromycin, tetracycline, and chloramphenicol.

Trials organised as part of the United Kingdom national external quality assessment scheme for microbiology (UKNEQAS)[1] have highlighted problems with susceptibility testing, especially with more delicate organisms.[2,3] In view of increased resistance of *Streptococcus pneumoniae* to antimicrobial agents[4] a trial was organised in March 1987 to investigate the standard of performance of susceptibility testing of *S pneumoniae* and factors affecting the results in a large number of laboratories in the United Kingdom.

Material and methods

The design and organisation of the trial was as described previously.[2,3]

Six recent clinical isolates of *S pneumoniae* from the United Kingdom were distributed as freeze dried cultures to participants in the UKNEQAS who were asked to test and report susceptibility to any of four named antimicrobial agents that they would normally test, and to complete a questionnaire on methodology. Correct results of sensitive or resistant (and moderately resistant for penicillin) were based on minimum inhibitory concentration (MIC) determinations in the Division of Microbiological Reagents and Quality Control (DMRQC) and the Antibiotic Reference Laboratory (ARL) (table). An agar dilution method based on that of Ericsson and Sherris,[5] using Oxoid direct sensitivity test agar supplemented with 5% lysed horse blood, was used.

For chloramphenicol, erythromycin, and tetracycline, participants' results recorded as sensitive or resistant were regarded as correct if the same as the designated correct result, and as incorrect if different. Results recorded as "intermediate" or "moderately resistant" were not assessed. The reference laboratories' MIC results for penicillin were evaluated on the basis of the commonly used three category classification[4,6]; strains with MICs of $<0{\cdot}1$ mg/l, $0{\cdot}1$–1 mg/l, and >1 mg/l were regarded as sensitive, moderately resistant, or resistant, respectively. On this basis, two strains were classified as sensitive, and reports of sensitive were considered to be correct and resistant or moderately resistant to be wrong. Four strains were classified as moderately resistant and reports of resistant or moderately resistant were considered to be correct, and sensitive to be wrong.

The ratios of correct:incorrect results achieved by laboratories using different methods was tested by the χ^2 test. The analysis was restricted to results of the 95% of laboratories using disc methods alone. Unless otherwise stated, the numbers of correct and incorrect results were the combined totals from all strains with all antimicrobial agents. 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

Accepted for publication 25 November 1987

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

Strain of S pneumoniae and antimicrobial agents	*Reference laboratory results Modal MICs (mg/l)*	*Designated correct result*	*No of laboratories reporting strains as*			*Percentage correct*
			Sensitive	*Intermediate/ moderately resistant*	*Resistant*	
MQCL 1441:						
Penicillin	0·25	MR	95	171	103	74
Tetracycline	64	R	9	0	357	98
Chloramphenicol	16	R	146	5	211	58
Erythromycin	0·25	S	367	0	3	99
MQCL 1442:						
Penicillin	0·25	MR	56	145	167	85
Tetracycline	0·5	S	358	0	6	98
Chloramphenicol	2	S	359	0	1	99
Erythromycin	0·12	S	367	0	1	99
MQCL 1443:						
Penicillin	1	MR	14	52	301	96
Tetracycline	32	R	11	0	352	97
Chloramphenicol	16	R	15	3	341	95
Erythromycin	0·25	S	367	0	0	100
MQCL 1444:						
Penicillin	0·01	S	360	5	4	98
Tetracycline	0·5	S	362	0	3	99
Chloramphenicol	2	S	358	0	3	99
Erythromycin	0·12	S	367	0	2	99
MQCL 1445:						
Penicillin	0·01	S	362	4	1	99
Tetracycline	64	R	13	1	349	96
Chloramphenicol	2	S	356	0	3	99
Erythromycin	0·25	S	365	0	2	99
MQCL 1446:						
Penicillin	1	MR	15	40	315	96
Tetracycline	64	R	5	0	361	99
Chloramphenicol	16	R	16	2	345	95
Erythromycin	32	R	7	0	363	98

S = sensitive; R = resistant; MR = moderate resistance

unequivocally stated, have not been included in text or tables.

Results

Results of susceptibility tests on at least one strain were received from 370(91%) laboratories, and 351(87%) returned the questionnaire on methods.

The table 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 5%. Some 70% of laboratories were correct in more than 95% of their reports and 85% were correct in more than 90% of their reports.

METHOD OF TESTING

A disc method was used by 332(95%) laboratories, a minimum inhibitory concentration (MIC) method by one (0·3%), a breakpoint method by six (2%) and the API ABT method by one (0·3%). Combinations of more than one method were used by 11 laboratories of which six (2%) used disc positive MIC methods, and five (1%) used disc positive breakpoint methods.

ASSOCIATION BETWEEN METHODS AND RESULTS

Significant association between methods used and error rates was found only with the following:

Media

Laboratories using DST Oxoid (n = 100, 2235 correct results, 93 incorrect results) made fewer errors than those using Isosensitest Oxoid (n = 75, 1690 correct results, 95 incorrect results) (χ^2 4·08, $p < 0·05$). Laboratories using general purpose growth media (n = 75, 1715 correct results, 60 incorrect results) made fewer errors than those using DST Gibco (n = 28, 634 correct results, 38 incorrect results) (χ^2 6·56, $p < 0·05$) and those using Isosensitest Oxoid (n = 75, 1690 correct results, 95 incorrect results) (χ^2 8·05, $\chi^2 < 0·01$).

Standardisation of inoculum

Laboratories standardising the inoculum (n = 232, 5242 correct results, 251 incorrect results) made proportionally fewer errors than those not standardising the inoculum (n = 103, 2206 correct results, 133 incorrect results) (χ^2 4·38, $p < 0·05$).

Application of inoculum
Laboratories inoculating by swab (n = 168, 3800 correct results, 174 incorrect results) made proportionally fewer errors than those using a loop (n = 55, 1212 correct results, 84 incorrect results) (χ^2 9·28, $p < 0·01$).

Use of controls
Laboratories using controls (n = 271, 6085 correct results, 296 incorrect results) made proportionally fewer errors than those not using controls (n = 60, 1339 correct results, 88 incorrect results) (χ^2 5·82, $p < 0·05$).

Measurement of zone sizes
Laboratories measuring zones always, if the test zone was less than the control or if doubtful (n = 182, 4148 correct results, 169 incorrect results), made proportionally fewer errors than those never measuring zones (n = 116, 2540 correct results, 160 incorrect results) (χ^2 15·03, $p < 0·001$).

Interpretation of results
Laboratories interpreting results by visual comparison of the test zone with the control zone (n = 169, 3793 correct results, 185 incorrect results) made proportionally fewer errors than those making visual assessment of the test zone without comparison with the control zone (n = 66, 1470 correct results, 101 incorrect results) (χ^2 7·28, $p < 0·01$).

Laboratories interpreting results by measured comparison of the test zone with the control zone (n = 70, 1816 correct results, 83 incorrect results) made proportionally fewer errors than those making visual assessment of the test zone without comparison with the control zone (n = 66, 1470 correct results, 101 incorrect results) (χ^2 7·25, $p < 0·01$).

Antibiotic used for testing susceptibility to penicillin
Laboratories using oxacillin, methicillin, or cloxacillin (n = 21, 122 correct results, four incorrect results) made proportionally fewer errors than those using penicillin alone (n = 309, 1666 correct results, 167 incorrect results) (χ^2 5·21, $p < 0·05$).

Disc content
Laboratories using high content penicillin discs (>1 unit) n = 120, 584 correct results, 109 incorrect results) made proportionally more errors than those using 1 unit discs (n = 151, 846 correct results, 50 incorrect results) (χ^2 44·69, $p < 0·001$) and those using <1 unit discs (n = 54; 309 correct results, 12 incorrect results) (χ^2 30·01, $p < 0·001$).

Laboratories using low content (1, 2, or 5 μg) erythromycin discs (n = 235, 1396 correct results, five incorrect results) made proportionally fewer errors than those using high content (10 or 15 μg) discs (n = 80, 462 correct results, six incorrect results) (χ^2 5·13, $p < 0·05$).

Laboratories using low content (1, 5, or 10 μg) tetracycline discs (n = 273, 1598 correct results, 27 incorrect results) made proportionally fewer errors than those using high content (25, 30, or 50 μg) discs (n = 37, 203 correct results, 10 incorrect results) (χ^2 = 8·78, $p < 0·01$).

Laboratories using low content (2, 5, or 10 μg) chloramphenicol discs (n = 242, 1333 correct results, 97 incorrect results) made proportionally fewer errors than those using high content (25, 30, or 50 μg) discs (n = 40, 306 correct results, 53 incorrect results) (χ^2 23·78, $p < 0·001$).

Discussion

The only major difficulties with these strains were seen with the detection of moderate resistance to penicillin and resistance to chloramphenicol. Decreased susceptibility to penicillin was recognised in strains 1443 and 1446 (MICs 1 mg/l) by an average of 96% participants. Decreased susceptibility was less readily recognised in strains 1441 and 1442 (MICs 0·25 mg/l) by an average of only 80% participants. The use of oxacillin discs has been recommended for the detection of penicillin resistance in pneumococci[6-8] because oxacillin is less active against pneumococci than penicillin and zone sizes are much smaller, facilitating recognition of resistance. The 21 laboratories using methicillin, cloxacillin, or oxacillin made proportionally fewer errors in detecting resistance or moderate resistance to penicillin than those using high content penicillin discs alone. The same effect is evident when low content penicillin discs were used, laboratories using 1 unit discs or lower made proportionally fewer errors than those using >1 unit discs.

We deposited strain 1442 (MIC 0·25 mg/l) in the National Collection of Type Cultures (12140) for use as a control to ensure that such decreased susceptibility can be recognised.

Resistance to chloramphenicol in strains 1443 and 1446 was indicated in 95% of reports; only 58% detected resistance in strain 1441, although the MIC of all three strains (16 mg/l) was the same. Difficulties in the detection of resistance to chloramphenicol in strains of *Haemophilus influenzae* have previously been reported in United Kingdom laboratories.[2] Laboratories using low content chloramphenicol discs (2, 5, or 10 μg) made proportionally fewer errors than those using high content discs (25, 30, or 50 μg). Association between disc content and results was also found with erythromycin and tetracycline, with proportionally fewer errors being made by those using low content discs. Similar associations have been

found with *H influenzae* and *Neisseria gonorrhoeae*.[2,3]

Other associations between methods and results generally agreed with those of previous surveys.[2,3] Because these associations generally reflect what would be regarded as good laboratory practice, we recommend that laboratories review their methods accordingly.

Despite several decades of experience of appropriate antimicrobial treatment in serious pneumococcal disease mortality remains high with estimated case fatalities of 20% for bacteraemia and 32% for meningitis.[9] High mortality is often associated with a wide variety of underlying and predisposing conditions in individual patients. Against this background, failure to detect reduced susceptibility to penicillin in laboratory tests, especially in cases of meningitis, may pass unnoticed in individual cases.

There is considerable geographic variation in the incidence of antimicrobial resistance in *S pneumoniae*.[4] In the United Kingdom resistance to antimicrobial agents other than tetracycline has been rare but appears to be increasing.[10] Such resistant strains have caused major clinical problems in other parts of the world.[4] Failure to recognise increased resistance to penicillin in the moderately resistant strains by an average of 12% of laboratories is disturbing. The use of the term "moderate resistance" to penicillin with pneumococci should be used with extreme care as this may be interpreted by clinicians as indicating that infections are likely to respond to high doses of penicillin. This seems true of treatment of bacteraemia without meningitis where serum concentrations of penicillin well in excess of MICs may be obtained.[4] It is not true of treatment of meningitis caused by strains with penicillin MICs of 0·1–1 mg/l, because much lower concentrations of penicillin are found in cerebrospinal fluid and treatment failures are common.[4]

We thank other members of the antibiotic susceptibility testing subcommittee of the steering committee for national external quality assessment in microbiology for advice. Members of the subcommittee are: I D Farrell (chairman), J J S Snell (secretary), D F J Brown, P Donnelly, R George, and A C Scott.

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