DRUG SENSITIVITY TESTS FOR TUBERCLE BACILLI

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You have heared from Dr. Narasirahan about the various methods of determining the sensitivity of tubercle bacilli to antituberculosis drugs. You have also seen from Dr. Kulkarni's presentation that the agreement between duplicate tests on the same culture or between tests on two cultures obtained from a patient at almost the same time is only 80%—90% for streptomycin or isoniazid, and even less for PAS or thiocetazone, if one considers only patients with drug-resistance. Such variation also occurs in drug-sensitive cultures, as can be seen from the results of sensitivity tests on the standard strain, H37Rv, which is usually employed as a control. The MIC of this strain in the standard test varies from 2—32 ug/ml streptomycin, the mode being 8 ug/ml. If such a 4-fold difference in the MIC can occur with one strain set up with the same method by the same technician, one may expect similar variation in the MIC of test strains obtained from patients. The interpretation of the result of a single test on a patient's strain therefore becomes difficult. For the interpretation of such a result to be meaningful, one must obtain precise definitions of drug resistance for each method, taking into consideration variations resulting from the errors of the test,

Mitchison in 1962 defined resistance as "a decrease in sensitivity of sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come into contact with the drugs". Sensitivity tests are designed with the object of detecting such a decrease in sensitivity. The most efficient criterion of resistance may be slightly different from one laboratory to another. Further, measures of sensitivity are expressed on different scales by the 3 methods of sensitivity tests. Comparison of such methods can be valid only if objective methods of calibration of sensitivity tests are employed.

In the past, a common approach was to test a sample of strains isolated before the start of treatment with a drug, and therefore presumed to be sensitive strains. Strains were considered resistant if they were slightly more resistant $han the great majority of sensitive strains. This procedure has the inherent defect that arbitrary criteria are to be employed to remove from the population of sensitive strains those which are considered to have natural or primary resistance.

A second approach is to consider the results of the treatment of patients who have varying degree of sensitivity at the start of treatment and to try to determine the level of sensitivity at which there is a change in the therapeutic response. This, however, is not feasible since it is not the practice to treat a tuberculosis patient with one drug alone.

The best approach is to compare the sensitivity of a sample of strains from untreated patients (a predominantly sensitive sample) with a sample of strains from patients who have been treated with the drug for at least a few months (a predominantly resistant sample). The criterion of resistance that discriminates between the two samples with the greatest efficiency can then be chosen. Even with this method, some misclassification of sensitive strains as resistant, and vice versa, can occur. To avoid misclassification of sensitive strains as resistant, it is preferable to choose a sensitive strain, although it may occasionally fail to detect strains with border-line degrees of resistance.

We have employed this approach in our laboratory for the purpose of comparison of various methods of testing mycobacterial drug-sensitivity.

An example of sensitivity test of dihydrostreptomycin is shown in Table I. The test was set up by using a standard suspension S_t and 4 successive 10-fold dilutions, S_2, S_3, S_4, and S_5. The readings have been taken at 40 days, and the growth on dihydrostreptomycin slopes has been expressed as a proportion of the growth on the drug-free slopes. This design also permits interpretation of the results as MICs, if read horizontally. This basic design has been used in our laboratory for all comparative studies with streptomycin, isoniazid, ethambutol, pyrazinamide and PAS.

Dihydrostreptomycin sensitivity tests were performed on 960 presumably sensitive (PR) strains obtained during chemotherapy with regimens containing streptomycin. Table 2 presents the sensitivity of these cultures as assessed by the proportion resistant to dihydrostreptomycin 4 ug/ml. Comparing the sensitivity of the PS and the PR populations at the various levels of resistance to dihydrostreptomycin 4 ug/ml, the highest discrimination (last

TABLE 1

Dihydrostreptomycin proportion sensitivity lest—example

Suspension	Drug- free		Dihydrostrep. Cone. (ug/ml)			
			2	4	1	16
Standard (Si)	3 +	(50,000)	2+-	2+	*11*	0
1 in 10 (S_1)	3-1-	(5,000)	2+	*26*	*2*	
1 in 103 (S_3)	2+ 2+	(500)	*32*	4	0	
1 in 103 (S_4)	*46* *54*	(50)	4	1	0	
1 in 10* (S_5)	8 8					
Proportion resistant			6.4%	0.52%	0.02%	

TABLE 2

Proportion resistant to 4 ug\m\ Dihydrosireplomycin

Proportion resistant	PS (%)	PR (%)	Difference
1% or more	16	81	65
5% or more	10	72	62
10% or more	9	69	60
25% or more	7	61	54
50% or more	5	47	42
No. of cultures	960	734	

column) occurs with a definition of 1 % or more; such a definition, however, would misclassify 16% of PS cultures as resistant, and is therefore unacceptable. A definition of 25% or more would label only 7% of such strains as resistant, and also provide a high degree of discrimination between the two populations, and is therefore acceptable. This approach was utilised to define resistance by other methods of testing.

Definitions of resistance were similarly derived by similar process for MIC and RR for streptomycin and MIC proportions on dihydrostreptomycin (Table 3). It will be noted that all measures classified less than 10% of the PS population as resistant. Definitions based on MIC or RR of streptomycin, or MIC of dihydrostreptomycin were as satisfactory as the best proportion, namely >1% on 8 ug/ml.

The patients whose pretreatment cultures were included in this study were all prescribed regimens of intermittent chemotherapy containing streptomycin as one of the drugs. The response to treatment for a period of 12 months could be assessed in about 840 patients. In these patients it was possible to study the prognostic significance of pretreatment resistance to streptomycin & dihydrostreptomycin, as defined by the discrimination approach (Table 4). Considering a definition of MIC >32 ug/ml. 52% of patients having resistant bacilli had an unfavourable response, compared with 16% of the sensitive patients, a highly significant difference ($P<0.001$). Differences in the response of the sensitive and the resistant populations are highly significant for each of the remaining definitions. Thus, the definitions obtained by employing the discrimination approach are clinically meaningful.

TABLE 3

Definitions of resistance to streptomycin

Definition	% classified as resistant		Difference
	PS	PR (6-12)	
Strep			
MIC >32 ug/ml	6	69	63
RR >4	7	71	64
Dihydrostrep			
MIC > 32 ug/ml	5	65	60
>50%on 2 ug/ml	10	61	51
>25% on 4 ug/ml	7	61	54
>1%on 1 ug/ml	8	70	62
No. of cultures	960	739	

TABLE 4

Prognostic significance of resistance to streptomycin

Definition	Patients with an unfavourable response %	
	Sensitive	Resistant
MIC DS> 32 ug/ml	16	52
MIC Strep. > 32 ug/ml	16	44
RR Strep>4	16	42
>50% on DS 2 ug/ml	16	36
> 5% on DS 4 ug/ml	16	38
>l%on DS 8 ug/ml	16	46
No of patients	778-812	29-72

The cultures tested for streptomycin sensitivity were also tested for their susceptibility to isoniazid. The definitions of isoniazid resistance which discriminate most efficiently between the PS and the PR populations are presented in Table 5. All the measures, including the MIC method, appear to be of equal efficiency in detecting acquired resistance. Correlating the pretreatment isoniazid resistance with response to treatment, the proportion of patients with unfavourable response among the sensitive group was found to be 16%, compared to 55-76% among those with resistance, a highly significant difference, providing evidence that for isoniazid also, the definitions of resistance are clinically meaningful.

The discrimination approach was also applied to pretreatment cultures and for cultures isolated from patients who had been treated

TABLE 5

Definitions of resistance to isoniazid

Definition	%classified as resistant		Difference
	PS	PR	
MIC > 1 ug/ml	5	64	59
> 5% on 0.1 ug/ml	6	66	60
>0.5% on 0.2 ug/ml	6	65	59
> 0.1% on 0.2 ug/ml	6	63	57
No. of cultures	959	740	

TABLE 6

Definitions of resistance to ethambutol

Definition	% classified as resistant		Difference
	PS	PR (4-12)	
S_1-20 col. MIC>8 ug/ml	1	40	39
S_2-l0col. MIC >8 ug/ml	1	42	41
S_3-10col. MIC >2.8 ug/ml	1	42	41
>25% OQ 1.4 ug/ml	0	27	37
>5% on 2.0 ug/ml	0	43	43
>0.5% on 2,8 ug/ml	1	43	42
No. of cultures	81	87	

with ethambutol for various periods. The relative efficiencies of the various proportion measures of resistance are shown in Table 6. Definitions of proportions on 2 ug/ml or on 2,8 ug/ml are equally efficient. The MIC definitions of resistance are also presented in Table 6. It may be noted that a definition of MIC 8 ug/ml or more is as efficient as the proportion definitions shown earlier.

Information on the clinical significance of these measures of resistance is not as yet available. This information will be available when all the patients complete their scheduled course of chemotherapy.

Earlier studies from this Centre had shown that sensitivity tests for PAS, employing an MIC or an RR type of test, was unsatisfactory for Indian cultures of tubercle bacilli, largely because of a high percentage of pretreatment cultures from Indian patients having low susceptibility to PAS. It was felt that the proportion test may prove more efficient. A comparative study was therefore undertaken on pretreatment cultures (PS) and on cultures isolated from patients while receiving treatment with PAS plus isoniazid daily or twice a week.

Table 7 presents the definitions of resistance to PAS. The best definition is proportion on

TABLE 7

Definitions of resistanee to PAS

Definition	% classified as resistant		Difference
	PS	PR (7-12)	
MIC >2 ug/ml	6	28	22
RR>4	5	31	26
> 10% on 0.25 ug/ml	7	26	19
>5%on0.5 ug/ml	5	28	23
>l%onl ug/ml	7	39	32
No. of cultures	235	218	

TABLE 8

Definitions of resistance to pyrazinamide

Definition	% classified as resistant		Difference
	PS	PR (4-12)*	
MIC@> 100 ug/ml	8	48	40
> 20% on 25 ug/ml	5	46	41
>5%on 50 ug/ml	5	48	43
>l%on 100 ug/ml	5	50	45
No. of cultures	268	101	

•Obtained from patients during the 4th and the 12th months of chemotherapy with daily regimens which included pyrazinamide
@Employing a 1 in 10 dilution and a 10-colony end-point

1 ug/ml, with a difference of 32%. We have not been able to study the clinical significanse, as any deficiency of this drug is compensated to a large extent by the highly active companion drug, isoniazid.

Table 8 presents definition of pyrazinamide resistance, derived by application of the discrimination approach. The best definition is proportion on 100 ug/ml; however, the MIC definition is almost as efficient, and has the great merit of simplicity.

So far, I have presented data on indirect sensitivity tests. As Dr. Narasimhan said earlier, such tests take at least 2 months for results to be available. Direct tests provide information on drug-sensitivity in 2-4 weeks time, and would therefore be useful in the management of cases. I shall briefly present data on a direct sensitivity test developed by my colleague, Sara Mathew.

In this test, 6 swabs are prepared from each sputum, and transferred to test-tubes half-filled with 1% sterile solution of cetrimide. After 60 minutes, the swabs are drained of excess fluid, and are smeared on the entire surface of

TABLE 9

Direct sensitivity results by swab method
Isoniazid sensitivity results by indirect and swab method

Indirect Test	Swab Test	Swab test read at :					
		3 weeks		4 weeks		8 weeks	
Sensitive	Sensitive	57	94%	55	93%	61	95%
Resistant	Resistant	121		130		134	
Resistant	Sensitive	9	5%	8	4%	6	3%
Sensitive	Resistant	2	1%	5	3%	5	2%
Total specimens		189		198		206	

TABLE 10

Direct Sensitivity results by swab method Streptomycin sensitivity test by indirect and swab methods

Indirect Test	Swab Test	Swab test read at :					
		3 weeks		4 weeks		8 weeks	
Sensitive	Sensitive	70	82%	70	88%	72	90%
Resistant	Resistant	83		104		113	
Resistant	Sensitive	30	16%	17	9%	11	
Sensitive	Resistant	4	2%	6	3%	10	
Total specimens		198		197		206	

2 drug-free L-J slopes, and 2 slopes each containing 16 ug/ml streptomycin on 0.2 ug/ml of isoniazid. The slopes are then incubated up to 8 weeks, and read each week. Any growth on 16 ug/ml or growth on 0.2 ug/ml is taken as indication of resistance.

The results of direct sentitivity tests on 206 sputum specimens were compared with those of indirect sensitivity tests on cultures obtained from the 206 specimens. Table 9 shows the extent of agreement between the results of the direct and the indirect sensitivity tests for isoniazid. The agreement is of the order of 93% at 4 weeks, and 95% at 8 weeks. The corresponding figures for streptomycin were 88% at 4 weeks and 90% at 8 weeks (Table 10).

Thus, the swabs sensitivity test gave results which were closely similar to those of the indirect sensitivity tests for streptomycin and isoniazid. The test is simple, easy to set up and economical. The test can therefore be set up in any laboratory which has facilities for culturing sputum. Even so, a period of 4-8 weeks must elapse before the test result is available. This period could perhaps be curtailed by the application of slide culture techniques in sensitivity test procedures.

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