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Direct Test For Determining Sensitivity Of M. Tuberculosis To Streptomycin

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For a total of 400 sputum specimens, the sensitivity of M. **tuberculosis** to streptomycin was determined by direct inoculation of the sputum sediment on to drug-free and drug-containing slopes of Lowenstein-Jensen medium, and also by a standard indirect test. Agreement between the two methods in the classification of strains as sensitive or resistant was of the order of 90%. The optimal time for reading the direct test is 6 weeks.

Introduction

An earlier report from this Centre (Devaki, Mohan and Gangadharam 1969) described a direct sensitivity test for isoniazid using Lowenstein-Jensen medium, and demonstrated close agreement between the findings of that test and those of a standard indirect sensitivity test. A direct sensitivity test for streptomycin was subsequently developed along the same lines. This paper presents the findings of a concurrent comparison of this test and a standard indirect test for streptomycin.

Material and Methods

In all, 513 sputum specimens, of which approximately one-third were from patients who were known to be excreting streptomycin-resistant organisms, were included in this investigation ; of these, 399 were positive and 114 were negative by direct smear examination.

Smear and culture examination : Direct smears were examined by fluorescence microscopy (Holst, Mitchison and Radhakrishna 1959). For culture, 5 ml of sputum was treated with twice the volume of 4 % sodium hydroxide and mechanically shaken in the incubator (37°C) for 20 min. It was then centrifuged for 15 min, and the deposit suspended in sterile distilled water and recentrifuged. A loopful of the sediment was inoculated on each of 2 slopes of Lowenstein-Jensen medium (Cruickshank 1965) using a 5-mm diameter loop. The slopes were then incubated at 37°C, examined weekly for growth of tubercle bacilli (or presence of contamination), and reported as negative if no growth was present by 8-9 weeks.

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Indirect sensitivity test : As soon as the culture became positive, an indirect sensitivity test was set up according to procedures described previously (Tuberculosis Chemotherapy Centre, Madras 1959), and employing streptomycin concentrations (pre-inspissation) of 4, 8, 16, 32 and 64 µg/ml. The extent of growth on the drug-free and the drug-containing slopes of medium was read at the end of 4 weeks of incubation, and the MIC, i.e. the minimum concentration inhibiting growth (defined as 20 colonies or more), was determined. If the growth on the control (drug-free) slope was 100 colonies or less, the test result was ignored and the test repeated. The standard sensitive strain, H37Rv, was also set up with each batch of tests, as a control. The sensitivity test result for each test strain was expressed as a resistance ratio (RR), i.e. the ratio of the MIC of the test strain to the MIC of H37Rv. If the RR was 4, the test was repeated.

Direct sensitivity test : The sediment left over in the bottle after setting up the culture was inoculated on to 7 slopes of Lowenstein-Jensen medium with a 5-mm loop. One of these slopes contained a pre-inspissation concentration of 8 µg/ml of streptomycin, 2 contained 16 µg/ml, 2 contained 32 µg/ml of streptomycin and the remaining 2 slopes, which were drug-free, served as controls.

All the slopes were incubated at 37°C and were read weekly for 8 weeks. Growth typical of **Mycobacterium tuberculosis** was recorded as 3+ if it was confluent, 2+ if there were more than 100 colonies and 1+ if there were 100-20 colonies ; the number of colonies was recorded if it was less than 20.

Definitions of resistance

Indirect sensitivity test-: *(a)* RR of 8 or more, or *(b)* RR of 4 followed by 4 or more in the repeat test.

Direct sensitivity test : *(a)* Growth of 20 or more colonies on 1 (or both) of the slopes containing 16 µg/ml of streptomycin, irrespective of the growth on the drug-free slopes, or *(b)* growth of 1-19 colonies on **each** of the two slopes containing 16 µg/ml of streptomycin and growth of 100 colonies or less on at least one of the drug-free slopes.

Results

Losses due to culture negativity (i.e. absence of growth on the drug-free slopes) or contamination occurred in similar proportions with the indirect test and the direct test, and were of the order of 8 % for smear-positive specimens and 60 % for smear-negative specimens.

In the case of the direct test, the percentage of specimens with growth on the drug-free slopes increased from week to week and stabilized at 4 weeks ; consequently, the comparisons with the indirect test are presented only from 4 weeks onwards..

In all, 400 specimens (358 smear-positive, 42 smear-negative) had a sensitivity test result by both the indirect method and the direct method (at 4, 5, 6, 7 or 8 weeks), and are considered in the analyses below.

Smear-positive specimens : The Table presents the findings in smear-positives sputum specimens. The classification (as streptomycin-sensitive or streptomycin-resistant) based on the direct test result at 4 weeks was identical with that based on the indirect test result for 312 (88%) of 354 specimens. Of the remaining 42 specimens, 36 were classfied as resistant by the indirect test but as sensitive by the direct test, and conversely 6 were classified as sensitive by the indirect test but as resistant by the direct test; the difference was highly significant (P=0.00001). Thus, although the extent of agreement between the two tests was high, there was definite evidence that the direct test was under-estimating the number of resistant strains at 4 weeks (This may also be seen from the fact that 219 (97%) of 225 strains classified as sensitive by the indirect test were classified as sensitive by the direct test also, whereas only 93 (72 %) of 129 strains classified as resistant by the indirect test were classified as resistant by the direct test). This tendency was present at 5 weeks also, but the corresponding contrast (20 compared to 9) just bordered on significance (P = 0.06). At 6 and 7 weeks, the extent of agreement between the two tests was 93 % and, furthermore, the disagreements were evenly distributed. 'Finally, at 8 weeks, the proportion of specimens with an identical classification by the two tests was 94%, but there was a slight suggestion that the direct test was over-estimating the number of resistant strains (P=0.2).

Table. Classification of smear-positive sputum specimens as streptomycin-sensitive or streptomycin-resistant by the indirect and direct sensitivity test methods

Classification based on :		Direct test read at :									
		4 weeks		5 weeks		6 weeks		7 weeks		8 weeks	
Indirect test	Direct test	Number of specimens	%	Number of specimens	%	Number of specimens	%	Number of specimens	%	Number of specimens	%
Sensitive	Sensitive	219	88	217	92	215	93	213	93	211	94
Resistant	Resistant	93		110		117		119		122	
Resistant	Sensitive	36	10	20	6	13	4	11	3	8	2
Sensitive	Resistant	6	2	9	3	11	3	14	4	15	4
	Total	354	100	356	101	356	100	357	100	356	100

Of 130 specimens resistant to streptomycin by the **indirect** test, 122 (94%) were classified as resistant by the direct test, 52 (40 %) by 2 weeks, 41 (32%) at 3 or 4 weeks, 24 (18 %) at 5 or 6 weeks and the remaining 5 (4 %) at 7 or 8 weeks ; the average interval was 3.3 weeks.

Smear-negative specimens : Since the number of smear-negative specimens in the analysis was small (see page 355) the findings are briefly described here, but not tabulated. The proportion of specimens with an identical classfication by the direct and indirect tests was not high at 4 weeks (78 %) and 5 weeks (72%), the direct test

showing definite evidence ($P < 0.01$) of under-estimating the number of resistant strains. At 6 weeks, however, agreement was obtained in 35 (88 %) of 40 specimens ; the remaining 5 specimens were classified as sensitive by the direct test and as resistant by the indirect test. Finally, the proportion with an identical classification by the two tests was 90% at both 7 weeks and 8 weeks.

Findings with the direct test on slopes containing streptomycin 8 µg/ml and 32 µg/ml: The findings on the slope containing 8 µg/ml and on the two slopes containing 32 µg/ml are not presented here, as definitions of resistance based on them were not very satisfactory; thus, the former over-estimated the number of resistant strains while the latter under-estimated them.

Discussion

Encouraging findings have been obtained with the direct streptomycin sensitivity test, employing Lowenstein-Jensen medium, described in this paper, especially in smear-positive specimens. Thus, in the classification of strains as streptomycin-sensitive or streptomycin-resistant, the extent of agreement between the direct test and a standard indirect test was of the order of 90 %. This figure is very similar to the agreement obtained (86 % to 91%) between duplicate indirect sensitivity tests on the **same culture** (Gangadharam 1965). The optimal time for reading the direct test is 6 weeks. At earlier weeks, the extent of agreement was appreciably lower in resistant strains than in sensitive strains, indicating that there was a strong tendency for the direct test to under-estimate the number of resistant strains.

Since direct tests are set up in one stage, they could be useful in situations where losses due to culture negativity are expected to be small-for instance, in patients with a positive sputum smear. However, in most laboratories, it would still be necessary to have facilities for indirect tests, since setting up direct tests on specimens that are negative on smear would not be economical.

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