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Early results from indirect drug susceptibility test for tubercle bacilli

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The indirect susceptibility test results on L-J medium for tubercle bacilli against streptomycin, isoniazid and rifampicin were read at the end of 2 wk and compared with the results at 4 wk. It was found that drug resistance could be correctly predicted in over 70 per cent of cultures including multi-drug resistant tuberculosis (MDR TB) strains at the end of 2 wk. The susceptibility to para-nitrobenzoic acid (PNB) read at 2 wk was able to distinguish non-tuberculous mycobacteria from *Mycobacterium tuberculosis* cultures. The early detection of resistance by this procedure requires only minimum inputs, and can benefit the majority of patients harbouring drug resistant tubercle bacilli.

Key words Early detection of resistance - indirect sensitivity tests

Lowenstein Jensen (L-J) medium is routinely used for the isolation and drug susceptibility testing of tubercle bacilli in most developing countries including India. This procedure requires about 8-10 wk for the final results. Clinicians however, cannot afford to wait that long to initiate treatment. Since there is a 13-25 per cent of initial drug resistance and a growing pool of acquired resistance in the community[1], early information oh drug susceptibility will enable timely intervention in the management of patients with resistant organisms.

Although some of the newer methods including BACTEC radiometric method have successfully reduced the time for culture and sensitivity tests to 2-3 wk[2], the high cost of equipment and medium that must necessarily be imported, put these methods beyond the reach of most laboratories. The Tuberculosis Research Centre (TRC), Chennai, has standardised the direct sensitivity method on L-J medium for streptomycin (S), isoniazid (H) and rifampicin (R) and reduced the time taken for reporting of results to 3-5 wk in smear positive specimens[3-5]. For smear negative specimens, however, one has to depend on the conventional indirect sensitivity method[6]. We therefore attempted to reduce the duration of the standard indirect test from 4 to 2 wk by advancing the reading by 2 wk. This paper details the controlled comparison of the 2 and 4 wk results of sensitivity tests for S, H and R, highlighting the early detection of resistance.

Material & Methods

The successive conventional indirect sensitivity tests, done routinely over a period of three months on 795 cultures of mycobacteria, formed the study material. The tests were read at the end of 2 and 4 wk of incubation, independently by two readers. The tests were considered valid only if there was a growth of 100 colonies or more on the drug-free medium, at both time points. The cultures were then identified as *Mycobacterium tuberculosis* or as non-tuberculous mycobacteria

(NTM), based on their colony morphology, susceptibility to 500 mg/l of para-nitro benzoic acid (PNB), niacin production[7] and stability of catalase at 68°C[7,8].

Cultures were classified as resistant to H if the minimal inhibitory concentration (MIC) was ≥ 1 mg/l and to R if it was ≥ 128 mg/l[6]. Definition for S resistance is normally based on the resistance ratio (RR), a measure that could not be used at 2 wk as the standard strain *M. tuberculosis* H37Rv often failed to give an end point within that time. Therefore, a MIC of ≥ 64 mg/l was taken as indicative of resistance both at 2 and 4 wk for the purpose of this analysis. This was to ensure that only truly resistant cultures were classified as resistant, since the next lower MIC of 32 might include some sensitive or doubtfully resistant isolates.

Results & Discussion

The comparison of results based on 2 and 4 wk readings were available for 649 cultures (368 for S, 533 for H and 499 for R). Further, 69 strains with no result at 2 wk due to inadequate growth on the drug free medium and 28 identified as NTM, were excluded and analysed separately.

At 4 wk 82 cultures were resistant to S, 184 were resistant to H and 74 to R. The proportion of resistant cultures that could be detected based on readings at 2 wk were 58 of 82 (71%), 150 of 184 (82%) and 57 of 74 (77%) to S, H and R respectively. Thus the majority of resistant cultures including MDR TB strains could be detected early by reading the tests at 2 wk. In the remaining resistant cultures, the resistant organism apparently took a longer time to produce visible colonies on the drug containing medium. Examining the growth on the PNB medium inoculated along with the sensitivity tests, at 2 wk, helped to ascertain the identity of *M. tuberculosis* strains in 100 per cent, since all of them were sensitive to PNB. Of the 28 cultures identified as NTM strains at 4 wk, 26 were found to be resistant to PNB at 2 wk. Thus, susceptibility to PNB at 500 mg/l in L-J medium was found to be an ideal method to identify *M. tuberculosis* at 2 wk.

Of the 746 cultures with results at 4 wk, 69 (9.2%) had inadequate growth on the drug free medium making the test invalid at 2 wk; this included 29 tested against S, 60 against H and 39 against R. Analysis not tabulated here revealed that 50 per cent isolates tested against H were resistant to the drug at 4 wk though the converse was not true, suggesting that a small proportion of H resistant strains was slow to grow even on a drug free medium. This should be borne in mind while interpreting sensitivity tests of shorter duration whatever may be the method used.

The purpose of reading the tests at 2 wk was to report drug resistance if any, early, for the benefit of the patient. Cultures that appear to be sensitive at 2 wk should be incubated further since a small proportion of them are likely to grow slowly on the drug containing medium. For this reason, no test should be reported as sensitive based on the 2 wk reading.

Smear positive specimens can be subjected to direct sensitivity tests in order to obtain early results, whereas pauci-bacillary specimens can be tested for drug susceptibility only after the primary cultures are obtained. For these category of samples, early reading of the indirect tests at 2 wk is advantageous. This study has shown that without any additional inputs of material or equipment, over 70 per cent of resistance to S, H and R could be detected at the end of 2 wk of setting up the tests. The PNB susceptibility helped to distinguish the NTM at the same time. Since all the R resistant cultures are invariably resistant to H also, this intervention ensures the early detection of drug resistance including MDR TB strains and thereby benefits the individual patient and also the community.

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