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Commentary

Two speedier phenotypic methods on drug susceptibility testing of *Mycobacterium tuberculosis*

The introduction of drugs beginning with streptomycin for the treatment of tuberculosis (TB) and the subsequent emergence of drug resistant *Mycobacterium tuberculosis* strains has made the testing for susceptibility of the latter a basic necessity. The World Health Organization (WHO) recognized the importance of these laboratory issues even in the early sixties and conducted extensive studies by involving laboratories from both disease endemic developing countries (DEDCs) and disease non endemic countries to standardize the susceptibility (DEDCs) testing procedures for *M. tuberculosis* for all the three methods that were in vogue, *viz*., absolute concentration method, resistance ratio method (RR) and the proportion susceptibility testing method (PST). Two reports by Canetti *et al*[1,2] at different periods delineated in precise terms the criteria of resistance by all these three methods and recommended Lowenstein-Jenson (LJ) medium as the standard culture medium for any susceptibility testing. The resurgence of TB worldwide in the recent past has been accompanied by an increase in the incidence of drug resistant TB, notably multi drug resistant tuberculosis (MDR - TB), *i.e*., strains resistant to at least isoniazid (INH) and rifampicin. Since the dreadful combination of TB with human immunodeficiency virus (HIV) infection, spread of MDR-TB with a global increase in HIV infection at the same time has become a major public health problem. In particular, in DEDCs; it has become a necessity to invent simple, rapid identification and susceptibility testing of such suspected resistant isolates. The newer rapid automated liquid culture methods such as BACTEC 460 TB, MGIT960, MB Bact *etc*., along with probe based methods of identification of species and detection of drug resistance with low turn around time are routinely available in most of the developed countries[3]. In DEDCs, such an approach is simply not feasible since there exists a considerable resource constraints both in terms of manpower and materials. However, in this setting, rapid diagnosis of resistance to the two key drugs, namely INH and rifampicin, is vital for the management of MDR-TB patients. In this context, developing low cost, speedier, at the same time technically less demanding simpler methods for the detection of resistant strains cannot be overemphasized.

As early as 1969 and 1970, promising results were published from the Tuberculosis Research Centre, Chennai of direct sensitivity tests from the concentrated sputum deposit and also by a swab culture method that can be performed even without the use of a centrifuge[4]. Later, a number of low cost colorimetric antimicrobial susceptibility testing of *M. tuberculosis* assays such as tetrazolium bromide (MTT assay), alamar blue assay, nitrate reductase assay and other simpler methods such as microscopic detection of cord-like growth (MODS) and E tests were developed and applied successfully in these settings[5]. However, these assays may have basic limitations since Mycobacteria other than tuberculosis (MOTT) can also produce cord factor and INH can interfere with formazan production in MTT assay and give rise to false resistant results. Besides, use of liquid medium in microtitre plates is associated with biohazard and cross contamination, and E test requires technical expertise in the preparation of inoculum for seeding and the danger of aerosol spread if not carried out in biosafety cabinets. One of the recent rapid techniques *i.e*., the nitrate reductase assay is based on the biochemical properties of *M. tuberculosis* to reduce the nitrate to nitrite in the substrate by using the nitrate reductase enzyme. Since most of the nonpathogenic mycobacteria lack this enzyme, the detection of *M.tuberculosis* is fairly accurate by this method. The presence of nitrite is detected by a colour change to pink upon addition of specific reagents that can be seen visually. The test is simple, rapid and is highly reproducible and fairly comparable to the BACTEC 460 TB system, in detecting MDR-TB

strains, and they are also equally rapid in that results are available within 7-14 days[3]. It can be done in most laboratories, having facility for culture and does not require any expensive reagents or chemicals. Likewise, the E test also can be performed in laboratories where culture facilities are already available[5]. However, unlike direct susceptibility test, growth of organisms is required for the performance of both these tests. And also at least one identification test method must be employed along with these tests to differentiate *M. tuberculosis.*

Although all these phenotypic methods as referred in this issue by Sethi *et al*[6] and Sumathi *et al*[7] may yield a quick answer in determining resistance, none of these are one hundred per cent perfect like PST method[1]. That is why the WHO advocates PST as a standard method for the conduction of global drug resistance surveillance with an External Quality Assurance Programme[8]. Efforts are underway in strengthening Mycobacteriology laboratory services in India. The Indian Council of Medical Research has taken up a lead in establishing good quality laboratory services in eight medical colleges in India with India Clen (INCLEN) initiative by allowing Tuberculosis Research Centre (TRC), Chennai to take part in this activity. Likewise, TRC is also serving as a National Reference Centre and also as a WHO Supra National Reference Laboratory (SNRL) for this region in streamlining the drug susceptibility testing activities as a part of its goal in strengthening laboratory services.

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