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DRUG RESISTANT TUBERCULOSIS IN INDIA: CURRENT TRENDS & FUTURE PROSPECTS

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ABSTRACT

Tuberculosis (TB) is a public health emergency that continues to kill nearly 1.5 million people each year worldwide. India has the highest TB burden with an estimated incidence of 2.2 million cases out of a global incidence of 8.7 million cases. Drug-resistant TB, known since the introduction of anti-tubercular drugs and evolving from being Mono-Resistant to Multi-Drug Resistant (MDR), extensively Drug Resistant (XDR) & recently Totally Drug Resistant (TDR) is now well established throughout the world. Approximately 3% of all newly diagnosed patients have MDR-TB and the resistance rates are ten times higher in previously treated cases. India is one of highest MDR-TB burden countries in the world, with an estimated 99,000 incident MDRTB cases. One TB/MDR-TB/XDR-TB case if not detected and treated early can potentially infect 15-20 persons annually. This, in combination with the AIDS pandemic, poses a great challenge for treating physicians & global TB control efforts emphasizing the need for rapid & accurate diagnosis of TB and drug-resistant TB. The purpose of this article is to review the conventional & the newer rapid tools for TB diagnosis & their utility in the resource-constrained, high-burden settings.

INTRODUCTION

Tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis (MTB) remains a major public health burden in most of the developing countries despite recent progress in control efforts worldwide. As per the WHO Global TB Report 2011, there were an estimated 8.7 million incident cases & 1.4 million deaths due to TB in 2011. India is the highest TB burden country with an estimated incidence figure of 2.2 million cases. It is estimated that about 40% of Indian population is infected with the TB bacillus.[1,2] Factors contributing to high prevalence of TB in developing countries& problems in its control include co-infection with HIV, emergence of multi-drug resistant tuberculosis, inadequate treatment, continuing poverty , malnutrition, overcrowding, armed conflict &increasing number of displaced persons. WHO1993 declared TB a global public health emergency & in order to ensure complete cure& prevent multidrug resistance, recommended Directly Observed Therapy Short course (DOTS) that consists of an initial 2 months intensive treatment with rifampicin(R), isoniazid(H), pyrazinamide(Z)& ethambutol(E) thrice weekly followed by a 4 months continuation phase of rifampicin & isoniazid for new cases. Streptomycin is added in re treatment cases. In India, Revised National Tuberculosis Control Program (RNTCP), based on the DOTS strategy that began as a pilot project in 1993 was launched as a national program in 1997 and the entire country was covered under DOTS by 24th March 2006. [3] The program has achieved treatment success rate of more than 85% and new smear positive (NSP) case detection rate (CDR) of 70%. The DOTS strategy has been able to standardize drug regimens, prevent misuse of drugs and avoid emergence of drug resistance. [2]

Currently, WHO 2010 recommends that national TB programs need only three standard regimens: [4]

-new patient regimen containing six months of rifampicin: IP (2) H3R3Z3E3/CP (4) H3R3

-retreatment regimen with first line drugs: IP (2) HRZES /1HRZE/CP (5) HRE

-MDR regimen, IP (6/9)Km Lvx ETo E Cs Z CP(18) Lvx ETo E Cs

(K, Lvx, Eto & Cs stand for Kanamycin,Levofloxacin, Ethionamide & cycloserine respectively.)

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EVOLUTION OF DRUG RESISTANT TB

The causative agent of TB was identified by Robert Koch in 1882. BCG vaccine became available for prophylactic use in humans thirty -nine years later in the year 1921. Introduction of antibiotics like Streptomycin (1947), PAS (1948) in quick succession followed by Rifampicin (1959) & Isoniazid (1962) was a revolution in TB chemotherapy resulting in drastic reduction in morbidity & mortality which was maintained for several decades. MTB, however, exhibits a high degree of intrinsic resistance to most antibiotics & chemo therapeutic agents due to low permeability of their cell walls. Also, MTB mutants resistant to any single drug are normally present in a large bacterial population, irrespective of exposure to anti tubercular drugs. Consequently, mono-therapy & improperly administered 2 drug therapy will select for drug resistant mutants that will continue multiplying unchecked. A serious consequence of this has been the emergence of MDRTB which is defined as TB where bacilli are resistant to isoniazid & rifampicin with or without resistance to other first line drugs. The Global estimation of proportion MDRTB in new cases& re-treatment cases is 3.4% & 20% respectively. It is slightly lesser in India, being 2-3%n in new cases and 14-17% in re treatment.[1,2]. Another worrisome condition is XDR-TB which is a subset of MDR-TB where the bacilli, in addition to being resistant to rifampicin & isoniazid are also resistant to fluroquinolones & any of the 2nd line injectable drugs (kanamycin, capreomycin or amikacin). In the recent years, two new terms extensively drug resistant tuberculosis (XDR) & totally drug resistant tuberculosis (TDR) have been described by different researchers (Italy 2007, Iran 2009& Mumbai, India 2011) although they have not been recognized by the WHO. These terms have been used for MTB strains that are resistant to all 1st line drugs & 2nd line anti- TB drugs.

These difficult to treat, drug resistant forms of TB are increasingly seen in Asia, Eastern Europe, South America& sub Saharan Africa disrupting the TB & HIV control program. Each year an estimated half million MDR-TB cases develop of which only 7% are diagnosed because of lack of laboratory capacity. Consequently, patients with drug resistant TB may be inappropriately treated& drug resistant MTB strains may spread in the community unchecked. Presently, there are 27 ”high burden” countries for MDR-TB with at least 4,000 cases of MDR-TB each year, & or at 10% of newly registered cases are of MDR-TB.[1,2]

CHALLENGES IN MDRTB MANAGEMENT

MDR-TB is established worldwide especially in HIV infected persons. Since 1st line drugs fail, 2nd linedrugs (quinolones, aminoglycosides, PAS, ethionamide, thiacetazone, cycloserine & capreomycin are used. It requires longer duration of treatment, has lower cure rates& even higher default rates besides being much more expensive. Also, infection control of MDR-TB is more difficult to implement due to long time taken by conventional drug susceptibility test (DST) results. Thus accurate & rapid diagnosis of drug resistant TB followed by adequate initial chemotherapy is of paramount importance for prevention of MDRTB & its progression to XDR& TDR.

CONVENTIONAL DIAGNOSTIC TECHNIQUES

Sputum smear microscopy for acid-fast bacilli (AFB), which is more specific, inexpensive, relatively rapid to perform & has less inter & intra-reader variability than X-ray is the key diagnostic tool for TB detection in RNTCP.[5] However, a minimum of 10^5 bacilli must be present per milliliter of sputum to be readily seen in a direct smear denoting a low sensitivity of 50-70% which can be enhanced by 10% by the use of fluorescence microscopy.[6] Equipment costs limit the wider use of fluorescent microscopes in poor resource- settings unless several smears are to be examined daily. Use of light-emitting diode (LED) bulbs allow fluorescent microscopes at a much lower cost; field-level evaluation showed promising results and this technology is now being widely scaled up.[7,8] RNTCP in India has established a nationwide laboratory network, encompassing over 13,000 designated sputum Microscopy Centers (DMCs), which are being supervised by Intermediate Reference Laboratories (IRL) at State level, and National Reference Laboratories (NRL) & Central TB division at the National level for quality assured sputum smear examination in

India. The conventional method of culture of MTB using solid medium such as the Lowenstein-Jenson (L-J) medium is highly specific and much more sensitive than smear microscopy but is mainly used for diagnosis of drug resistant TB as it is time consuming (6-8 weeks). Drug susceptibility testing (DST) using the indirect 1% proportion method is the accepted gold standard. This method was developed in the 1960's & is still being used particularly in the developing countries as it is inexpensive & easily accessible.

Commercial automated liquid culture DST methods such as MGIT 960 are highly accurate and have a relatively short turnaround time of 1-2 weeks due to sensitive automation& relatively faster growth of MTB in liquid culture[9]. It has been standardized for all 1st-line & most 2nd line drugs. This is the only rapid culture system currently endorsed by WHO for drug resistance surveillance as it has been demonstrated to be equivalent to the proportion method.[10] The automated systems ,however require the use of expensive equipment& reagents, thus increasing the cost per test.

NEWER RAPID DIAGNOSTICS

Several newer rapid diagnostic tools have been developed for faster and more economical CDST. These are either culture based (phenotypic NRA&MODS) or molecular (genotypic LIPA).

Non-commercial solid culture Nitrate Reductase Assay (NRA) which utilizes the property of MTB to reduce nitrate as an indicator of growth, before colonies can be seen macroscopically, reduces the turnaround time to results compared with conventional methods.[11] The WHO recommends that the NRA be used as a direct test on smear positive sputum specimens or as an indirect test on MTb isolates grown from conventional solid culture as it is 97% sensitive & 99% specific for detection of INH resistance.[12]

Non-commercial liquid culture method e.g. Microscopic Observation Drug Susceptibility (MODS) using 7H9 medium in micro titer wells & inverted microscope for detection of micro colonies, cord formation for early detection growth. It appears to have higher sensitivity, shorter time to culture positivity and is more cost effective than regular L-J medium.[13] INH and RIF can be incorporated in the testing process to enable MDR- TB detection.[14] The in-house MODS &NRA received WHO approval by virtue of similar accuracy to commercial liquid culture systems and could be implemented in high-burden, low-income settings with minimum cost; however, these tests require extensive operator training, standardization and quality assurance before implementation.[12]

The most popular genotypic method is the Line Probe Assay based on in-situ hybridization on nitro cellulose strips of specific genetic targets for resistance genes showing high sensitivity (>95%) and specificity (100%) when culture isolates were used. INNOLipaRifTB ®(Innogenetics, Ghent, Belgium) and Genotype® MTBDR plus kit (Hain Lifescience, Nehren, Germany) are the widely recommended formats. Results are available within 48 hours but there is requirement for expensive equipment, reagent, expertise& bio safety measures.[15] The WHO has endorsed the use of line probe assays, which can detect both M. tuberculosis complex as well as isoniazid and rifampicin resistance on smear-positive sputum or on early positive growth on culture.[16] Line probe assays are being used in conjunction with culture in the Intermediate Reference Laboratories set up by the Revised National TB Control Program (RNTCP) in India.[17]

IS THE GENE-XPERT CARVING A NICHE?

Newer tool under evaluation is the Gene Xpert MTb/Rif Assay (Cepheid, California, USA), a completely closed fully automated system that performs both sample preparation & real time PCR, producing results in less than 2 hours .It is capable of detecting the MTB & rifampicin resistance simultaneously and thus MDR-TB screening.[18] It is fast, accurate, easy& safe to operate with minimal biohazard component, but is expensive. It is designed for direct genotypic DST from unprocessed sputum or sediment from a concentrated specimen. Sample reagent is poured into the sample tube, incubated for 15 minutes, pipetted into the Xpert cartridge &inserted into the Gene Xpert machine for processing. It appears to be most field friendly of all the tests currently available, with very high sensitivity &

specificity and is currently recommended by WHO in settings with high MDR-TB burden.[19] RNTCP has initiated the evaluation of the Cartridge Based Nucleic Acid Amplification test (GeneXpert TB/RIF) in line with the global consultation guidelines to gather evidence for use within the country in various settings.[20]

WORK-FLOW FOR MDRTB DIAGNOSIS IN RNTCP

MDR-TB is a laboratory diagnosis so whenever a MDR-TB suspect is identified by persistence of sputum smear positivity at the end of 5th month of treatment, sputum is collected & transported in cold chain within 72 hours from the DMC to RNTCP accredited CDST usually an IRL which provides culture confirmation of species of MTB & DST for at least isoniazid & rifampicin. Presently,3 technologies are available for the latter viz. the conventional solid egg based Lowenstein-Jensen(LJ) media, the automated liquid culture(MGIT) & Line Probe Assay. The TAT is 84 days, 42 days &72 hours respectively. Wherever available, LPA is preferred diagnostic method followed by liquid CDST& then solid CDST. Confirmed MDR-TB case is then put on MDR regimen after pre treatment evaluation by the DOTS plus committee. Sputum smear microscopy & liquid/solid culture are used to monitor patients throughout treatment as per schedule. Patients on MDR regimen whose 6th month culture result is positive is considered as XDR suspect .Culture isolate is sent by the CDST laboratory to the respective NRL which will perform 2nd line DST for at least Kanamycin, Capreomycin & ofloxacin and inform the results to the referring laboratory as soon as possible.

CONCLUSION

MDR-TB is a public health problem of grave importance that needs to be addressed on a war footing in terms of early detection& appropriate treatment for its prevention and effective control. The role of smear microscopy as the initial test for detection of TB is known since the time of Koch& of the several methods available for diagnosis of drug resistance, molecular techniques when combined with conventional culture methods at the outset have already proved their utility in rapid screening of patients at risk of MDR-TB. Newer technologies are being developed& evaluated for accurate, accessible & affordable diagnosis which if followed by strict patient compliance to proper anti-tubercular treatment can go a long way in curbing the menace of MDR-TB.

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ERRATUM FOR VOLUME : III

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ERRATUM FOR VOLUME : II

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Title: Hand Dimensions as Predictor of Stature – An Anthropometric Study in a Sample of South Indian Population

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The columns of the Table 1 were printed in a very negligible size.

Table 1 : Hand dimensions and height of the total sample (n=180)

Measurements(cms)	Mean ± SD (range)	
	Right	Left
Hand length	18.199± 1.07 (15.70-21.20)	18.22 ± 1.12 (15.30-21.40)
Hand breadth	7.947 ± 6.73 (6.20-10.10)	7.725 ± 0.69 (6.40-9.40)
Palm length	10.442 ± 0.70 (8.80-12.60)	10.468 ± 0.74 (7.40-12.30)
Height	165.827 ± 8.99 (147.00 – 191.00)	