ANTI-TUBERCULOSIS DRUG RESISTANCE SURVEILLANCE

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Resistance to anti-tuberculosis drugs has been documented since the 1940s, when the first medicines for tuberculosis were introduced. Systematic monitoring of development of drug resistance was initiated through a global project in 1994 through a network of quality assured supranational reference laboratories. At present nearly 60% of all countries in the world have implemented surveillance activities that are being disseminated at a global level by the World Health Organization through their Global Anti-tuberculosis Drug Resistance Reports. Systematic surveillance for TB drug resistance monitoring is the best way to document its presence and has been very difficult to establish in most of the high-burden countries as the major obstacle to the expansion of routine surveillance activities has been the lack of laboratory capacity needed to detect resistance. In the past 15 years, special surveys have been the most common approach to investigate the frequency and patterns of drug-resistant tuberculosis. The advent of newer more rapid tools to detect drug resistance and with increased laboratory capacity, routine surveillance linked to patient care, which represents the best approach to monitor drug resistance, now has the possibility of becoming a reality even in resource-limited countries. Several countries across the globe have been reporting drug resistance data derived from their country specific survey methods. India has in the past reported drug resistance from sub-national level surveys and has currently commenced its first national level survey. The survey will provide a statistically representative national estimate of the prevalence of anti-tuberculosis drug resistance among new and previously treated patients in India, and will contribute to a more accurate estimate of anti-tuberculosis drug resistance globally. In addition India is moving towards systematic surveillance of drug resistance as it is already offering drug resistance testing to all treatment experienced patients and is moving towards universal DST by 2019.

The story of anti-tuberculosis chemotherapy is a miniature of the history of anti-infective chemotherapy. In the first half of the twentieth century the problem of tuberculosis appeared insolvable due to the lipid-rich cell wall that was believed to make chemotherapy impossible *(1)*. This gloomy view seemed to be confirmed when the first antibiotics developed, sulfonamides and penicillin, had no useful activity against *Mycobacterium tuberculosis*. With this in mind it is easy to understand the early euphoria surrounding Albert Schatz and Selman Waksman's discovery of streptomycin while working at Rutgers University in New Jersey *(2)* and Harold Lehmann's discovery of para-amino salicylic acid (PAS) shortly afterwards *(3)*.

Drug-resistant TB was recognized shortly after the introduction of effective anti-TB chemotherapy, with the description of streptomycin resistance by Pyle in 1947 *(4)*. In 1948, the British Medical Research Council (MRC) published its ground breaking report of streptomycin therapy for pulmonary TB and noted that mortality was similar in treated and untreated patients *(5)*. Among patients who had been treated with streptomycin, however, most who died had experienced a relapse that was the result of streptomycin-resistant strains. The recognition of this phenomenon led to the principle of multi-agent chemotherapy for TB, which was proved effective in a subsequent trial by the MRC *(6)*. Resistance to anti-TB drugs continued to be recognized as a sporadic clinical problem through the 1960s, 1970s and 1980s, but little attention was paid to the problem by researchers or public health officials. The emergence of multi-drug-resistant TB (MDR-TB) in the United States in the early 1990s led to renewed interest in

this topic *(7)*. During that period, a number of MDR-TB cases, defined as disease caused by strains resistant to at least isoniazid and rifampicin, were identified in epidemics in New York, New Jersey and Florida. The majority of these cases were the result of micro-epidemics with direct transmission among persons in hospital, jails, and homeless shelters, particularly among people with HIV infection *(7-9)*. The mortality in MDR-TB has been reported to be high both in HIV-infected and uninfected individuals *(10-14)*. Aggressive public health interventions at a cost of tens of millions of dollars helped to quickly contain these outbreaks, but not before the loss of many lives *(15)*.

In subsequent years, drug resistant TB, especially MDR-TB, has been recognized as a potentially catastrophic challenge to global public health. Major outbreaks of MDR-TB have been reported in the former Soviet Union, and low levels of MDR-TB in countries with high rates of TB, such as Peru, have resulted in large numbers of patients with disease. As a consequence, drug resistant TB now constitutes a global problem *(16)*.

The circumstances in which drug resistance emerges are well known and have been so since shortly after the first clinical trials became available and their lessons were digested *(17)*. In recent years the molecular basis for the mechanism of action of anti-tuberculosis agents and the way in which the organisms become resistant have begun to be unravelled.

Although management of TB has faced many challenges in the past, today there are two monumental threats to global TB control: the HIV epidemic and the increasing prevalence of drug resistance. HIV infection is contributing to large escalations in the incidence of TB in countries most heavily affected by AIDS, notably sub-Saharan Africa *(18)*. Resistance to anti-TB drugs, a problem recognized in the very early days of the chemotherapeutic era has also emerged as a serious problem. TB drug resistance is characterized by both the types of drugs to which the bacteria lack susceptibility and the manner in which resistance was acquired. Resistance to single agents is the most common type; resistance to multiple agents is less frequent but of greater concern. By convention, "multi-drug resistance" is defined as resistance to at least isoniazid and rifampicin.

An understanding of the molecular basis of drug resistance may contribute to the development of new drugs. *M. tuberculosis* is often acquired early in life with acute infection and with developing immunity, granuloma formation, and calcification. This is followed by a long latent period, which continues until reactivation occurs in a proportion of the individuals. This means that individual

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strains of *M. tuberculosis* have little opportunity to interact and exchange genetic information with other strains compared with, for example, organisms that colonize the nasopharynx or the gastrointestinal tract. In these locations, other bacteria may transmit antibiotic resistance determinants through transmissible genetic elements, transposons, integrons and plasmids, by transduction or transformation. This option is not available for *M. tuberculosis*, so resistance can only occur through chromosomal mutation although rarely movement of mobile genetic elements, such as the insertion sequence IS6110, has been associated with new resistance emerging through the inactivation of critical genes *(19, 20)*.

In any prokaryotic genome mutations are constantly occurring due to base changes caused by exogenous agents, DNA polymerase errors, deletions, insertions and duplications. For prokaryotes there is a constant rate of spontaneous mutation of 0.0033 mutations/DNA replication that is uniform for a diverse spectrum of organisms *(21)*. The mutation rate for individual genes varies significantly between and within genes. The antibiotic resistance genes encoding fundamental replication functions of the organism such as *rpoB* and *gyrA* are typically highly conserved *(22, 23)*.

The genetic basis of resistance for some anti-tuberculosis agents is not fully known. For example, streptomycin resistance emerges through mutations in *rrs* and *rpsL* that produce an alteration in the streptomycin binding site, but these changes are identified in just over one-half of the strains studied to date *(24, 25)*. Thus there is a considerable amount of research into the mechanisms of resistance that is still required. It should be noted that in many cases mutations found in association with drug resistant organisms may cause different levels of resistance and also may not be directly related to the mechanism of resistance. Isoniazid-resistance is a case in point. Modification of KatG, partial or total deletions, point mutations, or insertions, leads to the abolition or diminution of catalase activity and

Table 1: Average proportions of cases of tuberculosis, new or previously treated that are multi-drug resistant, in regions of the World Health Organization (WHO) and the world, 1994-2000.

WHO region	New cases	Previously treated cases
African region	1.9	9.4
Region of the Americas	2.1	11.5
Eastern Mediterranean region	3.4	20.6
European region	12.1	36.5
South East Asia region	2.1	17.2
Western Pacific region	4.9	23.2
World	3.4	19.8

Source: Bull World Health Organ 2012

Table 2: Multi-drug resistant tuberculosis (MDR-TB) rate in new and previously treated cases. (India-sub national surveys)

Survey	New Cases	Previously treated cases
Gujarat, 2007–2008 (population – 56 million)	2.4%	17.4%
Maharashtra, 2008 (population – 108 million)	2.7%	14.0%
Andhra Pradesh, 2009 (population – 86 million)	1.8%	11.8%
Tamil Nadu, 2011 (population – 70 million)	1.8 %	11.2%
RNTCP - India routine surveillance data, 2007-13	NA	16%

Source: Protocol for the first Nationwide Anti-Tuberculosis Drug resistance survey, India, 2014-2015

confers high-level resistance to isoniazid *(26, 27)*. Catalase activity is essential in activating isoniazid to the active hydrazine derivative. A deficiency in enzyme activity produces high-level resistance and is found in more than 80% of isoniazid-resistant strains *(28)*. Alternatively, low-level resistance can be caused by point mutations in the regulatory region of *inhA* operon, resulting in over expression of *inhA*. Strains with this mutation have normal mycolic acid synthesis but low-level resistance to isoniazid. Point mutations in the regulatory region of *ahpC* have also been demonstrated; these are a compensation for the effects of absent or reduced catalase (KatG) function and do not directly result in resistance *(29, 30)*. Most pyrazinamide-resistant organisms have mutations in the pyrazinamidase gene, although the gene may also be inactivated through the insertion of IS6110 *(31)*. Pyrazinamide is essential in producing the active pyrazinoic acid derivative, and mutants are unable to produce an active drug. In addition to this, some resistant strains have no defined mutation *(32)*. The rate at which resistance emerges differs for all of the anti-tuberculosis agents, being highest for ethambutol and lowest for rifampicin and quinolones. The risks of mutation for most of the antibiotics used in tuberculosis treatment have been defined previously *(33)*; for rifampicin, isoniazid, streptomycin, and ethambutol, they are 3.32×10^{-9}, 2.56×10^{-8}, 2.29×10^{-8}, and 1.0×10^{-7} mutations per bacterium per cell division, respectively. The mutation rate, rather than the mutation frequency, is the most reliable measure, as it records the risk of mutation per cell division rather than the proportion of mutant cells.

It has been assumed that the risk that an organism will develop resistance to two agents is the product of the risks of developing resistance to each separately. For example the resistance risk for a combination of rifampicin, streptomycin, and isoniazid is10–25/bacterium/generation.

Global anti-Tuberculosis Drug Resistance Surveillance Project

In 1993, Tuberculosis was declared as a global emergency following which, in 1994, the Global Project on Anti-Tuberculosis Drug Resistance Surveillance was initiated by the World Health Organzation (WHO) and International Union against Tuberculosis and Lung Diseases, aiming to measure the magnitude of drug resistant tuberculosis and to monitor trends *(34)*. Since 1994, five global reports on anti-tuberculosis drug resistance surveillance have been published *(35-39)*. Drug resistance data have been systematically collected and analysed from 114 countries (59% of all countries of the world).

Worldwide, approximately 5% of new cases and 20% of previously treated cases had multi-drug resistant TB (MDR-TB), (Table 1). Extensively drug resistant TB (XDR-TB) has been reported by 92 countries, and the average proportion of MDR-TB cases with XDR-TB is 9%.

Since the beginning of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance, two main mechanisms to measure drug resistance have been used: the organization of special surveys (surveys are defined as discrete studies measuring drug resistance among a specially-designed sample of tuberculosis cases representative of an entire population of TB cases) on selected samples of patients, and the establishment of a surveillance system based on routine drug susceptibility testing of all patients.

In the past 15 years, surveys and surveillance have been largely relying on culture and drug susceptibility testing methods based on solid media, which are associated with a very long turn-around times for results (at least 3–4 months) and enormous workload for laboratory personnel. We are

now in a new era for tuberculosis and MDR-TB diagnosis resulting from the advent of technological advances that make it possible to detect tuberculosis and rifampicin resistance much more rapidly.

Types of TB drug resistance surveys:

1. Surveillance system based on routine drug susceptibility testing

A surveillance system based on routine DST of all TB cases is able to provide continuous information on drug resistance patterns among patient groups, and is therefore able to accurately detect trends, as well as localized outbreaks.

2. Periodic surveys

In resource constrained settings where capacity is currently not available for routine DST of all TB cases, surveys can be conducted to measure drug resistance among a sample of patients' representative of the geographically defined population under study. When properly constructed and periodically conducted, such surveys provide a sound estimation of the resistance profile of all TB cases in the population under study and can detect general trends over time.

3. Sentinel surveillance systems

Some countries with well-established laboratory networks have opted for a sentinel system for surveillance. This type of system continuously reports DST results of all TB cases from a selection of laboratory or hospital sites, and therefore can be useful in documenting trends and detecting outbreaks or localized epidemics of drug resistance. For countries where resources, the health-care system structure, or geographical features preclude routine DST of all patients or surveys of sampled patients, the establishment of a sentinel surveillance system may be an option. A sentinel system could be a useful interim approach for countries intending to expand routine DST to all retreatment cases while moving towards this goal.

4. Regimen surveys

"Regimen surveys" measure first-line and /or second-line drug resistance among a group of selected patients that cannot be considered representative of a patient population. These surveys can help determine the predominant patterns of drug resistance, and can be useful in providing guidance on appropriate regimens for MDR-TB treatment for particular patient groups. These include return cases after treatment failure, chronic cases and symptomatic contacts of MDR-TB cases. Regimen surveys should be conducted in the process of developing MDR-TB treatment programmes or within selected centres or diagnostic units that regularly address high-risk cases.

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Indian surveys

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Anti-tuberculosis drug resistance among new and previously untreated TB cases, a proxy indicator for primary or initial drug resistance, suggests tuberculosis transmission. Anti-tuberculosis drug resistance among previously treated TB cases, a proxy indicator for acquired drug resistance, suggests failure of effective management in the prior TB episode.

Although the country had conducted several district level surveys in the past, it has also conducted four state level surveys using the WHO guidelines for Drug Resistance Survey, beginning in 2007 (Table 2). However acknowledging that India needs to move towards systematic surveillance, and as part of the scaling up of DR TB services all treatment experienced patients are being tested for drug resistance. India is also planning to move towards universal DST for all TB cases by 2019 as articulated in its National Strategic Plan and the Revised National Laboratory Scale up Plan 2015–2019 in line with post 2015 strategy.

In the interim, in order to plan, strategize and refine the quality of services for DR TB, data on the rates of drug resistance at a National level has been recognized as vital and towards this goal, India has initiated a National TB Drug Resistance Survey. This will be the first such survey that will be conducted in India as there has been no attempt previously as this was an enormous task and fraught with many challenges like the population to be covered, sampling strategy to include all geographical regions, number of patients to be screened, number of drug susceptibility testing to be undertaken to name a few. More than 5,000

patients from 120 clusters representing the country are expected to be enrolled for the survey. The samples collected would be subjected to a 13 drug DST (five first-line drugs and eight second-line drugs) using liquid culture systems. The survey will provide a statistically representative national estimate of the prevalence of anti-tuberculosis drug resistance among new and previously treated patients in India, and will contribute to a more accurate estimate of anti-tuberculosis drug resistance globally.

At a global level, India is the first among both the 22 high-burden TB and first among the 27 high MDR TB burden countries and this survey is considered ground breaking as it will provide a unique data set for both national and global level information on drug resistant TB and management.

Dr Kuldeep Singh Sachdeva is an expert in tuberculosis and chest diseases and health management with more than 30 years of experience. He is currently serving as Additional Deputy Director General, Central TB Division, Ministry of Health and Family Welfare, Government of India. He has extensive experience in programmatic management of drug resistant tuberculosis and is overseeing the implementation of largest ever and nationally representative Drug Resistance Survey in India. He is a core group member of WHOs Global Drug Resistance Initiative (GDI) and also Vice Chair of the Regional Green Light Committee of WHO South East Region.

Dr S Anand, a graduate in Microbiology, obtained his Master's degree in Applied Microbiology with a University rank. He also has a Master's in Environment and Ecology, and M Phil as well as PhD in Biotechnology. He is currently working as a Consultant Microbiologist at the Central TB Division, Ministry of Health and Family Welfare, Government of India. He has served as Unit Head of the National Reference Laboratory at the National TB Institute, Bangalore. He has industrial R&D experience and has also spent over a decade in teaching undergraduate and post graduate students of Microbiology and Biotechnology.

Dr Ranjani Ramachandran is an expert in the field of TB bacteriology with a post-graduate in medical microbiology from Madras Medical College. She also has post-graduate degree in Internal Medicine from National Board of Examinations. She has pursued her doctoral research in the field of TB-HIV opportunistic infections. She started her career as a clinical scientist working in the field of TB research including randomized clinical trials, field surveys in TB-HIV and then moved to laboratory medicine, TB drug resistance surveys and research in evaluation of new diagnostic tools. She has more than two decades of experience of working in the National Institute of Research in Tuberculosis (ICMR) Chennai and then moved to the World Health Organization as Medical Officer TB labs in 2009 and is at present the Technical Officer (Labs) at the WHO India country Office for India since 2012.

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