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Ministry of Health & Family Welfare
Government of India

World Health Organization
India

Report of the
FIRST NATIONAL ANTI-TUBERCULOSIS DRUG RESISTANCE SURVEY
INDIA

2014–16

NATIONAL HEALTH MISSION

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Contents

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Abbreviations

CBNAAT	cartridge-based nucleic acid amplification test
CIF	clinical information form
DEFF	design effect
DMC	designated microscopy centre
DRS	drug resistance survey
DR-TB	drug-resistant TB
IQC	internal quality control
M. tuberculosis	*Mycobacterium tuberculosis*
MDR-TB	multidrug-resistant TB
MGIT	mycobacteria growth indicator tube
NDRS	National Anti-tuberculosis Drug Resistance Survey
NIRT	National Institute for Research in Tuberculosis
NTI	National Tuberculosis Institute
PLHIV	people living with human immunodeficiency virus
PMDT	programmatic management of drug-resistant TB
QA	quality assurance
RNTCP	Revised National Tuberculosis Control Programme
RR-TB	rifampicin-resistant TB
SRL	Supranational Reference Laboratory
STLS	senior TB laboratory supervisor
STS	senior treatment supervisor
TB	tuberculosis
TU	TB unit
WHO	World Health Organization
XDR-TB	extensively drug-resistant TB

Executive summary

India has more new tuberculosis (TB) patients annually than any other country globally, contributing to 27% of the world's TB burden. About 2.79 million TB patients are estimated to be added annually. The Revised National Tuberculosis Control Programme (RNTCP) notified around 1.94 million TB patients in 2016 *(1)*.

As per the Global TB Report 2017 *(1)*, worldwide approximately 4.1% of new TB patients and 19% of previously treated TB patients have multidrug resistant-TB (MDR-TB), i.e. TB resistant to at least two of the first-line drugs – isoniazid and rifampicin. Extensively drug-resistant TB (XDR-TB), defined as MDR-TB with additional resistance to at least one fluoroquinolone and one second line injectable drug, has been reported by 123 countries. The proportion of XDR-TB among MDR-TB patients is 6.2% worldwide. The estimated number of MDR/rifampicin resistant (RR)-TB in India is 147 000, accounting for one fourth of the global burden of MDR/RR-TB *(1).*

India initiated the programmatic management of drug resistant TB (PMDT) in 2007 to address the emerging problem of drug resistant-TB (DR-TB), and the national PMDT scale-up was achieved by March 2013. The treatment success rate among MDR-TB patients in India is consistently about 46% and the death rate is around 20%, as against the global level of treatment success rate of 52% and death rate of 17%. High rates of treatment failure and deaths are associated with fluoroquinolone resistance in the Indian cohort of MDR-TB patients *(2).*

India has sub-national data from state level anti-TB drug resistant surveys conducted in the past *(3)*; However the epidemiology of DR-TB in India has never been studied nationally. Knowing the epidemiology of DR-TB is essential to guide development of evidence-based strategies to combat DR-TB in India. In view of the above, the Government of India decided to conduct a National Anti-TB Drug Resistance Survey (NDRS) to know the prevalence of drug resistance among TB patients with particular focus on MDR-TB among both new and previously treated TB patients.

This is the largest ever NDRS conducted by any country in the world and the first ever survey having drug susceptibility testing (DST) for 13 anti-TB drugs using the automated liquid culture system, mycobacteria growth indicator tube (MGIT) 960®.

A total of 5280 sputum smear-positive pulmonary TB patients (3240 new and 2040 previously treated) diagnosed at the designated microscopy centres (DMCs) of RNTCP were enrolled in the survey.

The results of the survey showed that:

- MDR-TB is 6.19% (CI 5.54–6.90%) among all TB patients with 2.84% (CI 2.27–3.50%) among new and 11.60% (CI 10.21–13.15%) among previously treated TB patients.

- Among MDR-TB patients, additional resistance to any fluoroquinolones was 21.82% (17.33–26.87%), and 3.58% (1.8–6.32%) to any second-line injectable drugs.

- Among MDR-TB patients, additional resistance to at least one drug from each of the two classes, i.e. fluoroquinolone and second-line injectable drugs (XDR-TB) was 1.3% (0.36–3.30%).

- Any first- or second line drug resistance among all TB patients is 28.0% (CI 26.77–29.29%) with 22.54% (CI 21.10–24.10%) among new and 36.82% (CI 34.64–39.04%) among previously treated TB patients.

- Any isoniazid resistance is 11.06% (CI 9.97–12.22%) and 25.09% (CI 23.1–-27.11%) among new and previously treated TB patients, respectively.

- Any pyrazinamide resistance is 6.95% (CI 6.07–7.91%) and 8.77% (7.53–10.13%) among new and previously treated TB patients, respectively.

MDR-TB rates at the national level are still within the range of previous state-level surveys conducted in India. However, more than a quarter of TB patients in India have drug resistance to one or the other anti-TB drug. Fluoroquinolone resistance among MDR-TB patients is high and is similar to resistance rates reported by the RNTCP. The survey results clearly indicate that drug resistance is present in all settings, and the wide range of resistance patterns from any isoniazid resistance to XDR-TB needs to be addressed with strengthening of drug resistance surveillance, universal DST and appropriate DST guided treatment strategies.

1. Introduction

1.1 Country profile

With a population of 1.32 billion, India has the highest burden of tuberculosis (TB) and drug-resistant TB (DR-TB) in the world. The Global TB Report 2017 published by World Health Organization (WHO) estimates that India contributes 27% (2.79 million) and 25% (147 000) of the global burden of TB and multi-drug resistant TB (MDR-TB), respectively *(1)*. The Revised National Tuberculosis Control Programme (RNTCP) has notified 1.94 million patients in 2016 *(1)*. India has been locating and treating MDR-TB patients since 2007 and achieved complete geographical coverage of programmatic management of drug-resistant TB (PMDT) services in 2013.

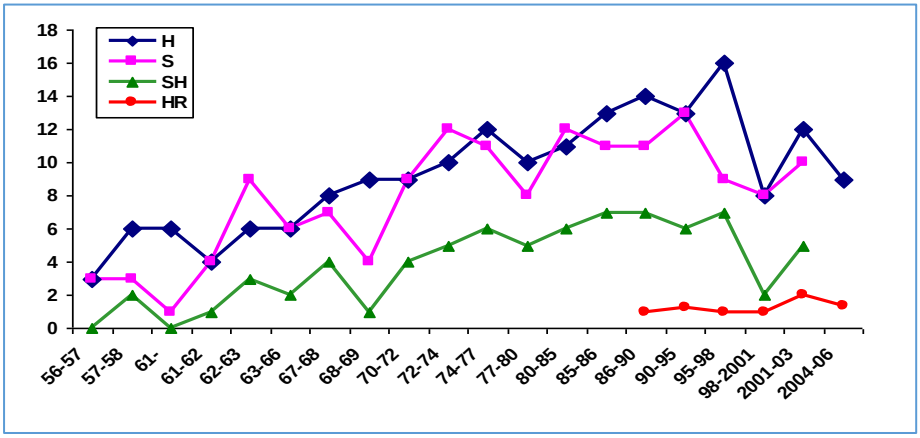
Up till 2016, 1 413 331 TB patients have been tested for drug resistance and 139 369 MDR/rifampicin-resistant (RR)-TB cases had been detected in India. Among them, 126 136 MDR/RR-TB and 6377 extensively drug-resistant (XDR)-TB patients have been put on treatment.

In 2016 alone, 580 438 TB patients have been tested, 37 358 MDR/RR-TB have been diagnosed and 32 914 MDR/RR-TB and 2475 XDR-TB patients have been put on treatment in India *(1)*. These numbers would increase in years to come as India has initiated implementation of the policy of universal drug-susceptibility testing (DST) in phases since mid-2017.

1.2 Statement of the problem

There have been a number of reports in the past on drug resistance in India A majority of them used non-standardized methodologies, biased samples, and were usually conducted at tertiary-level care facilities. However, there is data generated through clinical trials conducted in Tuberculosis Research Centre (now National Institute for Research in Tuberculosis [NIRT]), Chennai where rates of RR-TB and MDR-TB are available from the early 1980s from continuous surveillance of rifampicin resistance. This has been slowly increasing from 0% to 2% among new TB patients till date (Fig. 1). This graph shows the rates of drug resistance including MDR-TB. MDR-TB has been increasing from less than 1% in the early nineties to 2.0% in 2006. Since then, the rates of MDR-TB have been almost static. (Data from 2007 onwards is not shown in Fig. 1).

Fig. 1: Trend of first-line anti-TB drug resistance from historical surveys



H – Isoniazid; S- Streptomycin; SH – Streptomycin + Isoniazid; HR – Isoniazid + Rifampicin

In addition, valid state-level information about the extent of drug resistance is also available (Table 1). These drug resistance surveys (DRSs) were conducted in accordance with standard international protocol. Specimens were collected from a population-based sample of sputum smear-positive patients diagnosed at RNTCP designated microscopy centres (DMCs). Most of the state level surveys conducted in India have also reported patients of XDR-TB. State representative DRSs in Andhra Pradesh and Gujarat *(3)* showed 4–6% XDR-TB cases among MDR-TB isolates, with a high prevalence of fluoroquinolone resistance ranging from 21% to 25%.

Table 1: Rates of MDR-TB – state level surveys

Surveys, year and population	New patients	Previously treated patients
Tamil Nadu State, 1997–1998 (60 million)	3.4%	25.0%
Gujarat State, 2007–2008 (56 million) *(3)*	2.4%	17.4%
Maharashtra State, 2008 (108 million)	2.7%	14.0%
Undivided Andhra Pradesh State, 2009 (86 million)	1.8%	11.8%
Tamil Nadu State, 2011 (77 million) (unpublished)	1.8%	13.2%

Current knowledge on MDR-TB in India

During the initial phase of PMDT implementation, RNTCP offered DST to TB patients who were at the highest risk for MDR-TB, such as treatment failures and contacts of MDR-TB. As the diagnostic capacity increased, the offer of DST was expanded to cover TB patients with moderate/lower risk of MDR-TB. RNTCP continued to expand its DST coverage over the years to test most TB patients with upfront cartridge-based nucleic acid amplification test (CBNAAT) (Xpert MTB/RIF) followed by baseline second line DST, and has embarked upon phased implementation of universal DST since mid-2017.

Apart from DST, CBNAAT is also offered for diagnosis of TB in key populations like people living with human immunodeficiency virus (PLHIV), children, those with extra-pulmonary signs/symptoms and smear negative patients with chest X-ray suggestive of TB, for microbiological confirmation of TB.

The rates of MDR/RR-TB observed over the past decade in the programme as the DST offer expanded from highest risk cases to those at least risk of MDR-TB are shown in Table 2.

Table 2: MDR/RR-TB reported by RNTCP (PMDT services)

Rates of MDR/RR-TB reported under RNTCP – India's routine surveillance data	Among new TB patients	Among previously treated patients
2007–2012 (n = 144 326)	NA	19%
2013–2015 (n = 779 300)	5%	11%
2016 (n = 580 438)	4%	9%

Rationale

India is one of the highest burden countries for TB and MDR-TB. However, the epidemiology of DR-TB in India has never been studied nationally. This is essential to measure the DR-TB burden and to guide development of evidence-based strategies to combat DR-TB in India. In view of the above, the Government of India decided to conduct a National Anti-Tuberculosis Drug Resistance Survey (NDRS) to inform on the prevalence of drug resistance among TB patients, with particular focus on MDR-TB among both new and previously treated TB patients. Accordingly, the NDRS protocol was developed by National Tuberculosis Institute (NTI) and WHO for the RNTCP.

1.3 Aim and objectives

Aim

The aim of the survey was to find out the proportion of MDR-TB patients among new and previously treated TB patients diagnosed at RNTCP DMCs.

General objective

The general objective was to understand the epidemiology of DR-TB in the country to guide national policy and strategies to prevent further emergence and to control the problem.

Specific objectives

Primary objectives

- To determine the prevalence of MDR-TB among newly diagnosed sputum smear-positive TB patients;
- To determine the prevalence of MDR-TB among previously treated sputum smear-positive TB patients.

Secondary objectives

- To determine the prevalence of second line anti-TB drug resistance in *Mycobacterium tuberculosis (M. tuberculosis)* strains with confirmed resistance to isoniazid and rifampicin;
- To describe drug resistance patterns of *M. tuberculosis* strains collected from newly diagnosed sputum smear-positive TB patients;
- To describe drug resistance patterns of *M. tuberculosis* strains collected from previously treated sputum smear-positive TB patients.

2. Materials and methods

2.1 Study design

This was a cross-sectional study among all new smear-positive TB patients and all previously treated TB patients diagnosed in the RNTCP DMCs in India from August 2014 to July 2015.

2.2 Sample size determination

In 2012, RNTCP notified 1 467 585 total TB patients, of which 629 589 were new sputum smear-positive TB patients, 317 616 were smear-negative TB patients, 234 029 were new extra-pulmonary TB patients and 284 212 were previously treated TB patients of which 106 463 were relapse cases, 16 400 were treatment failures, 64 782 were TB patients who were lost to follow up and 96 567 were other retreatment TB patients. This data was used for arriving at the sample size for the survey.

The assumptions used to arrive at the sample size for new and previously treated patients to be enrolled in the survey are depicted in Table 3.

Table 3: Assumptions made to calculate the sample size for new and previously treated patients to be enrolled in the survey

Type of patient	New	Previously treated
Initial estimate of prevalence	0.03	0.15
Relative precision	34%	40%
Anticipated non-participation/loss rate	20%	
Design effect (due to cluster sampling)	2.5	

Previous state level studies have shown MDR-TB proportion among new and previously treated TB patients as 3% and 15%, respectively. Using these as initial prevalence, the sample size was obtained with a 34% relative precision in new TB patients and 40% relative precision in previously treated TB patients. Based on the analysis of state level DRSs (DMCs as sampling units) the design effect (DEFF) was reasonably fixed at 2.5, also accounting for high levels of heterogeneity among the TB units (TUs). An anticipated non-participation or loss rate was assumed to be 20%.

Thus, the sample size for new TB patients was derived as 3223 with a relative precision of 34%, i.e. (3% ± 1%) and a DEFF of 2.5 (with 20% loss included). For previously treated TB

patients with the proportion of MDR-TB at 15%, 40% relative precision, i.e. 17% ± 7% (10–24% in previously treated overall) and DEFF of 2.5, the sample size was derived as 1991.

More number of TUs would have be better for analytical purposes but may not have been practical. The fewer the number of respondents in each cluster, the lower the clustering effect, which would increase sample variance, thus effectively reducing the estimating power. Balancing both these factors, 120 TUs were chosen to account for analytic precision and logistic implementation. As the programme was under the process of further decentralization of TUs during the survey period, all the new TUs that fell under the original 120 TU clusters identified at baseline continued to contribute to the study.

Sampling strategy

The sampling strategy in this survey was a single-stage, weighted cluster sampling method, in which clusters were selected with probability proportional to size, with each cluster contributing a fixed number of new and previously treated TB patients. The cluster sampling methodology is appropriate for India because of the logistical challenges and laboratory capacity needed to cover all of the approximately over 14 000 DMCs in the country and around 1.5 million TB patients notified each year.

The primary sampling unit in the survey was the RNTCP-defined TU and survey participants were recruited from all DMCs in the selected TUs. Each TU represented a cluster. The proportion of urban TUs in the selection was 24%.

The following methodology was used to select the TU clusters. A list of all TUs in the country with the respective numbers of new sputum smear-positive TB patients, and previously treated TB patients registered in the first quarter (January–March) of 2012 was compiled. This data was annualized to calculate the estimated cumulative number of total TB patients. TUs were selected using a weighted-cluster sample technique based on new sputum smear-positive TB patients. Once TUs were selected, all DMCs in the selected TU would contribute until the enrolment of cumulative number of expected patients reached the required sample size. A total of 44 consecutive TB patients diagnosed at the DMCs from each selected TU were recruited to include 27 new TB patients and 17 previously treated TB patients per TU. Based on this, the total new and previously treated TB patients were rounded off to 3280 and 2040.

Those patients who met the inclusion criteria but could not be included in the survey for various reasons were replaced by consecutive patients diagnosed in the DMCs of the same TU according to the sampling procedure described.

Inclusion criteria

a) Newly diagnosed sputum smear-positive pulmonary TB patients with no history of prior treatment for TB, or a history of anti-TB treatment for less than 30 days. Enrolled patients should not have been initiated in a course of anti-TB treatment.
b) Diagnosed patients with sputum smear-positive pulmonary TB with a history of previous TB episode with more than 30 days of anti-TB treatment. This may include relapses, treatment after default, treatment after failure, or other patients who have claimed to have anti-TB treatment for more than 30 days. Enrolled patients should not have been initiated in a course of anti-TB treatment for the current episode.

Exclusion criteria

a) Patients with sputum smear-negative pulmonary TB
b) Patients with exclusively extra pulmonary TB
c) Patients diagnosed at a correctional facility (i.e., jails, prisons, asylums)
d) Persons unwilling or unable to give informed consent.

There were no age restrictions, provided the patient fulfilled the above criteria.

2.3 Definitions

Anti-TB drug resistance among new TB patients: Newly diagnosed sputum smear-positive pulmonary TB patients without a history of prior treatment for TB, or a history of treatment for less than 30 days. Enrolled patients should not have been initiated in a course of anti-TB treatment for the current episode.

Anti-TB drug resistance among previously treated TB patients: Diagnosed patients with sputum smear-positive pulmonary TB with a history of prior anti-TB therapy. This may include relapse, treatment after default and treatment after failure, or other patients who have claimed to have anti-TB treatment for greater than 30 days. Enrolled patients should not have been initiated in a course of anti-TB treatment for the current episode.

Multidrug resistant TB (MDR-TB): Patients with sputum smear-positive pulmonary TB with at least one *M. Tuberculosis* isolate with demonstrated resistance to at least isoniazid and rifampicin (with or without other first-line anti-TB drugs).

Extensively drug resistant TB (XDR-TB): Patients with sputum smear-positive pulmonary TB with at least one *M. Tuberculosis* isolate with demonstrated resistance to isoniazid, rifampicin, at least one fluoroquinolone (ofloxacin, levofloxacin or moxifloxacin), and at least one injectable second line drug (amikacin, capreomycin or kanamycin).

2.4 Training and data collection

The data collection formats included the clinical information form (CIF) and the diary of senior TB laboratory supervisors (STLS) for referral. A detailed CIF was designed for collecting data on socio-demography, occupation, income, symptoms, duration of illness, history of previous treatment, source of treatment, etc. The CIF was administered by the medical officer at the TU level along with which an informed consent for participation in the survey was also obtained from eligible patients.

Bar codes were applied to specimens, sputum containers, CIF and NDRS registers for automated aligning of all survey related data collection of individual patients from all the sites of survey.

A training video in English and local vernacular languages was developed for assisting the state level trainers trained at a series of national training of trainers to provide cascade training in the participating states, which gave a detailed account of all the survey procedures for data collection. In addition, dedicated in-house software was developed by NTI for real time data entry and validation.

2.5 Field activities

Consecutive sputum smear-positive TB patients diagnosed at each DMC of the selected TUs were recruited according to the sampling methodology based on categorization (new or previously treated). The senior treatment supervisor (STS) or STLS coordinated among the DMCs of the respective TUs to ensure that patients were enrolled in chronological order for the study. On identification of an eligible patient, he/she was given detailed information about the survey and informed consent was obtained from each patient. Two additional specimens were collected from the patient in falcon tubes (specimen C and D) and these were transported in a bio-safe container under cool chain to NTI along with CIF. CIF was filled in by the medical officer of the DMC and cross-checked by the STS or STLS to reconfirm the categorization. CIFs of 10% of enrolled patients were re-validated by district-level staff and another 10% by the RNTCP–WHO consultant as part of the data quality assurance procedure. Recruitment continued until the numbers of patients in each category were completed. Provider-initiated HIV counselling and testing was offered to all patients as per RNTCP guidelines.

The target transit time for receiving the specimens at NTI was fixed not to exceed 72 h; however, there was no rejection clause if received later than the target time. Attention was paid to transport logistics in order to minimize transportation time, prevent leakage and specimen contamination. Specimens were packed and transported as per RNTCP guidelines. Only fresh sputum specimens were transported and processed.

For patients eligible to screen for MDR-TB, two more specimens (E and F) were also collected for rapid molecular drug resistance testing as per the PMDT guidelines for routine care and MDR/RR-TB patients detected were initiated on an appropriate DR-TB regimen. DST results of the specimens processed under the NDRS were communicated by NTI to the respective district and DR-TB centre for appropriate treatment initiation or modification.

2.6 Laboratory procedures

All specimens were handled in a negative-pressure environment as per the international standards for biosafety and infection control for *M. tuberculosis*. Two sputum specimens from each study patient were decontaminated using N-acetyl-L-cysteine–sodium citrate–sodium-hydroxide (NALC-NaOH) procedure. Microscopy using auramine-O-phenol of the concentrated deposit smear was performed and inoculated onto 7 ml MGIT tubes as per the MGIT 960 System Manual *(4)* and RNTCP Laboratory manual *(5)*. Back up cultures for both specimens were maintained on LJ medium. One specimen was inoculated per tube. Tubes identified as positive by the MGIT system were further identified as M. tuberculosis-complex by using immune-chromatographic tests. DST was performed on only one positive-culture after identification as *M. tuberculosis* using the modified proportion sensitivity method for liquid culture system taking standard critical concentrations for isoniazid (0.1µg), rifampicin (1.0µg), streptomycin (1.0µg), ethambutol (5.0µg) and pyrazinamide (100µg) (as per the MGIT manual). For second line drugs, the drugs and concentrations as per the standard critical concentrations, i.e. kanamycin (2.5µg), amikacin (1.0µg), capreomycin (2.5µg), ofloxacin (2.0µg), levofloxacin (1.5µg), moxifloxacin (0.5µg), PAS (2.0µg) and ethionamide (5.0µg) were used *(6)*.

2.7 Quality assurance

Laboratory quality assurance (QA) (internal and external)

All laboratory procedures adhered to the internal quality control (IQC) procedures as per RNTCP laboratory manual, and in accordance with international standards times *(7)*. All data relating to the survey and records pertaining to IQC were maintained in separate registers – primary culture, identification and drug susceptibility testing, and IQC.

NTI participates in the regular annual proficiency panel exercise conducted by the Antwerp WHO Coordinating Supranational Reference Laboratory (SRL). In addition, 10% of all isolates were retested at NIRT (formerly TRC), also a WHO coordinating SRL for recording reproducibility. The results of the annual proficiency testing with Antwerp were 100% for all tested first- and second line drugs. The agreement for the drugs tested in the DRS as part of reproducibility was also complete.

2.8 Data management

NDRS – Laboratory Information Management System (NDRS-LIMS)

With the historical experience with the manual systems used in past subnational DRS surveys in India, NDRS was expected to offer several challenges in terms of logistics, training, implementation, data management and analysis. Further, the task of monitoring a survey of this magnitude for needful interventions and course corrections from NTI and keeping the central, state and WHO monitoring committees abreast with the progress on the field with periodic reports meant that the data collection and its reflection onto reports had to be as real time as possible.

Hence, a customized web-based application was designed, developed and hosted at NTI to cater to both the data collection and real time interaction with the 120 TUs for entering the external quality assessment (EQA) results and also to facilitate the survey monitoring by Central TB Division (CTD) and WHO. A state level survey monitoring committee was established in every state and was also provided individual login to access relevant information for real time monitoring of the survey. Apart from this, a customised survey specific laboratory information management system was also developed and hosted to facilitate a work flow integrated data capture mechanism. This ensured that real time reporting was available for necessary interventions by the data monitoring committee at NTI and in the states. Use of innovative tools like barcodes and optical mark recognition (OMR) sheets for result capture ensured an error-free and efficient data management process.

The applications were developed using open source software MySQL database and PHP programming language. The hosting, periodic backup, data security measures commensurate with the standards were deployed.

Customised unique specimen enrolment registers for each of the 120 TUs were designed with unique barcodes for 5280 patients. The date and time of specimen collection were recorded on these stickers and were affixed to the falcon specimen tubes before dispatch to NTI. 10 560 sputum smear examinations, culture examinations by both liquid and solid media and DSTs for all positive cultures had to be undertaken. In addition, Xpert MTB/RIF (GeneXpert) testing for paediatric patients was undertaken. It was essential to design a data management solution that was not only efficient, but also assisted the microbiologist to track the survey progress in terms of specimens received, specimens whose result was declared and specimens under process. Hence, a robust laboratory information management system was designed to facilitate the work process flow. All the stages of laboratory activities right from the specimen registration, specimen result declaration by lab technician and result verification by the microbiologist were captured in real time by a LAN based application. This brought in a decentralised yet effective mechanism of data

collection from the respective laboratory work benches, giving the supervisors an overview of the operations in real time. Also, pre-printed lab stationary was deployed. Only specimen and test specific result sheets were dynamically generated at the time of specimen registration with the unique barcodes printed. Thus, human errors in declaration of results to the wrong specimens were avoided as the process started by scanning of the barcodes. Also, the results were declared on optical mark recognition (OMR) sheets which were subsequently scanned and re-checked for any mismatch. These processes ensured a high degree of quality and accuracy in declaration of the laboratory results.

Multilingual video e-tutors on the standard operating procedures were prepared and used for training and as ready reference for all the survey personnel using the web portal.

Data analysis

The data was analysed.0 and Stata ver 12 statistical packages.

To ensure accuracy, double-data entry using SPSS ver 17was employed. Periodic data verification and validation exercises was conducted to ensure data accuracy

To calculate the prevalence of anti-tuberculosis drug resistance, the denominator was taken to be the number of patients with valid drug susceptibility results. However, it is also important to report the number of results missing as a result, for example, of contamination, or negative cultures.

A table comparing the number of patients enrolled from each diagnostic centre with the expected number of expected based on the sampling method was prepared. These were used to monitor enrolment and track specimens.

A table describing the proportion of patients with resistance to individual TB drugs, and to different combinations of TB drugs, among new and previously treated patients was prepared.

Proportion in age, sex, HIV status, type of re-treatment, source of previous treatment was stratified by categories of resistance.

Data was stored in the dedicated system with password protection and only accessible to authorized personnel. Backup data was also stored in a separate site and server for use in case of any fault in the primary PC.

Ethical issues and approval

Approval of the Institutional Ethics Committee of NTI was obtained. Informed consent was obtained from all patients enrolled for the study. The DST results of all patients were

communicated to the respective health facilities for standard of TB care as per national guidelines.

2.9 Limitations

The study did not include patients managed in the private sector who do not come in contact with the public health sector during the course of their disease, as getting an appropriate specimen from such patients was beyond the scope of the study.

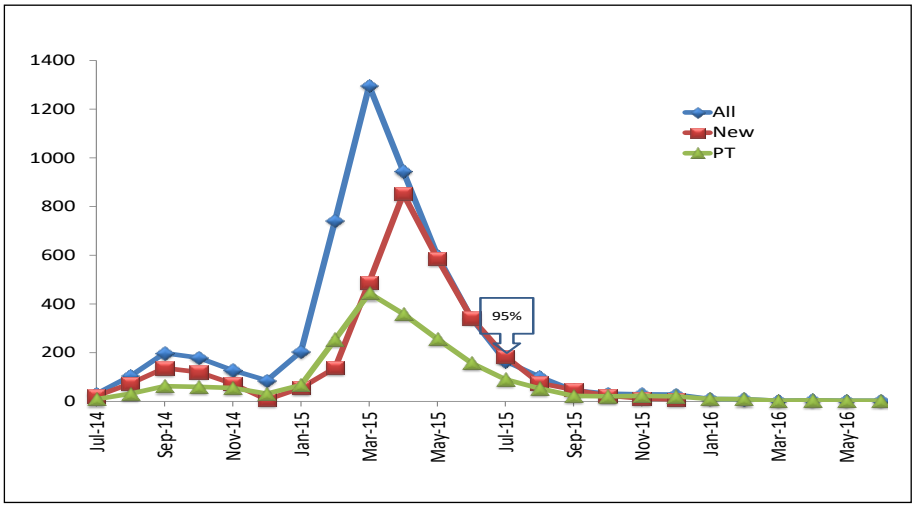
The survey was powered only for national prevalence of MDR-TB and not for state level prevalence.

3 Results, discussion and conclusion

3.1 Survey quality

The survey commenced in July 2014 and 95% of the enrolments were completed by July 2015. However, the rest of the 5% took longer and were completed only by May 2016. This longer intake period was significantly contributed to by paucity of previously treated TB patients diagnosed in certain DMCs, though they had such patients during the period of sample size determination period in 2012. After the initial conduct of three rounds of training of trainers, the survey commenced as per the agreed staggered timeline, with TUs which required 12 months or more starting to contribute to survey intake and those that could contribute within 15–30 days being at the tail end of the survey period. However, during the survey, intake was initially slow due to various reasons including some logistic issues contributing to delay in intake. This was compensated during the last quarter of the survey period (Fig. 2).

Fig. 2: Sample registration during the survey period



PT – Previously treated

Patients were enrolled from 120 TUs across the country. Of the 572 total DMCs within these 120 TUs, 530 DMCs participated in screening of patients to identify those eligible, while 524 (91.61%) DMCs contributed to survey samples.

Two specimens, A and B, were collected for routine management as per standard of care articulated in the RNTCP. After obtaining an informed consent, eligible patients were requested to provide two additional specimens (C and D) each from 5280 eligible patients. These were collected and transported to NTI Bangalore along with the completely filled CIFs, barcoded as per survey protocol. The specimens were from 3240 new TB patients and

2040 patients previously treated for TB. A total of 212 specimens were rejected due to various reasons and additional patients were enrolled as replacement.

The quality of both specimens was good for 98% of those collected with a minimal percentage of leakage or less than 2 ml volume observed in 1.0% each as shown in Table 4.

Table 4: Specimen quality

Specimen quality	Specimen C	Specimen D
Good	5140 (97.35%)	5164 (97.80%)
Leaked	57 (1.08%)	54 (1.02%)
Volume <2 ml	83 (1.57%)	62 (1.12%)
Total	5280	5280

Culture positivity of 93.9% was achieved in the liquid culture system with additional recovery of 2.2–2.5% from Löwenstein-Jensen (LJ) media backup, which further improved the culture recovery rates. DST was performed on culture growth from specimens of 3064 (94.57%) patients out of 3240 new TB patients and 1893 (92.79%) out of 2040 previously treated TB patients as shown in Table 5.

Table 5: Status of DST availability among enrolled patients

	New TB patients	Previously treated patients	All patients
Number enrolled	3240	2040	5280
DST Available	3064 (94.57%)	1893 (92.79%)	4957 (93.88%)

3.2 Participant profile

Age and gender distribution is shown in Tables 6 and 7. Seventy-two percent (72.01%) of the survey participants were males and 27.99% were females. Children in the 0–14 years age group contributed to 1.7% of the survey population. Though males were predominant in all age groups in terms of absolute numbers except in the 0–14 years age group, more than 60% of females were aged less than 34 years. The age and gender distribution of survey participants were similar to the distribution of smear positive TB patients registered in RNTCP in 2015.

Table 6: Gender distribution and type of patients among participants

Gender	New TB patients (%)	Previously treated patients (%)	All patients (%)
Female	1,013 (31.37%)	465 (22.79%)	1478 (27.99%)
Male	2227 (68.73%)	1575 (77.97%)	3802 (72.01%)
Total	3240 (100%)	2040 (100%)	5280 (100%)

Table 7: Age distribution and type of patients among participants

Age group (years)	Female (%)	Male (%)	Total (%)
(0–14)	69 (4.70%)	21 (0.60%)	90 (1.7%)
(15–24)	462 (31.30%)	636 (16.70%)	1098 (20.80%)
(25–34)	377(25.50%)	757 (19.90%)	1134 (21.50%)
(35–44)	211(14.30%)	752(19.80%)	963(18.20%)
(45–54)	155(10.50%)	752 (19.80%)	907(17.20%)
(55–64)	138(9.30%)	541 (14.20%)	679 (12.90%)
65+	66(4.50%)	343 (9.00%)	409 (7.70%)

3.3 MDR-TB/XDR-TB among new and previously treated TB patients

Among the 4958 TB patients with DST results, 28% had resistance to one or the other anti-TB drug, while 6.19% had MDR-TB. Among the 307 MDR-TB patients, 11 (3.58%) and 67 (21.82%) patients had additional resistance to any drug from second line injectable class and any drug from fluoroquinolone class, respectively; i.e. pre-XDR-TB. Thus, among the 78 preXDR-TB patients, most of the patients (67 [86%]) had additional fluoroquinolone resistance. XDR-TB among MDR-TB patients was 1.3%. (Table 8).

Table 8: MDR-TB/XDR-TB among new and previously treated TB patients

	New TB patients (95% CI)	Previously treated patients (95% CI)	All patients (95% CI)
DST results	3065	1893	4958
Susceptible	2374 (77.46%) (75.93–78.92%)	1196 (63.18%) (60.96–65.36%)	3570 (72.01%) (70.73–73.25%)
Any DR	691 (22.54%) (21.10–24.10%)	697 (36.82%) (34.64–39.04%)	1388 (28.00%) (26.77–29.29%)
MDR	87 (2.84%) (2.28–3.49%)	220 (11.62%) (10.21–13.15%)	307 (6.19%) (5.54–6.90%)
MDR + any SLI	6 (6.90%) (2.57–14.41%)	5 (2.27%) (0.74–5.22%)	11 (3.58%) (1.80–6.32%)
MDR + any FQ	21 (24.14%) (15.60–34.50%)	46 (20.91%) (15.73–26.89%)	67 (21.82%) (17.33–26.87%)
XDR	2 (2.30%) (0.28–8.06%)	2 (0.91%) (0.11–3.25%)	4 (1.30%) (0.36–3.30%)

Among the 3065 new TB patients subjected to DST for 13 drugs, 2374 (77.46%) were susceptible to all drugs tested, while 691 (22.54%) showed resistance to any drug. MDR-TB was detected in 87 (2.84%) of the new TB patients tested as shown in Table 8. Mono resistance to rifampicin was not observed among new TB patients, thereby indicating that rifampicin resistance was always accompanied by isoniazid resistance.

3.4 Individual drug resistance pattern among new and previously treated TB patients

Among the 1893 previously treated TB patients subjected to DST, 1196 (63.18%) were susceptible to all drugs while 697 (36.82%) showed resistance to any drug. MDR-TB was detected in 220 (11.62%) patients (Table 9). Mono resistance to rifampicin was observed in one patient (0.05%) among the previously treated TB patients.

Table 9*:* Individual drug resistance patterns

Drugs	New patients		Previously treated patients	
	% any resistance (95% CI)	% mono resistance (95% CI)	% any resistance (95% CI)	% mono resistance (95% CI)
Streptomycin	6.88 (6.01–7.84)	2.22 (1.73–2.81)	13.26 (11.76–14.87)	2.48 (1.83–3.29)
Isoniazid	11.06 (9.97–12.22)	3.85 (3.20–4.60)	25.09 (23.15–27.11)	7.61 (6.45–8.89)
Rifampicin	2.84 (2.28–3.49)	0 (0.0)	11.67 (10.26–13.21)	0.05 (0.001–0.29)
Ethambutol	2.28 (1.78–2.88)	0.23 (0.092–0.47)	7.03 (5.92–8.27)	0.21 (0.06–0.54)
Pyrazinamide	6.95 (6.07–7.91)	4.11 (3.44–4.88)	8.77 (7.53–10.13)	4.07 (3.22–5.06)
Kanamycin	1.01 (0.69–1.43)	0.03 (0.0–0.18)	1.01 (0.61–1.56)	0 (0.0)
Amikacin	0.98 (0.66–1.39)	0.07 (0.01–0.24)	1.01 (0.61–1.56)	0.05 (0.001–0.29)
Capreomycin	1.04 (0.72–1.47)	0.03 (0.02–0.18)	0.85 (0.48–1.37)	0 (0.0)
Ofloxacin	3.72 (3.08–4.45)	0.59 (0.35–0.93)	6.29 (5.23–7.48)	0.95 (0.56–1.50)
Levofloxacin	2.71 (2.16–3.35)	0.1 (0.02–0.29)	3.75 (2.94–4.71)	0 (0.0)
Moxifloxacin	2.58 (2.04–3.20)	0.07 (0.01–0.24)	4.01 (3.18–4.99)	0 (0.0)
Para-amino salicylic sodium	2.32 (1.81–2.91)	0.33 (0.16–0.60)	2.38 (1.74–3.17)	0.42 (0.18–0.83)
Ethionamide	2.54 (2.02–3.17)	0.33 (0.16–0.60)	3.06 (2.33–3.94)	0.26 (0.09–0.62)

Drug resistance patterns of M. tuberculosis strains collected from newly diagnosed sputum smear-positive TB patients

Resistance patterns to individual first-line drugs tested indicated highest resistance to isoniazid (any 11.06%, mono 3.85%) followed by resistance for pyrazinamide (any 6.95%, mono 4.11%), streptomycin (any 6.88%, mono 2.22%) and ethambutol (any 2.28%, mono 0.23%) as shown in Table 9.

Resistance patterns to individual second line drugs tested indicated highest resistance to ofloxacin (any 3.72%, mono 0.59%) followed by resistance for levofloxacin (any 2.71%, mono 0.1%), moxifloxacin (any 2.58%, mono 0.07%), ethionamide (any 2.54%, mono 0.33%), para-amino salicylic acid sodium (any 2.32%, mono 0.33%), capreomycin (any 1.04%, mono 0.03%), kanamycin (any 1.01%, mono 0.03%) and amikacin (any 0.98%, mono 0.33%) as shown in Table 9.

Drug resistance patterns of M. tuberculosis strains collected from previously treated sputum smear-positive TB patients

Resistance patterns to individual first-line drugs tested indicated highest resistance to isoniazid (any 25.09%, mono 7.61%) followed by resistance for streptomycin (any 13.26%, mono 2.48%), pyrazinamide (any 8.77%, mono 4.07%), and ethambutol (any 7.03%, mono 0.21%) as shown in Table 9.

Resistance patterns to individual second line drugs tested indicated highest resistance to ofloxacin (any 6.29%, mono 0.95%) followed by resistance for moxifloxacin (any 4.01%, mono 0.0%), levofloxacin (any 3.75%, mono 0.0%), ethionamide (any 3.06%, mono 0.26%), para-amino salicylic acid sodium (any 2.38%, mono 0.42%), amikacin (any 1.01%, mono 0.05%), kanamycin (any 1.01%, mono 0.0%) and capreomycin (any 0.85%, mono 0.0%) as shown in Table 9.

3.5 Additional first-line anti-TB drug resistance among confirmed MDR-TB patients

Among the 87 new TB patients with MDR-TB, any resistance to other first-line drugs was also observed to be high for streptomycin 70.1% (CI 59.35-79.46%), followed by ethambutol 45.98% (CI 35.23–57.0%), and the least for pyrazinamide 31.03% (21.55–41.86%).

Among the 220 previously treated TB patients with MDR-TB, any resistance to other first-line drugs was observed to be highest for streptomycin 59.09% (CI 52.28–65.65%), followed by ethambutol 46.36% (CI 39.64–53.19%) and the least for pyrazinamide 20.45% (CI 15.33–26.40%).

3.6 Additional second line anti-TB drug resistance among confirmed MDR-TB patients

Amongst the 87 new TB patients with MDR-TB, 21 (24.14%) had additional resistance to any fluoroquinolone and 6 (6.90%) to any second line injectable drugs. XDR-TB was observed in 2 (2.30%) patients among MDR-TB patients (Table 8). Further, any resistance to other second line drugs was observed to be highest for ofloxacin 24.14% (CI 15.6–34.5%), followed by moxifloxacin 18.39% (CI 10.89–28.14%), levofloxacin 17.24% (CI 9.98–26.84%), ethionamide and PAS 11.49% (CI 5.65–20.12%) each, amikacin and capreomycin 8.05% (CI 3.3–15.88%) each and the least for kanamycin 4.6% (CI 1.27–11.36%).

Among the previously treated TB patients with MDR-TB, 46 (20.91%) had additional resistance to any fluoroquinolone and 5 (2.27%) to any second line injectable drugs. XDR-TB was observed in 2 (0.91%) patients (Table 8). Further, any resistance to other second line drugs was observed to be highest for ofloxacin 21.36% (CI 16.14–27.38%), followed by moxifloxacin 15.45% (CI 10.95–20.92%), levofloxacin 14.09% (CI 9.78–19.40%), ethionamide 7.27% (CI 4.21–11.55%), PAS 4.09% (CI 1.89–7.62%), kanamycin and amikacin 2.27% (CI 0.74–5.22%) each and the least for capreomycin 1.81% (CI 0.49–4.59%).

3.7 DR-TB rates among states

Sample size for the survey was calculated for obtaining national level estimates only and State level inferences cannot be drawn directly from the survey. However, resistance to any drug as well as distribution of MDR among the States that participated suggests areas for focus action. State-wise drug resistance rates are given in Table 10 using the numbers collected from each state that provided specimens for the survey.

A state level analysis of drug resistance indicates that DR-TB is prevalent in all states, albeit with wide variations ranging from 18.42% in Himachal Pradesh to 36.84% in Jammu & Kashmir. In addition, this is an indication that screening of drug resistance has to be expanded to offer universal DST including expanded DST as envisaged in the updated PMDT guidelines. The second and most important activity is to strengthen drug resistance surveillance under the programme with inclusion of laboratories in the private sector as well. The state levels rates also give us the opportunity to plan and execute intervention prioritizing, based on the drug resistance trends observed.

Table 10: DR-TB rates among different states in India

State	New TB patients					Previously treated TB patients				
	Total DST result	MDR	% MDR	DR (other than MDR)	% DR (other than MDR)	Total DST result	MDR	% MDR	DR (other than MDR)	% DR (other than MDR)
Andhra Pradesh	183	1	0.55	40	21.86	114	9	7.89	25	21.93
Assam	79	2	2.53	20	25.32	48	2	4.17	16	33.33
Bihar	177	8	4.52	32	18.08	109	16	14.68	22	20.18
Chhattisgarh	47	2	4.26	9	19.15	33	2	6.06	11	33.33
Delhi	80	1	1.25	17	21.25	46	4	8.70	13	28.26
Gujarat	183	3	1.64	26	14.21	113	6	5.31	17	15.04
Haryana	53	0	0.00	6	11.32	31	4	12.90	11	35.48
Himachal Pradesh	23	1	4.35	2	8.70	15	0	0.00	4	26.67
Jammu & Kashmir	26	0	0.00	7	26.92	12	0	0.00	7	58.33
Jharkhand	103	2	1.94	13	12.62	62	7	11.29	13	20.97
Karnataka	128	0	0.00	37	28.91	81	1	1.23	24	29.63
Kerala	53	1	1.89	9	16.98	27	3	11.11	5	18.52
Madhya Pradesh	142	3	2.11	25	17.61	88	9	10.23	28	31.82
Maharashtra	259	20	7.72	50	19.31	161	19	11.80	41	25.47
Meghalaya	24	0	0.00	6	25.00	15	2	13.33	1	6.67
Nagaland	26	1	3.85	4	15.38	16	2	12.50	6	37.50
Orissa	106	0	0.00	27	25.47	66	7	10.61	17	25.76
Punjab	78	1	1.28	10	12.82	46	2	4.35	15	32.61
Rajasthan	179	5	2.79	34	18.99	116	15	12.93	33	28.45
Sikkim	25	1	4.00	6	24.00	17	3	17.65	2	11.76
Tamil Nadu	138	4	2.90	21	15.22	90	7	7.78	24	26.67
Telangana	53	0	0.00	16	30.19	33	5	15.15	7	21.21
Uttar Pradesh	640	29	4.53	133	20.78	399	79	19.80	96	24.06
Uttarakhand	54	1	1.85	12	22.22	30	6	20.00	7	23.33
West Bengal	205	1	0.49	42	20.49	125	10	8.00	32	25.60
Total	**3064**	**87**	**2.84**	**604**	**19.71**	**1893**	**220**	**11.62**	**477**	**25.20**

3.8 Conclusions

Key findings

- Among all TB patients tested, MDR-TB rate was 6.19% with 2.84% among new and 11.60% among previously treated TB patients.
- Any isoniazid resistance among new and previously treated TB patients was 11.06% and 25.09%, respectively.
- Any drug resistance among new TB patients was 22.54%, with 36.82% among previously treated TB patients and 28.02% among all patients.
- There was negligible rifampicin mono-resistance in the survey and isoniazid resistance was invariably associated with rifampicin resistance. Any pyrazinamide resistance was 6.95% and 8.77% among new and previously treated TB patients, respectively.
- Among MDR-TB patients, additional resistance to any fluoroquinolone was 21% and any second line drug resistance was 3.84%.
- Among MDR-TB patients, XDR-TB rate was 1.3%.
- There were wide variations in the state-wise levels of drug resistance (Table 10), highlighting that national level estimates tends to mask the local and focal epidemics that need to be addressed with specific interventions.

Key steps going forward

The key next steps are:

- Setting up and strengthening drug resistance surveillance including using state of art next generation sequencing. This will provide the programme with the trends of drug resistance, transmission patterns and mapping of hot spots in different states for better understanding of molecular epidemiology for TB surveillance.
- Rapidly scaling up universal DST and appropriate DST guided treatment.
- *Strengthening epidemiologic intelligence for specific interventions based on local epidemiological profile.*

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