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Assessment of burden of drug-resistant tuberculosis at a tertiary care center in northern India

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TITLE PAGE

Title: Assessment of burden of drug-resistant tuberculosis at a tertiary care center in northern India

Authors and affiliations: RichaMisra[a], VasudhaKesarwani[a], Alok Nath*[b]

[a]MD, Department of Microbiology, Division Mycobacteriology, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

[b]MD, DM, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

*__Corresponding author:__ Dr. Alok Nath, Professor, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow-226014,India.

Corresponding author E mail:

dralоksgpgi@gmail.com

Phone: +919839070065

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Abstract

Objectives: We aim to define the burden of rifampicin monoresistant tuberculosis at a tertiary care centre in Northern India as well as determine the second line drug susceptibilities in a subset of patients.

Methods: A total of 3045 pulmonary (n=1883) and extra-pulmonary (n=1162) samples from suspected tuberculosis patients were subjected to microscopy, culture and the Xpert MTB/RIF assay from March 2017 to June 2019.Second line drug susceptibility testing by version2 Line Probe Assay for Fluoroquinolones (FQs) and second line injectable drugs (SLIDs) was performed on 62 samples.

Results: Out of 3045 samples processed in our lab during the study period, 33.9% (1032/3045) were positive for MTBC and 21.6% were rifampicin mono-resistant (223/1032). The rate of rifampicin resistance in pulmonary samples was 22.1% (156/706) and in extrapulmonary cases it was 20.5% (67/326).Out of 62 cases included for second line testing, 37 were resistant to fluoroquinolones(77.4%)while 11 were extensively drug resistant(XDR).

Conclusions: India urgently needs to arrest an emerging multidrug-resistant tuberculosis epidemic to attain the Sustainable Development Goal (SDG) target of 2030. The majority of the isolates in our study were FQ resistant which is an exclusion criterion for the shorter MDR regimen recommended by World Health Organization.

Keywords: Tuberculosis; Multi-drug resistant; Xpert MTB/RIF assay; Line Probe Assay

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Article summary section

Strengths and limitations of this study

- We have not come across any study from India performed on such a large number of pulmonary as well as extrapulmonary samples performed by both conventional and molecular methods.
- Our study provides comprehensive recent data on the burden of drug resistant TB in India at a 1200 bed tertiary care centre.
- We could not perform liquid culture Drug Susceptibility Testing (DST) of the isolates and DNA sequencing

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INTRODUCTION

India has the highest Tuberculosis (TB) burden in the world and is home to 27% of the world's estimated 10.4 million annual tuberculosis cases.[1,2,3] As per WHO Global TB Report 1,30,000 cases of MDR-TB occurred in India in 2016.[1]The Programmatic Management of Drug Resistant TB (PMDT) guidelines were rolled out in 2005 and integrates all programme based strategies for DR-TB diagnosis, management and treatment under RNTCP.[4] In fact, the government of India in an ambitious move has changed the name of the national programme from RNTCP to NTEP, National Tuberculosis Elimination Programme in December 2019, to achieve the Sustainable Development Goal of ending TB by 2025.

India also has a complex as well as unorganized health-care system which includes the government sector, private sector and informal health care providers practicing non-allopathic schools of medicine such as ayurveda and homeopathy[2]. Though TB was made a notifiable disease in 2012, less than 40% cases from the private sector were notified to the government in 2017.[3]

The shorter drug regimen of 9-12 months for MDR-TB patients was introduced by World Health Organization (WHO), in May 2016.[5-7]It was recommended in patients who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded. However, drug susceptibility testing in India is technically challenging and requires specialist laboratory facilities and personnel that are still not widely available in the country.[8]

With this background, we aim to define the burden of rifampicin mono-resistant tuberculosis at a

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tertiary care referral medical center in northern India as well as determine the second line drug susceptibilities in a subset of patients.

METHODS

Study design and setting

This prospective observational study between March 2017 to June 2019 was conducted in the Mycobacteriology section of the Department of Microbiology at Sanjay Gandhi Postgraduate Institute of Medical Sciences, a 1200 bed tertiary care referral medical center in northern India. The study protocol was approved by the ethics committee of the Institute.

Clinical specimens

Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/ Fine Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected between March 2017 and June 2019 during the clinical routine. All samples were divided into 2 portions on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF assay while microscopy and culture was performed from the remaining sample. Direct smears were prepared from the specimens using Ziehl-Neelsen staining. All non-sterile clinical samples were processed using the *N*-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method. Samples were decanted following centrifugation, and sediments were re-suspended in 3 ml of phosphate buffer solution. Processed samples were used to inoculate either Lowenstein-Jensen (LJ) solid medium or BacT/Alert culture. Line probe assay *version2* (LPA*v2*) for second line testing was performed on either direct clinical samples if volume was adequate or on positive culture. Both Xpert MTB/RIF assay and LPA*v2* were performed according to the manufacturer's protocol.

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All cases detected positive by the Xpert MTB/RIF assay were grouped into (i) those with smear-positive and culture positive tuberculosis; (ii) those with smear-negative, culture-positive tuberculosis; (iii) those who were both smear and culture negative for tuberculosis but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or radiological findings (clinical tuberculosis).There was a sub group of samples that were culture positive but missed by the Xpert MTB/RIF assay.

Data collection

The medical records of patients were retrieved from the Hospital Information System. A senior resident extracted patient data prospectively from charts.

Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin resistant/multi-drug resistant/extensively drug resistant)[9]

A *bacteriologically confirmed TB case***:** One from whom a biological specimen was positive by smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert MTB/RIF assay.

*Pulmonary tuberculosis (PTB)***:** Any bacteriologically confirmed or clinically diagnosed case of TB involving lung parenchyma or tracheobronchial tree.

Extrapulmonary tuberculosis (EPTB): Any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges.

*Multidrug resistance TB (MDR)***:**A TB patient, whose biological specimen is resistant to both H and R with or without resistance to other first-line anti-TB drugs.

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Pre-XDR-TB: It is defined as TB with resistance to isoniazid and rifampicin and either a FQ or a second-line injectable agent but not both.

Extensive drug resistance (XDR): A MDR-TB patient whose biological specimen isadditionally resistant to at least a FQ and a SLI anti-TB drug.

Patient and public involvement

Patients were involved in the reporting of our research in this study.

RESULTS

During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with concomitant smear and culture inoculation on the same sample. All duplicate isolates were excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary) were detected for MTB complex. The assay failed to detect sixty nine samples that were culture positive. The MPT64 antigen test was positive on all these cultures. There were 806 (78.10%) males and 226 (21.89%) females among the positive specimens. The median age of patients was 32 years and nearly 43% patients were young adults in the age group of 30-45 yearsas shown in Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic biopsy) were the most common samples in extra-pulmonary cases that were positive. The sample distribution of positive specimens is shown in Figure 2. Out of 1032 samples detected positive by the CBNAAT assay, 507 and 517 specimens were smear and culture positive respectively. The rate of smear and culture positivity in pulmonary and extra-pulmonary cases was 54.1%, 54.3%, 38.3% and 40.7% respectively (Table-1). The results of conventional and molecular diagnostic testing by Xpert MTB/RIF assay of patients included in the study is shown in Figure 3.

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During the study period, we also recovered 35 isolates of Non-tuberculous Mycobacteria (NTM) from various pus and respiratory specimens. These were *Mycobacterium abscessus* (n=15), *Mycobacterium intracellulare* (7), *Mycobacterium fortuitum* (6), *Mycobacterium gordonae* (n=3) and *Mycobacterium simiae* (n=3).

Rifampicin monoresistance was detected in 223 out of 1032 samples (21.6%). It was 23.5% (n=166/706) and 17.4% (57/326) in pulmonary and extra-pulmonary cases respectively (Figure 4).

A summary of the performance data is shown in Table 2. Five hundred and seventeen samples were positive by culture resulting in an 86.6% agreement with the Xpert MTB/RIF assay. The assay had a 100% agreement for culture positive, smear positive specimens and 61.6% agreement for culture positive, smear negative specimens for the detection of *Mycobacterium tuberculosis.* Sixty nine samples that were culture positivetested negative by the Xpert MTB/RIF assay. We did not have any sample that was positive on both smear and culture but was negative by Xpert MTB/RIF assay. As shown in Table 2, we detected 413 more patients than we could have diagnosed by smear and/or culture alone.

Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing byLPA*v2.0* for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure 5, majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR) while 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that was aminoglycoside resistant but FLQ sensitive.

DISCUSSION

Multidrug-resistant tuberculosis is one of the greatest public health challenges worldwide.[1,10-12]

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To the best of our knowledge, ours is the first study from India to determine the burden of drug resistant tuberculosis by testing such a large number of pulmonary and extrapulmonary clinical samples. As per WHO Global TB Report, 2019 the best estimate of total TB incidence for India is 199 cases per 100,000 population which translates to around 9.9 cases of MDR-TB per 100000 population annually.[1] However, the estimates of TB incidence and mortality for India are interim, pending results from the national TB prevalence survey planned for 2019/2020.

In 2016, the male to female ratio for TB stood between 1.07 to 2.25, with women accounting for 40% of new cases. In our study, it was 3.5. Studies have shown that women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as high burden of household work, illiteracy, restricted mobility as well as lack of autonomy. There is also a high level of stigma associated with the disease among unmarried females. In addition, malnutrition, especially anemia is prevalent in more than half of the women in India. All this increases the risk of TB disease in women.[13]

In 2010, the World Health Organization (WHO) recommended the GeneXpert MTB/RIF assay for initial diagnosis of MDR-TB or HIV-associated tuberculosis.[14]In 2014, WHO expanded this recommendation for use in all patients. The accuracy of the MTB/RIF test to detect the presence of tuberculosis in smear-positive cases has been reported to be between 98% to 100%. For smear negative specimens, Zeka et al have reported sensitivities of 68.6% and 47.7% in pulmonary and extrapulmonary samples respectively.[15]In the present study, the sensitivity of the test was nearly 87% and it rose to 100% for smear-positive specimens. In all studies, the sensitivity of the MTB/RIF test for pulmonary specimens is higher than that for extrapulmonary specimens which may or may not be statistically significant as reported by various authors.[16]This could be because of the high smear-negative rate for non-respiratory specimens.Sixty nine specimens that were

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culture positive tested negative by the GeneXpert assay in our study (Table 2) resulting in a specificity of 61.6% (69/112).All these samples were smear negative. We also detected 413 cases (40% of 1032 positives) by the GeneXpert assay that were missed by both smear as well as culture. Xpert achieved higher diagnostic yield than microscopy and increased TB case finding by a factor of about 2. Boehme et al in a performance study on the use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance concluded that use of this test reduced the median time to detection and treatment for smear-negative tuberculosis from 56 days to 5 days.[17]In another study by Kim et al, on 321 patients the turn around time (TAT) for treatment between patients diagnosed with rifampicin-resistant TB using the Xpert assay and those diagnosed without the assay (phenotypic DST group) were compared.[18] It was 64 vs. 2 days ($p< 0.001$) from initial evaluation to commencing second-line anti-tuberculosis treatment.Usingphenotypic DST as the gold standard, Xpert sensitivity and specificity for diagnosis of rifampicin resistance was 100% and 98.7% respectively.

The overall rate of resistance to rifampicin in our study was 21.6%.Goyal et al published a recent systematic review of 75 epidemiological studies for the prevalence of DRTB in India across 2 decades, from 1995 to 2015.[19]Comparative analysis revealed aworsening trend in DR-TB between the two study decades, 37.7% vs 46.1% respectively. The prevalence of pre-XDR TB was 7.9% with 66.3% resistance to fluoroquinolones.

It is estimated that in India, by 2032, 85% of multidrug-resistant tuberculosis infections would be from primary transmission, compared with only 15% in 2012.[19] In the *Lancet Public Health,* Law et al have created a dynamic model of the tuberculosis epidemic in India, which they use to estimate the incidence of drug susceptible tuberculosis and multidrug-resistant tuberculosis over the next 20 years.[19] They have analyzed the emergence of drug resistance in all major health-care

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sectors in India including the country's burgeoning private sector. Private clinics in India are often used by patients seeking TB treatment and they administer regimens that are not recommended by standard guidelines. This not only results in suboptimal outcome but also potentially generates MDR TB. They conclude that as multidrug resistant tuberculosis transitions from an acquired condition to a primarily transmitted disease, improving the effectiveness of drug-susceptible tuberculosis treatment can no longer contain the spread of the epidemic. This epidemiological shift has profound resource implications since the cost of treatment of multidrug-resistant tuberculosis treatment can exceed that of first-line tuberculosis therapy by a factor of ten or more. A robust public health response is needed which includes a strong surveillance system, drug susceptibility testing for all patients with tuberculosis and rapid linkage to effective treatment throughout the course of the disease.

In a similar study, Suen et al sought to evaluate the effectiveness of two disease control strategies on reducing the prevalence of MDR TB in India.[21]One by improving treatment of non-MDR TB cases and second by shortening the infectious period between activation of MDR TB and initiation of effective treatment. They examine the implication of India's MDR TB epidemic from 1996-2038 for the effectiveness of public health interventions by using a dynamic transmission model calibrated to Indian demography and epidemiology. They have concluded that strategies that disrupt MDR transmission by shortening the time between MDR activation and treatment are projected to provide greater reductions in MDR prevalence compared with improving non-MDR treatment quality.

In our study, we could put up SLD testing for only 62 cases (27.8%) out of 223 i.e. less than 30%. The remaining cases were either sterile on culture or had inadequate volume of sample to put up the test directly.The PMDT guidelines in India outline the steps of specimen referral for

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TB patients in the government sector in India for both first and second line DST by LPA.[4]As per the operational process, two fresh sputum specimens need to be collected at designated collection centers by trained personnel and transported in a cool chain on the same day to the nearest CBNAAT lab for all eligible patients.At the CB-NAAT sites one specimen will be utilized to perform Gene Xpert assay and the second specimen will be transported to LPA lab for either first line (FL) testing if INH resistance is suspected or second line (SL) DST. At the LPA lab, the second specimen will be tested as applicable and processed further for Liquid Culture DST. Out of 62 cases put up for SLD testing in our study, only 14 were sensitive to both second line agents. Nearly 77.4% cases were resistant to FQs. FQ resistance is a defining feature of extensively drug-resistant Tuberculosis (XDR-TB).A recent study from India, assessed the proportion of rifampin-resistant TB patients in the state of Uttar Pradesh who would be eligible for a shorter regimen under the NTEP setting.[22]Of 541 conclusive LPA-SLD results, the proportion of strains resistant to only fluoroquinolone was 50.1% while 8.3% were resistant to both fluoroquinolones and SLIDs.Eleven cases in our study were extensively drug resistant.According to the data reported on XDR-TB from India, it varied from 0.3 to 60 per cent of MDR-TB cases.[2]The results of our study underscore the fact that resistance to second-line anti-tubercular drugs should be routinely assessed in areas endemic for TB.In another study by Chee et al, from Singapore, only 30% of patients with MDR-PTB from South-east Asia were eligible for the WHO shorter MDR-TB treatment regimen.[23]

The high rates of drug resistance observedin the present study may be due to the fact that ours is atertiary care hospital and we see patients after the referringhospital has already tried and failed to control infection using a combination of different anti-tubercular agents. Moreover, facilities for microbiological studies at the first contact physician/surgeon are usually not available in

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district hospitals/smaller citiesin India. Indiscriminate antimicrobial therapy, without establishing the etiology of infection results in incomplete treatment and misdiagnosis. In addition, many of these antimicrobial agents have antitubercular activity also. McDowell and Pai in an ethnographic study on the mismanagement of empirical TB treatment in India showed that all non-specialist private practitioners began antibiotic treatment, especially quinolones, for persistent cough before prescribing a test.[24]

Subbaraman et al in a systematic review and meta-analysis have created a 'cascade of care' model that focuses on the government run national programme, NTEP (previously known as RNTCP).[25]The purpose of their study was to estimate how many TB patients in India's national TB program are not being detected, not enrolling for treatment, not completing treatment, and not surviving without TB recurrence for 1-y after completing treatment. The results of their study show that pre-treatment loss to follow-up of diagnosed patients and post-treatment TB recurrence were major points of attrition in the new smear-positive TB cascade. Out of 2,700,000 prevalent TB patients in India only 39% achieved the optimal outcome of 1-y recurrence-free survival.

CONCLUSION

In conclusion,we have not come across any study from India performed on such a large number of pulmonary as well as extrapulmonary samples performed by both conventional and molecular methods.Our study provides comprehensive data on the burden of drug resistant TB in India at a 1200 bed tertiary care centre.The Sustainable Development Goal (SDG) target to end the tuberculosis epidemic by 2030 seems bleak.Notification data from low-income and middle-income countries, are prone to under-reporting and cannot beinterpreted without additional information on casedetection rate.The DR-TB diagnostic algorithm as given in the PMDT guidelines recommends SL – LPA testing for all RR TB cases diagnosed by the CBNAAT assay.

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However, it is labor intensive and requires trained manpower .Severe lack of Microbiology laboratories providing universal DST and visual interpretation of bands is a huge limitation especially in smear negative and extra-pulmonary cases with inadequate sample volumes as has been our experience even with version 2 of the test. The treatment algorithm recommends the shorter MDR TB regimen in all pulmonary cases of RR TB patients. However, the exclusion criterion is second-line drug resistance. Our data shows that out of 62 cases, 77% patients were resistant tofluoroquinolones. Though notification of all TB cases has been made mandatory by a gazette circular issued by the government in March 2018, the 'missing million' still remains a huge challenge. Our data provide a strong rationale for the implementation of evidence based strategies such as strengthening of laboratories, involvement of private sector, active case finding and strict compliance of treatment by directly observed therapy.

Contributors RM drafted the manuscript. VK compiled the data. All authors critically revised the manuscript, gave final approval and agreed to be accountable for all aspects of work.

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Competing interests None declared.

Patient consent for publication Not required

Ethics approval The study protocol was approved by the ethics committee of the Institute.

Data availability statement Data may be obtained by e mailing drricha1976@gmail.com

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Table 1. Smear and culture results among samples positive by Xpert MTB /RIF assay (n=1032)

Sample (N=1032)	Smear positive (%)	Culture positive (%)
Pulmonary(n=706)	382(54.1%)	384(54.3%)
Extra-pulmonary(n=326)	125(38.3%)	133(40.7%)
Total	507(49.1%)	517(50%)

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Table 2: Comparison of Gene Xpert MTB / RIF positive, MTB culture positive results with smear results

SmearMTB culture + Gene Xpert+	MTB culture + Gene Xpert-	MTB culture– Gene Xpert+	MTB culture– Gene Xpert–	Total
Positive	336	0	171	0	507
Negative	112	69	413	1944	2538
Total	448	69	584	1944	3045

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Figure legends

Figure 1. Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)

Figure 2. Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)

Figure 3. Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay of samples included in the study (n=3045)

Figure 4. Distribution of samples among rifampicin resistant cases (n=223)

Figure 5: Results for second line susceptibility testing performed by line probe assay (LPA) version2

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Figure 1

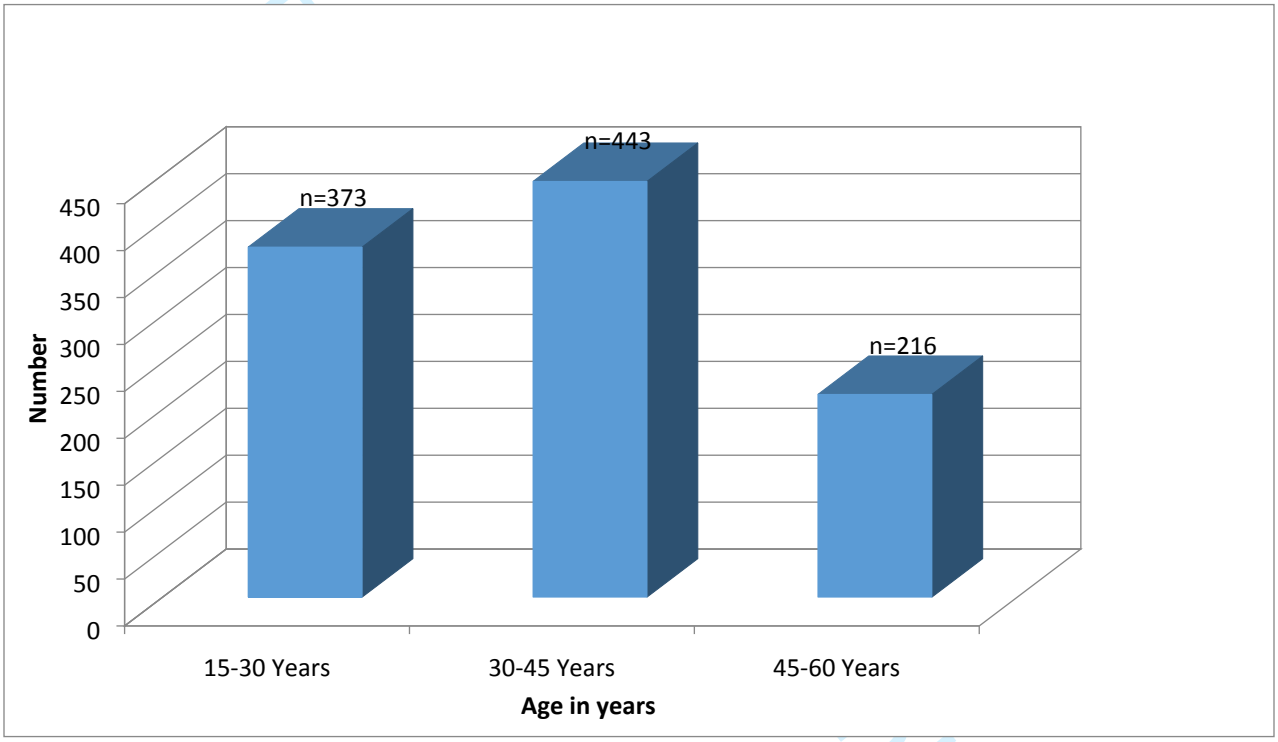
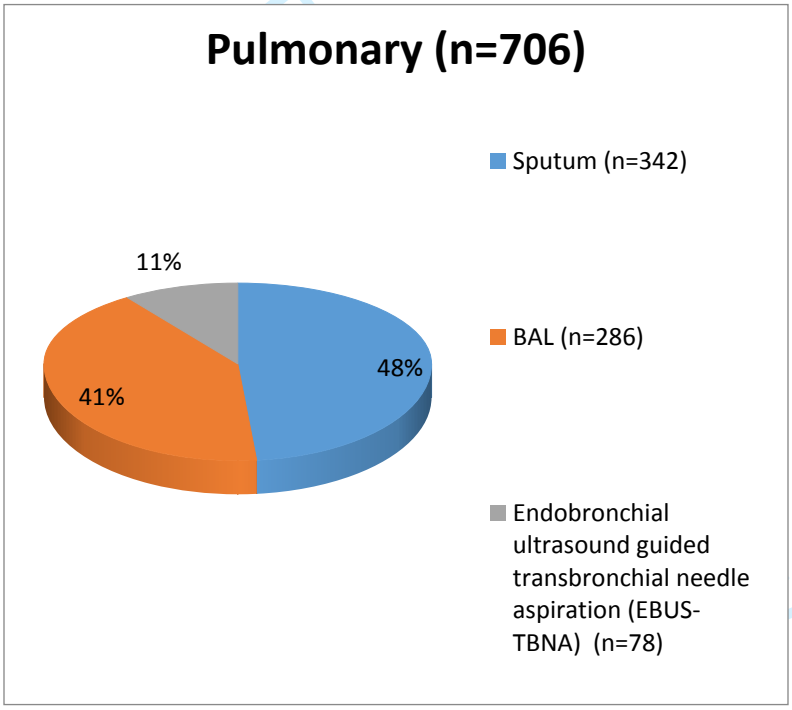

n=373
n=443
n=216
450
400
350
300
250
200
150
100
50
0
Number
15-30 Years
30-45 Years
45-60 Years
Age in years

Figure 2


Pulmonary (n=706)
Sputum (n=342)
BAL (n=286)
Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) (n=78)
48%
41%
11%

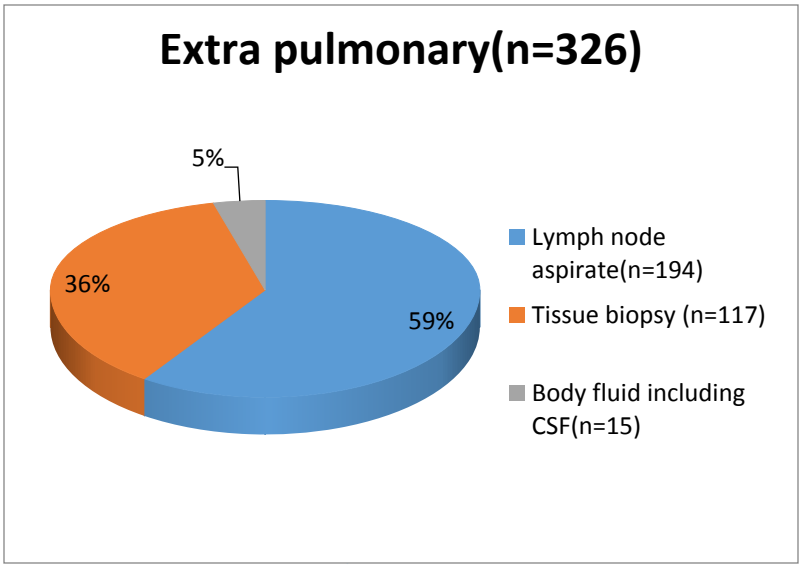


Figure 3

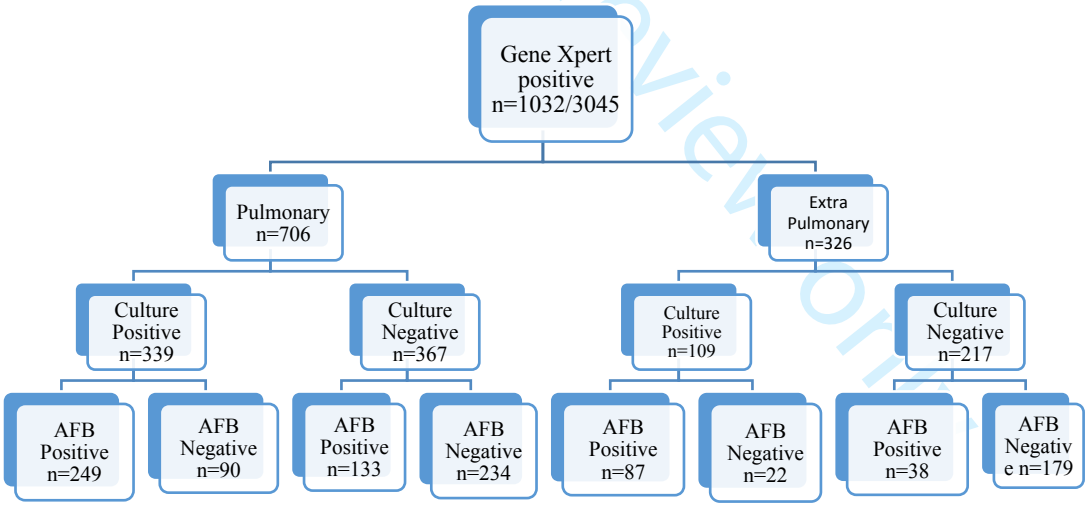


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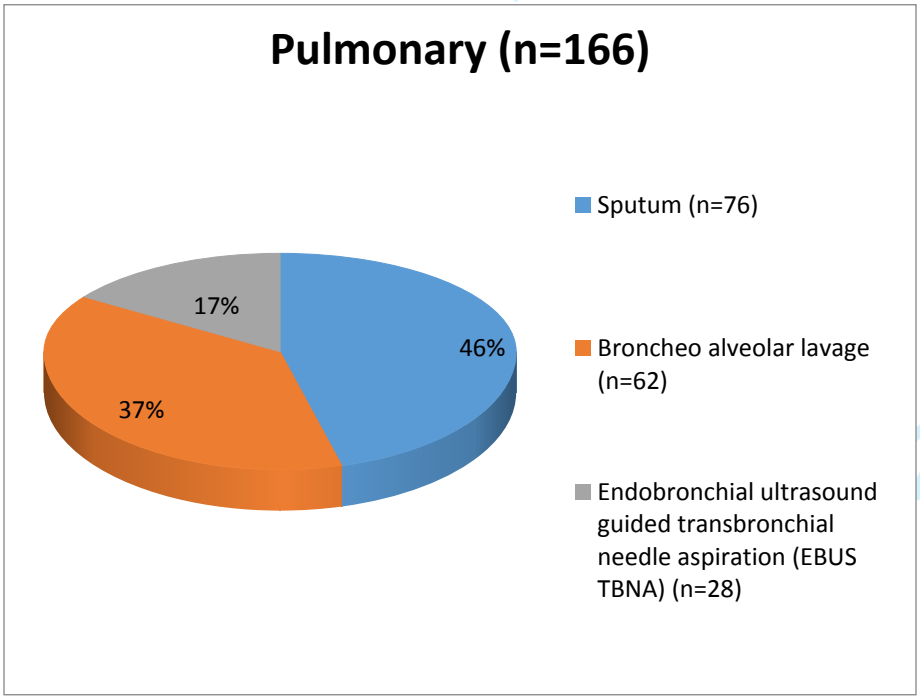
Figure 4



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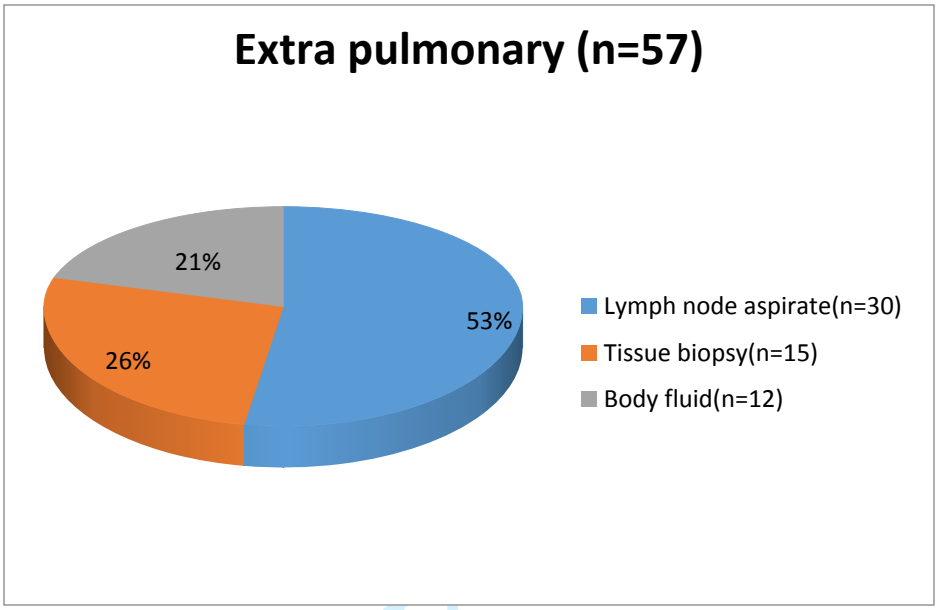

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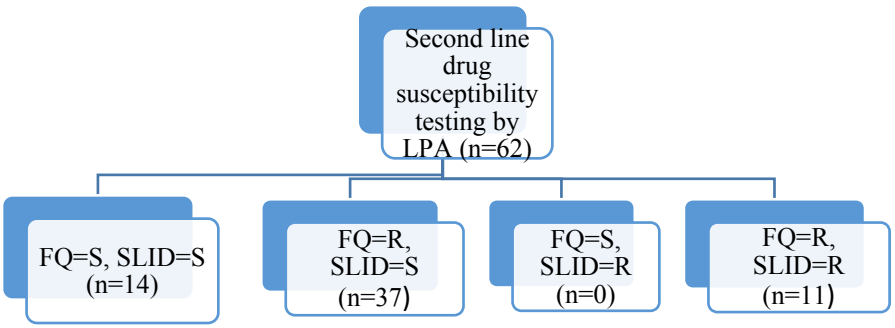

S = Sensitive; R = Resistant; FQ = Fluoroquinolones; SLID = Second line injectable drugs

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Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a community-based prospective single centre cohort study

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TITLE PAGE

Title: Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a community-based prospective single centre cohort study

Authors and affiliations: RichaMisra[a], VasudhaKesarwani[a], Alok Nath*[b]

[a]MD, Department of Microbiology, Division Mycobacteriology, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

[b]MD, DM, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

*__Corresponding author:__ Dr. Alok Nath, Professor, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow-226014, India.

Corresponding author E mail:

dralоksgpgi@gmail.com

Phone: +919839070065

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Abstract

Objectives: We aim to define the burden of rifampicin monoresistant tuberculosis at a tertiary care centre in Northern India as well as determine the second line drug susceptibilities in a subset of patients.

Methods: A total of 3045 pulmonary (n=1883) and extra-pulmonary (n=1162) samples from suspected tuberculosis patients were subjected to microscopy, culture and the Xpert MTB/RIF assay from March 2017 to June 2019.Second line drug susceptibility testing by version2 Line Probe Assay for fluoroquinolones (FQs) and second line injectable drugs (SLIDs) was performed on 62 samples.

Results: Out of 3045 samples processed in our lab during the study period, 36.1% (1101/3045) were positive for MTBC and 21.6% were rifampicin mono-resistant (223/1032). The rate of rifampicin resistance in pulmonary samples was 23.5% (166/706) and in extrapulmonary cases it was 17.4% (57/326).Out of 62 cases included for second line testing, 48 were resistant to FQs (77.4%)while 11 were extensively drug resistant(XDR).

Conclusions: India urgently needs to arrest an emerging multidrug-resistant tuberculosis epidemic with associated resistance to fluoroquinolones. A robust surveillance system is needed to execute the National strategic Plan (NSP) for 2017-2025.

Keywords: Tuberculosis; Multi-drug resistant; Xpert MTB/RIF assay; Line Probe Assay

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Article summary section

Strengths and limitations of this study

- Study has a large sample size of 3045 samples.
- 1162 extrapulmonary samples have been included such as EBUS-TBNA and biopsies
- Both Xpert MTB/RIF assay and Line probe assay have been performed
- MGIT-DST was not performed
- DNA squencing was not done on drug-resistant isolates

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INTRODUCTION

India has the highest Tuberculosis (TB) burden in the world and is home to 27% of the world's estimated 10.4 million annual tuberculosis cases.[1,2,3] The menace of drug resistant-TB (DR-TB), prompted the government to initiate the programmatic management of drug resistant TB (PMDT) in 2007 which integrates all programme based strategies for DR-TB diagnosis, management and treatment under the NTEP, National Tuberculosis Elimination Programme (renamed in December 2019)[4]. India also has a complex as well as unorganized health-care system which includes the government sector, private sector and informal health care providers practicing non-allopathic schools of medicine such as ayurveda and homeopathy[2]. Though TB was made a notifiable disease in 2012, less than 40% cases from the private sector were notified to the government in 2017[3].

The shorter drug regimen of 9-12 months for MDR-TB patients was introduced by World Health Organization (WHO), in May 2016 and updated in June 2020.[5,6]It was recommended in patients who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded. However, drug susceptibility testing in India is technically challenging and requires specialist laboratory facilities and personnel that are still not widely available in the country.[4]

With this background, we aim to define the burden of rifampicin mono-resistant tuberculosis at a tertiary care referral medical center in northern India as well as determine the second line drug susceptibilities in a subset of patients.

METHODS

Study design and setting

This prospective observational study between March 2017 to June 2019 was conducted in the Mycobacteriology section of the Department of Microbiology at Sanjay Gandhi Postgraduate

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Institute of Medical Sciences, a 1200 bed tertiary care referral medical center in northern India. Institutional ethics committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) approved the study protocol (IEC code 2017-37-IMP-EXP) and waiver of consent was obtained.

Clinical specimens

Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/ Fine Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected between March 2017 and June 2019 during the clinical routine. All samples were divided into 2 portions on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF assay while microscopy and culture was performed from the remaining sample. Direct smears were prepared from the specimens using Ziehl-Neelsen staining. All non-sterile clinical samples were processed using the *N*-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method. Samples were decanted following centrifugation, and sediments were re-suspended in 3 ml of phosphate buffer solution. Processed samples were used to inoculate either Lowenstein-Jensen (LJ) solid medium or BacT/Alert culture. Line probe assay *version2* (LPA*v2*) for second line testing was performed on either direct clinical samples if volume was adequate or on positive culture. Both Xpert MTB/RIF assay and LPA*v2* were performed according to the manufacturer's protocol.

All cases detected positive by the Xpert MTB/RIF assay were grouped into (i) those with smear-positive and culture positive tuberculosis; (ii) those with smear-negative, culture-positive tuberculosis; (iii) those who were both smear and culture negative for tuberculosis but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or radiological findings (clinical tuberculosis).

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There was a sub group of samples that were culture positive but missed by the Xpert MTB/RIF assay. We put up the TB Ag MPT64 Rapid test (SD BIOLINE) on all these positive cultures.

Data collection

The medical records of patients were retrieved from the Hospital Information System. A senior resident extracted patient data prospectively from charts.

Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin resistant/multi-drug resistant/extensively drug resistant)[7]

A*bacteriologically confirmed TB case*: One from whom a biological specimen was positive by smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert MTB/RIF assay.

Pulmonary tuberculosis (PTB): Any bacteriologically confirmed or clinically diagnosed case of TB involving lung parenchyma or tracheobronchial tree.

Extrapulmonary tuberculosis (EPTB): Any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges.Concomitant pulmonary lesions were ruled out in all cases by appropriate investigations and review of case files.

Multidrug resistance TB (MDR):A TB patient, whose biological specimen is resistant to both H and R with or without resistance to other first-line anti-TB drugs.

Pre-XDR-TB: It is defined as TB with resistance to isoniazid and rifampicin and either a FQ or a second-line injectable agent but not both.

*Extensive drug resistance (XDR):*A MDR-TB patient whose biological specimen isadditionally resistant to at least a FQ and a SLI anti-TB drug.

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Patient and public involvement

Patients were involved in the reporting of our research in this study.

RESULTS

During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with concomitant smear and culture inoculation on the same sample. All duplicate isolates were excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary) were detected for MTB complex. The assay failed to detect sixty nine samples that were culture positive. The MPT64 antigen test was positive on all these cultures. There were 806 (78.10%) males and 226 (21.89%) females among the positive specimens. The median age of patients was 32 years and nearly 43% patients were young adults in the age group of 30-45 yearsas shown in Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic biopsy) were the most common samples in extra-pulmonary cases that were positive. The sample distribution of positive specimens is shown in Figure 2. Out of 1032 samples detected positive by the CBNAAT assay, 507 and 517 specimens were smear and culture positive respectively. The rate of smear and culture positivity in pulmonary and extra-pulmonary cases was 54.1%, 54.3%, 38.3% and 40.7% respectively (Table-1). The results of conventional and molecular diagnostic testing by Xpert MTB/RIF assay of patients included in the study is shown in Figure 3.

During the study period, we also recovered 34 isolates of Non-tuberculous Mycobacteria (NTM) from various pus and respiratory specimens. These were *Mycobacterium abscessus* (n=15), *Mycobacterium intracellulare*(7), *Mycobacterium fortuitum* (6), *Mycobacterium gordonae*(n=3) and *Mycobacterium simiae*(n=3).

Rifampicin monoresistance was detected in 223 out of 1032 samples (21.6%). It was 23.5% (n=166/706) and 17.4% (57/326) in pulmonary and extra-pulmonary cases respectively

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(Figure 4).A summary of the performance data is shown in Table 2. Five hundred and seventeen samples were positive by culture resulting in an 86.6% agreement with the Xpert MTB/RIF assay. The assay had a 100% agreement for culture positive, smear positive specimens and 61.6% agreement for culture positive, smear negative specimens for the detection of *Mycobacterium tuberculosis.* Sixty nine samples that were culture positivetested negative by the Xpert MTB/RIF assay. We did not have any sample that was positive on both smear and culture but was negative by Xpert MTB/RIF assay. As shown in Table 2, we detected 413 more patients than we could have diagnosed by smear and/or culture alone.

Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing byLPA*v2.0* for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure 5, majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR) while 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that was aminoglycoside resistant but FLQ sensitive.

Discussion

Multidrug-resistant tuberculosis is one of the greatest public health challenges worldwide. To the best of our knowledge, ours is the first study from India to determine the burden of drug resistant tuberculosis by testing such a large number of pulmonary and extra-pulmonary clinical samples. As per WHO Global TB Report, 2020 the three countries with the largest share of the global burden were India (27%), China (14%) and the Russian Federation (8%)[1].

The results of the national anti-tuberculosis drug resistance survey has shown that the incidence of TB is highest in the 25–34 year age group in India[8]. We however documented a slightly higher age group in our study. Our cohort was dominated by males and nearly 43% patients were young adults in the age group of 30-45 years. The high frequency of the disease among the younger population may facilitate the transmission of TB in the community due to

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greater mobility of youth. A gender analysis of the TB epidemic shows thatTB affects different genders differently. In 2016, about 40% of the 2.79 million new cases of TB in India were among women and the male to female ratio for TB stood between1.07 to 2.25 with women accounting for 40% of new cases. In our study, it was 3.5. Studies have shown that women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as high burden of household work, illiteracy, restricted mobility as well as lack of autonomy. There is also a high level of stigma associated with the disease among unmarried females[9].

WHO's current policies and guidance recommend that the Xpert MTB/RIF assay may be used as an initial diagnostic test in individuals suspected of having MDR-TB. About 36% of the samples included in our study were positive for *Mycobacterium tuberculosis* complex and the overall rate of resistance to rifampicin was 21.6%.We assessed the burden of tuberculosis in a large cohort of consecutive patients in our hospital thereby eliminating any selection bias in the study population. We also recovered 34 isolates of Non-tuberculous Mycobacteria (NTM) from various samples and all these isolates were negative by the Xpert MTB/RIF assay.

In a study carried out in Mumbai, India's commercial capital and one of the most densely populated and congested cities, Udwadia et al tested 1539 samples at a tertiary care private hospital and reported MDR-TB in 30.14% of cases[10]. In another retrospective study from South India, Shivekar et al performed the MTBDRplus assay on 20245 specimens obtained from presumptive MDR-TB cases during a 6-year study period from 2013 to 2018.Based on the*rpoB*gene, true resistance, hetero-resistance, and inferredresistance to rifampicin was found in 38%, 29.3%, and 32.7% of the 1582 MDR cases, respectively.[11]Goyal et al published a recent systematic review of 75 epidemiological studies for the prevalence of DRTB in India across 2 decades, from 1995 to 2015.Comparative analysis revealed a worsening trend in DR-TB between the two study decades, 37.7% vs 46.1% respectively. The

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countrywide prevalence of MDR-TB also increased fromthe earlier decade at 14.9% to 27.9% in decade 2.[12]However, the report of the first national anti-tuberculosis drug resistance survey in India conducted during 2014-2016 concluded that among all TB patients tested, the MDR-TB rate was 6.19% with 2.84% among new and11.60% among previously treated TB patients. The survey has probably under-estimated the true burden of resistance in India since it excluded both smear-negative TB cases as well as extrapulmonary TB and did not include the private sector.[8]

We also attempted to find the overall agreement of the Xpert MTB/RIF assay compared to culture in our study cohort. In the present study, the sensitivity of the test was nearly 87% and it rose to 100% for smear-positive specimens. The accuracy of the MTB/RIF test to detect the presence of tuberculosis in smear-positive cases has been reported to be between 98% to 100%.[13]For smear negative specimens, Zeka et al have reported sensitivities of 68.6% and 47.7% in pulmonary and extra-pulmonary samples respectively.[14] Sixty nine specimens that were culture positive tested negative by the Xpert MTB/RIF assay in our study resulting in a specificity of 61.6% (69/112).All these samples were smear negative. We also detected 413 cases (40% of 1032 positives) by the Xpert MTB/RIF assay that were missed by both smear as wellas culture. The assay achieved higher diagnostic yield than microscopy and increased TB case finding by a factor of about 2.

The results of second line testing in our study revealed 77.4% resistance to fluroquinolones among RR isolates which is higher than other studies reported from India. Sethi et al in a retrospective study from a tertiary care center in northern India have documented an overall rate of 38.6% FQ resistance among 863 rifampicin-resistant TB isolates.[15] In another study from eight health care facilities in greater Mumbai between 2005 and 2013,Dalal et al investigated the trends over time of patterns of drug resistance in a sample of MDR-TB patients. Between 2005–2007 and 2011–2013, patients with ofloxacin and moxifloxacin

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resistance significantly increased from 57.6% to 75.3% and from 60.0% to 69.5% ($p<0.05$).[16] A meta-analysis by Ho et al has concluded that globally FQ resistance in MTB is largely confined to MDR strains and knowledge of the global extent of this resistance pattern is currently hampered by the absence of surveillance studies in the majority of regions where TB is endemic.[17]

Updated WHO guidelines, published in June 2020, recommend that for patients with MDR-TB and additional fluoroquinolone resistance, a regimen composed of bedaquiline, pretomanid, and linezolid may be used under operational research conditions (6-9 months).[5] Chee et al in a study conducted between 2002-2016 on 280 patients have demonstrated that only about 30% of patients with MDR pulmonary TB diagnosed in their study cohort from South-east Asia were eligible for the WHO shorter MDR-TB treatment regimen.[18]In a similar study from northern India, Singh and Jain have explored the eligibility of the shorter regimen in MDR patients under the programmatic setting. Outof 541 conclusive LPA-SLD results, the proportion of strains resistant to only fluoroquinolones was nearly 50% while 8.3% were resistant to both fluoroquinolones and SLIDs. [19]Eleven cases in our study were extensively drug resistant.

The high rates of drug resistance observed in our study may be due to the fact that ours is a tertiary care hospital in the state of Uttar Pradesh which has over 20% of the total number of notified cases of TB in India. We see patients after the referring hospital has already tried and failed to control infection using a combination of different anti-microbial agents. Since facilities for microbiological studies are usually not available in district hospitals/smaller cities in India, the first contact physician/surgeon/referral facility are compelled to initiate broad-spectrum antibiotics. Indiscriminate antimicrobial therapy without establishing the etiology of infection selects out the resistant strains. McDowell and Pai in an ethnographic study on the mismanagement of empirical TB treatment in India have demonstrated that all

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non-specialist private practitioners began antibiotic treatment, especially quinolones, for persistent cough before prescribing a test.[20]Their results underscore the fact that inappropriate prescribing practices in India's burgeoning private sector including easy, over-the-counter access to fluoroquinolones need to be halted as soon as possible.

The alarming rate of drug resistance in our study to rifampicin as well as fluoroquinolones has important implications for implementation of government strategies to control the TB epidemic in India. Firstly, standardized regimens containing a FQ to treat MDR –TB cases carry a high risk of being sub-optimal and resulting in treatment failure. Secondly, with such high rates of drug resistance India will have to equip itself with enough Mycobacteriology laboratories offering culture and drug susceptibility testing (C-DST) to both first as well as second-line agents. Currently, the focus is to roll out sufficient number of GeneXpert MTB/RIF assay machines to diagnose rifampicinresistant strains of *Mycobacterium tuberculosis*. However, this strategy may mask the diagnosis of pre-XDR TB. A high rate of FQ resistance has also been noted in newly diagnosed MDR/RR TB cases, which might be due to transmission of the drug-resistant strains. [15]It is estimated that in India, by 2032, 85% of multidrug-resistant tuberculosis infections would be from primary transmission, compared with only 15% in 2012. In the *Lancet Public Health,* Law et al have created a dynamic model of the tuberculosis epidemic in India, which they use to estimate the incidence of drug susceptible tuberculosis and multidrug-resistant tuberculosis over the next 20 years[2]. They have analyzed the emergence of drug resistance in all major health-care sectors in India. Private clinics in India are often used by patients seeking TB treatment and they administer regimens that are not recommended by standard guidelines. This not only results in suboptimal outcome but also potentially generates MDR TB. They conclude that as multidrug resistant tuberculosis transitions from an acquired condition to a primarily transmitted disease, improving the effectiveness of drug-susceptible tuberculosis treatment can no longer

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contain the spread of the epidemic. This epidemiological shift has profound resource implications since the cost of treatment of multidrug-resistant tuberculosis treatment can exceed that of first-line tuberculosis therapy by a factor of ten or more.

In addition, notification data from low-income and middle-income countries, are prone to underreporting and cannot be interpreted without additional information on case detection rate. The DR-TB diagnostic algorithm as given in the PMDT guidelines recommends SL – LPA testing for all RR TB cases diagnosed by the CBNAAT assay. However, it is labor intensive and requires trained manpower. Severe lack of Microbiology laboratories providing universal DST and visual interpretation of bands is a huge limitation especially in smear negative and extra-pulmonary cases with inadequate sample volumes as has been our experience even with version 2 of the test.

There were several limitations to this study. One of the methodological limitations of our study was that we could not perform liquid culture DST as well as sequencing and confirm the results of the drug resistant isolates. Another limitation was that we did not differentiate between new and previously treated TB cases. Most of the DR-TB patients in our cohort at the time of diagnosis were attached to the PMDT follow up for further evaluation and management except for some who insisted on institutional management. We could therefore put up SL-DST for only 62 cases. We also did not receive any grant for this study and hence could not put up FL-LPA on the 69 culture positive isolates that tested negative by the Xpert MTB/RIF assay. In addition, a study of risk factors in such a high burden setting would have allowed us to offer more useful remedies to policy makers.

CONCLUSION

In conclusion, we have not come across any prospective study from India on such a large number of pulmonary as well as extrapulmonary samples performed by both conventional and molecular methods. Our study provides comprehensive data on the high burden of drug

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resistant TB in India at a 1200 bed tertiary care center in northern India. The need of the hour is to have enough Mycobacteriology laboratories offering both first and second line DST under the NTEP umbrella.The high rates of FQ resistance documented in our study should prompt policy makers to tightly regulate them as reserve drugs, otherwise the ambitious goal of the Government of India to eliminate tuberculosis by 2025 seems bleak.

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Contributors RM conceptualized the manuscript, designed the methods, supervised the study and wrote the manuscript. VK curated the data. AN supervised the study and edited the manuscript. All authors have reviewed the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study protocol was approved by the Instituitional Ethics Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences SGPGIMS: (IEC 2017-37-IMP-EXP).Written informed consent was obtained from each participant.

Data availability statement Data are available upon reasonable request.

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20. McDowell A, Pai M. Treatment as diagnosis and diagnosis as treatment: empirical management of presumptive tuberculosis in India .*Int J tubercLung Dis. 2016;*20:536–543.

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Table 1. Smear and culture results among samples positive by Xpert MTB /RIF assay (n=1032)

Sample (N=1032) (%)	Smear positive	Culture positive (%)
Pulmonary(n=706)	382(54.1%)	384(54.3%)
Extra-pulmonary(n=326)	125(38.3%)	133(40.7%)
Total	507(49.1%)	517(50%)

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Table 2: Comparison of Gene Xpert MTB / RIF positive, MTB culture positive results with smear results

SmearMTB culture + Gene Xpert+	MTB culture + Gene Xpert-		MTB culture– Gene Xpert+	MTB culture– Gene Xpert–	Total
Positive	336	0	171	0	507
Negative	112	69	413	1944	2538
Total	448	69	584	1944	3045

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Figure legends

Figure 1. Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)

Figure 2. Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)

Figure 3. Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay of samples included in the study (n=3045)

Figure 4. Distribution of samples among rifampicin resistant cases (n=223)

Figure 5: Results for second line susceptibility testing performed by line probe assay (LPA) version2

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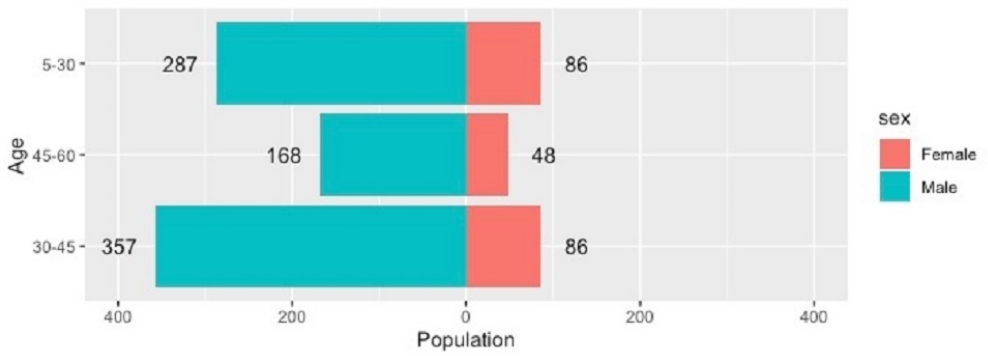

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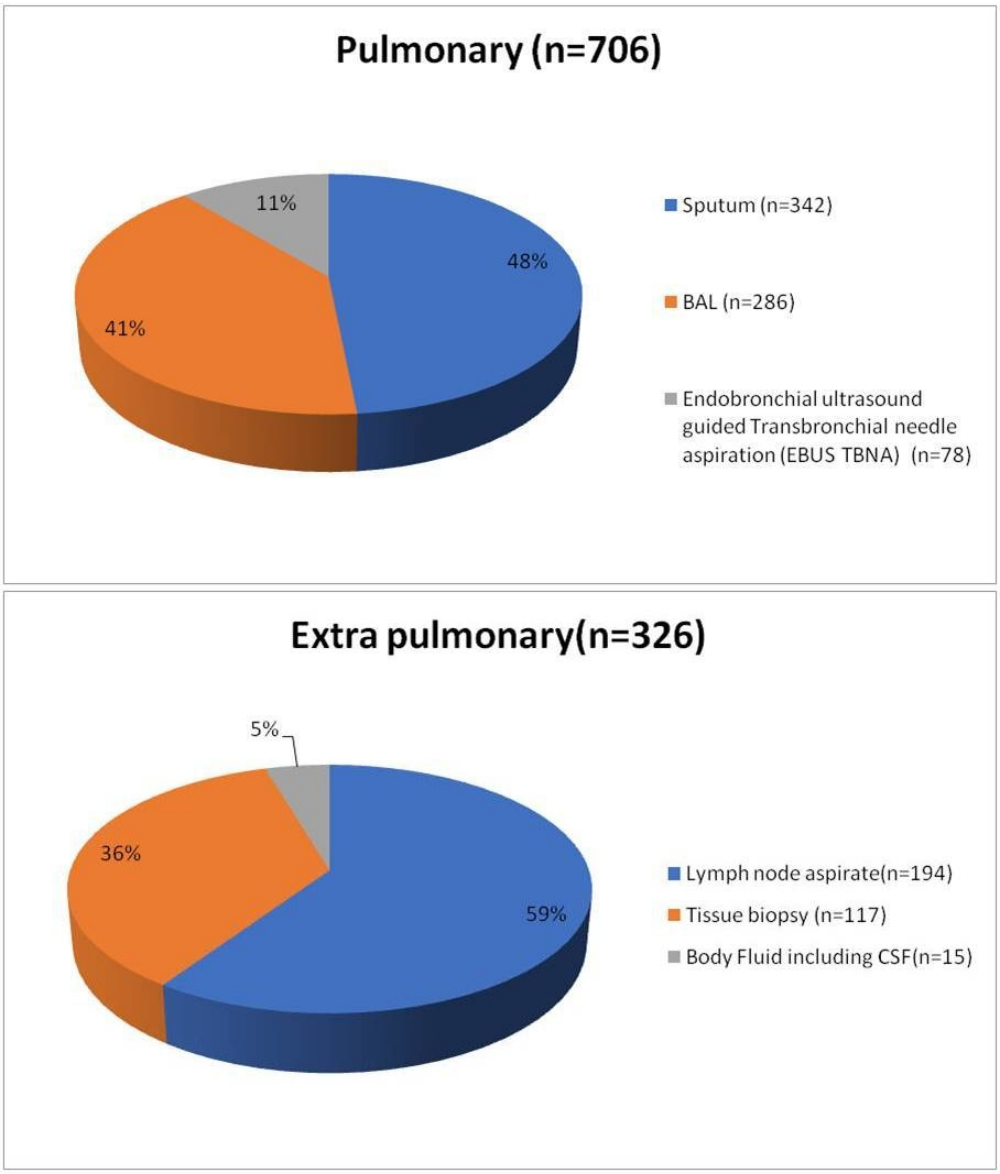

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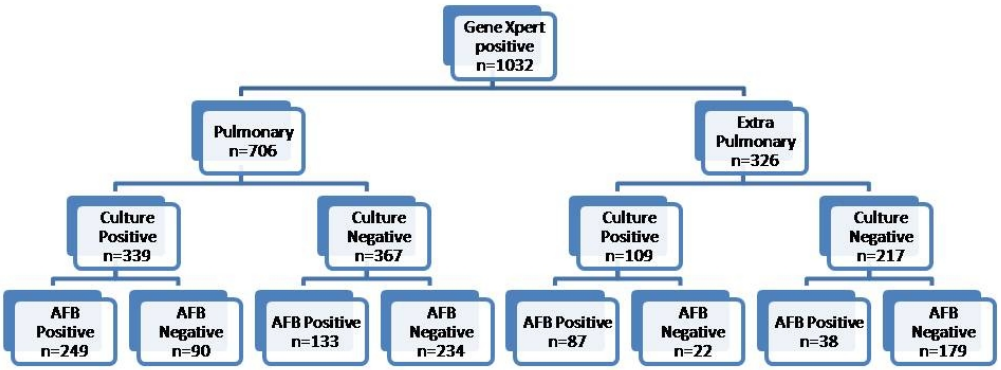

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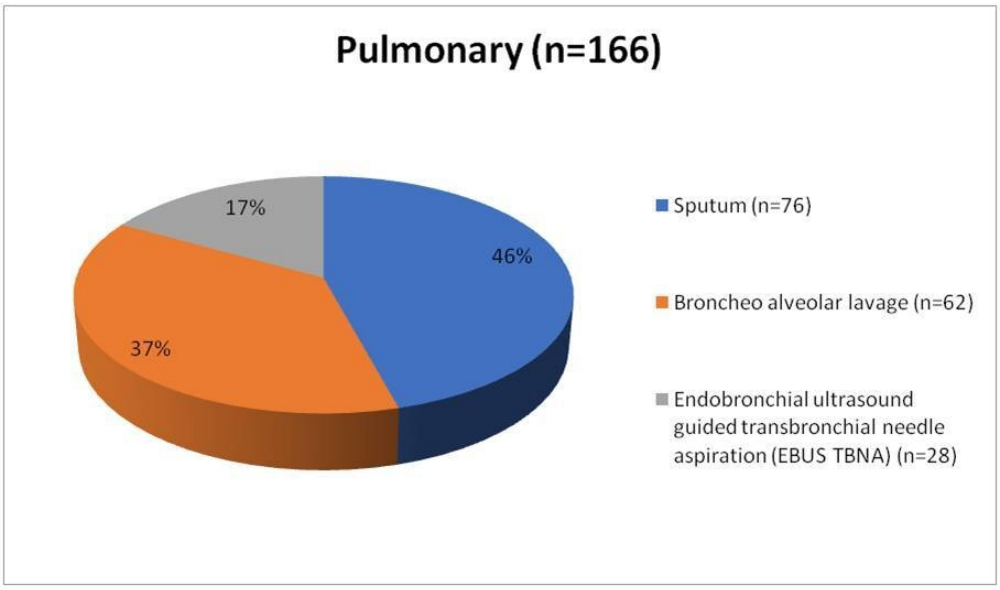

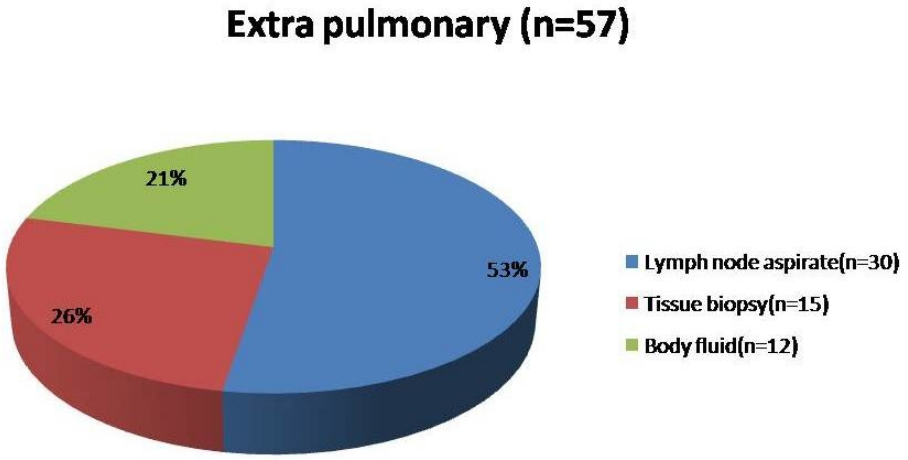

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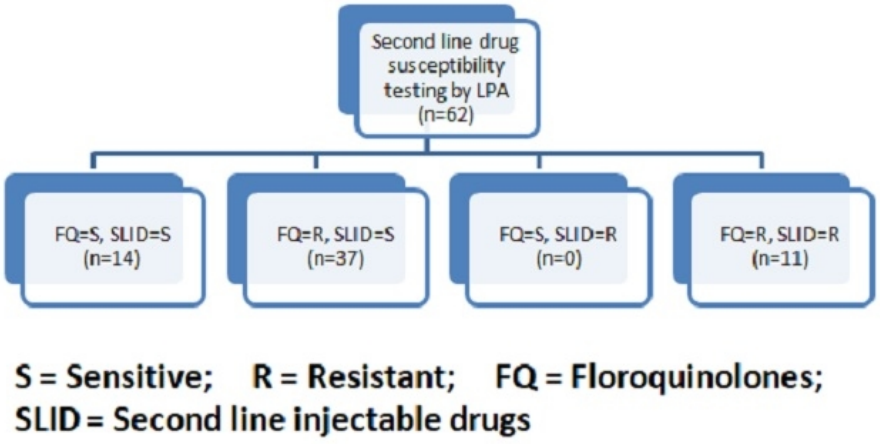

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #/Line no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	#1/2-3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	#2/23-38
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	#4/72-87
Objectives	3	State specific objectives, including any prespecified hypotheses	#4/88-90
Methods			
Study design	4	Present key elements of study design early in the paper	#4/93-96
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	#4/93-97 #6/124-125
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	#6/128-142
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	#7/166-181
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*NA*
Bias	9	Describe any efforts to address potential sources of bias	#9/203-205
Study size	10	Explain how the study size was arrived at	#5/100-104
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	#8/168-175
		(*b*) Describe any methods used to examine subgroups and interactions	#7/152-153
		(*c*) Explain how missing data were addressed	#6/120-122
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	#8/168-175
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	#6/126-142
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	#7/159-161 #8/177-178
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	#7/152-154
		(b) Indicate number of participants with missing data for each variable of interest	#6/1120-122 #13/307-310
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	#7/152-154
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	#7/168-172
Discussion			
Key results	18	Summarise key results with reference to study objectives	#13/314-321
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	#11/251-258 #13/304-312
Generalisability	21	Discuss the generalisability (external validity) of the study results	#13/317-321
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	#13/307-312

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a prospective single centre cohort study

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TITLE PAGE

Title: Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a prospective single centre cohort study

Authors and affiliations: RichaMisra[a], VasudhaKesarwani[a], Alok Nath*[b]

[a]MD, Department of Microbiology, Division Mycobacteriology, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

[b]MD, DM, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences,Lucknow-226014,India

*__Corresponding author:__ Dr. Alok Nath, Professor, Department of Pulmonary Medicine, Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow-226014, India.

Corresponding author E mail:

dralоksgpgi@gmail.com

Phone: +919839070065

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Abstract

Objectives: We aim to define the burden of rifampicin monoresistant tuberculosis at a tertiary care centre in Northern India as well as determine the second line drug susceptibilities in a subset of patients.

Methods: A total of 3045 pulmonary (n=1883) and extra-pulmonary (n=1162) samples from likely tuberculosis patients were subjected to microscopy, culture and the Xpert MTB/RIF assay from March 2017 to June 2019.Second line drug susceptibility testing by version2 Line Probe Assay for fluoroquinolones (FQs) and second line injectable drugs (SLIDs) was performed on 62 samples.

Results: Out of 3045 samples processed in our lab during the study period, 36.1% (1101/3045) were positive for MTBC and 21.6% were rifampicin mono-resistant (223/1032). The rate of rifampicin resistance in pulmonary samples was 23.5% (166/706) and in extrapulmonary cases it was 17.4% (57/326).Out of 62 cases included for second line testing, 48 were resistant to FQs (77.4%) while 11 were extensively drug resistant(XDR).

Conclusions: India urgently needs to arrest an emerging multidrug-resistant tuberculosis epidemic with associated resistance to fluoroquinolones. A robust surveillance system is needed to execute the National strategic Plan (NSP) for 2017-2025.

Keywords: Tuberculosis; Multi-drug resistant; Xpert MTB/RIF assay; Line Probe Assay

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Article summary section

Strengths and limitations of this study

- Study has a large sample size of 3045 samples.
- 1162 extrapulmonary samples have been included such as EBUS-TBNA and biopsies
- Both Xpert MTB/RIF assay and Line probe assay have been performed
- MGIT-DST was not performed
- DNA squencing was not done on drug-resistant isolates

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INTRODUCTION

India has the highest Tuberculosis (TB) burden in the world and is home to 26% of the world's estimated 10.4 million annual tuberculosis cases.[1] The menace of drug resistant-TB (DR-TB), prompted the government to initiate the programmatic management of drug resistant TB (PMDT) in 2007 which integrates all programme based strategies for DR-TB diagnosis, management and treatment under the NTEP, National Tuberculosis Elimination Programme (renamed in December 2019)[2]. India also has a complex as well as unorganized health-care system which includes the government sector, private sector and informal health care providers practicing non-allopathic schools of medicine such as ayurveda and homeopathy[3]. Though TB was made a notifiable disease in 2012, less than 40% cases from the private sector were notified to the government in 2017[4].

The shorter drug regimen of 9-12 months for MDR-TB patients was introduced by World Health Organization (WHO), in May 2016 and updated in June 2020.[5,6]It was recommended in patients who have not been previously treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable agents has been excluded. However, drug susceptibility testing in India is technically challenging and requires specialist laboratory facilities and personnel .The TB laboratory network has been expanded over the years to provide better access to quality assured diagnostic services. Laboratory services are now being provided free of cost to patients attending public health facilities as well as those referred from the private sector.[7]

With this background, we aim to define the burden of rifampicin mono-resistant tuberculosis at a tertiary care referral medical center in northern India as well as determine the second line drug susceptibilities in a subset of patients.

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METHODS

Study design and setting

This prospective observational study between March 2017 to June 2019 was conducted in the Mycobacteriology section of the Department of Microbiology at Sanjay Gandhi Postgraduate Institute of Medical Sciences, a 1200 bed tertiary care referral medical center in northern India. Institutional ethics committee of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) approved the study protocol (IEC code 2017-37-IMP-EXP) and waiver of consent was obtained.

Clinical specimens

Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/ Fine Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected between March 2017 and June 2019 during the clinical routine. All samples were divided into 2 portions on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF assay while microscopy and culture was performed from the remaining sample. Direct smears were prepared from the specimens using Ziehl-Neelsen staining. All non-sterile clinical samples were processed using the *N*-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method. Samples were decanted following centrifugation, and sediments were re-suspended in 3 ml of phosphate buffer solution. Processed samples were used to inoculate either Lowenstein-Jensen (LJ) solid medium or BacT/Alert culture. Line probe assay *version2* (LPA*v2*) for second line testing was performed on either direct clinical samples if volume was adequate or on positive culture. Both Xpert MTB/RIF assay and LPA*v2* were performed according to the manufacturer's protocol.

All cases detected positive by the Xpert MTB/RIF assay were grouped into (i) those with

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smear-positive and culture positive tuberculosis; (ii) those with smear-negative, culture-positive tuberculosis; (iii) those who were both smear and culture negative for tuberculosis but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or radiological findings (clinical tuberculosis).

There was a sub group of samples that were culture positive but missed by the Xpert MTB/RIF assay. We put up the TB Ag MPT64 Rapid test (SD BIOLINE) on all these positive cultures.

Data collection

The medical records of patients were retrieved from the Hospital Information System. A senior resident extracted patient data prospectively from charts.

Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin resistant/multi-drug resistant/extensively drug resistant)[8]

A*bacteriologically confirmed TB case***:** One from whom a biological specimen was positive by smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert MTB/RIF assay.

*Pulmonary tuberculosis (PTB)***:** Any bacteriologically confirmed or clinically diagnosed case of TB involving lung parenchyma or tracheobronchial tree.

Extrapulmonary tuberculosis (EPTB): Any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges.Concomitant pulmonary lesions were ruled out in all cases by appropriate investigations and review of case files.

Multidrug resistance TB (MDR)**:**A TB patient, whose biological specimen is resistant to both H and R with or without resistance to other first-line anti-TB drugs.

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Pre-XDR-TB: It is defined as TB with resistance to isoniazid and rifampicin and either a FQ or a second-line injectable agent but not both.

*Extensive drug resistance (XDR):*A MDR-TB patient whose biological specimen isadditionally resistant to at least a FQ and a SLI anti-TB drug.

Patient and public involvement

Patients were involved in the reporting of our research in this study.

RESULTS

During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with concomitant smear and culture inoculation on the same sample. All duplicate isolates were excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary) were detected for MTB complex. The assay failed to detect sixty nine samples that were culture positive. The MPT64 antigen test was positive on all these cultures. There were 806 (78.10%) males and 226 (21.89%) females among the positive specimens. The median age of patients was 32 years and nearly 43% patients were young adults in the age group of 30-45 yearsas shown in Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic biopsy) were the most common samples in extra-pulmonary cases that were positive. The sample distribution of positive specimens is shown in Figure 2. Out of 1032 samples detected positive by the CBNAAT assay, 507 and 517 specimens were smear and culture positive respectively. The rate of smear and culture positivity in pulmonary and extra-pulmonary cases was 54.1%, 54.3%, 38.3% and 40.7% respectively (Table-1). The results of conventional and molecular diagnostic testing by Xpert MTB/RIF assay of patients included in the study is shown in Figure 3.

During the study period, we also recovered 34 isolates of Non-tuberculous Mycobacteria (NTM) from various pus and respiratory specimens. These were *Mycobacterium abscessus*

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(n=15), *Mycobacterium intracellulare*(7), *Mycobacterium fortuitum* (6), *Mycobacterium gordonae*(n=3) and *Mycobacterium simiae*(n=3).

Rifampicin monoresistance was detected in 223 out of 1032 samples (21.6%). It was 23.5% (n=166/706) and 17.4% (57/326) in pulmonary and extra-pulmonary cases respectively (Figure 4).A summary of the performance data is shown in Table 2. Five hundred and seventeen samples were positive by culture resulting in an 86.6% agreement with the Xpert MTB/RIF assay. The assay had a 100% agreement for culture positive, smear positive specimens and 61.6% agreement for culture positive, smear negative specimens for the detection of *Mycobacterium tuberculosis.* Sixty nine samples that were culture positivetested negative by the Xpert MTB/RIF assay. We did not have any sample that was positive on both smear and culture but was negative by Xpert MTB/RIF assay. As shown in Table 2, we detected 413 more patients than we could have diagnosed by smear and/or culture alone.

Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing byLPA*v2.0* for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure 5, majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR) while 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that was aminoglycoside resistant but FLQ sensitive.

Discussion

Multidrug-resistant tuberculosis is one of the greatest public health challenges worldwide. To the best of our knowledge, ours is the first study from India to determine the burden of drug resistant tuberculosis by testing such a large number of pulmonary and extra-pulmonary clinical samples. As per Global TB Report 2020, eight countries accounted for two thirds of the global total: India (26%), Indonesia (8.5%), China (8.4%), the Phillippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%).[1]

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The results of the national anti-tuberculosis drug resistance survey has shown that the incidence of TB is highest in the 25–34 year age group in India[9]. We however documented a slightly higher age group in our study. Our cohort was dominated by males and nearly 43% patients were young adults in the age group of 30-45 years. The high frequency of the disease among the younger population may facilitate the transmission of TB in the community due to greater mobility of youth. A gender analysis of the TB epidemic shows that TB affects different genders differently. In 2016, about 40% of the 2.79 million new cases of TB in India were among women and the male to female ratio for TB stood between1.07 to 2.25 with women accounting for 40% of new cases. In our study, it was 3.5. Studies have shown that women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as high burden of household work, illiteracy, restricted mobility as well as lack of autonomy. There is also a high level of stigma associated with the disease among unmarried females[10].

WHO's current policies and guidance recommend that the Xpert MTB/RIF assay may be used as an initial diagnostic test in individuals likely of having MDR-TB. About 36% of the samples included in our study were positive for *Mycobacterium tuberculosis* complex and the overall rate of resistance to rifampicin was 21.6%.We assessed the burden of tuberculosis in a large cohort of consecutive patients in our hospital thereby eliminating any selection bias in the study population. We also recovered 34 isolates of Non-tuberculous Mycobacteria (NTM) from various samples and all these isolates were negative by the Xpert MTB/RIF assay.

In a study carried out in Mumbai, India's commercial capital and one of the most densely populated and congested cities, Udwadia et al tested 1539 samples at a tertiary care private hospital and reported MDR-TB in 30.14% of cases[11]. In another retrospective study from South India, Shivekar et al performed the MTBDRplus assay on 20245 specimens obtained from presumptive MDR-TB cases during a 6-year study period from 2013 to 2018.Based on the*rpoB*gene, true resistance, hetero-resistance, and inferredresistance to rifampicin was

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found in 38%, 29.3%, and 32.7% of the 1582 MDR cases, respectively.[12]Goyal et al published a recent systematic review of 75 epidemiological studies for the prevalence of DRTB in India across 2 decades, from 1995 to 2015.Comparative analysis revealed a worsening trend in DR-TB between the two study decades, 37.7% vs 46.1% respectively. The countrywide prevalence of MDR-TB also increased from the earlier decade at 14.9% to 27.9% in decade 2.[13]However, the report of the first national anti-tuberculosis drug resistance survey in India conducted during 2014-2016 concluded that among all TB patients tested, the MDR-TB rate was 6.19% with 2.84% among new and11.60% among previously treated TB patients. The survey has probably under-estimated the true burden of resistance in India since it excluded both smear-negative TB cases as well as extrapulmonary TB and did not include the private sector.[9]

We also attempted to find the overall agreement of the Xpert MTB/RIF assay compared to culture in our study cohort. In the present study, the sensitivity of the test was nearly 87% and it rose to 100% for smear-positive specimens. The accuracy of the MTB/RIF test to detect the presence of tuberculosis in smear-positive cases has been reported to be between 98% to 100%.[14]For smear negative specimens, Zeka et al have reported sensitivities of 68.6% and 47.7% in pulmonary and extra-pulmonary samples respectively.[15] Sixty nine specimens that were culture positive tested negative by the Xpert MTB/RIF assay in our study resulting in a specificity of 61.6% (69/112).All these samples were smear negative. We also detected 413 cases (40% of 1032 positives) by the Xpert MTB/RIF assay that were missed by both smear as wellas culture. The assay achieved higher diagnostic yield than microscopy and increased TB case finding by a factor of about 2.

The results of second line testing in our study revealed 77.4% resistance to fluroquinolones among RR isolates which is higher than other studies reported from India. Sethi et al in a retrospective study from a tertiary care center in northern India have documented an overall

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rate of 38.6% FQ resistance among 863 rifampicin-resistant TB isolates.[16] In another study from eight health care facilities in greater Mumbai between 2005 and 2013, Dalal et al investigated the trends over time of patterns of drug resistance in a sample of MDR-TB patients. Between 2005–2007 and 2011–2013, patients with ofloxacin and moxifloxacin resistance significantly increased from 57.6% to 75.3% and from 60.0% to 69.5% ($p<0.05$).[17] A meta-analysis by Ho et al has concluded that globally FQ resistance in MTB is largely confined to MDR strains and knowledge of the global extent of this resistance pattern is currently hampered by the absence of surveillance studies in the majority of regions where TB is endemic.[18]

Updated WHO guidelines, published in June 2020, recommend that for patients with MDR-TB and additional fluoroquinolone resistance, a regimen composed of bedaquiline, pretomanid, and linezolid may be used under operational research conditions (6-9 months).[5] Chee et al in a study conducted between 2002-2016 on 280 patients have demonstrated that only about 30% of patients with MDR pulmonary TB diagnosed in their study cohort from South-east Asia were eligible for the WHO shorter MDR-TB treatment regimen.[19]In a similar study from northern India, Singh and Jain have explored the eligibility of the shorter regimen in MDR patients under the programmatic setting. Out of 541 conclusive LPA-SLD results, the proportion of strains resistant to only fluoroquinolones was nearly 50% while 8.3% were resistant to both fluoroquinolones and SLIDs. [20]Eleven cases in our study were extensively drug resistant.

The high rates of drug resistance observed in our study may be due to the fact that ours is a tertiary care hospital in the state of Uttar Pradesh which has over 20% of the total number of notified cases of TB in India. We see patients after the referring hospital has already tried and failed to control infection using a combination of different anti-microbial agents. Since facilities for microbiological studies are usually not available in district hospitals/smaller

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cities in India, the first contact physician/surgeon/referral facility are compelled to initiate broad-spectrum antibiotics. Indiscriminate antimicrobial therapy without establishing the etiology of infection selects out the resistant strains. McDowell and Pai in an ethnographic study on the mismanagement of empirical TB treatment in India have demonstrated that all non-specialist private practitioners began antibiotic treatment, especially quinolones, for persistent cough before prescribing a test.[21]Their results underscore the fact that inappropriate prescribing practices in India's burgeoning private sector including easy, over-the-counter access to fluoroquinolones need to be halted as soon as possible.

The alarming rate of drug resistance in our study to rifampicin as well as fluoroquinolones has important implications for implementation of government strategies to control the TB epidemic in India. Firstly, standardized regimens containing a FQ to treat MDR –TB cases carry a high risk of being sub-optimal and resulting in treatment failure. Secondly, with such high rates of drug resistance India will have to equip itself with enough Mycobacteriology laboratories offering culture and drug susceptibility testing (C-DST) to both first as well as second-line agents. Currently, the focus is to roll out sufficient number of GeneXpert MTB/RIF assay machines to diagnose rifampicin resistant strains of *Mycobacterium tuberculosis*. However, this strategy may mask the diagnosis of pre-XDR TB. A high rate of FQ resistance has also been noted in newly diagnosed MDR/RR TB cases, which might be due to transmission of the drug-resistant strains. [3]It is estimated that in India, by 2032, 85% of multidrug-resistant tuberculosis infections would be from primary transmission, compared with only 15% in 2012. In the *Lancet Public Health,* Law et al have created a dynamic model of the tuberculosis epidemic in India, which they use to estimate the incidence of drug susceptible tuberculosis and multidrug-resistant tuberculosis over the next 20 years[3]. They have analyzed the emergence of drug resistance in all major health-care sectors in India. Private clinics in India are often used by patients seeking TB treatment and they administer

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regimens that are not recommended by standard guidelines. This not only results in suboptimal outcome but also potentially generates MDR TB. They conclude that as multidrug resistant tuberculosis transitions from an acquired condition to a primarily transmitted disease, improving the effectiveness of drug-susceptible tuberculosis treatment can no longer contain the spread of the epidemic. This epidemiological shift has profound resource implications since the cost of treatment of multidrug-resistant tuberculosis treatment can exceed that of first-line tuberculosis therapy by a factor of ten or more.

In addition, notification data from low-income and middle-income countries, are prone to underreporting and cannot be interpreted without additional information on case detection rate. The DR-TB diagnostic algorithm as given in the PMDT guidelines recommends SL – LPA testing for all RR TB cases diagnosed by the CBNAAT assay. However, it is labor intensive and requires trained manpower. Severe lack of Microbiology laboratories providing universal DST and visual interpretation of bands is a huge limitation especially in smear negative and extra-pulmonary cases with inadequate sample volumes as has been our experience even with version 2 of the test.

There were several limitations to this study. One of the methodological limitations of our study was that we could not perform liquid culture DST as well as sequencing and confirm the results of the drug resistant isolates. Another limitation was that we did not differentiate between new and previously treated TB cases. Most of the DR-TB patients in our cohort at the time of diagnosis were attached to the PMDT follow up for further evaluation and management except for some who insisted on institutional management. We could therefore put up SL-DST for only 62 cases. We also did not receive any grant for this study and hence could not put up FL-LPA on the 69 culture positive isolates that tested negative by the Xpert MTB/RIF assay. In addition, a study of risk factors in such a high burden setting would have allowed us to offer more useful remedies to policy makers.

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CONCLUSION

In conclusion, we have not come across any prospective study from India on such a large number of pulmonary as well as extrapulmonary samples performed by both conventional and molecular methods. Our study provides comprehensive data on the high burden of drug resistant TB in India at a 1200 bed tertiary care center in northern India. The need of the hour is to have enough Mycobacteriology laboratories offering both first and second line DST under the NTEP umbrella.The high rates of FQ resistance documented in our study should prompt policy makers to tightly regulate them as reserve drugs, otherwise the ambitious goal of the Government of India to eliminate tuberculosis by 2025 seems bleak.

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Contributors RM conceptualized the manuscript, designed the methods, supervised the study and wrote the manuscript. VK curated the data. AN supervised the study and edited the manuscript. All authors have reviewed the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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Ethics approval The study protocol was approved by the Instituitional Ethics Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences. Written informed consent was obtained from each participant.

Data availability statement Data are available upon reasonable request.

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Table 1. Smear and culture results among samples positive by Xpert MTB /RIF assay (n=1032)

Sample (N=1032) (%)	Smear positive	Culture positive (%)
Pulmonary(n=706)	382(54.1%)	384(54.3%)
Extra-pulmonary(n=326)	125(38.3%)	133(40.7%)
Total	507(49.1%)	517(50%)

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Table 2: Comparison of Gene Xpert MTB / RIF positive, MTB culture positive results with smear results

SmearMTB culture + Gene Xpert+	MTB culture + Gene Xpert-	MTB culture– Gene Xpert+	MTB culture– Gene Xpert–	Total
Positive	336	0	171	0	507
Negative	112	69	413	1944	2538
Total	448	69	584	1944	3045

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Figure legends

Figure 1. Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)

Figure 2. Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)

Figure 3. Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay of samples included in the study (n=3045)

Figure 4. Distribution of samples among rifampicin resistant cases (n=223)

Figure 5: Results for second line susceptibility testing performed by line probe assay (LPA) version2

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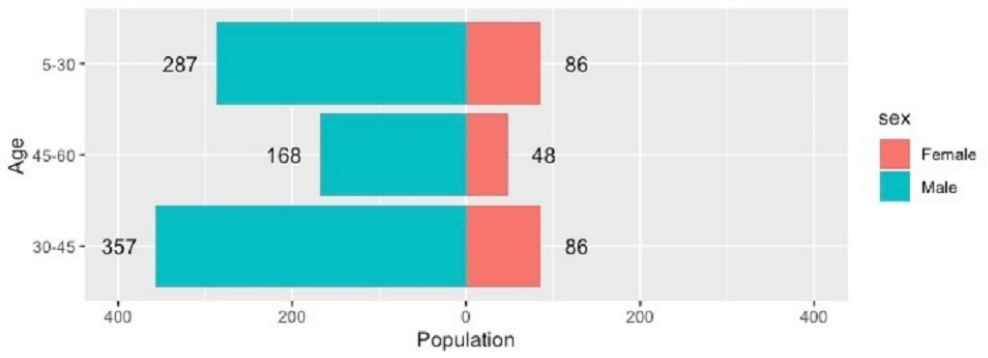

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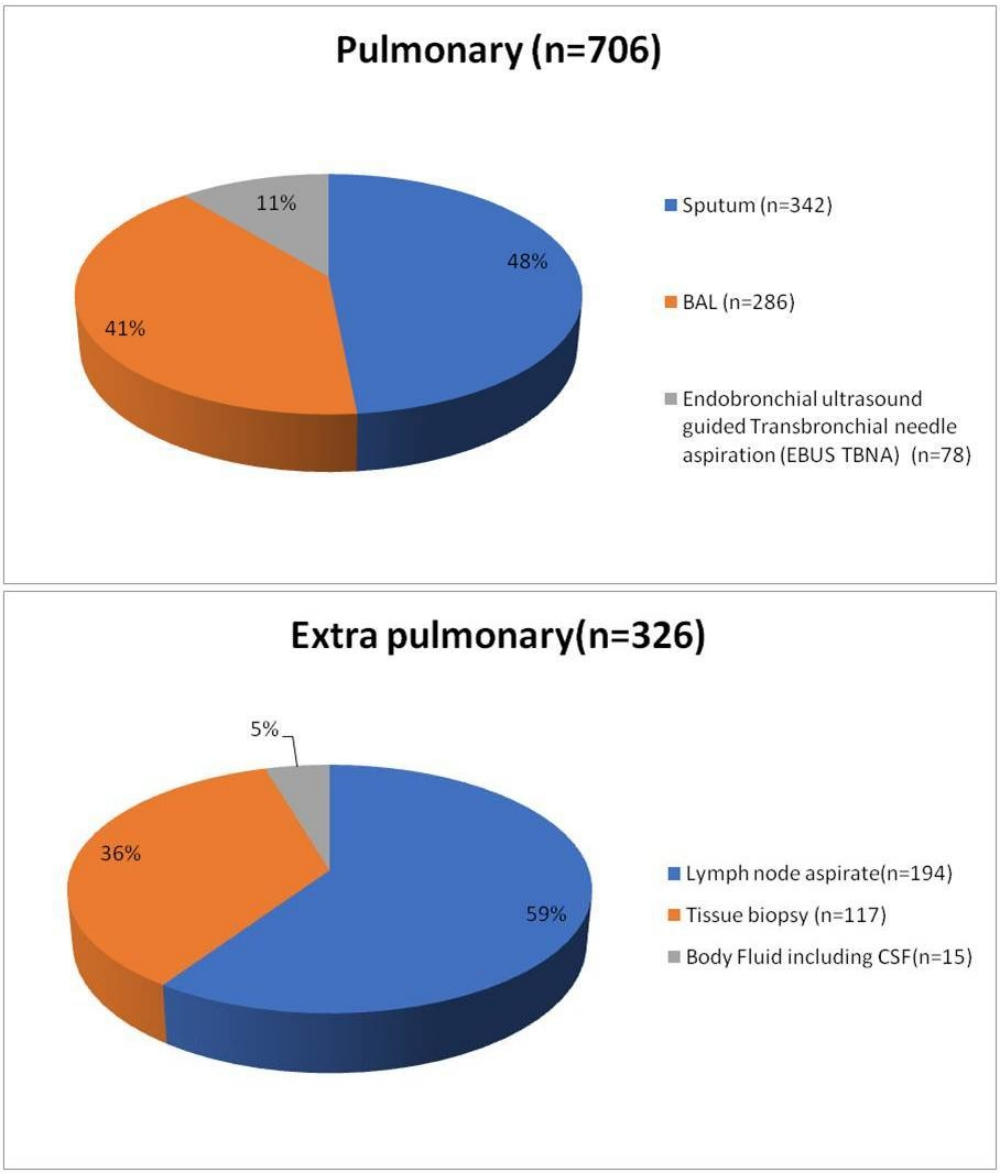

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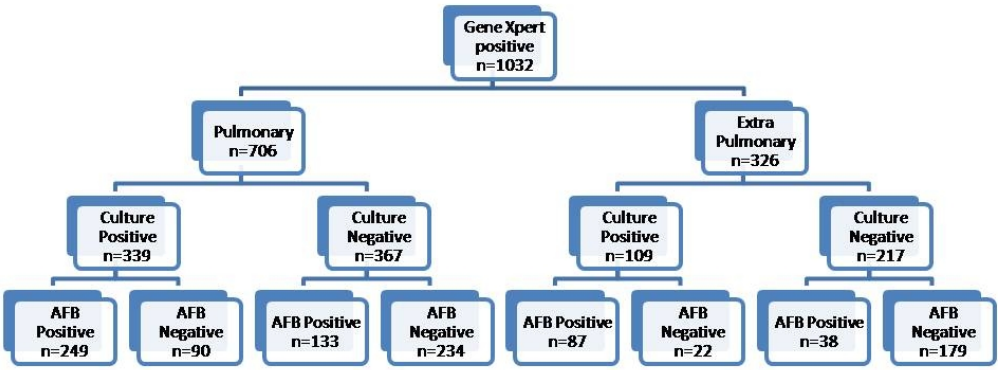

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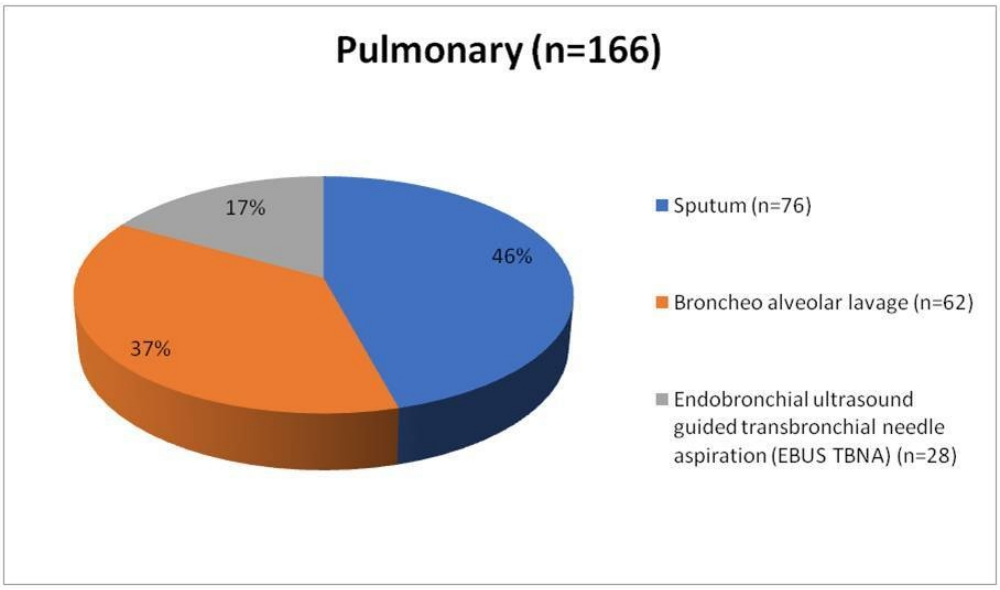

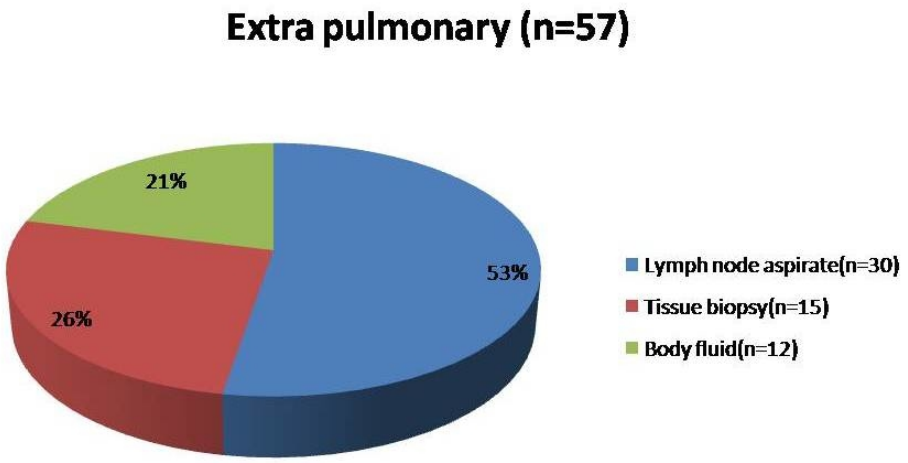

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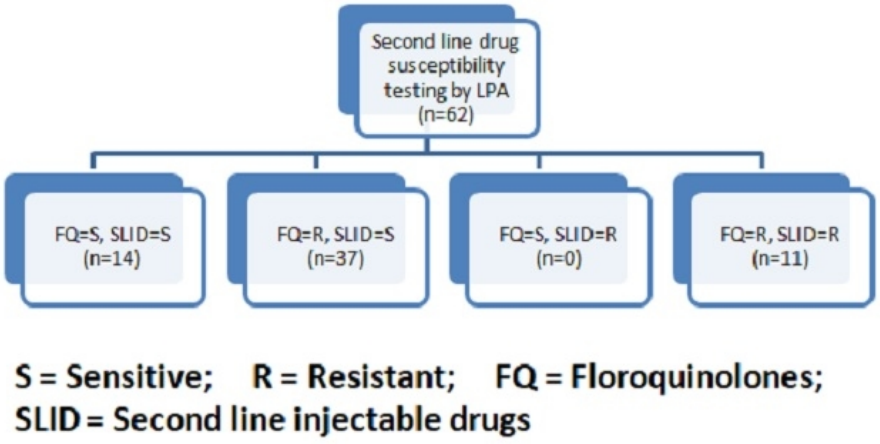

59x29mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #/Line no.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	#1/2-3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	#2/23-38
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	#4/72-87
Objectives	3	State specific objectives, including any prespecified hypotheses	#4/88-90
Methods			
Study design	4	Present key elements of study design early in the paper	#4/93-96
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	#4/93-97 #6/124-125
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	#6/128-142
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	#7/166-181
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*NA*
Bias	9	Describe any efforts to address potential sources of bias	#9/203-205
Study size	10	Explain how the study size was arrived at	#5/100-104
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	NA
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	#8/168-175
		(*b*) Describe any methods used to examine subgroups and interactions	#7/152-153
		(*c*) Explain how missing data were addressed	#6/120-122
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	#8/168-175
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	#6/126-142
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	#7/159-161 #8/177-178
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	#7/152-154
		(b) Indicate number of participants with missing data for each variable of interest	#6/1120-122 #13/307-310
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	#7/152-154
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	#7/168-172
Discussion			
Key results	18	Summarise key results with reference to study objectives	#13/314-321
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	#11/251-258 #13/304-312
Generalisability	21	Discuss the generalisability (external validity) of the study results	#13/317-321
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	#13/307-312

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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