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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

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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 version 2 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

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

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 2016.¹The 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 homeopathy². 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 2016.⁵⁻⁷It 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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3 tertiary care referral medical center in northern India as well as determine the second line drug
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5 susceptibilities in a subset of patients.
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8 **METHODS**

9 **Study design and setting**

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

23
24 Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752
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26 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided
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28 transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/ Fine
29
30 Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected between
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32 March 2017 and June 2019 during the clinical routine. All samples were divided into 2 portions
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34 on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF assay while
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36 microscopy and culture was performed from the remaining sample. Direct smears were prepared
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38 from the specimens using Ziehl-Neelsen staining. All non-sterile clinical samples were processed
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40 using the *N*-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method. Samples were
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42 decanted following centrifugation, and sediments were re-suspended in 3 ml of phosphate buffer
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44 solution. Processed samples were used to inoculate either Lowenstein-Jensen (LJ) solid medium
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46 or BacT/Alert culture. Line probe assay *version2* (LPA_{v2}) for second line testing was performed
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48 on either direct clinical samples if volume was adequate or on positive culture. Both Xpert
49
50 MTB/RIF assay and LPA_{v2} were performed according to the manufacturer's protocol.
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3 All cases detected positive by the Xpert MTB/RIF assay were grouped into (i) those with smear-
4 positive and culture positive tuberculosis; (ii) those with smear-negative, culture-positive
5 tuberculosis; (iii) those who were both smear and culture negative for tuberculosis but who were
6 nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or radiological
7 findings (clinical tuberculosis). There was a sub group of samples that were culture positive but
8 missed by the Xpert MTB/RIF assay.
9

16 **Data collection**

17
18
19 The medical records of patients were retrieved from the Hospital Information System. A senior
20 resident extracted patient data prospectively from charts.
21

22 **Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin 23 resistant/multi-drug resistant/extensively drug resistant)⁹**

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25
26 *A bacteriologically confirmed TB case:* One from whom a biological specimen was positive by
27 smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert
28 MTB/RIF assay.
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32 *Pulmonary tuberculosis (PTB):* Any bacteriologically confirmed or clinically diagnosed case of
33 TB involving lung parenchyma or tracheobronchial tree.
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37 *Extrapulmonary tuberculosis (EPTB):* Any bacteriologically confirmed or clinically diagnosed
38 case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen,
39 genitourinary tract, skin, joints and bones, meninges.
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43 *Multidrug resistance TB (MDR):* A TB patient, whose biological specimen is resistant to both H
44 and R with or without resistance to other first-line anti-TB drugs.
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3 *Pre-XDR-TB*: It is defined as TB with resistance to isoniazid and rifampicin and either a FQ or a
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5 second-line injectable agent but not both.
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8 *Extensive drug resistance (XDR)*: A MDR-TB patient whose biological specimen is additionally
9
10 resistant to at least a FQ and a SLI anti-TB drug.
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12 **Patient and public involvement**

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14 Patients were involved in the reporting of our research in this study.
15

16 **RESULTS**

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18 During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens
19
20 (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with
21
22 concomitant smear and culture inoculation on the same sample. All duplicate isolates were
23
24 excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary) were
25
26 detected for MTB complex. The assay failed to detect sixty nine samples that were culture
27
28 positive. The MPT64 antigen test was positive on all these cultures. There were 806 (78.10%)
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30 males and 226 (21.89%) females among the positive specimens. The median age of patients was
31
32 32 years and nearly 43% patients were young adults in the age group of 30-45 years as shown in
33
34 Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic biopsy) were the
35
36 most common samples in extra-pulmonary cases that were positive. The sample distribution of
37
38 positive specimens is shown in Figure 2. Out of 1032 samples detected positive by the CBNAAT
39
40 assay, 507 and 517 specimens were smear and culture positive respectively. The rate of smear
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42 and culture positivity in pulmonary and extra-pulmonary cases was 54.1%, 54.3%, 38.3% and
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44 40.7% respectively (Table-1). The results of conventional and molecular diagnostic testing by
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46 Xpert MTB/RIF assay of patients included in the study is shown in Figure 3.
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3 During the study period, we also recovered 35 isolates of Non-tuberculous Mycobacteria (NTM)
4 from various pus and respiratory specimens. These were *Mycobacterium abscessus* (n=15),
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6 *Mycobacterium intracellulare* (7), *Mycobacterium fortuitum* (6), *Mycobacterium gordonae* (n=3)
7
8 and *Mycobacterium simiae* (n=3).
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11
12 Rifampicin monoresistance was detected in 223 out of 1032 samples (21.6%). It was 23.5%
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14 (n=166/706) and 17.4% (57/326) in pulmonary and extra-pulmonary cases respectively (Figure
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17 4).
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19 A summary of the performance data is shown in Table 2. Five hundred and seventeen samples
20
21 were positive by culture resulting in an 86.6% agreement with the Xpert MTB/RIF assay. The
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23 assay had a 100% agreement for culture positive, smear positive specimens and 61.6%
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25 agreement for culture positive, smear negative specimens for the detection of *Mycobacterium*
26
27 *tuberculosis*. Sixty nine samples that were culture positive tested negative by the Xpert MTB/RIF
28
29 assay. We did not have any sample that was positive on both smear and culture but was negative
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31 by Xpert MTB/RIF assay. As shown in Table 2, we detected 413 more patients than we could
32
33 have diagnosed by smear and/or culture alone.
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37 Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing
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39 by LPA v2.0 for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure 5,
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41 majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were
42
43 sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR) while
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45 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that was
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47 aminoglycoside resistant but FLQ sensitive.
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51 **DISCUSSION**

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54 Multidrug-resistant tuberculosis is one of the greatest public health challenges worldwide.^{1,10-12}
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3 To the best of our knowledge, ours is the first study from India to determine the burden of drug
4 resistant tuberculosis by testing such a large number of pulmonary and extrapulmonary clinical
5 samples. As per WHO Global TB Report, 2019 the best estimate of total TB incidence for India
6 is 199 cases per 100,000 population which translates to around 9.9 cases of MDR-TB per 100000
7 population annually.¹ However, the estimates of TB incidence and mortality for India are
8 interim, pending results from the national TB prevalence survey planned for 2019/2020.
9

10
11 In 2016, the male to female ratio for TB stood between 1.07 to 2.25, with women accounting for
12 40% of new cases. In our study, it was 3.5. Studies have shown that women may be diagnosed
13 late or not diagnosed at all due to socio-cultural barriers such as high burden of household work,
14 illiteracy, restricted mobility as well as lack of autonomy. There is also a high level of stigma
15 associated with the disease among unmarried females. In addition, malnutrition, especially
16 anemia is prevalent in more than half of the women in India. All this increases the risk of TB
17 disease in women.¹³

18
19 In 2010, the World Health Organization (WHO) recommended the GeneXpert MTB/RIF assay
20 for initial diagnosis of MDR-TB or HIV-associated tuberculosis.¹⁴In 2014, WHO expanded this
21 recommendation for use in all patients. The accuracy of the MTB/RIF test to detect the presence
22 of tuberculosis in smear-positive cases has been reported to be between 98% to 100%. For smear
23 negative specimens, Zeka et al have reported sensitivities of 68.6% and 47.7% in pulmonary and
24 extrapulmonary samples respectively.¹⁵In the present study, the sensitivity of the test was nearly
25 87% and it rose to 100% for smear-positive specimens. In all studies, the sensitivity of the
26 MTB/RIF test for pulmonary specimens is higher than that for extrapulmonary specimens which
27 may or may not be statistically significant as reported by various authors.¹⁶This could be because
28 of the high smear-negative rate for non-respiratory specimens. Sixty nine specimens that were
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3 culture positive tested negative by the GeneXpert assay in our study (Table 2) resulting in a
4 specificity of 61.6% (69/112). All these samples were smear negative. We also detected 413 cases
5 (40% of 1032 positives) by the GeneXpert assay that were missed by both smear as well as
6 culture. Xpert achieved higher diagnostic yield than microscopy and increased TB case finding
7 by a factor of about 2. Boehme et al in a performance study on the use of the Xpert MTB/RIF
8 test for diagnosis of tuberculosis and multidrug resistance concluded that use of this test reduced
9 the median time to detection and treatment for smear-negative tuberculosis from 56 days to 5
10 days.¹⁷ In another study by Kim et al, on 321 patients the turn around time (TAT) for treatment
11 between patients diagnosed with rifampicin-resistant TB using the Xpert assay and those
12 diagnosed without the assay (phenotypic DST group) were compared.¹⁸ It was 64 vs. 2 days ($p <$
13 0.001) from initial evaluation to commencing second-line anti-tuberculosis
14 treatment. Using phenotypic DST as the gold standard, Xpert sensitivity and specificity for
15 diagnosis of rifampicin resistance was 100% and 98.7% respectively.

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33 The overall rate of resistance to rifampicin in our study was 21.6%. Goyal et al published a recent
34 systematic review of 75 epidemiological studies for the prevalence of DRTB in India across 2
35 decades, from 1995 to 2015.¹⁹ Comparative analysis revealed a worsening trend in DR-TB
36 between the two study decades, 37.7% vs 46.1% respectively. The prevalence of pre-XDR TB
37 was 7.9% with 66.3% resistance to fluoroquinolones.

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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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3 sectors in India including the country's burgeoning private sector. Private clinics in India are
4 often used by patients seeking TB treatment and they administer regimens that are not
5 recommended by standard guidelines. This not only results in suboptimal outcome but also
6 potentially generates MDR TB. They conclude that as multidrug resistant tuberculosis transitions
7 from an acquired condition to a primarily transmitted disease, improving the effectiveness of
8 drug-susceptible tuberculosis treatment can no longer contain the spread of the epidemic. This
9 epidemiological shift has profound resource implications since the cost of treatment of
10 multidrug-resistant tuberculosis treatment can exceed that of first-line tuberculosis therapy by a
11 factor of ten or more. A robust public health response is needed which includes a strong
12 surveillance system, drug susceptibility testing for all patients with tuberculosis and rapid
13 linkage to effective treatment throughout the course of the disease.
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28 In a similar study, Suen et al sought to evaluate the effectiveness of two disease control strategies
29 on reducing the prevalence of MDR TB in India.²¹ One by improving treatment of non-MDR TB
30 cases and second by shortening the infectious period between activation of MDR TB and
31 initiation of effective treatment. They examine the implication of India's MDR TB epidemic
32 from 1996-2038 for the effectiveness of public health interventions by using a dynamic
33 transmission model calibrated to Indian demography and epidemiology. They have concluded
34 that strategies that disrupt MDR transmission by shortening the time between MDR activation
35 and treatment are projected to provide greater reductions in MDR prevalence compared with
36 improving non-MDR treatment quality.
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49 In our study, we could put up SLD testing for only 62 cases (27.8%) out of 223 i.e. less than
50 30%. The remaining cases were either sterile on culture or had inadequate volume of sample to
51 put up the test directly. The PMDT guidelines in India outline the steps of specimen referral for
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3 TB patients in the government sector in India for both first and second line DST by LPA.⁴As per
4 the operational process, two fresh sputum specimens need to be collected at designated collection
5 centers by trained personnel and transported in a cool chain on the same day to the nearest
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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 observed in the present study may be due to the fact that ours is a tertiary care hospital and we see patients after the referring hospital 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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3 district hospitals/smaller cities in India. Indiscriminate antimicrobial therapy, without establishing
4 the etiology of infection results in incomplete treatment and misdiagnosis. In addition, many of
5 these antimicrobial agents have antitubercular activity also. McDowell and Pai in an
6 ethnographic study on the mismanagement of empirical TB treatment in India showed that all
7 non-specialist private practitioners began antibiotic treatment, especially quinolones, for
8 persistent cough before prescribing a test.²⁴

9
10 Subbaraman et al in a systematic review and meta-analysis have created a ‘cascade of care’
11 model that focuses on the government run national programme, NTEP (previously known as
12 RNTCP).²⁵ The purpose of their study was to estimate how many TB patients in India’s national
13 TB program are not being detected, not enrolling for treatment, not completing treatment, and
14 not surviving without TB recurrence for 1-y after completing treatment. The results of their study
15 show that pre-treatment loss to follow-up of diagnosed patients and post-treatment TB recurrence
16 were major points of attrition in the new smear-positive TB cascade. Out of 2,700,000 prevalent
17 TB patients in India only 39% achieved the optimal outcome of 1-y recurrence-free survival.

35 CONCLUSION

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37 In conclusion, we have not come across any study from India performed on such a large number
38 of pulmonary as well as extrapulmonary samples performed by both conventional and molecular
39 methods. Our study provides comprehensive data on the burden of drug resistant TB in India at a
40 1200 bed tertiary care centre. The Sustainable Development Goal (SDG) target to end the
41 tuberculosis epidemic by 2030 seems bleak. Notification data from low-income and middle-
42 income countries, are prone to under-reporting and cannot be interpreted without additional
43 information on case detection rate. The DR-TB diagnostic algorithm as given in the PMDT
44 guidelines recommends SL – LPA testing for all RR TB cases diagnosed by the CBNAAT assay.

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3 However, it is labor intensive and requires trained manpower .Severe lack of Microbiology
4 laboratories providing universal DST and visual interpretation of bands is a huge limitation
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6 especially in smear negative and extra-pulmonary cases with inadequate sample volumes as has
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8 been our experience even with version 2 of the test. The treatment algorithm recommends the
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10 shorter MDR TB regimen in all pulmonary cases of RR TB patients. However, the exclusion
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12 criterion is second-line drug resistance. Our data shows that out of 62 cases, 77% patients were
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14 resistant to fluoroquinolones. Though notification of all TB cases has been made mandatory by a
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16 gazette circular issued by the government in March 2018, the ‘missing million’ still remains a
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18 huge challenge. Our data provide a strong rationale for the implementation of evidence based
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20 strategies such as strengthening of laboratories, involvement of private sector, active case finding
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22 and strict compliance of treatment by directly observed therapy.
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28 **Contributors** RM drafted the manuscript. VK compiled the data. All authors critically revised
29
30 the manuscript, gave final approval and agreed to be accountable for all aspects of work.
31
32

33 **Funding** None
34

35 **Competing interests** None declared.
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38 **Patient consent for publication** Not required
39

40 **Ethics approval** The study protocol was approved by the ethics committee of the Institute.
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42 **Data availability statement** Data may be obtained by e mailing drricha1976@gmail.com
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12 **Table 1.** Smear and culture results among samples positive by Xpert MTB /RIF assay (n=1032)
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| 17 Sample (N=1032) | 18 Smear positive (%) | 19 Culture positive (%) |
|----------------------------------|------------------------------|--------------------------------|
| 20 Pulmonary(n=706) | 21 382(54.1%) | 22 384(54.3%) |
| 23 Extra- 24 pulmonary(n=326) | 25 125(38.3%) | 26 133(40.7%) |
| 27 Total | 28 507(49.1%) | 29 517(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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12 **Figure legends**
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17 **Figure 1.** Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)
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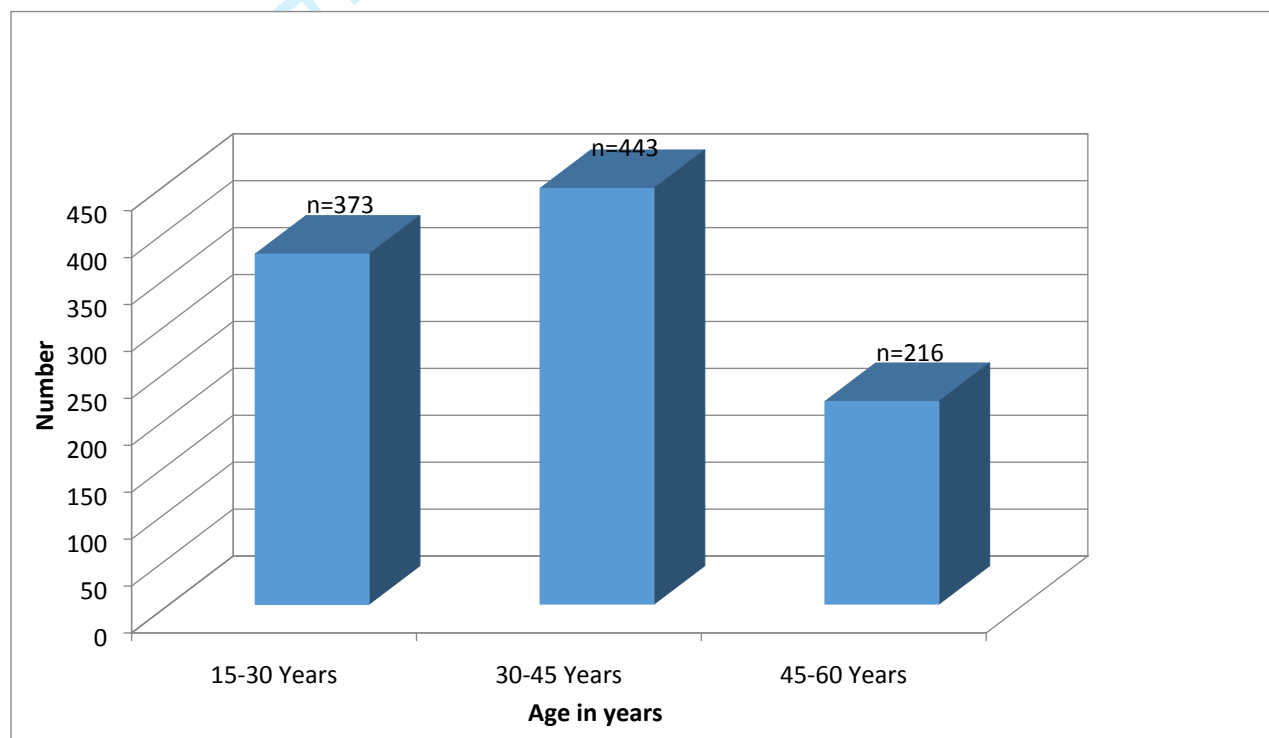
21 **Figure 2.** Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)
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26 **Figure 3.** Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay of
27 samples included in the study (n=3045)
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33 **Figure 4.** Distribution of samples among rifampicin resistant cases (n=223)
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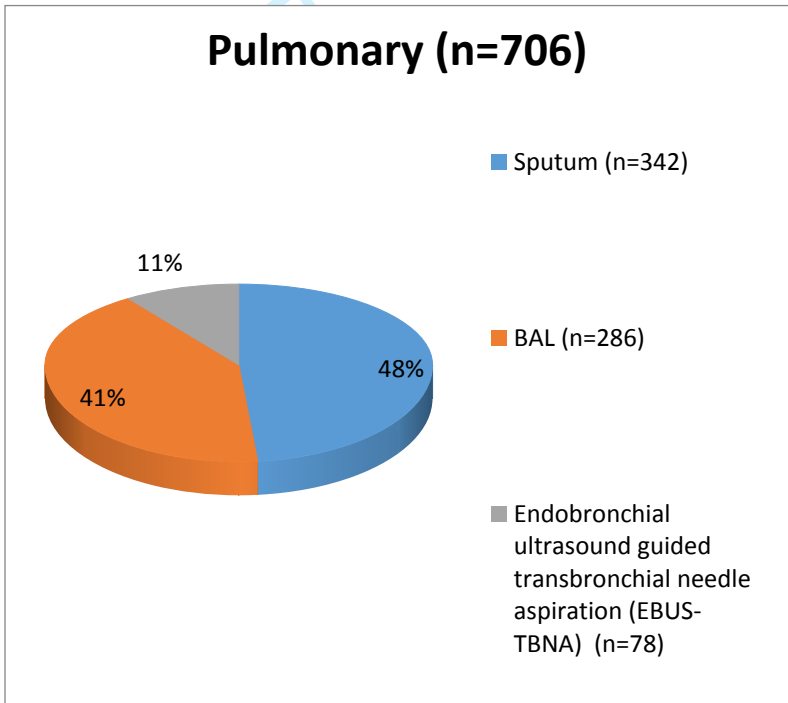
38 **Figure 5:** Results for second line susceptibility testing performed by line probe assay (LPA)
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Figure 1



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Figure 2



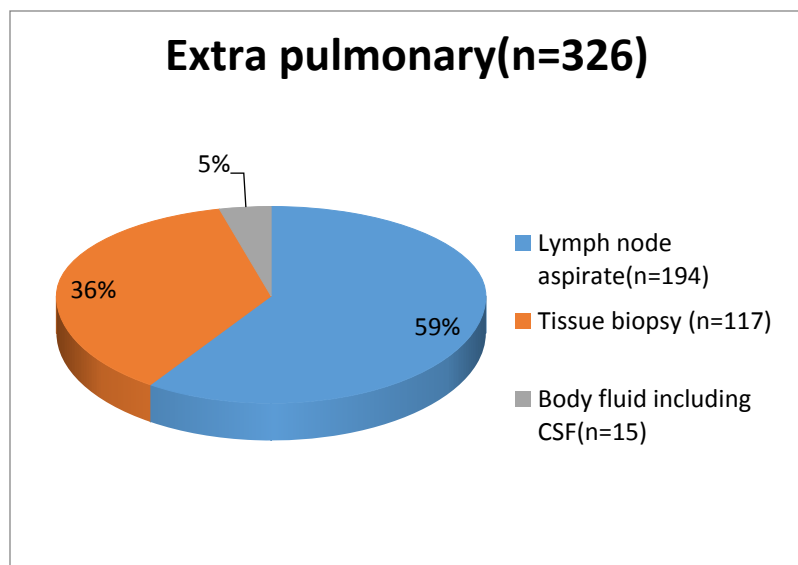
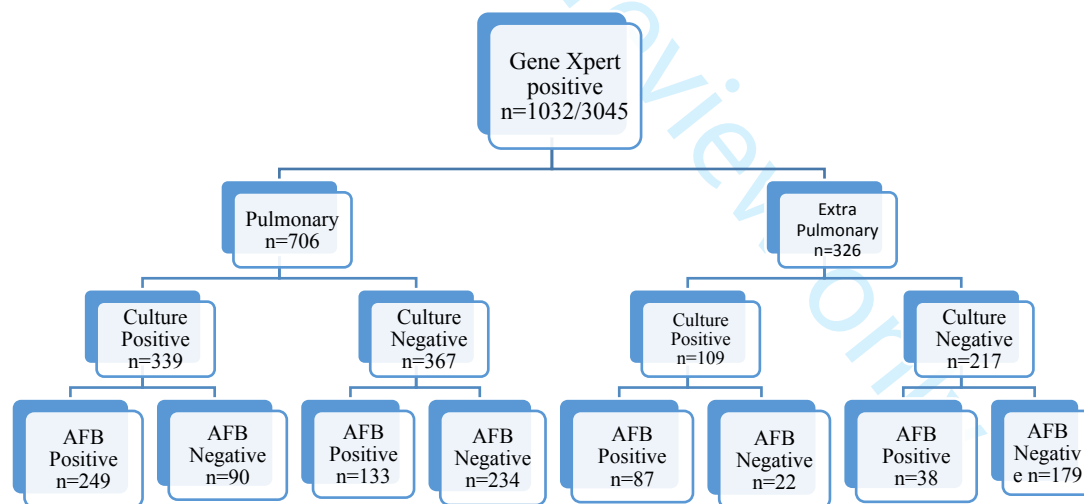


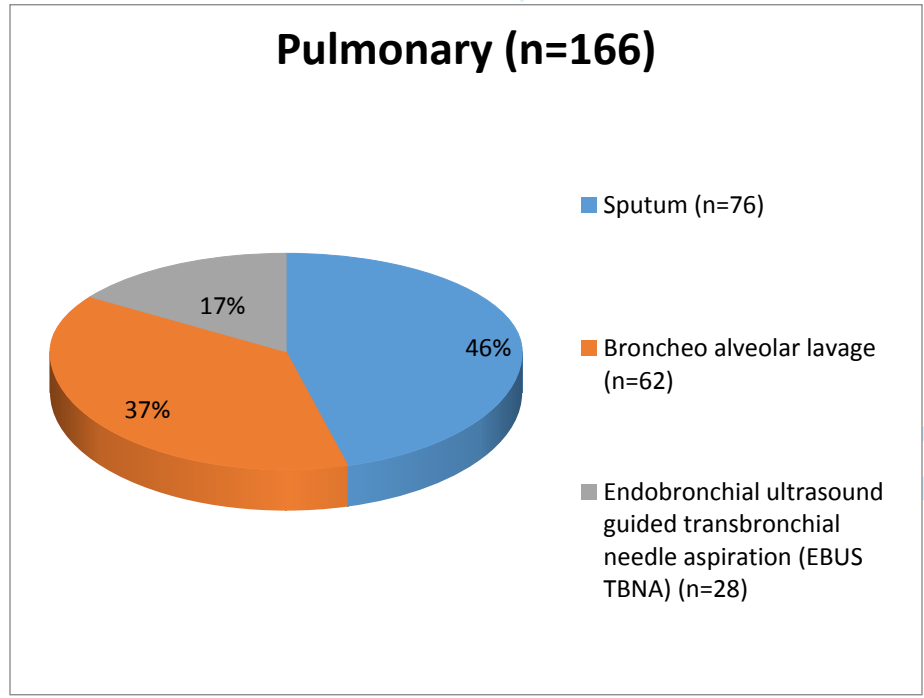
Figure 3



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Figure 4



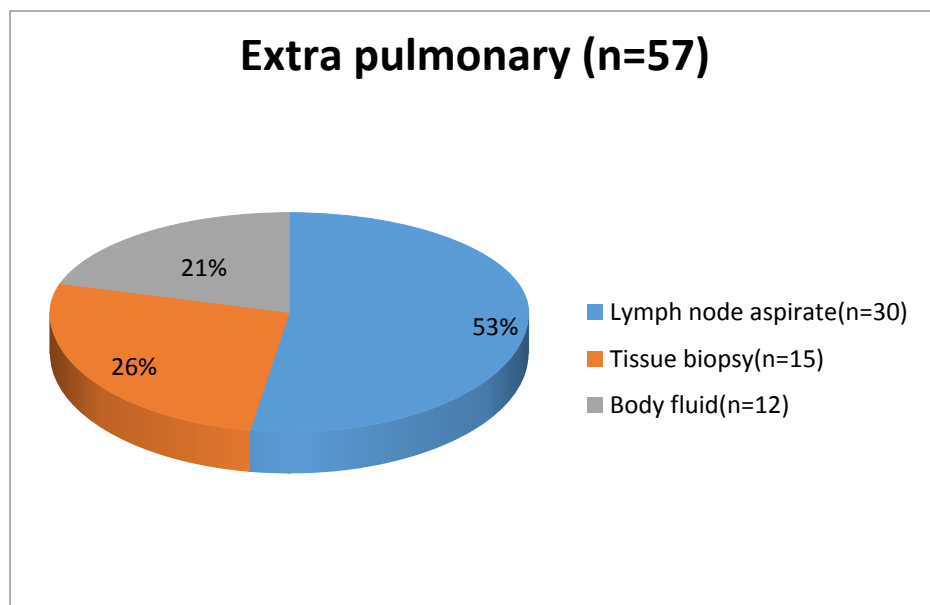
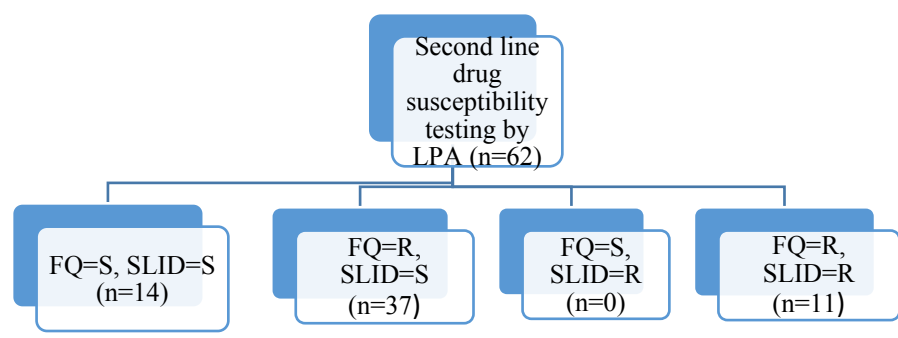


Figure 5

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

BMJ Open

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

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

26 **Methods:** A total of 3045 pulmonary (n=1883) and extra-pulmonary (n=1162) samples from
27 suspected tuberculosis patients were subjected to microscopy, culture and the Xpert
28 MTB/RIF assay from March 2017 to June 2019. Second line drug susceptibility testing by
29 version 2 Line Probe Assay for fluoroquinolones (FQs) and second line injectable drugs
30 (SLIDs) was performed on 62 samples.

31 **Results:** Out of 3045 samples processed in our lab during the study period, 36.1%
32 (1101/3045) were positive for MTBC and 21.6% were rifampicin mono-resistant (223/1032).
33 The rate of rifampicin resistance in pulmonary samples was 23.5% (166/706) and in
34 extrapulmonary cases it was 17.4% (57/326). Out of 62 cases included for second line testing,
35 48 were resistant to FQs (77.4%) while 11 were extensively drug resistant (XDR).

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

39
40 **Keywords:** Tuberculosis; Multi-drug resistant; Xpert MTB/RIF assay; Line Probe Assay

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6 **Article summary section**
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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 sequencing was not done on drug-resistant isolates

71 INTRODUCTION

72 India has the highest Tuberculosis (TB) burden in the world and is home to 27% of the
73 world's estimated 10.4 million annual tuberculosis cases.^{1,2,3} The menace of drug resistant-
74 TB (DR-TB), prompted the government to initiate the programmatic management of drug
75 resistant TB (PMDT) in 2007 which integrates all programme based strategies for DR-TB
76 diagnosis, management and treatment under the NTEP, National Tuberculosis Elimination
77 Programme (renamed in December 2019)⁴. India also has a complex as well as unorganized
78 health-care system which includes the government sector, private sector and informal health
79 care providers practicing non-allopathic schools of medicine such as ayurveda and
80 homeopathy². Though TB was made a notifiable disease in 2012, less than 40% cases from
81 the private sector were notified to the government in 2017³.

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

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

91 METHODS

92 Study design and setting

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

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3 95 Institute of Medical Sciences, a 1200 bed tertiary care referral medical center in northern
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5 96 India. Institutional ethics committee of Sanjay Gandhi Postgraduate Institute of Medical
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7 97 Sciences (SGPGIMS) approved the study protocol (IEC code 2017-37-IMP-EXP) and waiver
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9 98 of consent was obtained.
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13 99 **Clinical specimens**

14
15 100 Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752
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17 101 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided
18
19 102 transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/
20
21 103 Fine Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected
22
23 104 between March 2017 and June 2019 during the clinical routine. All samples were divided into
24
25 105 2 portions on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF
26
27 106 assay while microscopy and culture was performed from the remaining sample. Direct smears
28
29 107 were prepared from the specimens using Ziehl-Neelsen staining. All non-sterile clinical
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31 108 samples were processed using the *N*-acetyl-L-cysteine-sodium citrate-NaOH (NALC-NaOH)
32
33 109 method. Samples were decanted following centrifugation, and sediments were re-suspended
34
35 110 in 3 ml of phosphate buffer solution. Processed samples were used to inoculate either
36
37 111 Lowenstein-Jensen (LJ) solid medium or BacT/Alert culture. Line probe assay *version2*
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39 112 (LPA_{v2}) for second line testing was performed on either direct clinical samples if volume
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41 113 was adequate or on positive culture. Both Xpert MTB/RIF assay and LPA_{v2} were performed
42
43 114 according to the manufacturer's protocol.
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45 115 All cases detected positive by the Xpert MTB/RIF assay were grouped into (i) those with
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47 116 smear-positive and culture positive tuberculosis; (ii) those with smear-negative, culture-
48
49 117 positive tuberculosis; (iii) those who were both smear and culture negative for tuberculosis
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51 118 but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or
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53 119 radiological findings (clinical tuberculosis).
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3 120 There was a sub group of samples that were culture positive but missed by the Xpert
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5 121 MTB/RIF assay. We put up the TB Ag MPT64 Rapid test (SD BIOLINE) on all these
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8 122 positive cultures.
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10 11 123 **Data collection**

12
13 124 The medical records of patients were retrieved from the Hospital Information System. A
14
15 125 senior resident extracted patient data prospectively from charts.

16 17 126 **Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin** 18 19 20 127 **resistant/multi-drug resistant/extensively drug resistant)⁷**

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22
23 128 *Abacteriologically confirmed TB case:* One from whom a biological specimen was positive
24
25 129 by smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert
26
27
28 130 MTB/RIF assay.

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31 131 *Pulmonary tuberculosis (PTB):* Any bacteriologically confirmed or clinically diagnosed case
32
33 132 of TB involving lung parenchyma or tracheobronchial tree.

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36 133 *Extrapulmonary tuberculosis (EPTB):* Any bacteriologically confirmed or clinically
37
38 134 diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes,
39
40 135 abdomen, genitourinary tract, skin, joints and bones, meninges. Concomitant pulmonary
41
42 136 lesions were ruled out in all cases by appropriate investigations and review of case files.

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46 137 *Multidrug resistance TB (MDR):* A TB patient, whose biological specimen is resistant to both
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48 138 H and R with or without resistance to other first-line anti-TB drugs.

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51 139 *Pre-XDR-TB:* It is defined as TB with resistance to isoniazid and rifampicin and either a FQ
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53 140 or a second-line injectable agent but not both.

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56 141 *Extensive drug resistance (XDR):* A MDR-TB patient whose biological specimen
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58 142 is additionally resistant to at least a FQ and a SLI anti-TB drug.
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143 Patient and public involvement

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

145 RESULTS

146 During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens
147 (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with
148 concomitant smear and culture inoculation on the same sample. All duplicate isolates were
149 excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary)
150 were detected for MTB complex. The assay failed to detect sixty nine samples that were
151 culture positive. The MPT64 antigen test was positive on all these cultures. There were 806
152 (78.10%) males and 226 (21.89%) females among the positive specimens. The median age of
153 patients was 32 years and nearly 43% patients were young adults in the age group of 30-45
154 years as shown in Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic
155 biopsy) were the most common samples in extra-pulmonary cases that were positive. The
156 sample distribution of positive specimens is shown in Figure 2. Out of 1032 samples detected
157 positive by the CBNAAT assay, 507 and 517 specimens were smear and culture positive
158 respectively. The rate of smear and culture positivity in pulmonary and extra-pulmonary
159 cases was 54.1%, 54.3%, 38.3% and 40.7% respectively (Table-1). The results of
160 conventional and molecular diagnostic testing by Xpert MTB/RIF assay of patients included
161 in the study is shown in Figure 3.

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

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

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3 168 (Figure 4). A summary of the performance data is shown in Table 2. Five hundred and
4
5 169 seventeen samples were positive by culture resulting in an 86.6% agreement with the Xpert
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7 170 MTB/RIF assay. The assay had a 100% agreement for culture positive, smear positive
8
9 171 specimens and 61.6% agreement for culture positive, smear negative specimens for the
10
11 172 detection of *Mycobacterium tuberculosis*. Sixty nine samples that were culture positive tested
12
13 173 negative by the Xpert MTB/RIF assay. We did not have any sample that was positive on both
14
15 174 smear and culture but was negative by Xpert MTB/RIF assay. As shown in Table 2, we
16
17 175 detected 413 more patients than we could have diagnosed by smear and/or culture alone.
18
19 176 Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing
20
21 177 by LPA v2.0 for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure
22
23 178 5, majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were
24
25 179 sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR)
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27 180 while 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that
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29 181 was aminoglycoside resistant but FLQ sensitive.

182 Discussion

183 Multidrug-resistant tuberculosis is one of the greatest public health challenges worldwide. To
184 the best of our knowledge, ours is the first study from India to determine the burden of drug
185 resistant tuberculosis by testing such a large number of pulmonary and extra-pulmonary
186 clinical samples. As per WHO Global TB Report, 2020 the three countries with the largest
187 share of the global burden were India (27%), China (14%) and the Russian Federation (8%)¹.
188 The results of the national anti-tuberculosis drug resistance survey has shown that the
189 incidence of TB is highest in the 25–34 year age group in India⁸. We however documented a
190 slightly higher age group in our study. Our cohort was dominated by males and nearly 43%
191 patients were young adults in the age group of 30–45 years. The high frequency of the disease
192 among the younger population may facilitate the transmission of TB in the community due to

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3 193 greater mobility of youth. A gender analysis of the TB epidemic shows that TB affects
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5 194 different genders differently. In 2016, about 40% of the 2.79 million new cases of TB in India
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8 195 were among women and the male to female ratio for TB stood between 1.07 to 2.25 with
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10 196 women accounting for 40% of new cases. In our study, it was 3.5. Studies have shown that
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12 197 women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as
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14 198 high burden of household work, illiteracy, restricted mobility as well as lack of autonomy.
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16
17 199 There is also a high level of stigma associated with the disease among unmarried females⁹.
18
19 200 WHO's current policies and guidance recommend that the Xpert MTB/RIF assay may be
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21 201 used as an initial diagnostic test in individuals suspected of having MDR-TB. About 36% of
22
23 202 the samples included in our study were positive for *Mycobacterium tuberculosis* complex and
24
25 203 the overall rate of resistance to rifampicin was 21.6%. We assessed the burden of tuberculosis
26
27 204 in a large cohort of consecutive patients in our hospital thereby eliminating any selection bias
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29 205 in the study population. We also recovered 34 isolates of Non-tuberculous Mycobacteria
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31 206 (NTM) from various samples and all these isolates were negative by the Xpert MTB/RIF
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33 207 assay.
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36 208 In a study carried out in Mumbai, India's commercial capital and one of the most densely
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38 209 populated and congested cities, Udwardia et al tested 1539 samples at a tertiary care private
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40 210 hospital and reported MDR-TB in 30.14% of cases¹⁰. In another retrospective study from
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42 211 South India, Shivekar et al performed the MTBDRplus assay on 20245 specimens obtained
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44 212 from presumptive MDR-TB cases during a 6-year study period from 2013 to 2018. Based on
45
46 213 the *rhoB* gene, true resistance, hetero-resistance, and inferred resistance to rifampicin was
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48 214 found in 38%, 29.3%, and 32.7% of the 1582 MDR cases, respectively.¹¹ Goyal et al
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50 215 published a recent systematic review of 75 epidemiological studies for the prevalence of
51
52 216 DRTB in India across 2 decades, from 1995 to 2015. Comparative analysis revealed a
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54 217 worsening trend in DR-TB between the two study decades, 37.7% vs 46.1% respectively. The
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3 218 countrywide prevalence of MDR-TB also increased from the earlier decade at 14.9% to 27.9%
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5 219 in decade 2.¹² However, the report of the first national anti-tuberculosis drug resistance survey
6
7 220 in India conducted during 2014-2016 concluded that among all TB patients tested, the MDR-
8
9 221 TB rate was 6.19% with 2.84% among new and 11.60% among previously treated TB
10
11 222 patients. The survey has probably under-estimated the true burden of resistance in India since
12
13 223 it excluded both smear-negative TB cases as well as extrapulmonary TB and did not include
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15 224 the private sector.⁸

16
17 225 We also attempted to find the overall agreement of the Xpert MTB/RIF assay compared to
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19 226 culture in our study cohort. In the present study, the sensitivity of the test was nearly 87% and
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21 227 it rose to 100% for smear-positive specimens. The accuracy of the MTB/RIF test to detect the
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23 228 presence of tuberculosis in smear-positive cases has been reported to be between 98% to
24
25 229 100%.¹³ For smear negative specimens, Zeka et al have reported sensitivities of 68.6% and
26
27 230 47.7% in pulmonary and extra-pulmonary samples respectively.¹⁴ Sixty nine specimens that
28
29 231 were culture positive tested negative by the Xpert MTB/RIF assay in our study resulting in a
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31 232 specificity of 61.6% (69/112). All these samples were smear negative. We also detected 413
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33 233 cases (40% of 1032 positives) by the Xpert MTB/RIF assay that were missed by both smear
34
35 234 as well as culture. The assay achieved higher diagnostic yield than microscopy and increased
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37 235 TB case finding by a factor of about 2.

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39 236 The results of second line testing in our study revealed 77.4% resistance to fluoroquinolones
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41 237 among RR isolates which is higher than other studies reported from India. Sethi et al in a
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43 238 retrospective study from a tertiary care center in northern India have documented an overall
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45 239 rate of 38.6% FQ resistance among 863 rifampicin-resistant TB isolates.¹⁵ In another study
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47 240 from eight health care facilities in greater Mumbai between 2005 and 2013, Dalal et al
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49 241 investigated the trends over time of patterns of drug resistance in a sample of MDR-TB
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51 242 patients. Between 2005–2007 and 2011–2013, patients with ofloxacin and moxifloxacin
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3 243 resistance significantly increased from 57.6% to 75.3% and from 60.0% to 69.5% ($p < 0.05$).¹⁶

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5 244 A meta-analysis by Ho et al has concluded that globally FQ resistance in MTB is largely

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7 245 confined to MDR strains and knowledge of the global extent of this resistance pattern is

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9 246 currently hampered by the absence of surveillance studies in the majority of regions where

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11 247 TB is endemic.¹⁷

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13 248 Updated WHO guidelines, published in June 2020, recommend that for patients with MDR-

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15 249 TB and additional fluoroquinolone resistance, a regimen composed of bedaquiline,

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17 250 pretomanid, and linezolid may be used under operational research conditions (6-9 months).⁵

18
19 251 Chee et al in a study conducted between 2002-2016 on 280 patients have demonstrated that

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21 252 only about 30% of patients with MDR pulmonary TB diagnosed in their study cohort from

22
23 253 South-east Asia were eligible for the WHO shorter MDR-TB treatment regimen.¹⁸In a similar

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25 254 study from northern India, Singh and Jain have explored the eligibility of the shorter regimen

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27 255 in MDR patients under the programmatic setting. Out of 541 conclusive LPA-SLD results, the

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29 256 proportion of strains resistant to only fluoroquinolones was nearly 50% while 8.3% were

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31 257 resistant to both fluoroquinolones and SLIDs.¹⁹Eleven cases in our study were extensively

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33 258 drug resistant.

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35 259 The high rates of drug resistance observed in our study may be due to the fact that ours is a

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37 260 tertiary care hospital in the state of Uttar Pradesh which has over 20% of the total number of

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39 261 notified cases of TB in India. We see patients after the referring hospital has already tried and

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41 262 failed to control infection using a combination of different anti-microbial agents. Since

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43 263 facilities for microbiological studies are usually not available in district hospitals/smaller

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45 264 cities in India, the first contact physician/surgeon/referral facility are compelled to initiate

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47 265 broad-spectrum antibiotics. Indiscriminate antimicrobial therapy without establishing the

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49 266 etiology of infection selects out the resistant strains. McDowell and Pai in an ethnographic

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51 267 study on the mismanagement of empirical TB treatment in India have demonstrated that all

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3 268 non-specialist private practitioners began antibiotic treatment, especially quinolones, for
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5 269 persistent cough before prescribing a test.²⁰Their results underscore the fact that inappropriate
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7 270 prescribing practices in India's burgeoning private sector including easy, over-the-counter
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9 271 access to fluoroquinolones need to be halted as soon as possible.
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12 272 The alarming rate of drug resistance in our study to rifampicin as well as fluoroquinolones
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14 273 has important implications for implementation of government strategies to control the TB
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16 274 epidemic in India. Firstly, standardized regimens containing a FQ to treat MDR –TB cases
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18 275 carry a high risk of being sub-optimal and resulting in treatment failure. Secondly, with such
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20 276 high rates of drug resistance India will have to equip itself with enough Mycobacteriology
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22 277 laboratories offering culture and drug susceptibility testing (C-DST) to both first as well as
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24 278 second-line agents. Currently, the focus is to roll out sufficient number of GeneXpert
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26 279 MTB/RIF assay machines to diagnose rifampicinresistant strains of *Mycobacterium*
27
28 280 *tuberculosis*. However, this strategy may mask the diagnosis of pre-XDR TB. A high rate of
29
30 281 FQ resistance has also been noted in newly diagnosed MDR/RR TB cases, which might be
31
32 282 due to transmission of the drug-resistant strains. ¹⁵It is estimated that in India, by 2032, 85%
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34 283 of multidrug-resistant tuberculosis infections would be from primary transmission, compared
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36 284 with only 15% in 2012. In the *Lancet Public Health*, Law et al have created a dynamic model
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38 285 of the tuberculosis epidemic in India, which they use to estimate the incidence of drug
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40 286 susceptible tuberculosis and multidrug-resistant tuberculosis over the next 20 years². They
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42 287 have analyzed the emergence of drug resistance in all major health-care sectors in India.
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44 288 Private clinics in India are often used by patients seeking TB treatment and they administer
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46 289 regimens that are not recommended by standard guidelines. This not only results in
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48 290 suboptimal outcome but also potentially generates MDR TB. They conclude that as multidrug
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50 291 resistant tuberculosis transitions from an acquired condition to a primarily transmitted
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52 292 disease, improving the effectiveness of drug-susceptible tuberculosis treatment can no longer
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3 293 contain the spread of the epidemic. This epidemiological shift has profound resource
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5 294 implications since the cost of treatment of multidrug-resistant tuberculosis treatment can
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8 295 exceed that of first-line tuberculosis therapy by a factor of ten or more.

9
10 296 In addition, notification data from low-income and middle-income countries, are prone to
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12 297 underreporting and cannot be interpreted without additional information on case detection
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14 298 rate. The DR-TB diagnostic algorithm as given in the PMDT guidelines recommends SL –
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17 299 LPA testing for all RR TB cases diagnosed by the CBNAAT assay. However, it is labor
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19 300 intensive and requires trained manpower. Severe lack of Microbiology laboratories providing
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21 301 universal DST and visual interpretation of bands is a huge limitation especially in smear
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23 302 negative and extra-pulmonary cases with inadequate sample volumes as has been our
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25
26 303 experience even with version 2 of the test.

27
28 304 There were several limitations to this study. One of the methodological limitations of our
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30 305 study was that we could not perform liquid culture DST as well as sequencing and confirm
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32 306 the results of the drug resistant isolates. Another limitation was that we did not differentiate
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34 307 between new and previously treated TB cases. Most of the DR-TB patients in our cohort at
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36 308 the time of diagnosis were attached to the PMDT follow up for further evaluation and
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38 309 management except for some who insisted on institutional management. We could therefore
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40 310 put up SL-DST for only 62 cases. We also did not receive any grant for this study and hence
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42 311 could not put up FL-LPA on the 69 culture positive isolates that tested negative by the Xpert
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44 312 MTB/RIF assay. In addition, a study of risk factors in such a high burden setting would have
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46
47 313 allowed us to offer more useful remedies to policy makers.

51 314 **CONCLUSION**

52
53 315 In conclusion, we have not come across any prospective study from India on such a large
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55 316 number of pulmonary as well as extrapulmonary samples performed by both conventional
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57 317 and molecular methods. Our study provides comprehensive data on the high burden of drug
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3 318 resistant TB in India at a 1200 bed tertiary care center in northern India. The need of the hour
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5 319 is to have enough Mycobacteriology laboratories offering both first and second line DST
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7 320 under the NTEP umbrella. The high rates of FQ resistance documented in our study should
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9 321 prompt policy makers to tightly regulate them as reserve drugs, otherwise the ambitious goal
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11 322 of the Government of India to eliminate tuberculosis by 2025 seems bleak.
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16
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19 325

20 326 **Contributors** RM conceptualized the manuscript, designed the methods, supervised the
21
22 327 study and wrote the manuscript. VK curated the data. AN supervised the study and edited the
23
24 328 manuscript. All authors have reviewed the final version of the manuscript.
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28
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32 333 **Competing interests** None declared.
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34 334

35 335 **Patient consent for publication** Not required.
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38 337 **Ethics approval** The study protocol was approved by the Institutional Ethics Committee of
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40 338 Sanjay Gandhi Post Graduate Institute of Medical Sciences SGPGIMS: (IEC 2017-37-IMP-
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42 339 EXP). Written informed consent was obtained from each participant.
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45 341 **Data availability statement** Data are available upon reasonable request.
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3 **424 Table 1.** Smear and culture results among samples positive by Xpert MTB /RIF assay

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5 **425** (n=1032)

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8 **426**

| 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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3 **443 Table 2:** Comparison of Gene Xpert MTB / RIF positive, MTB culture positive results with
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6 **444** smear results

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| | 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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3 **462 Figure legends**
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8 **464 Figure 1.** Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)
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12 **466 Figure 2.** Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)
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17 **468 Figure 3.** Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay
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19 469 of samples included in the study (n=3045)
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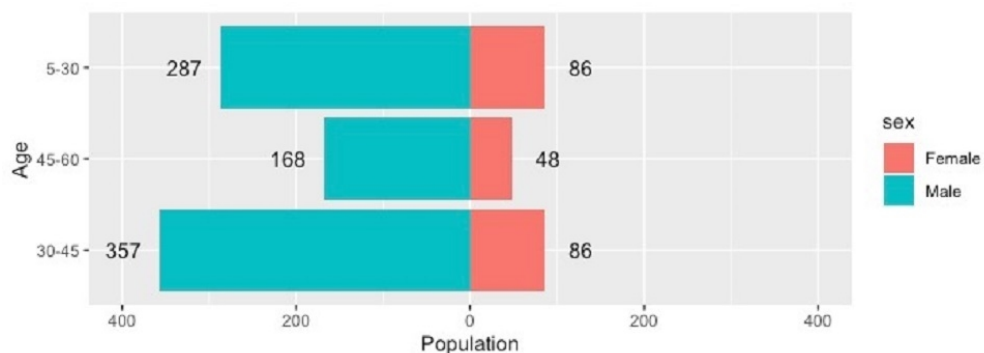
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24 **471 Figure 4.** Distribution of samples among rifampicin resistant cases (n=223)
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28 **473 Figure 5:** Results for second line susceptibility testing performed by line probe assay (LPA)
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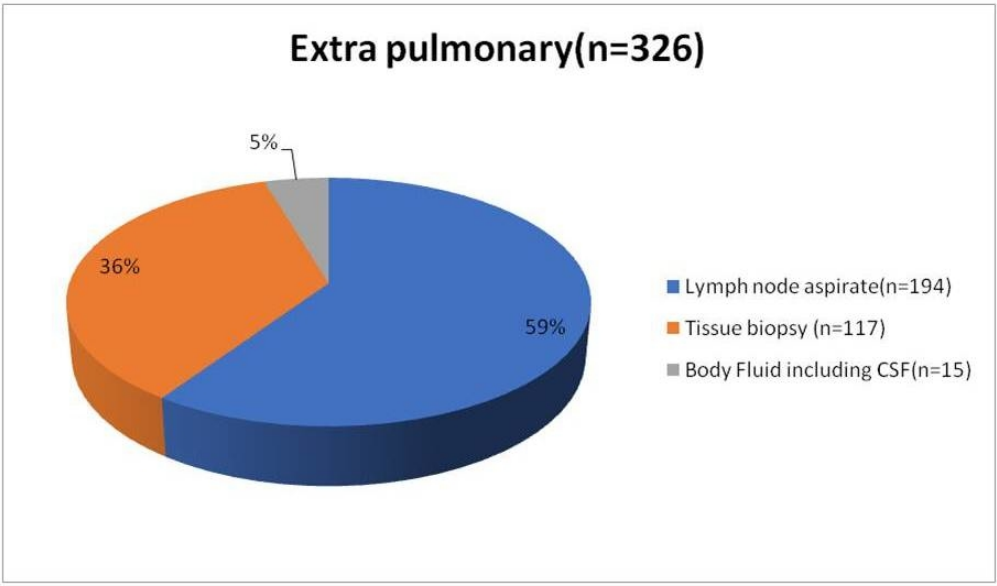
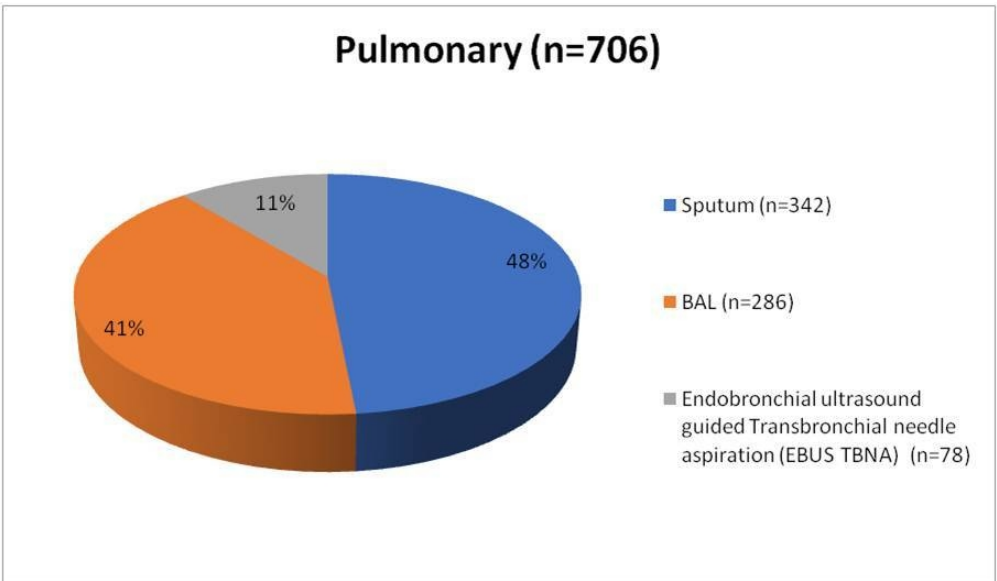
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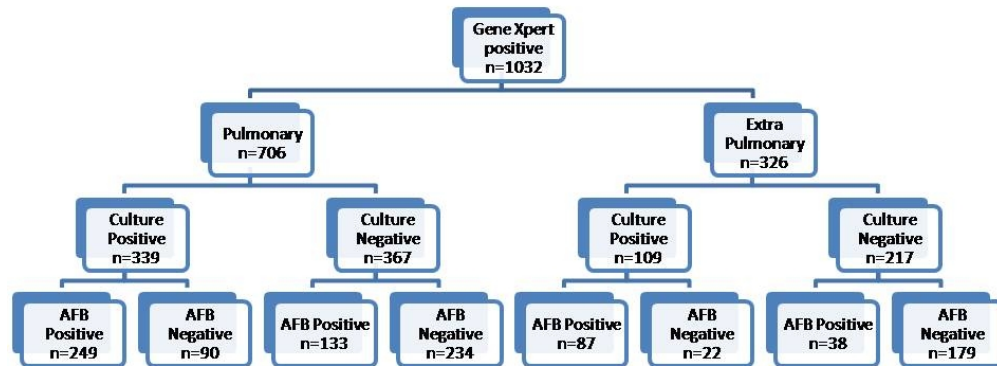


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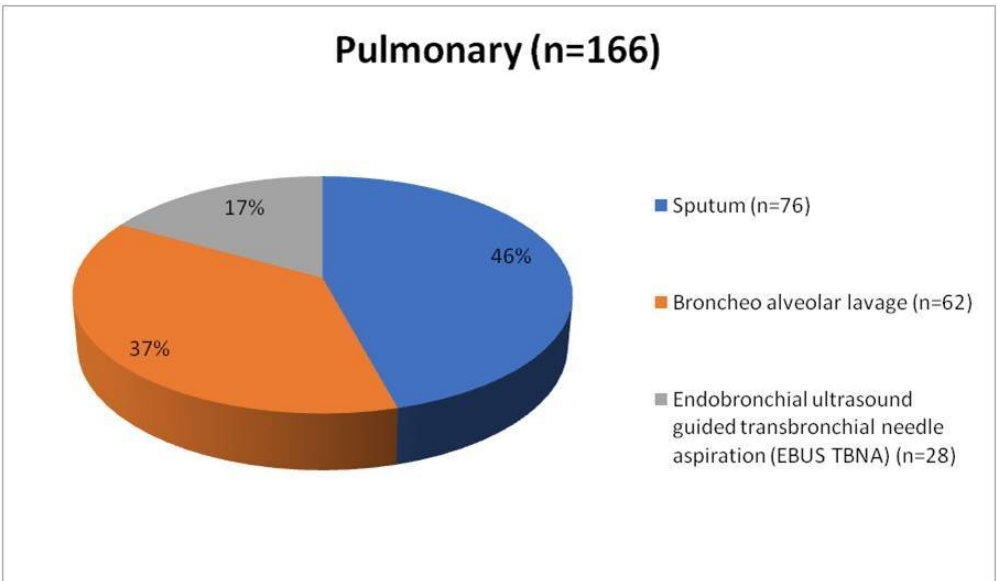


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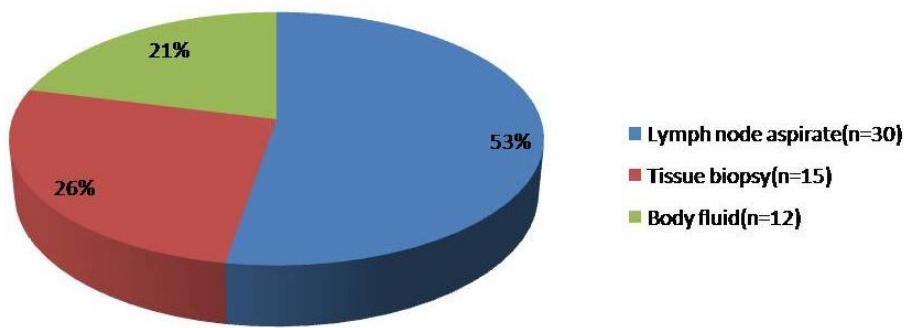


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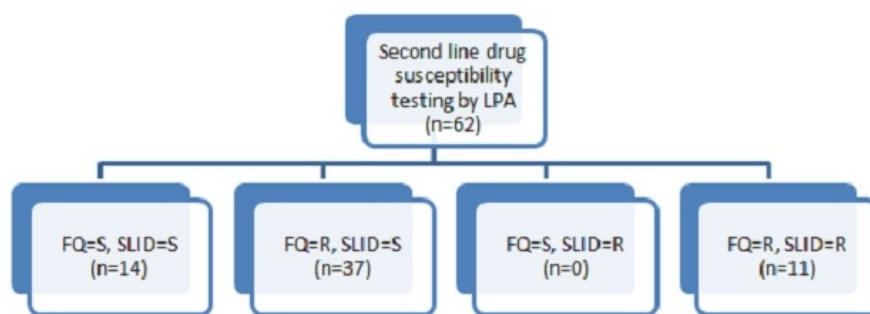
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Extra pulmonary (n=57)



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

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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.

BMJ Open

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

| | |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2020-044096.R2 |
| Article Type: | Original research |
| Date Submitted by the Author: | 26-Mar-2021 |
| Complete List of Authors: | Misra, Richa; SGPGIMS Kesarwani, Vasudha ; SGPGIMS Nath, Alok; SGPGIMS, |
| Primary Subject Heading: | Epidemiology |
| Secondary Subject Heading: | Public health |
| Keywords: | Tuberculosis < INFECTIOUS DISEASES, Epidemiology < TROPICAL MEDICINE, MICROBIOLOGY, Public health < INFECTIOUS DISEASES |
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3 **TITLE PAGE**
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5
6 **Title:** Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in
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8 northern India: a prospective single centre cohort study
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10
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22 Abstract

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

26 **Methods:** A total of 3045 pulmonary (n=1883) and extra-pulmonary (n=1162) samples from
27 likely tuberculosis patients were subjected to microscopy, culture and the Xpert MTB/RIF
28 assay from March 2017 to June 2019. Second line drug susceptibility testing by version 2 Line
29 Probe Assay for fluoroquinolones (FQs) and second line injectable drugs (SLIDs) was
30 performed on 62 samples.

31 **Results:** Out of 3045 samples processed in our lab during the study period, 36.1%
32 (1101/3045) were positive for MTBC and 21.6% were rifampicin mono-resistant (223/1032).
33 The rate of rifampicin resistance in pulmonary samples was 23.5% (166/706) and in
34 extrapulmonary cases it was 17.4% (57/326). Out of 62 cases included for second line testing,
35 48 were resistant to FQs (77.4%) while 11 were extensively drug resistant (XDR).

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

39
40 **Keywords:** Tuberculosis; Multi-drug resistant; Xpert MTB/RIF assay; Line Probe Assay

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6 **Article summary section**
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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 sequencing was not done on drug-resistant isolates

71 INTRODUCTION

72 India has the highest Tuberculosis (TB) burden in the world and is home to 26% of the
73 world's estimated 10.4 million annual tuberculosis cases.¹ The menace of drug resistant-TB
74 (DR-TB), prompted the government to initiate the programmatic management of drug
75 resistant TB (PMDT) in 2007 which integrates all programme based strategies for DR-TB
76 diagnosis, management and treatment under the NTEP, National Tuberculosis Elimination
77 Programme (renamed in December 2019)². India also has a complex as well as unorganized
78 health-care system which includes the government sector, private sector and informal health
79 care providers practicing non-allopathic schools of medicine such as ayurveda and
80 homeopathy³. Though TB was made a notifiable disease in 2012, less than 40% cases from
81 the private sector were notified to the government in 2017⁴.

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

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

94 **METHODS**

95 **Study design and setting**

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

102 **Clinical specimens**

103 Three thousand forty five pulmonary and extrapulmonary samples (930 sputum, 752
104 bronchoalveolar lavage, 146 EBUS-TBNA (endobronchial ultrasound with real-time guided
105 transbronchial needle aspiration), 54 bronchial/tracheal aspirate, 429 lymph node aspirates/
106 Fine Needle Aspiration Cytology(FNAC), 367 biopsies, 338 pus and 29 CSF were collected
107 between March 2017 and June 2019 during the clinical routine. All samples were divided into
108 2 portions on receipt in the laboratory. One aliquot was used to perform the Xpert MTB/RIF
109 assay while microscopy and culture was performed from the remaining sample. Direct smears
110 were prepared from the specimens using Ziehl-Neelsen staining. All non-sterile clinical
111 samples were processed using the *N*-acetyl-L-cysteine-sodium citrate-NaOH (NALC-NaOH)
112 method. Samples were decanted following centrifugation, and sediments were re-suspended
113 in 3 ml of phosphate buffer solution. Processed samples were used to inoculate either
114 Lowenstein-Jensen (LJ) solid medium or BacT/Alert culture. Line probe assay *version2*
115 (LPA_{v2}) for second line testing was performed on either direct clinical samples if volume
116 was adequate or on positive culture. Both Xpert MTB/RIF assay and LPA_{v2} were performed
117 according to the manufacturer's protocol.

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

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3 119 smear-positive and culture positive tuberculosis; (ii) those with smear-negative, culture-
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5 120 positive tuberculosis; (iii) those who were both smear and culture negative for tuberculosis
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7
8 121 but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or
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10 122 radiological findings (clinical tuberculosis).

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13 123 There was a sub group of samples that were culture positive but missed by the Xpert
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15 124 MTB/RIF assay. We put up the TB Ag MPT64 Rapid test (SD BIOLINE) on all these
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17
18 125 positive cultures.

19 20 21 126 **Data collection**

22
23 127 The medical records of patients were retrieved from the Hospital Information System. A
24
25 128 senior resident extracted patient data prospectively from charts.

26 27 28 129 **Classifications and definitions including RR-TB/MDR-TB/XDR-TB(rifampicin** 29 30 130 **resistant/multi-drug resistant/extensively drug resistant)⁸**

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32
33 131 *Abacteriologically confirmed TB case:* One from whom a biological specimen was positive
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35 132 by smear microscopy, culture or WRD (WHO approved rapid diagnostic test) such as Xpert
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37
38 133 MTB/RIF assay.

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41 134 *Pulmonary tuberculosis (PTB):* Any bacteriologically confirmed or clinically diagnosed case
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43 135 of TB involving lung parenchyma or tracheobronchial tree.

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46 136 *Extrapulmonary tuberculosis (EPTB):* Any bacteriologically confirmed or clinically
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48 137 diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes,
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50 138 abdomen, genitourinary tract, skin, joints and bones, meninges. Concomitant pulmonary
51
52 139 lesions were ruled out in all cases by appropriate investigations and review of case files.

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56 140 *Multidrug resistance TB (MDR):* A TB patient, whose biological specimen is resistant to both
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58 141 H and R with or without resistance to other first-line anti-TB drugs.

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3 142 *Pre-XDR-TB*: It is defined as TB with resistance to isoniazid and rifampicin and either a FQ
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5 143 or a second-line injectable agent but not both.

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7 144 *Extensive drug resistance (XDR)*: A MDR-TB patient whose biological specimen
8
9 145 is additionally resistant to at least a FQ and a SLI anti-TB drug.

12 146 **Patient and public involvement**

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14
15 147 Patients were involved in the reporting of our research in this study.

17 148 **RESULTS**

18
19 149 During the 27 month study period, 1883 pulmonary and 1162 extra-pulmonary specimens
20
21 150 (n=3045) were subjected to the GeneXpert MTB/RIF assay in our laboratory along with
22
23 151 concomitant smear and culture inoculation on the same sample. All duplicate isolates were
24
25 152 excluded. One thousand thirty two (33.8%) samples (706 pulmonary, 326 extra-pulmonary)
26
27 153 were detected for MTB complex. The assay failed to detect sixty nine samples that were
28
29 154 culture positive. The MPT64 antigen test was positive on all these cultures. There were 806
30
31 155 (78.10%) males and 226 (21.89%) females among the positive specimens. The median age of
32
33 156 patients was 32 years and nearly 43% patients were young adults in the age group of 30-45
34
35 157 years as shown in Figure 1. Lymph node aspirates/FNAC and tissue biopsy (including colonic
36
37 158 biopsy) were the most common samples in extra-pulmonary cases that were positive. The
38
39 159 sample distribution of positive specimens is shown in Figure 2. Out of 1032 samples detected
40
41 160 positive by the CBNAAT assay, 507 and 517 specimens were smear and culture positive
42
43 161 respectively. The rate of smear and culture positivity in pulmonary and extra-pulmonary
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45 162 cases was 54.1%, 54.3%, 38.3% and 40.7% respectively (Table-1). The results of
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47 163 conventional and molecular diagnostic testing by Xpert MTB/RIF assay of patients included
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49 164 in the study is shown in Figure 3.

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51 165 During the study period, we also recovered 34 isolates of Non-tuberculous Mycobacteria
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53 166 (NTM) from various pus and respiratory specimens. These were *Mycobacterium abscessus*
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167 (n=15), *Mycobacterium intracellulare*(7), *Mycobacterium fortuitum* (6), *Mycobacterium*
168 *gordoniae*(n=3) and *Mycobacterium simiae*(n=3).

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

179 Out of 223 rifampicin resistant cases, we could put up second line drug susceptibility testing
180 by LPA v2.0 for 62 cases (n=40, pulmonary and n=22, extra-pulmonary). As shown in Figure
181 5, majority of our patients (77.4%) were resistant to FLQs (n=48/62). Only 14 patients were
182 sensitive to both FLQ and SLID. Thirty seven cases were resistant to FQs only (Pre-XDR)
183 while 11 were resistant to both classes of drugs (XDR). We did not recover any isolate that
184 was aminoglycoside resistant but FLQ sensitive.

185 Discussion

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

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3 192 The results of the national anti-tuberculosis drug resistance survey has shown that the
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5 193 incidence of TB is highest in the 25–34 year age group in India⁹. We however documented a
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7 194 slightly higher age group in our study. Our cohort was dominated by males and nearly 43%
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9 195 patients were young adults in the age group of 30-45 years. The high frequency of the disease
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11 196 among the younger population may facilitate the transmission of TB in the community due to
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13 197 greater mobility of youth. A gender analysis of the TB epidemic shows that TB affects
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15 198 different genders differently. In 2016, about 40% of the 2.79 million new cases of TB in India
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17 199 were among women and the male to female ratio for TB stood between 1.07 to 2.25 with
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19 200 women accounting for 40% of new cases. In our study, it was 3.5. Studies have shown that
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21 201 women may be diagnosed late or not diagnosed at all due to socio-cultural barriers such as
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23 202 high burden of household work, illiteracy, restricted mobility as well as lack of autonomy.
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25 203 There is also a high level of stigma associated with the disease among unmarried females¹⁰.
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27 204 WHO's current policies and guidance recommend that the Xpert MTB/RIF assay may be
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29 205 used as an initial diagnostic test in individuals likely of having MDR-TB. About 36% of the
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31 206 samples included in our study were positive for *Mycobacterium tuberculosis* complex and the
32
33 207 overall rate of resistance to rifampicin was 21.6%. We assessed the burden of tuberculosis in a
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35 208 large cohort of consecutive patients in our hospital thereby eliminating any selection bias in
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37 209 the study population. We also recovered 34 isolates of Non-tuberculous Mycobacteria (NTM)
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39 210 from various samples and all these isolates were negative by the Xpert MTB/RIF assay.
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41 211 In a study carried out in Mumbai, India's commercial capital and one of the most densely
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43 212 populated and congested cities, Udwardia et al tested 1539 samples at a tertiary care private
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45 213 hospital and reported MDR-TB in 30.14% of cases¹¹. In another retrospective study from
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47 214 South India, Shivekar et al performed the MTBDRplus assay on 20245 specimens obtained
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49 215 from presumptive MDR-TB cases during a 6-year study period from 2013 to 2018. Based on
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51 216 the *poB* gene, true resistance, hetero-resistance, and inferred resistance to rifampicin was
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3 217 found in 38%, 29.3%, and 32.7% of the 1582 MDR cases, respectively.¹²Goyal et al
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5 218 published a recent systematic review of 75 epidemiological studies for the prevalence of
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7 219 DRTB in India across 2 decades, from 1995 to 2015.Comparative analysis revealed a
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10 220 worsening trend in DR-TB between the two study decades, 37.7% vs 46.1% respectively. The
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12 221 countrywide prevalence of MDR-TB also increased from the earlier decade at 14.9% to
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14 222 27.9% in decade 2.¹³However, the report of the first national anti-tuberculosis drug resistance
15
16 223 survey in India conducted during 2014-2016 concluded that among all TB patients tested, the
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18 224 MDR-TB rate was 6.19% with 2.84% among new and 11.60% among previously treated TB
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20 225 patients. The survey has probably under-estimated the true burden of resistance in India since
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22 226 it excluded both smear-negative TB cases as well as extrapulmonary TB and did not include
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24 227 the private sector.⁹

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28 228 We also attempted to find the overall agreement of the Xpert MTB/RIF assay compared to
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30 229 culture in our study cohort. In the present study, the sensitivity of the test was nearly 87% and
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32 230 it rose to 100% for smear-positive specimens. The accuracy of the MTB/RIF test to detect the
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34 231 presence of tuberculosis in smear-positive cases has been reported to be between 98% to
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36 232 100%.¹⁴For smear negative specimens, Zeka et al have reported sensitivities of 68.6% and
37
38 233 47.7% in pulmonary and extra-pulmonary samples respectively.¹⁵ Sixty nine specimens that
39
40 234 were culture positive tested negative by the Xpert MTB/RIF assay in our study resulting in a
41
42 235 specificity of 61.6% (69/112).All these samples were smear negative. We also detected 413
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44 236 cases (40% of 1032 positives) by the Xpert MTB/RIF assay that were missed by both smear
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46 237 as well as culture. The assay achieved higher diagnostic yield than microscopy and increased
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48 238 TB case finding by a factor of about 2.

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51 239 The results of second line testing in our study revealed 77.4% resistance to fluoroquinolones
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53 240 among RR isolates which is higher than other studies reported from India. Sethi et al in a
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55 241 retrospective study from a tertiary care center in northern India have documented an overall
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3 242 rate of 38.6% FQ resistance among 863 rifampicin-resistant TB isolates.¹⁶ In another study
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5 243 from eight health care facilities in greater Mumbai between 2005 and 2013, Dalal et al
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7 244 investigated the trends over time of patterns of drug resistance in a sample of MDR-TB
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9 245 patients. Between 2005–2007 and 2011–2013, patients with ofloxacin and moxifloxacin
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11 246 resistance significantly increased from 57.6% to 75.3% and from 60.0% to 69.5% ($p<0.05$).¹⁷
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13 247 A meta-analysis by Ho et al has concluded that globally FQ resistance in MTB is largely
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15 248 confined to MDR strains and knowledge of the global extent of this resistance pattern is
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17 249 currently hampered by the absence of surveillance studies in the majority of regions where
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19 250 TB is endemic.¹⁸
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21 251 Updated WHO guidelines, published in June 2020, recommend that for patients with MDR-
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23 252 TB and additional fluoroquinolone resistance, a regimen composed of bedaquiline,
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25 253 pretomanid, and linezolid may be used under operational research conditions (6-9 months).⁵
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27 254 Chee et al in a study conducted between 2002-2016 on 280 patients have demonstrated that
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29 255 only about 30% of patients with MDR pulmonary TB diagnosed in their study cohort from
30
31 256 South-east Asia were eligible for the WHO shorter MDR-TB treatment regimen.¹⁹In a similar
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33 257 study from northern India, Singh and Jain have explored the eligibility of the shorter regimen
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35 258 in MDR patients under the programmatic setting. Out of 541 conclusive LPA-SLD results,
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37 259 the proportion of strains resistant to only fluoroquinolones was nearly 50% while 8.3% were
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39 260 resistant to both fluoroquinolones and SLIDs. ²⁰Eleven cases in our study were extensively
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41 261 drug resistant.
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43 262 The high rates of drug resistance observed in our study may be due to the fact that ours is a
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45 263 tertiary care hospital in the state of Uttar Pradesh which has over 20% of the total number of
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47 264 notified cases of TB in India. We see patients after the referring hospital has already tried and
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49 265 failed to control infection using a combination of different anti-microbial agents. Since
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51 266 facilities for microbiological studies are usually not available in district hospitals/smaller
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3 267 cities in India, the first contact physician/surgeon/referral facility are compelled to initiate
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5 268 broad-spectrum antibiotics. Indiscriminate antimicrobial therapy without establishing the
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7 269 etiology of infection selects out the resistant strains. McDowell and Pai in an ethnographic
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10 270 study on the mismanagement of empirical TB treatment in India have demonstrated that all
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12 271 non-specialist private practitioners began antibiotic treatment, especially quinolones, for
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14 272 persistent cough before prescribing a test.²¹ Their results underscore the fact that inappropriate
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16 273 prescribing practices in India's burgeoning private sector including easy, over-the-counter
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18 274 access to fluoroquinolones need to be halted as soon as possible.

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21 275 The alarming rate of drug resistance in our study to rifampicin as well as fluoroquinolones
22
23 276 has important implications for implementation of government strategies to control the TB
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25 277 epidemic in India. Firstly, standardized regimens containing a FQ to treat MDR –TB cases
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27 278 carry a high risk of being sub-optimal and resulting in treatment failure. Secondly, with such
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29 279 high rates of drug resistance India will have to equip itself with enough Mycobacteriology
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31 280 laboratories offering culture and drug susceptibility testing (C-DST) to both first as well as
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33 281 second-line agents. Currently, the focus is to roll out sufficient number of GeneXpert
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35 282 MTB/RIF assay machines to diagnose rifampicin resistant strains of *Mycobacterium*
36
37 283 *tuberculosis*. However, this strategy may mask the diagnosis of pre-XDR TB. A high rate of
38
39 284 FQ resistance has also been noted in newly diagnosed MDR/RR TB cases, which might be
40
41 285 due to transmission of the drug-resistant strains. ³It is estimated that in India, by 2032, 85%
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43 286 of multidrug-resistant tuberculosis infections would be from primary transmission, compared
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45 287 with only 15% in 2012. In the *Lancet Public Health*, Law et al have created a dynamic model
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47 288 of the tuberculosis epidemic in India, which they use to estimate the incidence of drug
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49 289 susceptible tuberculosis and multidrug-resistant tuberculosis over the next 20 years³. They
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51 290 have analyzed the emergence of drug resistance in all major health-care sectors in India.
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53 291 Private clinics in India are often used by patients seeking TB treatment and they administer
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3 292 regimens that are not recommended by standard guidelines. This not only results in
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5 293 suboptimal outcome but also potentially generates MDR TB. They conclude that as multidrug
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7 294 resistant tuberculosis transitions from an acquired condition to a primarily transmitted
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9 295 disease, improving the effectiveness of drug-susceptible tuberculosis treatment can no longer
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11 296 contain the spread of the epidemic. This epidemiological shift has profound resource
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13 297 implications since the cost of treatment of multidrug-resistant tuberculosis treatment can
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15 298 exceed that of first-line tuberculosis therapy by a factor of ten or more.

16
17 299 In addition, notification data from low-income and middle-income countries, are prone to
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19 300 underreporting and cannot be interpreted without additional information on case detection
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21 301 rate. The DR-TB diagnostic algorithm as given in the PMDT guidelines recommends SL –
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23 302 LPA testing for all RR TB cases diagnosed by the CBNAAT assay. However, it is labor
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25 303 intensive and requires trained manpower. Severe lack of Microbiology laboratories providing
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27 304 universal DST and visual interpretation of bands is a huge limitation especially in smear
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29 305 negative and extra-pulmonary cases with inadequate sample volumes as has been our
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31 306 experience even with version 2 of the test.

32
33 307 There were several limitations to this study. One of the methodological limitations of our
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35 308 study was that we could not perform liquid culture DST as well as sequencing and confirm
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37 309 the results of the drug resistant isolates. Another limitation was that we did not differentiate
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39 310 between new and previously treated TB cases. Most of the DR-TB patients in our cohort at
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41 311 the time of diagnosis were attached to the PMDT follow up for further evaluation and
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43 312 management except for some who insisted on institutional management. We could therefore
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45 313 put up SL-DST for only 62 cases. We also did not receive any grant for this study and hence
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47 314 could not put up FL-LPA on the 69 culture positive isolates that tested negative by the Xpert
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49 315 MTB/RIF assay. In addition, a study of risk factors in such a high burden setting would have
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51 316 allowed us to offer more useful remedies to policy makers.
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3 **317 CONCLUSION**
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5 **318** In conclusion, we have not come across any prospective study from India on such a large
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7 **319** number of pulmonary as well as extrapulmonary samples performed by both conventional
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9 **320** and molecular methods. Our study provides comprehensive data on the high burden of drug
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11 **321** resistant TB in India at a 1200 bed tertiary care center in northern India. The need of the hour
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13 **322** is to have enough Mycobacteriology laboratories offering both first and second line DST
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15 **323** under the NTEP umbrella. The high rates of FQ resistance documented in our study should
16
17 **324** prompt policy makers to tightly regulate them as reserve drugs, otherwise the ambitious goal
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19 **325** of the Government of India to eliminate tuberculosis by 2025 seems bleak.
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25
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30
31 **329 Contributors** RM conceptualized the manuscript, designed the methods, supervised the
32
33 **330** study and wrote the manuscript. VK curated the data. AN supervised the study and edited the
34
35 **331** manuscript. All authors have reviewed the final version of the manuscript.
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41
42 **334** agency in the public, commercial or not – for- profit sectors.
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45 **335**
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47 **336 Competing interests** None declared.
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49 **337**

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51 **338 Patient consent for publication** Not required.
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3 340 **Ethics approval** The study protocol was approved by the Institutional Ethics Committee of
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5 341 Sanjay Gandhi Post Graduate Institute of Medical Sciences. Written informed consent was
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7 342 obtained from each participant.
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12 344 **Data availability statement** Data are available upon reasonable request.
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3 **439 Table 1.** Smear and culture results among samples positive by Xpert MTB /RIF assay

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| 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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3 **458 Table 2:** Comparison of Gene Xpert MTB / RIF positive, MTB culture positive results with
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5 smear results
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8 **460**

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10 **461**

| | 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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3 477 **Figure legends**
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8 479 **Figure 1.** Age distribution of cases positive by Xpert MTB/ RIF assay (n=1032)
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12 481 **Figure 2.** Distribution of samples positive by the Xpert MTB/RIF assay (n=1032)
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17 483 **Figure 3.** Results of conventional and molecular diagnostic testing by Xpert MTB/ RIF assay
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19 484 of samples included in the study (n=3045)
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23 486 **Figure 4.** Distribution of samples among rifampicin resistant cases (n=223)
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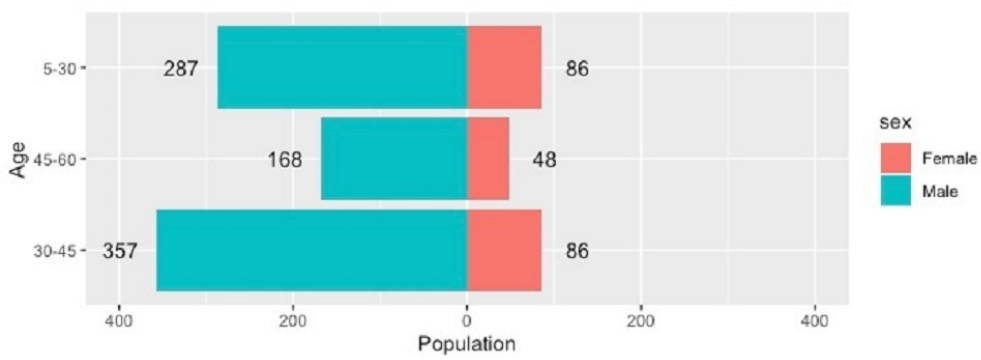
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28 488 **Figure 5:** Results for second line susceptibility testing performed by line probe assay (LPA)
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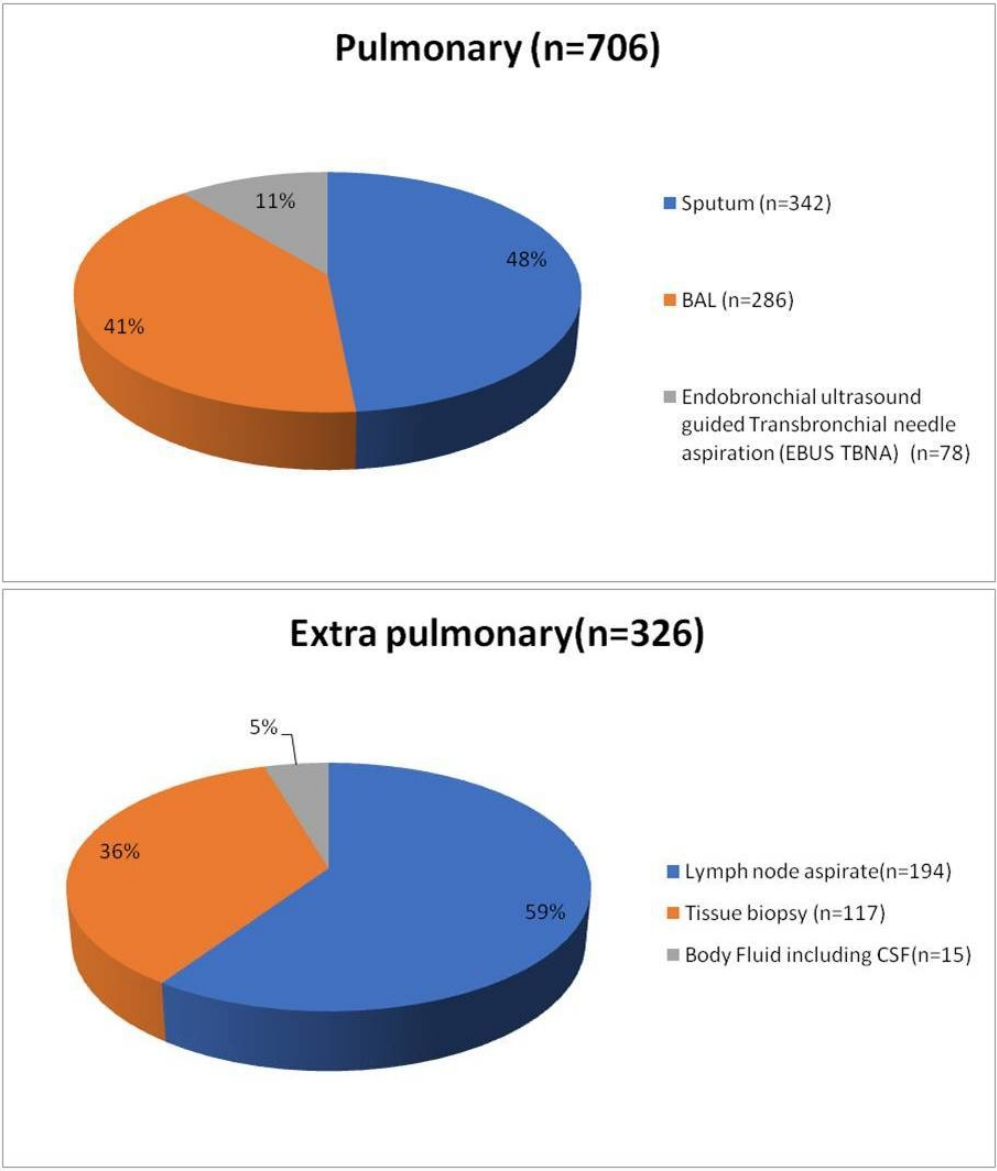
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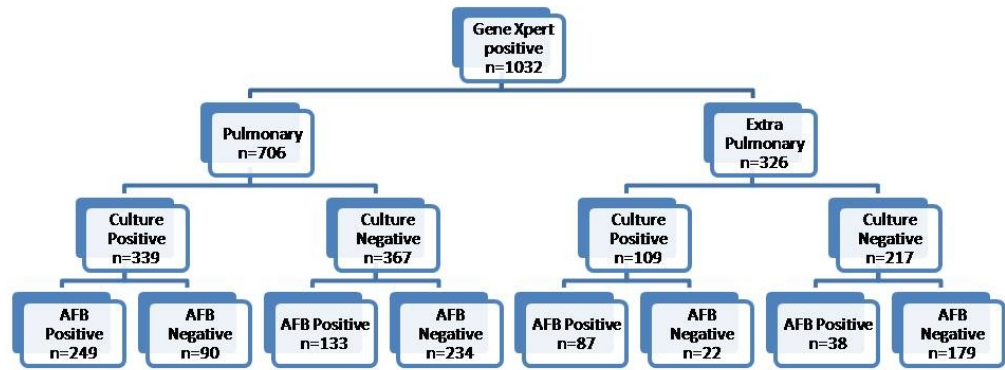
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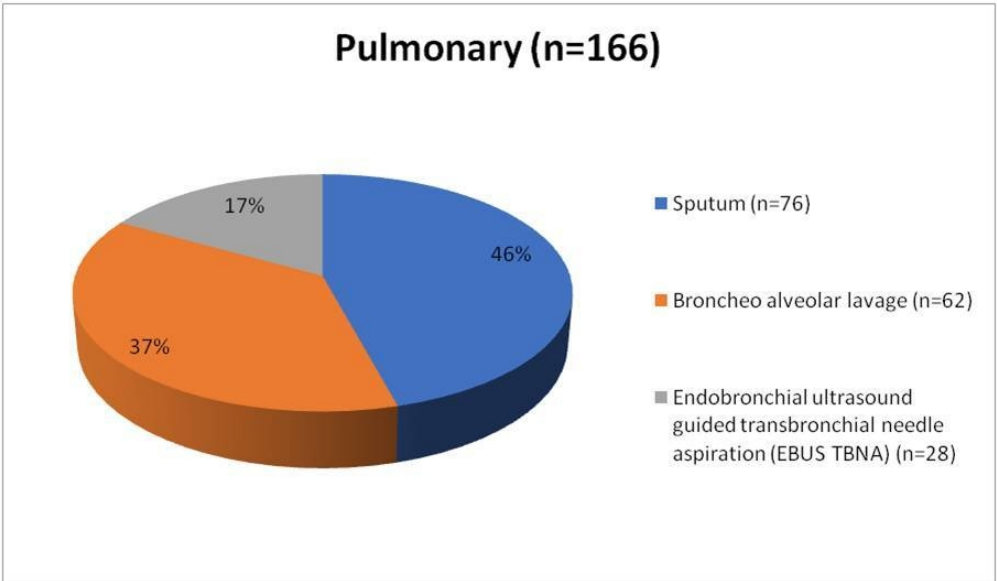
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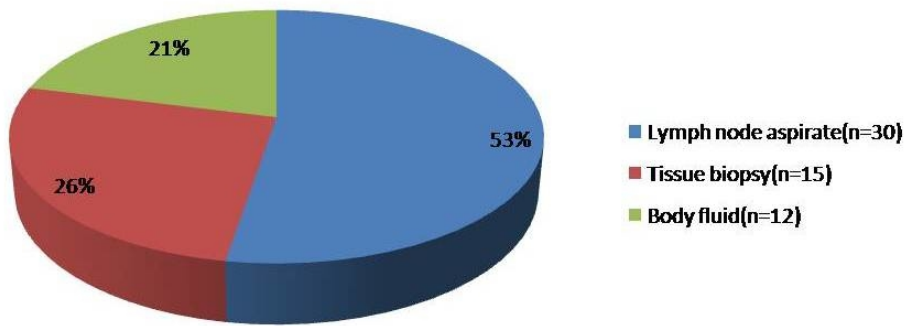


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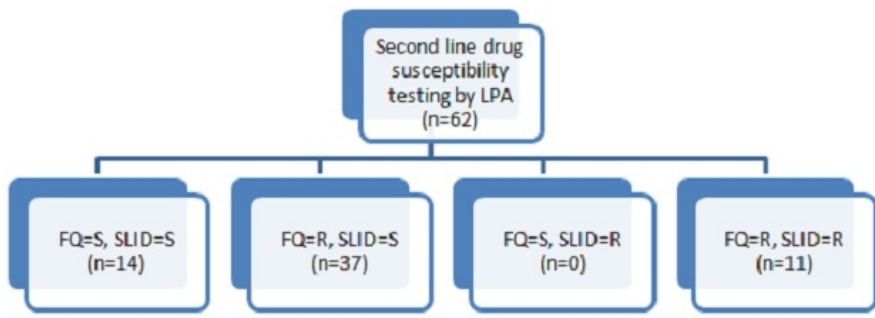


Extra pulmonary (n=57)



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

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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 (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram | #6/126-142 NA #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 (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount) | #7/152-154 #6/1120-122 #13/307-310 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 (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | NA #7/152-154 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.