PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a prospective single centre cohort study
AUTHORS	Misra, Richa; Kesarwani, Vasudha; Nath, Alok

VERSION 1 – REVIEW

REVIEWER	Bhatia, Vineet World Health Organization Regional Office for South-East Asia, Communicable Diseases
REVIEW RETURNED	12-Oct-2020

GENERAL COMMENTS	While the study tends to answer an important and topically relevant question, some revisions and clarifications on methodology will help the reader better understand the implications of the results. The comments are placed below: Overall 1. Methodology – The main questions are around sampling process at each stage of the study a. The selection process of patients/ samples in this study is not clear. Is the figure of 3045 indicative of all patients visiting the OPD or there were some screening criteria used? If so, what screening criteria and how many patients were left out? Was it a general OPD or a specialist OPD? b. It is stated that a total of 3045 ‘samples’ were tested. Whether it mean ‘samples’ or ‘patients’ needs to be clarified. c. It is not clear why out of the identified 223 Rifampicin resistant (RR) cases only 62 were tested for second-line resistance. Volume of sample and culture positivity do not appear to be sufficient reasons. A disaggregation of data is required to specify which patients got tested and whether the selection introduced a bias. d. The algorithm followed by the study to detect resistance appears different from the country's national guidelines on sputum examination for drug resistance. As per the narrative, only one sputum sample was collected and divided into two portions while the national guidelines advocate for collection of two samples simultaneously. e. There is no description of the laboratory where culture and the resistance tests were conducted and whether this is an accredited laboratory under the national network for the tests performed. f. It needs to be clarified if pulmonary concomitant lesions were ruled out in extra-pulmonary TB cases. This is not clear from case definitions presented in the narrative. g. It seems that there were significant number of discrepant results between culture results and GeneXpert assay - both ways. However, it is not clear how the discrepancies were sorted – apparently all

	samples found positive by any of the techniques were considered positive. However, was a possible cross-contamination ruled out for discordant results? Further, if a sample is culture positive and GeneXpert assay negative, was it subjected separately to testing for rifampicin resistance. 2. Discussions: These need to be more focused as per the objective of the study a. Presently the discussions are more around sensitivity of GeneXpert, diagnostic delays and other aspects of management. It does not appear that the study was originally designed or powered for this purpose. b. While this study certainly analyses samples from a large number of patients, this is not the only one to be published from a tertiary hospital. There should be comparison with results of other similar publications. c. A significant proportion of tested samples were found MTB+ve (~34% among tested), RR+ve (~22%) and resistant to second line drugs (>77% to FQ). This is unusual and hence all forms of potential bias need to be discussed. d. There is no mention of the drug resistance survey (DRS) carried out by the national programme completed in 2016. The DRS would also provided data on drug-resistance proportions in the community. 3. Conclusions: This should include the implications for the national programme as well as other tertiary hospitals because of high proportions of first- and second-line drug resistance expected in patients reporting to tertiary care hospitals. Specific comments on pages and references Page 6/28, lines • #9 – It doesn't look like that the reference article #2 cited in the sentence has the information on proportion of TB burden in India. • #10 and 11 o WHO provides estimates of RR and multidrug-resistant (MDR-TB) collectively, not just MDR-TB. This may be corrected in the manuscript while mentioning the burden o The incidence figures quoted for RR/MDR-TB in India are from Global TB Report 2019 for the reporting year 2018 and not 2016. This needs to be corrected in the text as well as reference • #13 an 14 – It is not clear why the text mentions of 20025 guidelines for programmatic management of drug-resistant TB (PMDT) • #33 – The information on <40% referral from private sector could not be located in the referenced document • #38-40 – The publications referenced for shorter regimen endorsed by WHO are research publication and are not in exact alignment with the regimen recommended. It is better to reference WHO guidelines on treatment of MDR-TB (latest being the 2020 update) • #49-50 – Status of laboratory facilities availablity if being quoted from a 2011 publication. A more recent publication should be used to comment on the availablity of laboratory services. Page 11/28, lines • #10 – The estimated incidence of RR and MDR-TB for India as per Global TB Report 2019 is 9.6/100,000 population. Page 12/28, lines • #47-54 – It seems that reference for this data should be 20 rather than 19, as is provided now. Additional comment: GeneXpert, Xpert MTB/Rif, WRD and CBNAAT

	has been used interchangeably throughout the document. This needs to be made consistent.

REVIEWER	Yu, Chuanhua Wuhan University, Department of Epidemiology and Biostatistics, School of Health Sciences
REVIEW RETURNED	09-Nov-2020

GENERAL COMMENTS	Assessment of burden of drug-resistant tuberculosis at a tertiary care center in northern India 1. Description of methods • 1. The sex difference in tuberculosis was most significant. Here we can add the specific values to supplement the gender difference. Especially in figure1, the comparison between gender and age can be added • Prospective observational research should be written in more detail in the research design part, such as how to analyze subgroup analysis, whether sensitivity analysis is required, etc. 2. Narrative structure • This study uses many pictures to show the disease burden of drug-resistant tuberculosis, but I think that if the corresponding results can be presented in the form of a table, it will help us to understand the disease burden of tuberculosis diseases more quantitatively. • In abstract, “MDR” is the first time to appear, so it should show the full name. • In Introduction “RNTCOP” is also the first time to appear, it should add the full name. 3. Tables and figures • It is too simple and monotonous to draw only the estimated value of the point. For table 1 and table 2, it is recommended to display the 95% uncertainty interval of the corresponding variable in the table rather than number and percent. • This results is presented by a lot of figures, which otherwise can be represented in some other comprehensive way, this can be summarized in table form for 2019 estimate with 95% UI and % change between 2017 and 2019, for tuberculosis with multiple disease aspects(e.g. death, prevalence, DALY etc.). • All ages trends for multiple aspects of single disease can be plot on single graph by multiple color lines, and the data provided in table1 and table 2 are too little, so that the analysis is not thorough enough 4. Future directions • The association of tuberculosis and other diseases with sociodemographic index over time may further explore the socioeconomic transition and change in disease risk. • The study of attributable risk factors to tuberculosis may offer the useful remedies or recommendation to policy makers for control of future disease burden. This paper should add many risk factors to analyze the disease burden. • The risk factors have many overlaying or correlation. For example, dietary risks, high fasting plasma glucose, high body mass index and low physical activity are themselves highly correlated. The relationship between tuberculosis and risk factor is not stressed and proved enough in this text. • Data availability could be provided and weighted in further studies • The discussions and suggestions come with little citations and proof. More proof and data could be added into the study to show correlation.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. Methodology – The main questions are around sampling process at each stage of the study

a. The selection process of patients/ samples in this study is not clear. Is the figure of 3045 indicative of all patients visiting the OPD or there were some screening criteria used? If so, what screening criteria and how many patients were left out? Was it a general OPD or a specialist OPD?

Response a: We included both in-patients as well as out-patients in our study cohort. Our teaching hospital is a 1200 bed tertiary care referral centre. We did not use any screening criterion for including patients in our study. All clinically suspected cases of both pulmonary and extra-pulmonary tuberculosis seen during the clinical routine were included in the study. We received pulmonary samples like BAL, EBUS-TBNA and cases of lymphadenitis from the Department of Pulmonary Medicine. Biopsy samples, pus and CSF were received from the Departments of Gastroenterology, Neurology and Pathology.

b. It is stated that a total of 3045 'samples' were tested. Whether it mean 'samples' or 'patients' needs to be clarified.

Response b: 3045 pulmonary and extra-pulmonary samples (not patients) were collected between March 2017 and June 2019 during the clinical routine. They were all non-duplicate. The sample distribution has been mentioned in the methods section on page number 5 and line numbers 100-104.

c. It is not clear why out of the identified 223 Rifampicin resistant (RR) cases only 62 were tested for second-line resistance. Volume of sample and culture positivity do not appear to be sufficient reasons. A disaggregation of data is required to specify which patients got tested and whether the selection introduced a bias.

Response c: The following chart shows the break-up samples included in the study.

Most of the DR-TB patients in our cohort at the time of diagnosis were attached to the PMDT follow up for further evaluation and management except for some who insisted on institutional management First and second line susceptibility testing by LPA is a paid test at our centre. We did not receive any grant for this study. Therefore we could test only those samples for which requisition forms were raised. We have added this as a limitation of the study on page number 13, line number 303-305.

d. The algorithm followed by the study to detect resistance appears different from the country's national guidelines on sputum examination for drug resistance. As per the narrative, only one sputum sample was collected and divided into two portions while the national guidelines advocate for collection of two samples simultaneously.

Response d: We collected two sputum samples as per the national guidelines but excluded duplicate isolates from the study. We have added this in the results section of the study on page number 7, line number 147-148.

e. There is no description of the laboratory where culture and the resistance tests were conducted and whether this is an accredited laboratory under the national network for the tests performed.

Response e: Ours is a tertiary care referral centre in northern India and our laboratory participates as a National Certified Diagnostic Laboratory for MDR-TB follow ups from 14 districts of Uttar Pradesh state under the National Tuberculosis Elimination Program (NTEP).We routinely perform both solid (in-houe prepared LJ medium) and liquid culture (BacT/Alert culture, bioMérieux as well as molecular diagnostic tests such as GeneXpert MTB/RIF assay and Line Probe Assay with quality control.

f. It needs to be clarified if pulmonary concomitant lesions were ruled out in extra-pulmonary TB cases. This is not clear from case definitions presented in the narrative.

Response f: We ruled out the presence or absence of concomitant pulmonary lesions in extra-pulmonary TB cases by appropriate investigations and review of case files. All our patients were clinically suspected cases of tuberculosis. We have added this in the definitions section under data collection (page number 6, line number 134-135)

g. It seems that there were significant number of discrepant results between culture results and GeneXpert assay - both ways. However, it is not clear how the discrepancies were sorted – apparently all samples found positive by any of the techniques were considered positive. However, was a possible cross-contamination ruled out for discordant results? Further, if a sample is culture positive and GeneXpert assay negative, was it subjected separately to testing for rifampicin resstance.

Response g: We considered a "bacteriologically confirmed TB case" as per WHO definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014 and January 2020)- reference 7, in which a WHO-approved rapid diagnostics (WRD) such as Xpert MTB/RIF assay has been included. We therefore considered any sample positive by either GeneXpert MTB/RIF assay or culture as a confirmed case of TB. In our study, 1032 samples tested positive by the GeneXpert MTB/RIF assay out of which 517 were culture positive as shown in Table 1. In addition, 69 samples that tested negative by the Xpert MTB/RIF assay were culture positive. We performed the MPT64 antigen test (SD BIOLINE TB Ag MPT64 Rapid) on all 69 cultures. This has been added in the methods section on page number 6, line number 121. The possibility of cross-contamination was ruled out as all processing was performed in a Biosafety cabinet with full infection control precautions. All cases included in our study were clinically suspected cases of tuberculosis seen during the clinical routine.

We could not perform 1st line LPA for these 69 isolates for determining rifampicin resistance (and INH as well) as we did not receive any grant for this study. We have added this as a limitation of the study on page number 13, line number 304-306.

2. Discussions: These need to be more focused as per the objective of the study
a. Presently the discussions are more around sensitivity of GeneXpert, diagnostic delays and other aspects of management. It does not appear that the study was originally designed or powered for this purpose.

Response a: The authors have modified the discussion section.
b. While this study certainly analyses samples from a large number of patients, this is not the only one to be published from a tertiary hospital. There should be comparison with results of other similar publications.

Response b: The authors have included a comparison with similar publications in the discussion section along with appropriate references (10, 11, 15, 16, 19)

c. A significant proportion of tested samples were found MTB+ve (~34% among tested), RR+ve (~22%) and resistant to second line drugs (>77% to FQ). This is unusual and hence all forms of potential bias need to be discussed.

Response c: We assessed the burden of tuberculosis as well as drug resistance in a large cohort of consecutive patients in our hospital thereby eliminating any selection bias in the study population. A comparison of data with studies from metro cities like Mumbai, Chandigarh in north India and South India (references 10, 11 and 15) has revealed a similar pattern of disease. The high rates of drug resistance observed in our study may be due to the fact that ours is a tertiary care hospital in the state of Uttar Pradesh which has over 20% of the total number of notified cases of TB in India and we see

patients after the referring hospital has already tried and failed to control infection using a combination of different anti-microbial agents. It is important to note that we have included both smear negative and extra-pulmonary samples which could be the reason for a high rate of disease burden and drug resistance.

d. There is no mention of the drug resistance survey (DRS) carried out by the national programme completed in 2016. The DRS would also provided data on drug-resistance proportions in the community.

Response d: The authors have included the results of DRS in the discussion section on page number 10, line number 247-252 (reference 8).

3. Conclusions: This should include the implications for the national programme as well as other tertiary hospitals because of high proportions of first- and second-line drug resistance expected in patients reporting to tertiary care hospitals.

Response: The authors have modified the conclusions appropriately (page number 13, line number 311-316).

Specific comments on pages and references

Page 6/28, lines

• **#9** – It doesn't look like that the reference article #2 cited in the sentence has the information on proportion of TB burden in India.

Response: We have modified the reference.

• **#10and11**

o WHO provides estimates of RR and multidrug-resistant (MDR-TB) collectively, not just MDR-TB. This may be corrected in the manuscript while mentioning the burden

Response: We have corrected this in the manuscript-Introduction section- page number 4, line number 72-73 and in the Discussion section- page number 8, line number 72-73.

o The incidence figures quoted for RR/MDR-TB in India are from Global TB Report 2019 for the reporting year 2018 and not 2016. This needs to be corrected in the text as well as reference

Response: We have included data from Global TB Report 2020 (reference 1).

- **#13 an 14** – It is not clear why the text mentions of 20025 guidelines for programmatic management of drug-resistant TB (PMDT)

Response: We have deleted this in the Introduction section**.**

- **#33** – The information on <40% referral from private sector could not be located in the referenced document

Response: We have corrected the reference (No.3).

- **#38-40** – The publications referenced for shorter regimen endorsed by WHO are research publication and are not in exact alignment with the regimen recommended. It is better to reference WHO guidelines on treatment of MDR-TB (latest being the 2020 update)

Response: We have added the update –reference 5.

- **#49-50** – Status of laboratory facilities availablity if being quoted from a 2011 publication. A more recent publication should be used to comment on the availablity of laboratory services.

Response: We have corrected the reference (No. 4)

Page 11/28, lines

- **#10** – The estimated incidence of RR and MDR-TB for India as per Global TB Report 2019 is 9.6/100,000 population.

Response: We have corrected this in the manuscript.

Page 12/28, lines

- **#47-54** – It seems that reference for this data should be 20 rather than 19, as is provided now.

Response: We have corrected the reference.

Additional comment: GeneXpert, Xpert MTB/Rif, WRD and CBNAAT has been used

interchangeably throughout the document. This needs to be made consistent.

Response: We have corrected this in the manuscript.

Reviewer: 2

1. Description of methods

- 1. The sex difference in tuberculosis was most significant. Here we can add the specific values to supplement the gender difference. Especially in figure1, the comparison between gender and age can be added

Response: We have modified Figure 1.

- Prospective observational research should be written in more detail in the research design part, such as how to analze subgroup analysis, whether sensitivity analysis is required, etc.

Response: The authors have not been able to do sub-group analysis. It was not one of the aims of our study.

2. Narrative structure

- This study uses many pictures to show the disease burden of drug-resistant tuberculosis, but I think that if the corresponding results can be presented in the form of a table, it will help us to understand the disease burden of tuberculosis diseases more quantitatively.

Response: The authors have represented the results in Tables 1 and 2.

- In abstract, “MDR” is the first time to appear, so it should show the full name.

Response: We have corrected this in the abstract.

- In Introduction “RNTCOP” is also the first time to appear, it should add the full name.

Response: We have corrected this in the Introduction section.

3. **Tables and figures**

- It is too simple and monotonous to draw only the estimated value of the point. For table 1 and table 2, it is recommended to display the 95% uncertainty interval of the corresponding variable in the table rather than number and percent.

Response: As we have not calculated a range of values the 95% uncertainty interval does not apply to our data.

- This results is presented by a lot of figures, which otherwise can be represented in some other comprehensive way, this can be summarized in table form for 2019 estimate with 95% UI and % change between 2017 and 2019, for tuberculosis with multiple disease aspects(e.g. death, prevalence, DALY etc.).

Response: Our study cohort is prospective and observational. We have not divided our data for comparison between time periods or as a range of values.

- All ages trends for multiple aspects of single disease can be plot on single graph by multiple color lines, and the data provided in table1 and table 2 are too little, so that the analysis is not thorough enough.

Response: We have suitably modified Figure 1 and Tables 1 and 2 represent the smear and culture results and their percentage agreement with the XpertMTB/RIF assay.

4. Future directions

- The association of tuberculosis and other diseases with sociodemographic index over time may further explore the socioeconomic transition and change in disease risk.

Response: The authors will attempt to analyse this in future.

- The study of attributable risk factors to tuberculosis may offer the useful remedies or recommendation to policy makers for control of future disease burden. This paper should add many risk factors to analyze the disease burden.

Response: The authors will definitely design a study of attributable risk factors to tuberculosis in future.

• The risk factors have many overlaying or correlation. For exmple, dietary risks, high fasting plasma glucose, high body mass index and low physical activity are themselves highly correlated. The relationship between tuberculosis and risk factor is not stressed and proved enough in this text.

Response: The authors agree with the reviewer. However, this was not one of the aims of the study.

• Data availability could be provided and weighted in further studies

Response: We agree with the reviewer.

• The discussions and suggestions come with little citations and proof. More proof and data could be added into the study to show correlation

Response: The authors have modified the discussion and conclusions.

VERSION 2 – REVIEW

REVIEWER	Bhatia, Vineet World Health Organization Regional Office for South-East Asia, Communicable Diseases
REVIEW RETURNED	13-Mar-2021

GENERAL COMMENTS	Thanks for the clarifications provided and changes made to the manuscript. A few follow-up and additional comments: 1. The title of the manuscript has now been changed and the words "community-based" added. It is not clear how this study can be classified as community-based because all data is emerging from the hospital which is unlikely to be representative of community picture. 2. It is still felt that a significant bias may have been introduced in the study because of inability to perform second-line DST on 62 out of 223 RR-TB patients. This means that around 70% of the sample was excluded. One of the ways to partly address this is to make a comparison of population characteristics of 223 RR-TB patients and the 62 patients who got tested. 3. Page 4, line 73, use of old publications (2 and 3) for referencing TB burden seems redundant 4. Page 4 line 87: For the status of laboratory services in India, please use the TB India 2020 report available at www.tbcindia.nic.in 5. Page 8, line 187: Please check the country wise burden again from WHO Global TB Report, 2020, page 23. 6. Page 10, line 219-221: While it is good to see referencing to drug-resistance survey (DRS) being added now, it is not clear how authors arrived at conclusion that there was an underreporting

	because of exclusion of smear negative and EP-TB cases. Some details of assumptions may be required here. 7. The word “suspected” for TB patients is considered as discriminatory and should be avoided. Better replace with terms such as “likely”, “presumptive”, etc.

VERSION 2 – AUTHOR RESPONSE

Reviewer1

Comment 1: The title of the manuscript has now been changed and the words “community-based” added. It is not clear how this study can be classified as community-based because all data is emerging from the hospital which is unlikely to be representative of community picture.

Response 1: We have modified the title of the study as “Assessment of burden of drug –resistant tuberculosis at a tertiary care centre in northern India: a prospective single centre cohort study” (Title page, page 1)

Comment 2: It is still felt that a significant bias may have been introduced in the study because of inability to perform second-line DST on 62 out of 223 RR-TB patients. This means that around 70% of the sample was excluded. One of the ways to partly address this is to make a comparison of population characteristics of 223 RR-TB patients and the 62 patients who got tested.

Response 2: We agree with the reviewer that we have not been able to perform SL-LPA on nearly 70% of the samples. However, the main aim of our study was to define the burden of rifampicin mono-resistant tuberculosis at our centre. This is predominantly a laboratory based study and we therefore did not collect the population characteristics or the demographic details with comorbidities of patients (such as smoking, alcohol, Diabetes mellitus etc). We have also mentioned this as a limitation of the study on Page 13, line no.317-322. In addition, the high rates of fluoroquinolone resistance (77.4%), documented in our study are representative of the prevalent patterns of drug resistance as cited in references 17 and 20.

Comment 3: Page 4, line 73, use of old publications (2 and 3) for referencing TB burden seems redundant.

Response 3: We have deleted references 2 and 3 for defining the burden of TB in India.

Comment 4: Page 4 line 87: For the status of laboratory services in India, please use the TB India 2020 report available at HYPERLINK "http://www.tbcindia.nic.in/" \t "_blank"www.tbcindia.nic.in

Response 4: We have mentioned the status of laboratory services in India from the TB India Report 2020 on page number 4, line numbers 92-95 and included it as a reference also (Reference number 7).

Comment 5: Page 8, line 187: Please check the country wise burden again from WHO Global TB Report, 2020, page 23.

Response 5: We have corrected the country wise burden from WHO Global TB Report 2020 (Page 9, line numbers 195-197).

Comment 6: Page 10, line 219-221: While it is good to see referencing to drug-resistance survey (DRS) being added now, it is not clear how authors arrived at conclusion that there was an underreporting because of exclusion of smear negative and EP-TB cases. Some details of assumptions may be required here.

Response 6: As mentioned in the Drug Resistance Survey 2016, it's primary objective was to determine the prevalence of MDR –TB among newly diagnosed and previously treated sputum smear positive TB patients (Page 6). They have not included sputum smear negative or extra pulmonary TB patients as well as patients diagnosed at correctional facilities (i.e., jails, prisons, asylums) and the private sector.

Chatterjee et al in their commentary on the challenge of drug-resistant tuberculosis in India (Reference number 4) have also documented this as a limitation of the survey.

Comment 7: The word "suspected" for TB patients is considered as discriminatory and should be avoided. Better replace with terms such as "likely", "presumptive", etc

Response 7: We have replaced the word "suspected" for TB patients with the term "likely" (Page 2, line 29 and Page 9, line 211).

VERSION 3 – REVIEW

REVIEWER	Bhatia, Vineet World Health Organization Regional Office for South-East Asia, Communicable Diseases
REVIEW RETURNED	30-Mar-2021

GENERAL COMMENTS	Thanks for addressing the earlier comments. It is suggested that a follow-on study with complete demographic data and wider use of SL-LPA may be conducted after mobilising necessary resources.