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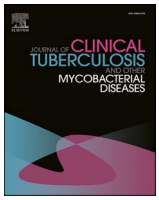

Detection of extensive drug resistance by the Xpert MTB/XDR assay in multidrug resistant tuberculosis cases at a tertiary care centre in northern India, and therapeutic decision making for the six-month BPaLM regimen

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A B S T R A C T

The Xpert MTB/XDR assay has been approved by World Health Organization (WHO) as a reflex test on sputum samples after testing for rifampicin resistance. Recently, the Union Health Ministry of India in September 2024 approved the introduction of the six-month BPaLM regimen under its National TB Elimination Program (NTEP). In this study, the Xpert MTB/XDR assay was used to detect extensive drug resistance in pulmonary and extra-pulmonary tuberculosis patients with positive result for MTBC, and RIF resistance by the Xpert MTB/RIF ULTRA assay. We also aimed to assess the eligibility of patients for the BPaLM regimen based on the drug susceptibility profile of this test in a high burden Indian setting.

We conducted a single centre prospective cohort study between January 2023 to August 2024 on 42 old, and 68 new patients presenting with MDR/RR tuberculosis. A total of 110 samples (82 pulmonary and 28 extra pulmonary samples) were included in the study. The Xpert MTB/XDR assay was used to determine the susceptibilities to isoniazid, fluoroquinolones, amikacin, kanamycin, capreomycin, and ethionamide.

Out of 110 samples processed, 13 samples were 'not detected' by the assay while three gave invalid results. Resistance to isoniazid, fluoroquinolones, amikacin, kanamycin, capreomycin and ethionamide was detected in 85/94 cases (90·42%), 74/94 cases (78·72%), 08/94 cases (8·5%), 13/94 cases (13·83%), 08/94 cases (8·5%), and 14/94 cases (14·89%) respectively.

With the updated definitions of drug-resistant TB and high burden of fluoroquinolone resistance the Xpert MTB/XDR assay has a limited application in India.

Detection of extensive drug resistance by the Xpert MTB/XDR assay in multidrug resistant tuberculosis cases at a tertiary care centre in northern India, and therapeutic decision making for the six-month BPaLM regimen.

1. Introduction

The World Health Organization (WHO) Global TB Report (2023) ranked India among the eight countries that accounted for 87 % of the world's TB cases in 2022, and two-thirds of the global total [1]. Molecular-based assays have become the mainstay of diagnosis for detection of tuberculosis as well as drug susceptibility testing [2,3]. Recently, a low complexity automated NAAT was endorsed by WHO as a reflex test that detects multiple mutations across several genes associated with resistance to isoniazid, ethionamide, fluoroquinolones, amikacin, kanamycin, and capreomycin [4,5,6]. Several studies have demonstrated that the assay has an accuracy of resistance detection between 95 % to 99 % for all drugs [7]. However, there are limited studies that have determined the rate of drug resistance by this test in India, and the usefulness of this assay in guiding therapeutic decision making in a high burden endemic TB setting.

The WHO in December 2022, released two new recommendations regarding the treatment regimens for multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) in infections without fluoroquinolone resistance: the use of the 6-month treatment regimen composed of bedaquiline, pretonamid, linezolid (600 mg) and moxifloxacin (BPaLM) and the use of the 9-month all oral regimen rather than the longer regimens [8].

Recently, in September 2024, the Government of India approved the introduction of the bedaquiline, pretomanid, linezolid (600 mg), and moxifloxacin (BPaLM) regimen under its National TB Elimination Program (NTEP) [9].

In this study, the Xpert MTB/XDR assay was used to detect extensive drug resistance in pulmonary and extra-pulmonary tuberculosis patients with positive result for MTBC, and RIF resistance by the Xpert MTB/RIF

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ULTRA assay. We also aimed to determine the eligibility of patients for the BPaLM regimen based on the drug susceptibility profiling of this test in a high burden Indian setting.

2. Methods

2.1. Study design and setting

We conducted a prospective single centre cross-sectional study between January 2023 to August 2024 at a 1500-bed tertiary care centre in northern India. Clinically suspected pulmonary and extrapulmonary tuberculosis patients with positive result for MTBC, and RIF resistance by the Xpert MTB/RIF ULTRA assay were included in the study. Written informed consent was obtained from all the participants. The study design was approved by the institutional ethics committee.

2.2. Clinical specimens and procedures

82 pulmonary (56 sputum, 25 bronchoalveolar lavage and one EBUS-TBNA (endobronchial ultrasound with real-time guided transbronchial needle aspiration) and 28 extrapulmonary samples (19 lymph node aspirates, seven CSF, one pleural fluid and one biopsy) were collected from both inpatients, and outpatients during the clinical routine. Ziehl-Neelsen staining, and Xpert MTB/XDR assay were performed directly on homogenized samples. Liquid and solid cultures were inoculated with 2 ml decontaminated specimen or directly in case of sterile body fluids.

3. Results

Between January 2023 to August 2024, 110 patients (82 pulmonary, and 28 extra-pulmonary) were screened for eligibility and samples were processed simultaneously for microscopy, culture and the Xpert MTB/XDR assay (Fig. 1). The median age of the patients was 26 years, and 51·8% were females. 53/82 pulmonary (64 %) and 13/28 extra-pulmonary samples (46·4%) were positive on microscopy while 28/62 pulmonary (45 %), and 08/19 extra-pulmonary samples (42·1%) grew on culture. Among the 110 samples undergoing Xpert MTB/XDR analysis, the assay failed to detect 13 specimens (seven sputum, two CSF, two BAL, one pleural fluid and one EBUS respectively) while non-determinate/invalid results were documented in three samples (one sputum, one BAL, and one pus respectively). The overall rate of invalid results was 2·72 %.

Fig. 2 is representing the results of expanded drug susceptibility testing to different drugs among 94 rifampicin-resistant patients included in the study for both pulmonary and extra-pulmonary infections. 85/94 (90·42 %) samples were resistant to isoniazid whereas 4/94 (4·25 %) were indeterminate, and 5/94 (5·32 %) were sensitive respectively. Low-level isoniazid resistance was absent in any of our samples and the majority of the isolates were sensitive to ethionamide (80/94, 85·1%).

Fluoroquinolone resistance was documented in 74/94 (78·72 %) of all the infections and in 71/85 (83·5%) of the multidrug-resistant cases. Additional resistance to at least one of the second-line injectable drugs (SLID) was detected in 13/74 (17·57 %) samples. We documented a total of 74 pre-XDR infections in our study cohort. Sensitive, resistant and indeterminate findings among the SLIDs were as follows: amikacin, 83/94 (88·3%), 3/94 (3·2%), and 8/94 (8·51 %); kanamycin, 78/94 (82·97 %), 3/94(3·2%), and 13/94 (13·83 %); capreomycin 84/94 (89·36 %), 2/94 (2·13 %) and 8/94 (8·51 %) respectively. We recovered only 16 isolates that were sensitive to both fluoroquinolones and SLIDs (one sample was indeterminate for amikacin and kanamycin but sensitive to capreomycin).

The rate of indeterminate results in 8/94 samples (three pulmonary and five extra-pulmonary samples) for all drugs is summarized in Table 1.

4. Discussion

In this single centre clinical study, the Xpert MTB/XDR assay was used to detect extensive drug resistance in 82 pulmonary and 28 extra-pulmonary tuberculosis patients with positive results for MTBC, and RIF resistance by the Xpert MTB/RIF ULTRA assay. Our study is unique as we included 28 extra pulmonary samples with interpretable results in 24 samples, though the assay is recommended to be used in unprocessed

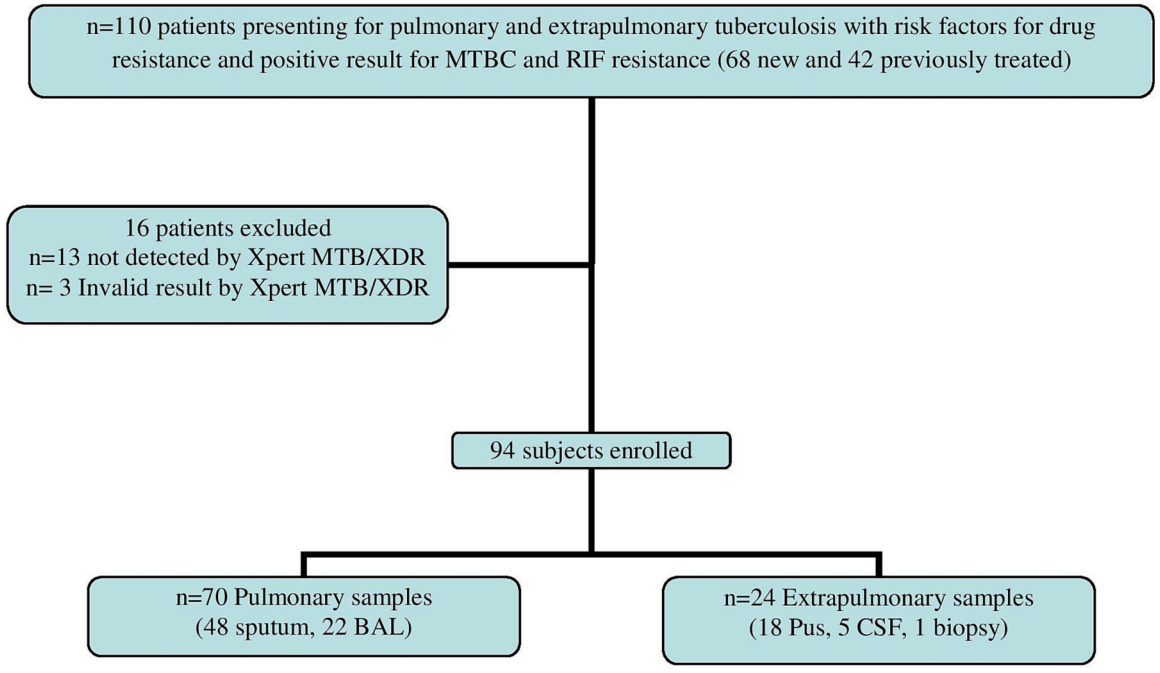

Fig. 1. Participant enrolment and exclusions.

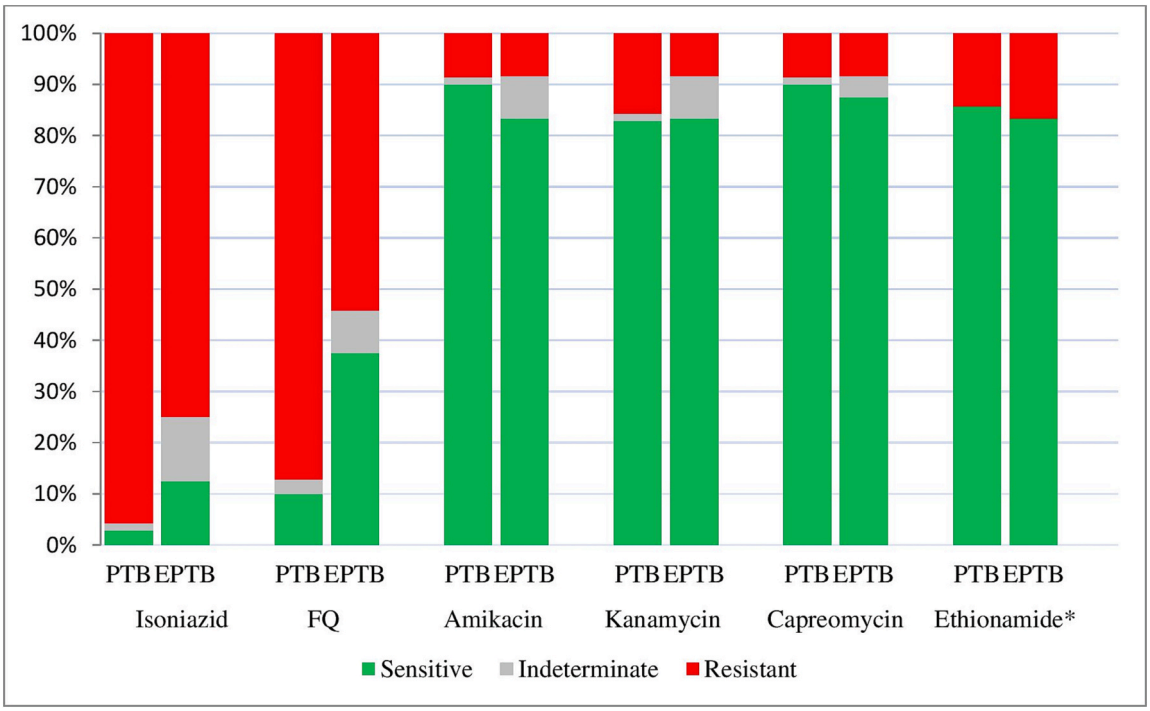

Fig. 2. Xpert MTB/XDR assay results and resistance rates to different drugs among 94 rifampicin-resistant isolates. *Indeterminate results for Ethionamide are not reported by the Xpert MTB/XDR assay.

Table 1
Overall Xpert MTB/XDR indeterminate rates in various pulmonary and extra pulmonary samples (n = 94*).

Drug	Number (%)
Isoniazid	04 of 94 (4·25 %); one pulmonary, three extrapulmonary
Fluroquinolones	04 of 94 (4·25 %); one pulmonary, three extrapulmonary
Amikacin	03 of 94 (3·19 %); one pulmonary, two extrapulmonary
Kanamycin	03 of 94 (3·19 %); one pulmonary, two extrapulmonary
Capreomycin	02 of 94 (2·12 %); one pulmonary, one extrapulmonary

* Among participants who had a documented valid Xpert MTB/XDR result with MTBC detected.

sputum or concentrated sputum sediments only. In addition, even with a limited sample size, our study is the first to document a female preponderance among drug resistant TB cases (51·8%). As per WHO Global TB Report 2023, the disease affects men more than women, and this finding is consistent with national TB prevalence surveys as well [1,10]. The higher number of women in our study (more than 50 %) reiterates the fact that females in India are diagnosed late, and neglected till the disease manifests as a drug-resistant inescapable form. It also reflects the lack of autonomy among women, and the high level of stigma associated with the disease among young unmarried females. The median age distribution of 26 years in our population was compatible with global reports that tuberculosis is prevalent in young adults across the world, and higher mobility of this population facilitates transmission of the disease [1,11].

In the current study, the Xpert MTB/XDR assay was used as a 'reflex' test to determine extensive drug resistance in rifampicin mono-resistant cases. We had only three samples (3·2%) that were non-determinate (invalid detection) while Penn-Nicholson et al have reported an overall rate of 2·96 % in a multi-centre diagnostic accuracy study on 611 samples [7]. In the same study, the rate of indeterminate results for various drugs was 3·5% or less while in our study it ranged from 2·12 % – 4·25 %. Recent studies have shown that compared to phenotypic drug susceptibility testing (pDST), and whole genome sequencing (WGS) the Xpert MTB/XDR assay has a specificity of >98 % for all the drugs, but sensitivity varied by drug target, ranging from 50 % for ethionamide to 100 % for aminoglycosides [7].

Eighty five out of 94 infections were multi-drug resistant in our study cohort i.e. additionally resistant to INH. However, low level INH resistance was not detected in any isolate. The majority of the MDR isolates were sensitive to ethionamide (71/85). Cao et al have reported that the assay targets mutations in the *inhA* promoter region whereas other possible gene mutations such as *ethA* can be associated with resistance to ethionamide but are not covered by the test [6]. Another possibility could be loss of function mutations, and presence of heteroresistance as documented by Chen et al [4].

The fluoroquinolone resistance pattern of *M. tuberculosis* isolates varies significantly across countries depending on the testing. In a recent multi-centre study from China on 497 patients of RR pulmonary TB have reported up to 23·9% resistance to fluoroquinolones by the Xpert MTB/XDR assay [4]. China is one of the eight countries that accounts for more than two-thirds of the global total of all estimated incident cases worldwide [1]. In our study, FQ resistance was detected in 87·14 % (61/70) and 54·16 % (13/24) of pulmonary, and extra-pulmonary isolates respectively. This is similar to the data published by the authors in 2021, and by Dalal et al in a metropolitan area of India [11,12]. The high rates of FQ resistance observed at our centre could be due to the fact that ours is a referral set up in one of the most populous states of the country with the highest burden of drug-resistant TB cases. In addition, Microbiology laboratories at the district level are usually lacking with indiscriminate empirical treatment of respiratory tract infections and persistent cough without establishing the aetiology of infections. Over the counter fluoroquinolones are easily available and rampantly prescribed by chemists as well as non-allopathic private practitioners as published by McDowell and Pai in a study on the mismanagement of empirical TB treatment in India [13].

We observed low rates of resistance to SLIDs ranging from 8-14 %. Since WHO has downgraded the role of second line injectable drugs and revised case definitions of DR-TB, the iteration of the Xpert MTB/XDR assay for tailoring regimens has been diluted. Even in India, the national program has transitioned to an all-oral regimen to minimize side effects

due to drug toxicities and improve patient adherence to treatment. In fact, the Indian government recently approved the BPaLM regimen with the aim of boosting the country's progress to achieve the ambitious goal of eliminating TB in India by 2025 [9]. However, our study shows that only 24·4% (23/94) of our study cohort was eligible for this regimen. We documented higher rates of FQ resistance in previously treated patients (82.8 %) as compared to new patients (76.3 %). Dheda et al in a recent review have identified locally specific risk factors for DR TB and noted that previous TB treatment is the only host-specific determinant of DR TB that has been consistently identified across different locales [14]. In addition, the WHO estimates that only one in three patients with MDR/RR-TB are detected and treated [1].

Suen et al in a dynamic transmission microsimulation model of the MDR-TB epidemic in India have proved that it has transitioned from a treatment-generated one to a transmission- generated epidemic [15]. Consequently, the transmission of MDR TB can be effectively curtailed only through the implementation of standardized treatment regimens that are based on rapid and precise drug susceptibility testing, coupled with rigorous monitoring of the public healthcare sector. As per recent WHO Global Tuberculosis Report 2023, the numbers of people being detected with MDR/RR TB and those enrolled on treatment fall far short of the incident cases each year and closing the gap requires improvement in detection of people with TB as well as testing for drug resistance [1]. Additionally, the results from a dynamic modelling study by Law et al predict that if tuberculosis management practices across sectors in India remain unchanged over next 20 years, then by 2032, an estimated 85 % of MDR cases will be primary drug-resistant compared to 15 % in 2012 [16]. This grim situation has been further aggravated by the recent COVID pandemic for nearly three years [17].

5. Conclusion

We have documented expanded drug susceptibility by the Xpert MTB/XDR assay in both pulmonary and extra pulmonary samples from India for the first time. Our study cohort had a female preponderance that has not been published previously. With the updated WHO definitions of drug-resistant TB and a high burden of fluoroquinolone resistance, the Xpert MTB/XDR assay has a limited application in India with only one-fourth of the population being eligible for the BPaLM regime as desired by the recent Government of India mandate. Diagnostic assays incorporating detection of susceptibility to bedaquiline, and linezolid with better understanding of mechanisms of resistance is urgently needed to expand drug susceptibility testing under the national program in India.

CRediT authorship contribution statement

Richa Misra: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Parijat Das:** Writing – review & editing, Investigation, Data curation. **Alok Nath:** Writing – review & editing, Validation, Supervision, Methodology. **Zafar Neyaz:** Writing – review & editing, Validation, Supervision, Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors read and approved the final version of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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