



Assessment of Conventional and Molecular Methods in the Routine Management of Tuberculosis in a High Tuberculosis Burden Setting

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Abstract

Background and aims: India is a high-burden tuberculosis (TB) region and a drug-resistance hotspot. Despite numerous reports of pulmonary tuberculosis (PTB) cases, there needs to be more literature available on the importance of diagnostic methods in the case of extrapulmonary tuberculosis (EPTB). A prospective study was performed from July 2017 to June 2018 to compare the efficacy of conventional and molecular methods in detecting PTB and EPTB cases.

Methods: Overall, 1000 presumptive PTB and 412 EPTB cases were subjected to staining (Ziehl-Neelsen [ZN] and fluorescent staining), culture, GeneXpert, and line probe assay (LPA).

Results: The sensitivity, specificity, and strength of association [i.e., kappa (k) value of light-emitting diode-fluorescent microscopy, ZN, and GeneXpert] were calculated using standard formulae using solid culture as the gold standard. The sensitivity of GeneXpert in smear-positive/culture-positive PTB was comparable with the smear-negative/culture-positive PTB cases (95.7% vs. 87.5%) with an overall sensitivity and specificity of 90.5% and 90.1% in EPTB cases, respectively. However, sensitivity was lower for pleural fluid (75%) and tissues (85.7%). In pulmonary instances, 10% (6.7% Rifampicin [RIF] + isoniazid [INH] resistant and 3.3% INH monoresistant) drug resistance was observed, and no drug resistance was found in extra-pulmonary samples.

Conclusion: Among conventional methods, fluorescent staining is more sensitive than ZN staining, while the sensitivity of GeneXpert varies w.r.t type of sample using culture positivity as the gold standard. In general, the present study suggests the promotion of universal drug susceptibility testing (DST) for all individuals with TB to control drug-resistant TB.

Keywords: RNTCP, Culture, Fluorescent staining, ZN staining, GeneXpert, LPA

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Introduction

The alarming incidence of pulmonary tuberculosis (PTB) and extrapulmonary TB (EPTB) cases is a global concern. Based on estimation, 1.5 million deaths (1.4-1.6 million) were reported in 2018 due to TB.¹ This problem is further compounded by the emergence of human immunodeficiency virus (HIV)-associated TB infection.¹ As per a recent estimate, about 70% of the 0.8 million EPTB cases out of a total of 5.4 million new TB cases were localized in Southeast Asian countries, with India ranking first having a maximum number (about 0.35 million) of EPTB cases.¹

Emerging drug resistance in the form of multidrug-resistant TB (MDR-TB) and monoresistant TB is a significant public health problem globally. Among monoresistance in TB, isoniazid (INH) resistance is reported to be the most common. According to the Global TB Report 2018, the prevalence of MDR-TB and INH-monoresistant TB is indicated to be 3.5% and 7.1% in new PTB cases, as well as 18% and 7.9%, respectively in previously treated PTB cases,

respectively.¹ However, limited information is available on drug resistance in EPTB from a high TB burden country such as India.² The reason could be difficulty obtaining specimens and limited laboratories offering culture and drug susceptibility testing (DST) for EPTB samples.

GenoType MTBDR assay, also termed line probe assay (LPA), is a molecular method that detects *Mycobacterium tuberculosis* complex (MTBC) even in paucibacillary cases but also detects mutations in genes responsible for drug resistance viz. *rpoB* (for Rifampicin [RIF] resistance), *katG* (for high-level INH resistance), and *inhA* (for low-levels of INH resistance) genes in less than two days. A new molecular method called GeneXpert MTB/RIF Assay detects *M. tuberculosis*, along with RIF resistance, an indicator of MDR-TB, within three hours. The later assay requires minimal biosafety infrastructure and training, unlike LPA, which is labor-intensive, time-consuming, and technically demanding. The present study sought to compare the performance of molecular methods, namely, GenoType MTBDR plus and GeneXpert with conventional

methods, staining and culture in PTB and EPTB samples.

Materials and Methods

A prospective study was performed from July 2017 to June 2018 in the Department of Microbiology at a Tertiary Care Hospital (HAHC) in Delhi, India, after approval by the institutional research and ethics committee with a patient information sheet provided to all patients, and informed consent was obtained from them for this study.

Study Subjects and Sample Size

The sample size was calculated using the standard formula but was later increased to 1 year. A total of 1000 and 412 consecutive new cases with presumptive diagnoses of PTB and EPTB as per revised National TB Control Programme (RNTCP) guidelines constituted the subjects of the present study.³ The sample size for the study was estimated using the following formula:

$$Z^2 \times \frac{\text{Prevalence}\%}{100} \times \frac{1 - \text{Prevalence}\%}{100} / d^2$$

where Z and d represent the standard deviation (1.96) and confidence interval (0.05), respectively. Prevalence was taken at 20% (as per previous studies)

But later, it was decided to include all the samples from the study duration (July 2017- June 2018).

Sample Collection and Transportation

Pulmonary Tuberculosis Cases

Patients were given a pre-sterilized universal container (100 mL) to collect the deep expectorated sputum sample (3-5 mL) on two occasions (one spot sample under supervision and the other early morning sample).

Extrapulmonary Tuberculosis Cases

These specimens were divided into aseptically collected specimens (sterile) [fluids such as cerebrospinal fluid (CSF), pleural, synovial, ascitic, and tissues (lymph node, tissue biopsies)] and specimens contaminated by normal flora or not collected aseptically [gastric lavage, urine, and pus].⁴ All the samples were collected according to RNTCP guidelines 2016.⁴

Transportation

Specimens were transported to the laboratory as soon as possible and, in case of delay, they were refrigerated at 4 °C to inhibit the growth of unwanted micro-organisms.^{3,4}

Laboratory Methods

Homogenization

It was performed only in aseptically collected EPTB samples using a homogenizer tube.⁵

Decontamination

All the clinical samples were decontaminated regardless of the homogenization step.

NALC-NaOH method⁶ for pulmonary samples

(sputum) and milder decontamination procedure as per RNTCP guidelines were used for EPTB samples.⁷ All decontaminated PTB and EPTB samples were divided into three aliquots and kept at 2-8 °C.

Smear Microscopy

The first aliquot of the decontaminated PTB or EPTB sample was utilized to prepare two smears (one for Ziehl-Neelsen [ZN] and the other for fluorescent staining). The staining procedure (both ZN and fluorescence staining) and interpretation of results were made according to RNTCP guidelines 2016⁷ (Figures 1 and 2).

Processing of Samples for Culture, GeneXpert Assay, and Line Probe Assay

All smear-positive and smear-negative PTB and EPTB samples were employed for culture and GeneXpert assay (Figures 1 and 2).

(I) Culture

One loopful of the re-suspended sediment from the first aliquot of the decontaminated sample was used to inoculate the Lowenstein Jensen (LJ) media slant. The solid cultures were read every week (up to 8 weeks) for growth, and any change observed during the incubation period was subjected to ZN staining. Positive ZN staining was interpreted as the specimen positive for acid-fast bacteria (AFB).

(II) GeneXpert

The second aliquot of the decontaminated sample was utilized to perform this assay according to the manufacturer's instructions.⁸ The results were interpreted as detected/not detected for MTB and detected/not detected/indeterminate for RIF resistance.

(III) Line Probe Assay

The third aliquot of decontaminated and GeneXpert positive PTB and EPTB samples were put up for LPA, which is based on the principle of DNA strip technology involving three steps,⁹ including DNA extraction using GenoLyse kit (GenoType® MTBDRplus kit- Hain Lifescience GmbH, Nehren, Germany, Cat No. 30496AM). The second step was multiplex amplification with biotinylated primers using a GXT DNA/RNA Extraction kit (GenoType® MTBDRplus kit- Hain Lifescience GmbH, Nehren, Germany, Cat. No. 30496AA). The third step was manual reverse hybridization of labeled amplification products with oligonucleotide probes immobilized on strips (GenoType® MTBDRplus kit- Hain Lifescience GmbH, Nehren, Germany, Cat No. 30496A) with a colorimetric detection of 27 reaction bands for the detection of MTBC and resistance to RIF and INH.

Statistics

The data were tabulated in a Microsoft Excel spreadsheet in the form of a master chart and were used for correlation. A one-way analysis of variance (ANOVA) test was utilized to

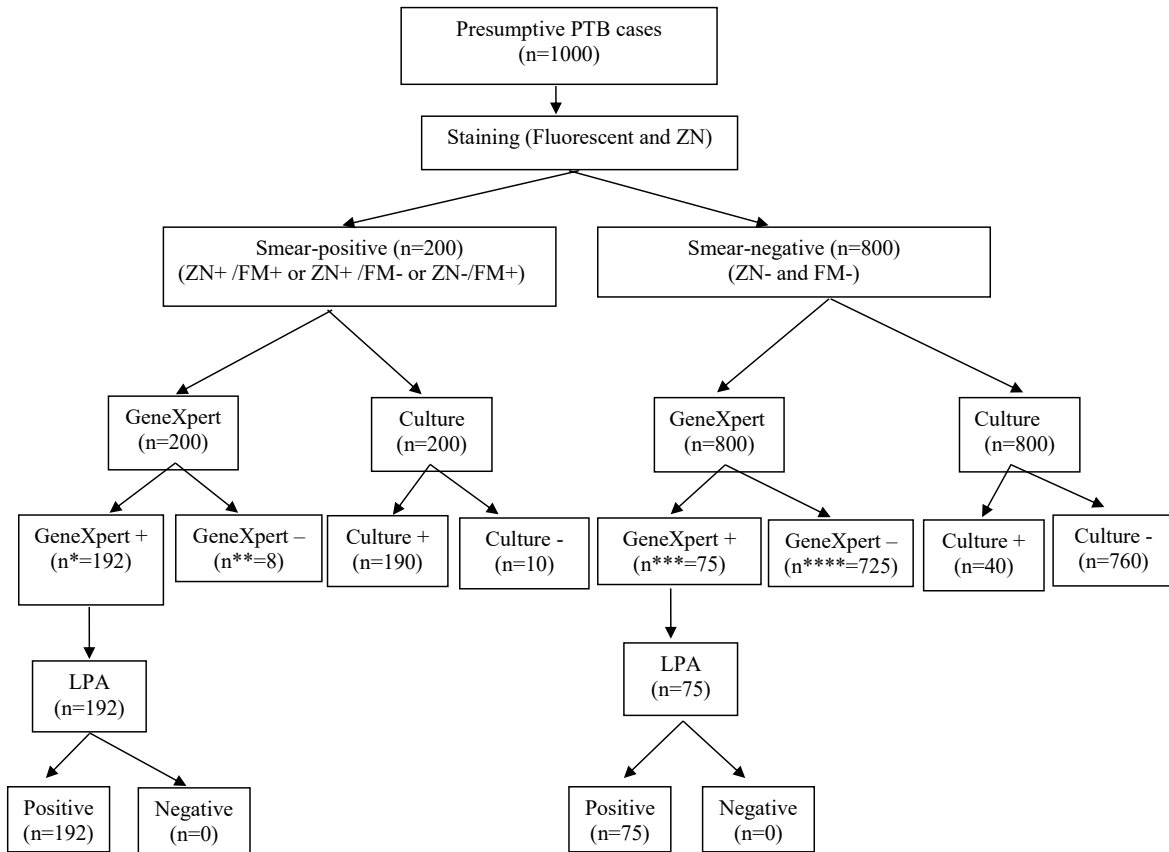


Figure 1. Flowchart for Processing of Presumptive PTB Samples and the Results. *Note.* PTB: Pulmonary tuberculosis; ZN: Ziehl-Neelsen; FM: Fluorescent microscopy; LPA: Line probe assay. * (182 culture-positive and 10 culture-negative cases); ** (8 culture-positive cases); *** (35 culture-positive and 40 culture-negative cases); **** (5 culture-positive and 720 culture-negative cases)

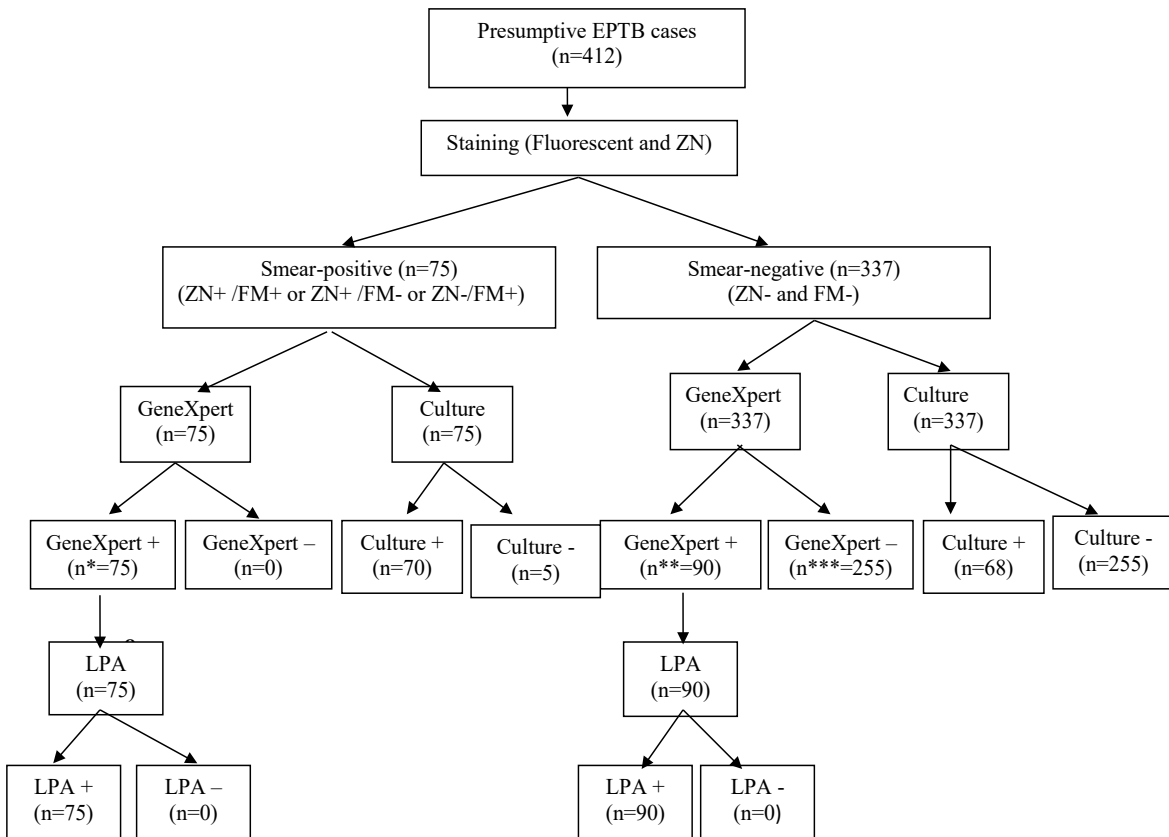


Figure 2. Flowchart for Processing of Presumptive EPTB Samples and the Result. *Note.* EPTB: Extrapulmonary tuberculosis; ZN: Ziehl-Neelsen; FM: Fluorescent microscopy; LPA: Line probe assay. * (65 culture-positive and 5 culture-negative cases); ** (60 culture-positive and 22 culture-negative cases); *** (8 culture-positive and 247 culture-negative cases)

calculate the *P* value to determine any significant statistical differences between mean ages and was performed in Microsoft Excel. The sensitivity, specificity, and strength of association [kappa (*k*) value of light-emitting diode-fluorescent microscopy (LED-FM), ZN, and GeneXpert] were measured using standard formulae mentioned below using culture positivity as the gold standard.¹⁰

$$\text{Sensitivity} = \frac{A}{A+C} \times 100$$

$$\text{Sensitivity} = \frac{D}{D+B} \times 100$$

where A, B, C, and D represent true positive, false positive, false negative, and true negative, respectively.

$$k = \frac{p_o - p_e}{1 - p_e}$$

where *p_o* and *p_e* denote relative observed agreement among raters and hypothetical probability of chance agreement, respectively.

Results

A total of 1000 consecutive new cases with the presumptive diagnosis of PTB and 412 samples of patients with the presumptive diagnosis of EPTB were enrolled in the study.

Distribution of Pulmonary Tuberculosis and Extrapulmonary Tuberculosis Samples

All 1000 PTB samples were sputum. The distribution of various types of EPTB samples was found to be maximum for pus (*n*=210, 50.9%), followed by tissues (*n*=77, 18.6%), synovial fluid (*n*=50, 12.1%), pleural fluid (*n*=30, 7.2%), gastric aspirate (*n*=20, 4.8%), and CSF (*n*=15, 3.6%) while being the least for urine (*n*=10, 2.4%).

Detection of AFB by Staining, Culture, GeneXpert, and Line Probe Assay

Detection by Staining

Out of the total of 1000 PTB smear samples, 200 (20%) were positive by light-emitting diode-fluorescent microscopy (LED-FM), and 110 (11%) were positive by the ZN method, thereby indicating a better detection rate of the former technique compared to later ($\chi^2=30$, $P \leq 0.005$). Out of 200 PTB smear-positive samples by LED-FM, 97 (48.5%), 39 (19.5%), 52 (26%), and 12 (6%) were 3+, 2+, 1+, and scanty, respectively, while out of 110 smear-positive samples by the ZN method, 66 (60%), 27 (24.5%), and 17 (15.4%) were 3+, 2+, and 1+, respectively, as per RNTCP guidelines on the grading of smears.⁸ Out of 412

EPTB smear samples, 75 (18.2%) were smear-positive by LED-FM, and 38 (9.2%) were smear-positive by the ZN method. Out of 75 EPTB smears positive by LED-FM, 18 (24%) and 57 (76%) cases were graded as 1+ and scanty, while out of 38 smears positive by ZN staining, 7 (18.4%) and 31 (81.5%) cases were graded as 1+ and scanty, respectively, as per RNTCP guidelines on the grading of smears⁷ (Table 1).

Detection by Solid Culture

All the clinical samples were decontaminated, based on which 90 (95%) cases were positive, and 10 (5%) cases were negative on solid culture (i.e., LJ medium). Among smear-positive extra-pulmonary samples (*n*=75), 70 (93.3%) and 5 (6.7%) were culture-positive and culture-negative cases, respectively. Among 800 smear-negative PTB samples, 40 (5%) were culture-positive, and 760 (95%) were negative. Among smear-negative EPTB samples (*n*=337), 68 (4.9%) were culture-positive, and 269 (95.1%) were culture-negative for AFB in the LJ medium as identified by ZN staining (Table 2).

Detection by GeneXpert

Out of 200 smear-positive PTB samples, 192 were GeneXpert-positive (177 RIF sensitive and 15 RIF resistant), and 8 were GeneXpert-negative, respectively. Among smear-positive EPTB samples (*n*=75), 70 were GeneXpert-positive and RIF sensitive, while five were GeneXpert-negative, respectively. Among 800 smear-negative PTB samples, 75 were GeneXpert-positive (72 RIF sensitive and 3 RIF resistant), and 725 were GeneXpert-negative, respectively. Among smear-negative EPTB samples (*n*=337), 90 were GeneXpert-positive and RIF sensitive, and 247 were GeneXpert-negative, respectively.

Detection by Line Probe Assay

All 267 GeneXpert-positive PTB samples (192 smear-positive and 75 smear-negative) were positive by LPA, in which 243 (91.1%) were sensitive to both RIF and INH (168 smear-positive and 68 smear-negative), 18 (6.7%)

Table 1. Staining Results by ZN and LED-FM

Cases	ZN		FM	
	Positive	Negative	Positive	Negative
PTB (<i>n</i> =1000)	110 (11%)	890 (89%)	200 (20%)	800 (80%)
EPTB (<i>n</i> =412)	38 (9.2%)	374 (90.7%)	75 (18.5)	337 (81.7%)

Note. ZN: Ziehl-Neelsen; LED-FM: Light-emitting diode-fluorescent microscopy; PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis.

Table 2. Comparison of Staining With Culture

Cases	Staining Positive and Culture Positive*	Staining Negative and Culture Positive**	Staining Positive and Culture Negative	Staining Negative and Culture Negative
PTB (<i>n</i> =1000)	190 (19%)	40 (4%)	10 (1%)	760 (76%)
EPTB (<i>n</i> =412)	70 (16.9%)	68 (16.5%)	5 (1.2%)	269 (65.2%)

Note. PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis.

* ZN+/FM+ or ZN+/FM- or ZN-/FM+

**ZN-/FM-

were resistant to both RIF and INH (15 smear-positive and three smear-negative), and 6 (2.2%) were INH mono-resistant (4 smear-positive and 2 smear-negative). All 83 GeneXpert-positive EPTB samples (38 smear-positive and 45 smear-negative) were positive by LPA and sensitive to RIF and INH.

Demographic Characteristics of Confirmed Pulmonary Tuberculosis and Extrapulmonary Tuberculosis Cases

Out of 445 TB cases confirmed by culture and GeneXpert, 62.9% (n=280) were PTB cases, and 37% (n=165) were EPTB cases. The most affected age group for PTB and EPTB was 20-40 years, followed by <20 years in EPTB and 41-50 years in PTB cases. Most EPTB cases belonged to bones and joints, followed by the pleural, abdomen, and urinary tract (Table 3). The least affected age group was >50 years in PTB and EPTB cases (Table 4). A one-way ANOVA test revealed no statistically significant difference between age group means [F (6, 28) = 1.19, P=0.33] in various TB cases (Table 4). Males (180/280, 64.2%) were more affected by PTB (P=0.000), while females were more affected by EPTB (96/165, 58.1%), the details of which are provided in Table 4.

Discussion

The lab diagnosis of TB is primarily based on direct microscopy in PTB cases and culture in paucibacillary PTB and EPTB cases. Culture is a more sensitive method than direct microscopy, which detects AFB only

when present at least 5000-10000 bacilli per milliliter of sputum.¹¹ Nevertheless, the latter test is simple and cheap and remains the most commonly used method for diagnosing TB in developing countries such as India for detecting infectious cases and assessing patient response to treatment. In the present study, the smear positivity rates by LED-FM in PTB and EPTB samples were higher (20% and 18.2%) than in ZN microscopy (11% and 9.2%). A low smear positivity rate of PTB in our study may be due to the earlier presentation of presumptive cases that are more paucibacillary.¹² Considering culture as the gold standard for the diagnosis of TB,¹⁰ the sensitivity of LED-FM was higher (82.6%) than the ZN method (47.8%), but specificity was approximately the same (98.7% for LED-FM and 100% for ZN) and in substantial agreement (k=0.75) with culture in PTB samples in the current study (Table 5). According to a systematic review of 45 relevant studies, FM was found to be, on average, 10% more sensitive with similar specificity compared to conventional microscopy in sputum samples.¹³ A similar high sensitivity of LED-FM to the ZN method (50.7% vs. 27.5%) with nearly the same specificity (98% vs. 100%) and the kappa value in substantial agreement (k=0.71) with culture was observed for EPTB samples in our study (Table 5), which is in accordance with the results reported by Adarsh et al¹⁴ (50% vs. 16.6% sensitivity and 100% vs. 100% specificity) and Munshi et al (45.2% vs. 33.9% sensitivity and 88.13% vs. 90.8% specificity).¹⁵ The high sensitivity of LED-FM may be attributed to the stronger affinity of auramine to

Table 3. Distribution of Positive EPTB and PTB Cases (n=445)

Type of Cases	Number of Cases (%)	Distribution of Cases and Their Mean Age in Years (Within Parenthesis) in Various Age Subgroups									
		<20		21-30		31-40		41-50		>50	
		Mean Age	No. of Cases	Mean Age	No. of Cases	Mean Age	No. of Cases	Mean Age	No. of Cases	Mean Age	No. of Cases
Pulmonary TB	280 (62s.9)	12	15	26	130	36	100	43	20	60	15
Bones and joints TB	94 (21.1)	16	14	24.5	33	28.5	31	43.6	9	57	7
Pleural TB	22 (4.9)	13	2	27.5	8	34.2	8	47	4	0	0
Urinary TB	4 (1)	0	0	0	0	37	2	45	1	61	1
Tuberculous meningitis	5 (1.1)	10	1	22	2	33	2	0	0	0	0
Abdominal TB	6 (1.3%)	11	1	24	3	37	2	0	0	0	0
Tissue	34 (7.7%)	17	2	26	15	36	12	44	5	0	0

Note. PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis; TB: Tuberculosis.

Table 4. Gender-wise Distribution of TB Patients

Age group (y)	PTB		P Value	EPTB		P Value
	Male (n, %)	Female (n, %)		Male (n, %)	Female (n, %)	
>20	7 (40%)	9 (60%)	0.001*	12 (60%)	8 (40%)	0.35
21-30	72 (55.4%)	58 (44.6%)		26 (42.6%)	35 (57.3%)	
31-40	80 (80%)	20 (20%)		22 (38.5%)	35 (61.40%)	
41-50	14 (70%)	6 (30%)		6 (31.5%)	13 (68.4%)	
>60	7 (50%)	7 (50%)		2 (28.5%)	5 (71.4%)	

Note. PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis.

*Statistically significant.

Table 5. Sensitivity, Specificity, and Kappa Value of FM and GeneXpert With Culture as the Gold Standard in PTB and EPTB Cases

	Sensitivity (%)	Specificity (%)	Kappa Value
FM (PTB)	82.6	98.1	0.75
FM (EPTB)	50.9	98	0.71
GeneXpert (PTB)	91.6	94.1	0.71
GeneXpert (EPTB)	90.5	90.1	0.71
GeneXpert (CSF)	100	100	-
GeneXpert (Gastric aspirate)	100	100	-
GeneXpert (Urine)	100	100	-
GeneXpert (Synovial fluid)	100	100	-
GeneXpert (Pus)	96.23	100	-
GeneXpert (Tissues)	85.71	100	-
GeneXpert (Pleural fluid)	75	100	-

Note. PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis; CSF: Cerebrospinal fluid; FM: Fluorescent microscopy.

the mycolic acid of tubercle bacilli than carbol fuchsin¹⁶ and a wider field of smear examination (under 40X compared to 100X in conventional light microscopy), and the appearance of AFB as bright rods against a dark background contributing to better detection. These results support its superior diagnostic performance¹⁷ and the target of its implementation by RNTCP in health facilities with higher workloads.¹⁸ However, an essential concern regarding LED-FM is false positivity (due to fluorescence by food particles and artifacts in the sample or any impurity in auramine stain). This further suggests ZN staining by LED-FM in all scanty and doubtful cases.¹⁹

The World Health Organization (WHO) has recently endorsed the routine use of two molecular methods (i.e., GeneXpert and LPA) for rapid diagnosis and simultaneous screening of MDR-TB in many countries.^{20,21} MDR-TB is defined as resistance to both RIF and INH. However, RIF resistance is considered by the WHO as an excellent surrogate marker for MDR-TB as mono-resistance to RIF in *M. tuberculosis* is rare.²² GeneXpert is the critical investigation in RNTCP that detects *M. tuberculosis* and RIF resistance due to mutation in the 81 base pair core regions of the *rpoB* gene.

In the present study, the sensitivity of GeneXpert in smear-positive/culture-positive PTB was comparable with smear-negative/culture-positive PTB (95.7% vs. 87.5%) with an overall specificity of 94.15%. In EPTB cases, sensitivity and specificity were 90.5% and 90.1%, respectively (Table 5). Previous studies reported the sensitivity of GeneXpert on direct sputum or decontaminated sputum samples to vary from 72-75% in smear-negative PTB cases, 98-100% in smear-positive PTB cases,²³⁻²⁵ 73-100% in EPTB cases, and specificity of 95-100%, respectively, in EPTB cases.²⁶⁻²⁸ The overall performance of GeneXpert was found to be in substantial agreement ($k=0.71$) with the gold standard method, namely, solid culture (Table 5).

In the current study, the performance of GeneXpert in detecting EPTB was found to vary concerning different specimens. Maximum sensitivity was observed in CSF,

gastric aspirate, urine, and synovial fluid, followed by pus, tissues, and least in the pleural fluid (Table 5). However, the specificity was the same for all types of EPTB samples compared to 81.2% and 66.7% in tissues, as well as 79.5% and 66.7% in CSF in other studies.^{29,30} Variations in the sensitivity of GeneXpert could be due to differences in patients' population, type, and quality of clinical specimens. The high specificity of GeneXpert could be due to the closed reaction chamber, which reduces cross-contamination and false positivity. Low sensitivity in the pleural fluid and tissues can be attributed to low bacillary load,³¹ as GeneXpert requires 131 bacilli/mL compared to culture with a minimum detection limit of 10 bacilli/mL.³² Although a high specificity gives clinicians confidence to confirm the diagnosis of TB when the GeneXpert test is positive, a negative result cannot rule out the diagnosis of TB because of low sensitivity. Therefore, the clinician should rely on the results of both culture and GeneXpert.

Despite a short turnaround time (less than 3 hours) and minimal training requirement, GeneXpert has a few limitations such as the limited shelf-life of the cartridges, restrictions in operating temperature and humidity, continuous electricity supply, unknown long-term robustness, and periodic calibration of the machine.³³

Our result indicates 37% of EPTB cases and 63% of PTB cases among all TB cases. This is in line with a study reported from Delhi in which the incidence of EPTB and PTB was 30.8% and 69.2%, respectively.³⁴ The most caseload was found in the 20-40 year-age group (Table 4) in both PB and EPTB. Similar results were reported by Bagchi et al and Sharma et al.^{34,35} A higher proportion of females were affected by EPTB, while males were more involved with PTB in the current study. However, as per reports from Delhi, males predominate in both PTB and EPTB cases.³⁴ The predilection towards women in EPTB cases could be attributed to self-neglect towards health and later presentation of active TB as EPTB.³⁶⁻³⁸

According to the WHO global TB report, the prevalence of MDR-TB in new and previously treated cases was 2.5-2.8% and 14-17%, respectively.³⁹ As a high TB burden country, India is also a hotspot region for MDR-TB infection.⁴⁰ A 12.9% prevalence of MDR-TB was reported from North India, with Delhi at 9.55%.³⁴ In the present study, a 6.7% prevalence of MDR-TB due to *rpoB* mutation probe 1 (mutation in D516V) and *KatG* mutation probe 1 (transformation in S315T1) was observed only in PTB cases. To date, the primary focus of drug-resistant TB in India has been mainly on RIF resistance. According to the first national TB drug resistance survey in India, INH monoresistance was the highest (4% in new cases and 8% in previously treated cases)³⁶ and the single most common anti-TB drug resistance due to two genes (i.e., *KatG* and *inhA*)³⁷ unlike RIF resistance (*rpoB* gene), but it has not received much attention yet. This could be due to complex diagnostic procedures using molecular techniques and their uncertain clinical implications. A 3.3% prevalence of INH monoresistant TB was found in PTB cases responsible for

KatG mutation probe 1 (mutation in S315T1). More than 90% of INH monoresistance in India is due to mutation in the *KatG* gene associated with high-level resistance and poor treatment outcomes,³⁸ and mutations in both *KatG* and *inhA* genes responsible for INH monoresistance lead to higher chances for the development of MDR-TB.³⁵

Among DR-TB cases in PTB samples, 75% were MDR-TB, and 25% were INH-mono-resistant TB. This percentage of INH-mono-resistance should be noticed and should be seriously taken immediately. INH-mono-resistant cases will be missed if not precisely looked for. This will result in misdiagnosis, leading to an increased risk of treatment failure or relapse and a greater propensity of progression toward MDR-TB.

A significant hurdle in controlling the INH monoresistance problem in India is the use of cartridge-based nucleic acid amplification test (CB-NAAT) in national programs, which does not identify it. As per RNTCP, if CB-NAAT does not detect RIF resistance, the patient should be looked at explicitly for INH monoresistance by LPA and treated accordingly. However, INH testing is still a severe challenge to India. The reason is a limitation of tools such as LPA and liquid DST to reference or centralized laboratories. There are still large numbers of INH-mono-resistant patients who are misdiagnosed currently and mismanaged subsequently. Therefore, the promotion of INH testing in all RIF-sensitive cases or, in general, universal DST on all MTB isolates, followed by individualized therapy, should be strictly performed to control the overall drug resistance to fulfill the ambitious goal of eliminating TB by 2025.

Conclusion

With the increasing prevalence of TB in India, the mainstay of its diagnosis, which is smear microscopy, does not detect drug resistance. MDR-TB is resistant to both RIF and INH. Novel molecular assays such as GeneXpert detect only RIF resistance, while only a few of them, including LPA, detect INH resistance, which is quite common and can lead to therapy failure or rapid progress to MDR-TB. India has set an ambitious goal of eliminating TB by 2025. Limiting the spread of TB, especially DR-TB, by fast diagnostic methods can be a powerful tool to achieve this goal and should not be considered an additional cost. This may be achieved by promoting universal DST for all positive cases. Finally, the physicians should be wide-awake in promptly identifying and treating INH-mono-resistant TB accordingly.

Authors' Contribution

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Formal analysis: Mehvash Haider.

Funding acquisition: Mehvash Haider

Investigation: Mehvash Haider, Rushna Wazahat.

Methodology: Mehvash Haider, Rushna Wazahat.

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Supervision: Mehvash Haider, Mridu Dudeja.

Validation: Mehvash Haider, Rushna Wazahat.

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