Journal of Tropical Pediatrics, 2024, **70**(5), fmae024
https://doi.org/10.1093/tropej/fmae024
Original paper

OXFORD

Xpert MTB/RIF assay in the diagnosis of pulmonary tuberculosis in children in tertiary care setting in South India

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ABSTRACT

Xpert MTB/RIF is recommended for the diagnosis of tuberculosis (TB) in children. We determined the performance of Xpert MTB/RIF in the diagnosis of pulmonary TB in children. The characteristics of children influencing Xpert MTB/RIF positivity were explored. Children aged <15 years with symptoms suggestive of pulmonary TB were prospectively enrolled from 2013 to 2019. Two sputum/early morning gastric aspirate specimens were collected for examination by smear (fluorescence microscopy), Xpert MTB/RIF, and culture [Mycobacteria growth indicator tube (MGIT)/Lowenstein–Jensen (LJ) medium]. Diagnostic performance of Xpert MTB/RIF was evaluated using LJ and or MGIT culture positivity as the reference standard. Sensitivity, specificity with 95% confidence interval (CI) were calculated. Stratified analysis was done; $P < .05$ was considered statistically significant. Of the total 1727 enrolled children, 1674 (97%) with complete results for at least one sputum/gastric aspirate sample were analyzed. The sensitivity of Xpert MTB/RIF was 68.5% in sputum and 53.6% in gastric aspirate while the specificity was 99% for both. The sensitivity compared to smear was 68.5% vs. 33.7% ($P < .001$) and 53.6% vs. 14.5%; ($P < .001$) in sputum and gastric aspirate, respectively. The sensitivity of Xpert MTB/RIF was 23.9% with decision to treat as reference standard. Xpert MTB/RIF positivity was significantly influenced by sex, age, nutritional status, chest X-ray abnormality, TB infection status, and symptoms suggestive of TB. Xpert MTB/RIF as an upfront test compared to smear improves diagnosis of pulmonary TB in children yet the sensitivity is suboptimal. Newer TB diagnostic tools with improved sensitivity is warranted in children.

LAY SUMMARY

We evaluated the performance of Xpert MTB/RIF in the diagnosis of pulmonary TB in children and explored the characteristics influencing Xpert MTB/RIF positivity. Sputum and or early morning gastric aspirate specimen was collected from children aged <15 years with symptoms suggestive of pulmonary TB. This was examined by smear (fluorescence microscopy), Xpert MTB/RIF, and culture (Mycobacteria growth indicator tube (MGIT)/Lowenstein–Jensen (LJ) medium). Diagnostic performance of Xpert MTB/RIF was evaluated using LJ and or MGIT culture positivity as the reference standard. Of the total 1727 enrolled children, 1674 (97%) with complete results for at least one sputum/gastric aspirate sample were analyzed. The sensitivity of Xpert MTB/RIF was 68.5% in sputum and 53.6% in gastric aspirate which was higher than smear and the specificity was 99%. The sensitivity of Xpert MTB/RIF was 23.9% with decision to treat for TB as reference standard. The Xpert MTB/RIF positivity was influenced by sex, age, nutritional status, chest X-ray abnormality, TB infection status, and symptoms suggestive of TB. Xpert MTB/RIF as an upfront test compared to smear improves the diagnosis of pulmonary TB in children yet the sensitivity is suboptimal. Newer TB diagnostic tools with improved sensitivity is warranted in children.

INTRODUCTION

Worldwide an estimated 10.6 million tuberculosis (TB) were reported in 2022, of which children (aged < 15 years) constituted 12% of the cases [1]. India accounted for 2.8 million TB cases in 2012 and children aged 0–14 years accounted for 5% [1]. Children are often under or over-diagnosed with TB due to difficulty in obtaining specimens for testing and lack of optimal diagnostic tools. In 2021, World Health Organization (WHO) recommended the use of Xpert MTB/RIF as the initial diagnostic test for all children with presumptive pulmonary TB [2]. Systematic review and meta-analysis on Xpert MTB/RIF in the diagnosis of pulmonary TB in children documented higher sensitivity of 36% to 44% with Xpert MTB/RIF compared to smear microscopy [3]. Xpert MTB/RIF is being used in the diagnosis of TB in low- and middle-income countries (LMIC) which contribute significantly to the TB burden. The TB Control Programme of India recommends Cartridge-based nucleic acid amplification test (CBNAAT) as the upfront diagnostic test for children with presumptive pulmonary TB [4]. Studies from India have reported on the use of Xpert MTB/RIF in the diagnosis of pulmonary TB in children under programmatic and hospital settings [5–11]. Data on the performance of TB diagnostics in children in whom the diagnosis of TB is a challenge is important from TB endemic country like India. Moreover, there is low certainty of evidence with Xpert MTB/RIF for accuracy in gastric aspirate [2]. We determined the diagnostic accuracy of Xpert MTB/RIF in sputum and or early morning gastric aspirate specimen in the diagnosis of pulmonary TB in children with presumptive TB who attended five tertiary care hospitals for care in south India. In addition, we explored the characteristics of children that influence Xpert MTB/RIF positivity in sputum and gastric aspirate specimens.

MATERIALS AND METHODS

Study design and participants

This prospective cohort study was done during November 2013 to December 2019 by the Indian Council for Medical Research (ICMR)—National Institute for Research in TB (NIRT) in collaboration with tertiary care hospitals in Chennai, Madurai, and Vellore in the state of Tamil Nadu. Consecutive children aged < 15 years with any one or more symptoms suggestive of TB and or abnormal chest radiograph were considered for the study. Symptoms suggestive of presumptive pulmonary TB include persistent, non-remitting cough of > 2 weeks not responding to a course of antibiotics, persistent unexplained fever of >1 week, presence of reduced playfulness or lethargy or loss weight of >5% from previous observation. Children currently on anti-TB treatment, with extra-pulmonary TB, unconscious or seriously ill were considered ineligible for the study.

The study was approved by the NIRT—Institutional Ethics Committee on 21 May 2012 (NIRT-IEC No: 2012 004). Written informed consent was obtained from the child's parent/legally authorized representative (LAR) prior to study-related procedures. Written assent was obtained from children aged >7 years.

Investigations

The following details were collected from the parent/LAR using a structured proforma: symptoms for pulmonary and extra-pulmonary TB, past TB treatment, contact with TB, immunosuppressive illness, and exposure to smoke. Physical examination was performed for the child. The child's height in cm, weight in kg, and Bacillus Calmette-Guerin (BCG) scar status were recorded. *Z* scores were calculated for children aged ≤5 years from the measured anthropometrics as follows: stunting (height for age *Z* score—HAZ), underweight (weight for age *Z* score-WAZ), and wasting

(weight for height *Z* score—WHZ). Body mass index (BMI) was calculated with height and weight measurements in children age >5 years.

Chest radiograph anterio-posterior (AP) view and right lateral view was taken. The chest radiograph was read using a structured proforma by two independent radiologists, and in case of discrepancy, an umpire reading was done. Chest radiograph was interpreted as suggestive of TB/abnormal/normal. Features suggestive of TB included hilar, para-tracheal adenopathy, consolidation, parenchymal infiltration, cavity, bronchiectasis, pleural effusion and pneumothorax. Tuberculin skin test (TST) with two tuberculin unit (TU)-purified protein derivative (PPD) was administered to the child. Blood was collected for complete hemogram and enzyme-linked immunosorbent assay (ELISA) test for human immunodeficiency virus (HIV).

Bacteriological tests

Two sputum specimens (one spot and one home) were collected from children who were able to expectorate sputum. Early morning gastric aspirate specimens on two consecutive mornings were collected from children unable to produce sputum. They were examined directly by fluorescence microscopy [12]. Xpert MTB/RIF was performed using 1 ml of the specimen as per the manufacturer's protocol [13]. Remaining specimen was subsequently processed with NaLC-NaOH (1% final concentration of NaOH) [14]. The pellet was used for raising culture on a set of in-house media including Löwenstein–Jensen (LJ) medium, LJ with 0.5% sodium pyruvate (LJ-P), selective Kirschner's liquid medium (SKLM), and commercial BACTEC MGIT (mycobacteria growth indicator tube) 960 (Becton Dickinson Diagnostic System, Sparks, MD) as per the standard protocol [14]. Drug susceptibility test (DST) to first-line TB drugs were done by MGIT 960 as per manufacturer's protocol [14].

Operational definition

Microbiologically confirmed TB: any one sputum/gastric aspirate specimen positive for TB by LJ or MGIT culture.

Clinically diagnosed TB: no microbiological confirmation and anti-TB treatment initiated based on persistence of symptoms with chest radiograph findings, TST results and or history of contact with TB.
Stunting: HAZ: ≤ −2SD
Underweight: WAZ: ≤ −2SD
Wasting: WHZ: ≤ −2SD
Chest X-ray
Suggestive of TB: any one of the chest X-ray features suggestive of TB
Abnormal: chest X-ray abnormal with features not suggestive of TB
Normal: normal chest X-ray
TST positive: an induration of ≥10 mm in HIV-uninfected and ≥5 mm in HIV-infected or severely malnourished with *Z* scores ≤ 3 SD (severely stunted/under-weight/wasted).
TST negative: an induration of <10 mm in HIV-uninfected and <5mm in HIV-infected or severely malnourished with *Z* scores ≤ 3 SD (severely stunted/under-weight/wasted).

Statistical analysis

Data were entered and double-verified in EpiData (EpiData Association, Odense, Denmark), The *Z* scores for weight and height were computed based on each child's age and gender using the EPI-NUT component of the EPI-INFO 2002 software package (version 3.4.3) from the CDC (based on India's National Center for Health Statistics reference median values). The statistical analysis was done using Stata V.15.0 (StataCorp, USA) and statistical significance was determined at $P < .05$.E

The data were described as frequency, percentage, and median with minimum and maximum range. Individuals with complete results for at least one sample were included in the analysis. For the sample-based diagnostic accuracy analysis, samples with indeterminate Xpert MTB/RIF test results and contaminated sputum cultures were excluded. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), positive-likelihood ratio (PLR), negative-likelihood ratio (NLR) with 95% confidence interval (CI) of Xpert MTB/RIF and smear were calculated with LJ and or MGIT culture as reference standard. The diagnostic accuracy of Xpert MTB/RIF and smear was calculated. The *Z*-test for two proportions was used for comparing the difference between the diagnostic accuracy. Chi-square test was used to assess the association between the factors and the diagnosis tests. We performed stratified analysis for the influence of age, sex, nutritional status, chest X-ray findings, TST, history of contact with TB, previous TB treatment, HIV status, BCG scar, symptoms suggestive of TB on Xpert MTB/RIF positivity in sputum and gastric aspirate specimens. $P < .05$ was considered statistically significant.

RESULTS

A total of 3172 children with presumptive TB were screened and 1727 were enrolled into the study (Fig. 1). The reason for nonenrolment to the study in the remaining 1445 include, not fulfilling presumptive TB symptom criteria ($n = 647$), unwilling for study procedures ($n = 735$), currently on anti-TB treatment ($n = 30$), and too sick ($n = 33$). Of the 1727 enrolled, 1674 (97%) children with complete results for smear, LJ culture, MGIT culture, Xpert MTB/RIF for at least one sputum/gastric sample were included in the analysis.

Baseline characteristics

Baseline characteristics of the 1674 children with presumptive TB is provided in Table 1. Overall, 887 (53%) were males. The mean age (range) in years was 6.1 (8 months to 15 years). The mean BMI (range) kg/m^2 in 945 children aged > 5 years was 13.6 (12.5–27.4). There were 28%, 40%, and 30% of children aged ≤ 5 years who had stunting, underweight, and wasting, respectively.

There were 441 children (26%) with chest radiograph abnormalities and 164 (10%) were suggestive of TB. TST positivity was observed in 484 (29%) and the presence of BCG scar was noted in 1617 (97%) children. Contact with TB was reported in 819 (49%), of which 232 (28%) [169 (73%) within household and 63 (27%) outside household] had contact within the past 2 years. There were 93 (6%) children who had received previous anti-TB treatment.

Specimens and bacteriology results

There were 821 (49%), 728 (43%), and 125 (8%) of the 1674 children from whom sputum, gastric aspirate, and sputum + gastric aspirate were collected, respectively (Table 2). Smear, LJ culture, MGIT culture, and

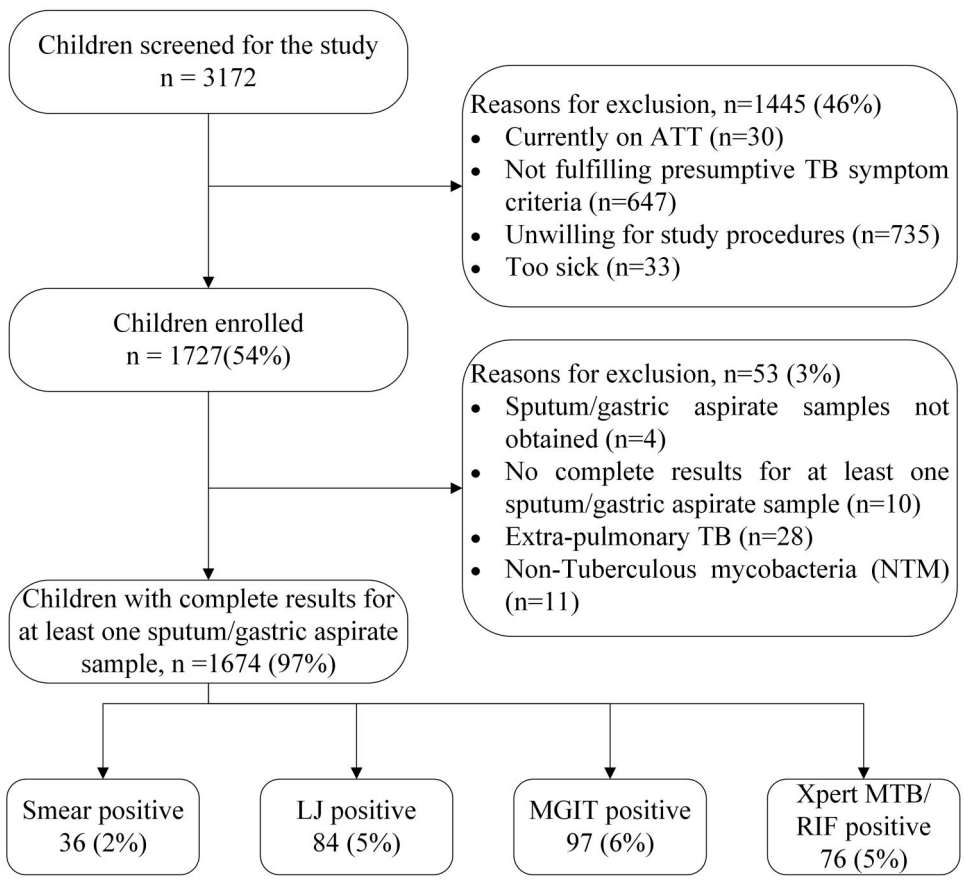

Figure 1. Flowchart from screening to microbiological confirmation in children with presumptive pulmonary TB. Lowenstein–Jensen (LJ)—solid culture; mycobacteria growth indicator tube (MGIT)—liquid culture.

Table 1. Characteristics of children with presumptive pulmonary TB ($N = 1674$).

Characteristics		Total *n* (%)
Sex	Female	787 (47)
	Male	887 (53)
Age in years	<1	65 (4)
	1–5	655 (39)
	5.1–10	710 (42)
	>10	244 (15)
Body mass index (BMI) (kg/m^2)[a] ($n = 954$)	<16	865 (91)
	16–18.49	56 (6)
	18.5–22.9	29 (3)
	>23	4 (<1)
Weight for age (WAZ)[b] ($n = 720$)	Normal	431 (60)
	Underweight	289 (40)
Height for age (HAZ)[b] ($n = 720$)	Normal	518 (72)
	Stunted	202 (28)
Weight for height (WHZ)[b] ($n = 720$)	Normal	507 (70)
	Wasted	213 (30)
Chest radiograph	Normal	1233 (74)
	Abnormal	277 (16)
	Suggestive of TB	164 (10)
Tuberculin skin test (TST)	Positive	484 (29)
	Negative	1190 (71)
History of contact with TB	No	855 (51)
	Yes	819 (49)
Previous TB treatment	No	1581 (94)
	Yes	93 (6)
HIV status	Reactive	24 (1)
	Nonreactive	1650 (99)
BCG scar	No	57 (3)
	Yes	1617 (97)

[a] BMI for children aged > 5 years.
[b] WAZ, WHZ, HAZ for children aged ≤ 5 years.
HIV, human immunodeficiency virus (HIV); Bacillus Calmette-Guerin, BCG.

Xpert MTB/RIF positivity was in 24 (3%), 41 (5%), 60 (7%), and 39 (5%), respectively in sputum, while in gastric aspirate specimens, it was 10 (1%), 35 (5%), 31 (4%), and 31 (4%), respectively. Overall positivity by smear, LJ culture, MGIT culture, and Xpert MTB/RIF in 1674 specimens was observed in 36 (2%), 84 (5%), 97 (6%), and 76 (5%), respectively (Fig. 1). The overall positivity by Xpert MTB/RIF compared to smear was 76 (5%) vs. 36 (2%), $P < .001$.

Diagnostic accuracy of Xpert MTB/RIF

Diagnostic accuracy of Xpert MTB/RIF using MGIT and or LJ culture positivity as reference standard in sputum/gastric aspirate sample is provided in Table 3. The sensitivity of Xpert MTB/RIF for sputum specimens was 68.5% (95% CI: 58.0%–77.8%) and specificity was 99.5% (95% CI: 99.0%–99.8%). In case of gastric aspirate specimens, the sensitivity of Xpert MTB/RIF was 53.6% (95% CI: 41.2%–65.7%) and specificity was 99.1% (95% CI: 98.4%–99.5%). The sensitivity of Xpert MTB/RIF compared to smear was 68.5% vs. 33.7%; $P < .001$ in sputum specimen and 53.6% vs. 14.5%; $P < .001$ in gastric aspirate while the specificity was about 99% in both. The sensitivity of Xpert MTB/RIF for sputum specimens compared to gastric aspirate specimens was 68.5% vs. 53.6%; $P = .078$.

The sensitivity of Xpert MTB/RIF with one sputum specimens compared to two was 55.6% (95% CI: 21.2%–86.3%) vs. 58.9% (95% CI: 45.0%–71.9%); $P = .849$ while it was 100% (95% CI: 29.2%–100%) vs. 46.7% (95% CI: 31.7%–62.1%); $P = .074$ for one vs. two gastric aspirate specimens (Table 4).

Of the 1674 children, a total 310 (18.5%) children were initiated on treatment for TB. Among the 310 children, 124 (40%) had microbiologically confirmed TB, and 186 (60%) were clinically diagnosed TB. The sensitivity and specificity of Xpert MTB/RIF with the decision to treat as reference standard was 23.9% (95%CI: 19.2%–29.0%) and 99.9% (95%CI: 99.5%–100%), respectively.

Concordance between Xpert MTB/RIF rifampicin susceptibility with culture-based drug susceptibility test

Concordance between Xpert MTB/RIF rifampicin susceptibility and LJ/MGIT culture-based drug susceptibility test was observed in 60 rifampicin-sensitive and one rifampicin-resistant strain (98%). One rifampicin-resistant and one indeterminate sample on Xpert MTB/RIF were found to be susceptible by MGIT DST.

Xpert MTB/RIF positivity based on characteristics of children

The Xpert MTB/RIF positivity in sputum specimen was significantly higher in females, children aged >10 years, underweight, stunted children, those with chest X-ray suggestive of TB, those with positive TST, children with fever and children with reduced playfulness/lethargy (Supplementary Table S1). In gastric aspirate specimen, the Xpert MTB/RIF positivity was significantly higher in children with chest X-ray suggestive of TB, positive TST, absence of BCG scar, those with fever and reduced playfulness/lethargy.

The diagnostic accuracy of Xpert MTB/RIF based on the characteristics of the children is provided in

Table 2. Sputum/gastric aspirate specimens and bacteriology test results ($N = 1674$).

Type of specimen	Number of specimen	Number of children with complete results	Positive result—*n* (%)			
			Smear	LJ	MGIT	Xpert MTB/RIF
Sputum	One	51	2 (4)	4 (8)	9 (18)	5 (10)
	Two	770	22 (3)	37 (5)	51 (7)	34 (4)
	Total	821	24 (3)	41 (5)	60 (7)	39 (5)
Gastric aspirate	One	45	2 (4)	2 (4)	1 (2)	4 (9)
	Two	683	8 (1)	33 (5)	30 (4)	27 (4)
	Total	728	10 (1)	35 (5)	31 (4)	31 (4)
Sputum and gastric aspirate	Two	125	2 (2)	8 (6)	6 (5)	6 (5)

Lowenstein–Jensen (LJ)—solid culture.
Mycobacteria growth indicator tube (MGIT)—liquid culture.

Table 3. Diagnostic accuracy of Xpert MTB/RIF and smear in sputum and gastric aspirate in children with presumptive pulmonary TB.

Specimen type	Xpert MTB/RIF		Culture positive		Smear microscopy		Culture positive	
			Yes	No			Yes	No
Sputum ($n = 1594$)	Xpert MTB/RIF Positive	Yes	63	7	Smear Positive	Yes	31	4
		No	29	1495		No	61	1498
	Sensitivity		68.5 (58.0–77.8)		Sensitivity		33.7 (24.2–44.3)	
	Specificity		99.5 (99.0–99.8)		Specificity		99.7 (99.3–99.9)	
	PPV		90.0 (80.5–95.9)		PPV		88.6 (73.3–96.8)	
	NPV		98.1 (97.3–98.7)		NPV		96.1 (95.0–97.0)	
	Accuracy		97.7 (96.9–98.4)		Accuracy		95.9 (94.8–96.8)	
	PLR		146.9 (69.3–311.7)		PLR		126.5 (45.6–350.8)	
	NLR		0.3 (0.2–0.4)		NLR		0.7 (0.6–0.8)	
Gastric aspirate ($n = 1470$)	Xpert MTB/RIF Positive	Yes	37	13	Smear Positive	Yes	10	3
		No	32	1388		No	59	1398
	Sensitivity		53.6 (41.2–65.7)		Sensitivity		14.5 (7.2–25.0)	
	Specificity		99.1 (98.4–99.5)		Specificity		99.8 (99.4–99.9)	
	PPV		74.0 (59.7–85.4)		PPV		76.9 (46.2–95.0)	
	NPV		97.7 (96.8–98.5)		NPV		96.0 (94.8–96.9)	
	Accuracy		96.9 (95.9–97.8)		Accuracy		95.8 (94.6–96.8)	
	PLR		57.8 (32.2–103.6)		PLR		67.7 (19.1–240.4)	
	NLR		0.5 (0.4–0.6)		NLR		0.9 (0.8–0.9)	

Positive predictive value (PPV), negative predictive value (NPV), positive-likelihood ratio (PLR), negative-likelihood ratio (NLR).
Sensitivity, specificity, PPV, NPV, and accuracy—expressed in percentage (95% CI).
The samples with valid results, i.e. either positive or negative in all the three tests were considered.

Supplementary Table S2. The sensitivity of Xpert MTB/RIF in sputum was high in age group <5 years and >10 years, undernutrition, contact with TB patient, Chest X-ray suggestive of TB, reduced playfulness and lethargy. In gastric aspirate, the sensitivity of Xpert MTB/RIF was high in females, age group <5 years and >10 years, undernutrition, chest X-ray suggestive of TB, persistent fever and weight loss/failure to thrive.

DISCUSSION

The 5% positivity with Xpert MTB/RIF in sputum, gastric aspirate specimen was observed to be higher than sputum smear positivity of 2%, but similar to that observed with solid LJ/liquid culture. Xpert MTB/RIF positivity was observed to be two times higher than that of smear microscopy in our study. This is similar to the previous study done under programmatic

Table 4. Diagnostic accuracy of Xpert MTB/RIF and smear based on number of sputum/gastric aspirate specimen in children with presumptive pulmonary TB.

No of specimen	Specimen type	Xpert MTB/RIF		Culture positive		Smear microscopy		Culture positive	
				Yes	No			Yes	No
One	Sputum ($n = 51$)	Xpert MTB/RIF Positive	Yes	5	0	Smear Positive	Yes	1	1
			No	4	42		No	8	41
		Sensitivity		55.6 (21.2–86.3)		Sensitivity		11.1 (0.3–48.3)	
		Specificity		100.0 (91.6–100.0)		Specificity		97.6 (87.4–99.9)	
		PPV		100.0 (47.8–100.0)		PPV		50.0 (1.3–98.7)	
		NPV		91.3 (79.2–97.6)		NPV		83.7 (70.3–92.7)	
		Accuracy		92.2 (81.1–97.8)		Accuracy		82.4 (69.1–91.6)	
		PLR		NA		PLR		4.7 (0.3–67.8)	
		NLR		0.4 (0.2–0.9)		NLR		0.9 (0.7–1.2)	
Two	Sputum ($n = 770$)	Xpert MTB/RIF Positive	Yes	33	1	Smear Positive	Yes	21	1
			No	23	713		No	35	713
		Sensitivity		58.9 (45.0–71.9)		Sensitivity		37.5 (24.9–51.5)	
		Specificity		99.9 (99.2–100.0)		Specificity		99.9 (99.2–100.0)	
		PPV		97.1 (84.7–99.9)		PPV		95.5 (77.2–99.9)	
		NPV		96.9 (95.3–98.0)		NPV		95.3 (93.6–96.7)	
		Accuracy		96.9 (95.4–98.0)		Accuracy		95.3 (93.6–96.7)	
		PLR		420.8 (58.6–3019.5)		PLR		267.8 (36.7–1954.0)	
		NLR		0.4 (0.3–0.6)		NLR		0.6 (0.5–0.8)	
One	Gastric aspirate ($n = 45$)	Xpert MTB/RIF Positive	Yes	3	1	Smear Positive	Yes	1	1
			No	0	41		No	2	41
		Sensitivity		100.0 (29.2–100.0)		Sensitivity		33.3 (0.8–90.6)	
		Specificity		97.6 (87.4–99.9)		Specificity		97.6 (87.4–99.9)	
		PPV		75.0 (19.4–99.4)		PPV		50.0 (1.3–98.7)	
		NPV		100.0 (91.4–100.0)		NPV		95.3 (84.2–99.4)	
		Accuracy		97.8 (88.2–99.9)		Accuracy		93.3 (81.7–98.6)	
		PLR		42.0 (6.1–291.3)		PLR		14.0 (1.1–172.7)	
		NLR		0.0		NLR		0.7 (0.3–1.5)	
Two	Gastric aspirate ($n = 683$)	Xpert MTB/RIF Positive	Yes	21	6	Smear Positive	Yes	6	2
			No	24	632		No	39	636
		Sensitivity		46.7 (31.7–62.1)		Sensitivity		13.3 (5.1–26.8)	
		Specificity		99.1 (98.0–99.7)		Specificity		99.7 (98.9–100.0)	
		PPV		77.8 (57.7–91.4)		PPV		75.0 (34.9–96.8)	
		NPV		96.3 (94.6–97.6)		NPV		94.2 (92.2–95.9)	
		Accuracy		95.6 (93.8–97.0)		Accuracy		94.0 (91.9–95.7)	
		PLR		49.6 (21.1–116.7)		PLR		42.5 (8.8–204.8)	
		NLR		0.5 (0.4–0.7)		NLR		0.9 (0.8–1.0)	
Two	Sputum + gastric aspirate ($n = 125$)	Xpert MTB/RIF Positive	Yes	4	2	Smear Positive	Yes	1	1
			No	6	113		No	9	114
		Sensitivity		40.0 (12.2–73.8)		Sensitivity		10.0 (0.3–44.5)	
		Specificity		98.3 (93.9–99.8)		Specificity		99.1 (95.3–100.0)	
		PPV		66.7 (22.3–95.7)		PPV		50.0 (1.3–98.7)	
		NPV		95.0 (89.3–98.1)		NPV		92.7 (86.6–96.6)	
		Accuracy		93.6 (87.8–97.2)		Accuracy		92.0 (85.8–96.1)	
		PLR		23.0 (4.8–110.5)		PLR		11.5 (0.8–170.4)	
		NLR		0.6 (0.4–1.0)		NLR		0.9 (0.7–1.1)	

Positive predictive value (PPV), Negative predictive value (NPV), Positive likelihood ratio (PLR), Negative likelihood ratio (NLR).
Sensitivity, specificity, PPV, NPV and accuracy—expressed in percentage (95% CI). NA—not applicable.

conditions in India which documented higher Xpert MTB/RIF positivity compared to smear microscopy (6.1% vs. 2.6%) and (4.9% vs. 1.1%) in sputum/induced sputum and gastric aspirate/lavage samples, respectively [6]. We observed that Xpert MTB/RIF had higher sensitivity than smear in sputum (68.5% vs. 33.7%) and gastric aspirate (53.6% vs. 14.5%) samples. Our findings indicate that Xpert MTB/RIF as an upfront test would detect at least twice as many cases as smear microscopy in children with presumptive pulmonary TB. Xpert MTB/RIF has been reported to have a rapid turn-around-time in programmatic settings with results available within 24 h for 90% of the children with presumptive TB [6]. Early diagnosis reduces the delay in TB treatment initiation and reduces morbidity and mortality due to disease.

The pediatric TB project of India which demonstrated the feasibility of upfront Xpert MTB/RIF in pediatric population reported MTB/RIF positivity of 6.1% in sputum/induced sputum and 4.9% in gastric aspirate/lavage samples [6]. This is almost similar to the Xpert MTB/RIF positivity of 5% in sputum and 4% in gastric aspirate specimens observed in our study. Earlier studies from Africa have reported Xpert MTB/RIF positivity of 7% in induced sputum and nasopharyngeal aspirate and 13% in induced sputum [15, 16]. A retrospective study from India documented 27.4% and 7.6% Xpert MTB/RIF positivity in induced sputum/sputum samples and gastric aspirate/lavage samples, respectively [5]. The lower Xpert MTB/RIF positivity in sputum observed in our study is because we collected expectorated and not induced sputum.

We observed that the sensitivity of Xpert MTB/RIF to be higher for sputum specimens as compared to gastric aspirate specimens (68.5% vs. 53.6%) though not statistically significant. A previous study from China documented Xpert MTB/RIF sensitivity of 33.3% and 57.1% in gastric lavage aspirate and sputum samples, respectively [17]. Another study from Sub-Saharan Africa in 930 children documented Xpert MTB/RIF sensitivity of 68.8% for gastric lavage aspirates and 90% for sputum samples [18]. A study from India in 70 children with presumptive pulmonary TB reported 75.0% and 63.64% sensitivity for gastric aspirate and induced sputum samples [11]. A systematic review and meta-analysis reported 62% and 66% sensitivity of Xpert MTB/RIF in expectorated or induced sputum and gastric lavage samples, respectively [3]. The subsequent systematic review and meta-analysis with 144 studies on the efficacy of Xpert MTB/RIF for the diagnosis of TB on various specimens also documented similar sensitivity in sputum and gastric juice specimens in pulmonary TB [19]. Nasopharyngeal aspirate as an alternate specimen for testing by Xpert MTB/RIF for the diagnosis of pulmonary TB in children has been evaluated and similar sensitivity was observed in 2 induced sputum and nasopharyngeal aspirate specimens [20]. This underscores the varied performance of Xpert MTB/RIF in biological specimens in the diagnosis of pulmonary TB in children and the need to optimize specimen collection to improve TB diagnosis in children.

Concordance for rifampicin susceptibility by Xpert MTB/RIF and culture DST was 98%. Since the detection of rifampicin resistance was negligible in our cohort, valid conclusion could not be drawn on the diagnostic accuracy of Xpert MTB/RIF for rifampicin resistance.

WHO recommended replacing smear microscopy with mWRD (WHO recommended molecular diagnostics) as the initial test for all patients with presumptive TB in 2021 [2]. These mWRDs have improved TB diagnosis in the recent years. Since 2021, TB programs in various countries especially in LMICs have widely adopted mWRDs in the diagnostic algorithm. Xpert MTB/RIF is one such mWRD that has replaced smear microscopy for TB diagnosis. The sensitivity of Xpert MTB/RIF though higher than smear, is still suboptimal in the diagnosis of pulmonary TB in children. In the context of improving TB diagnosis in children, newer diagnostics tools and specimens other than the routine (sputum, gastric/nasopharyngeal aspirate) are being evaluated. Xpert MTB/RIF Ultra assay with improvement in the lower limit of detection and improved detection of silent mutations in rifampicin resistance was developed to address the limitation of Xpert MTB/RIF [21]. The Cochrane systematic review in 2022 in TB diagnosis in children reported sensitivity of Xpert MTB/RIF Ultra assay as 75.3%, 70.4%, 56.1% and 43.7% in sputum, gastric aspirate, stool, and nasopharyngeal aspirate, respectively [22]. Non-invasive specimens which include stool and urine have been evaluated for TB diagnosis in children [23–25].

The study has inherent limitations that the number of children was not sufficient for the evaluation of the diagnostic accuracy of one sputum or gastric aspirate specimen and the 95% CI are wide. In addition, the findings for characteristics influencing Xpert MTB/RIF positivity have to be interpreted with caution as the sample may not be optimal for the subanalysis and the inclusion of children with any one or more symptoms suggestive of pulmonary TB based on the information provided by parent/guardian has its inherent limitations.

CONCLUSION

The findings of our study support earlier evidence that Xpert MTB/RIF improves the diagnosis of TB in children with presumptive pulmonary TB. However, the sensitivity is suboptimal for the diagnosis of TB in children. In children with presumptive TB, WHO has recommended Xpert Ultra with low certainty of evidence as the initial TB diagnostic test in children in sputum or nasopharyngeal aspirate [2]. Newer diagnostic tools with better sensitivity are warranted in children for the diagnosis of TB. The TB diagnostic landscape in promising with evaluation of various new TB diagnostic tools and specimens being evaluated for improving the diagnosis of TB in children.

ACKNOWLEDGEMENTS

The authors thank the staff of the bacteriology and other departments of the Indian Council of Medical Research—National Institute for Research in Tuberculosis (ICMR-NIRT) for their technical assistance. We thank the nursing staff of ICMR-NIRT—A. Gunasundari, Mangalambal, Valarmathi Nagarajan, Komathi Anbalagan, Shakila Shankar, A. Stella Mary, Hema Giranab, Seetha, S. Vaishnavi, R. Selvi, J. Jemima, Krishna Yadav, Supriya, M. Shanthi, M. Vedavalli, Rajalakshmi, and S. Yokeshwaran for valuable assistance in recruitment and follow-up of participants. We thank Dr Manjula Dutta, retired senior scientist, Dr Ranjani Ramachandran, WHO expert, and Dr Leeberk Raja for the support. We thank the doctors and nursing staff of ICMR-NIRT, Institute of Child Health and Hospital for Children, Chennai; Govt. Stanley Medical College Hospital, Chennai; Govt. Rajaji Hospital, Madurai; Govt. Vellore Medical College Hospital, Vellore; and Christian Medical College Hospital, Vellore for their support and co-operation. We would like to thank the children and their caregivers for their support and cooperation.

AUTHOR CONTRIBUTIONS

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SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Tropical Pediatrics* online.

FUNDING

This work was supported in part by Indian Council of Medical Research (ICMR) intramural funds and Model DOTS Project funded by the World Health Organization (WHO) and the United States Agency for International Development (USAID).

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Journal of Tropical Pediatrics, 2024, 70, 1–11
https://doi.org/10.1093/tropej/fmae024
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