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ORIGINAL RESEARCH

A Retrospective Study On Assessment Of Diagnostic Efficacy Of Xpert MTB/Rif Assay In Gastric Aspirate Samples At A Tertiary Care Hospital In North India

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Abstract
Background: Diagnosing pulmonary tuberculosis (TB) in children and non-sputum-producing patients is challenging. Gastric aspirate (GA) provides an alternative for detecting *Mycobacterium tuberculosis* (MTB). The Cartridge-Based Nucleic Acid Amplification Test (CBNAAT/Xpert MTB/RIF) is recommended as the initial MTB test. This retrospective study evaluated the diagnostic yield and efficacy of Xpert MTB/RIF on GA samples in a tertiary care hospital in New Delhi, comparing it with Ziehl–Neelsen (ZN) staining and Mycobacteria Growth Indicator Tube (MGIT™) rapid liquid culture. **Methods:** A retrospective analysis of 144 GA samples from 128 patients (aged <1 month to 83 years) with suspected TB between October 2022 and December 2024 was conducted. Samples were tested for MTB and rifampicin resistance using GeneXpert. Results were compared with ZN staining, MGIT culture, and Drug Sensitivity Testing (DST)/First Line Line Probe Assay (FLLPA). Sensitivity, specificity, and diagnostic yield were analyzed. The number of GA samples per patient was 1, 2 & 4. **Results:** Xpert MTB/RIF showed 100% sensitivity, 94.44% specificity, 55.56% positive predictive value (PPV), and 100% negative predictive value (NPV). Smear microscopy had a lower sensitivity (40%). MTB was confirmed in 6.49% of cultured GA samples. The diagnostic yield was higher in adults (21.43%) than children (4.62%). MTB detection increased with multiple GA samples: 3.51% (1 sample), 11.54% (2 samples), and 50% (4 samples). For rifampicin resistance, Xpert MTB/RIF had 50% sensitivity, 100% specificity, 100% PPV, and 66.67% NPV. **Conclusions:** Xpert MTB/RIF on GA is effective for early MTB and rifampicin resistance detection in pediatric and non-sputum-producing patients. Multiple GA samples improve diagnostic yield.

Keywords: *Mycobacterium tuberculosis*, Xpert MTB/RIF, GeneXpert, Gastric aspirate, tuberculosis diagnosis, rifampicin resistance, Molecular diagnostics, pediatric TB detection

Introduction

Tuberculosis (TB) is curable, preventable, present in all countries, and is a leading cause of death worldwide from a single infectious agent that claimed the lives of 1.25 million people in the year 2023, almost twice as many deaths as HIV/AIDS. With rising numbers since 2021, in 2023, about 10.8 million people fell ill with TB and 8.2 million people were newly diagnosed. About 87% of the global total was in 30 high-burden countries. India is one of the five countries that accounted for 56% of the worldwide total and with 26% is at the top followed by Indonesia (10%), China (6.8%), the Philippines (6.8%) and Pakistan (6.3%). 55% of men, 33% of women and 12% of children and young adolescents developed TB. Globally in 2023, 400,000 people developed MDR/RR-TB and only 44% were diagnosed and treated for multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB). The United Nations Sustainable Development Goals (SDGs) have set a target to end the TB epidemic by 2030. [1]

Globally, about 11% of all people with TB are children and young adolescents (aged below 15 years), with 1.1 million children becoming ill with TB every year, of which almost 50% are below 5 years of age and over half a million are older adolescents (aged 15–19 years). In children, there is a large case detection gap and only less than 50% of cases have

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been reported by National TB programs. Besides negative impact of COVID-19 pandemic on receipt of TB preventive Treatment (TPT) in children below 5 years of age, there are multiple reasons including challenges with specimen collection, bacteriological confirmation of TB in young children, due to the paucibacillary nature of TB disease in this age group and the lack of highly sensitive point-of-care tests. Children also have higher risk of developing TB disease, including severe forms of TB within a few months following exposure and infection. [2]

Tuberculosis remains a major public health concern in India with a disproportionately high burden of disease compared to global averages. Rapid and accurate diagnosis of TB is very critical and efforts towards early detection and treatment initiation, along with community engagement since 2015, have resulted in a decline of 16% in TB incidence and an 18% reduction in mortality due to TB.[3]

The standard diagnostic approach for pulmonary TB relies on sputum-based tests, including smear microscopy, Xpert MTB/RIF assay, and culture. However, obtaining sputum samples is often challenging in pediatric patients and individuals with minimal or non-productive cough.

Gastric aspirate GA) as an alternative sample has been reported to have the highest MTB detection rates (40–92%) compared to others namely, bronchoalveolar Lavage (BAL) (4-43%), nasopharyngeal aspirates (24–30%), laryngeal swab (27–63%), and with Induced Sputum (IS)(20–30%), particularly in young children who swallow their sputum rather than expectorate it. [4,5,6]

Smear Microscopy by Ziehl–Neelsen (ZN) staining to detect acid-fast bacilli, is a rapid and inexpensive method for diagnosis of TB but lacks sensitivity.[7]

The Xpert® MTB/RIF assay (Cepheid, USA) is an automated Cartridge based nucleic acid amplification test (CBNAAT) that simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and its Rifampicin resistance directly from clinical samples. It can be used on chemically inactivated specimen, is a simple and rapid molecular test that does not require special technical expertise and biosafety requirements, and results are available within 2 hours. This test has been recommended by the World Health Organization (WHO) for diagnosis of Pulmonary TB in adults (2010), children, and specific forms of extrapulmonary TB (2013). [1, 8].

Mycobacterium tuberculosis culture, considered as the gold standard, is time- consuming (takes 2–6 weeks), requires technical expertise, good laboratory infrastructure, and biosafety requirements. BD BACTEC™ Mycobacteria Growth Indicator Tube (MGIT™) automated mycobacterial detection system is an automated liquid culture system, designed and optimized for the rapid detection of mycobacteria from clinical specimens.[9]

The World Health Organization (WHO) has recommended low complexity Molecular assays, CBNAAT (Genexpert, True Nat) and moderate complexity automated Nucleic Acid Amplification Test (NAATs) as initial test for diagnosis of MTB replacing smear microscopy.[10] The National Tuberculosis Elimination Program (NTEP), also recommends upfront use of rapid molecular diagnostics. [CBNAAT (Genexpert/ True Nat)] wherever possible, for diagnosis of TB and early identification of resistance to treating drugs.

Studies on the diagnostic performance of the Xpert MTB/RIF assay on GA samples have shown varying results, with most conducted outside India. This retrospective study evaluated the diagnostic yield and effectiveness of the Xpert MTB/RIF assay on GA samples in a tertiary care hospital in New Delhi, comparing it with ZN staining and MGIT rapid liquid culture, the gold standard for MTB detection, and also rifampicin resistance. Additionally, it examined the correlation between diagnostic yield and the number of GA samples tested per patient. The findings would contribute to the growing evidence for molecular testing as the initial diagnostic method for TB in GA samples, and may guide evidence-based updates to laboratory protocols.

Materials and Methods

Study Design and Population

This retrospective study analyzed records of 144 gastric aspirate samples from 128 patients of all ages with suspected pulmonary TB, evaluated in the outpatient departments of a tertiary hospital in New Delhi, between October 2022 and December 2024. Patients were included based on symptoms indicative of TB, such as persistent cough, weight loss, fever, night sweats, and abnormal chest radiographs.

Sample Collection and Process

The treating physician collected early-morning gastric aspirate (GA) samples from fasting patients using a nasogastric tube and sent them to the microbiology department for analysis. Samples were processed using smear microscopy, the GeneXpert MTB/RIF assay, and liquid culture (BD BACTEC MGIT Mycobacterial Growth Indicator Tube).

Following the National Tuberculosis Elimination Program (NTEP) guidelines, CBNAAT testing was conducted on GA samples from children. Samples were divided into two parts. One part was centrifuged, and 0.5 ml of the deposit was treated with 1.5 ml of sample reagent for Xpert MTB/RIF testing on the GeneXpert Machine (Cepheid, USA), following the manufacturer's standard operating procedure (SOP). Invalid or erroneous results were rerun per the test algorithm. Two drops (approximately 200 µl) of the centrifuged sample were used for smear microscopy with Ziehl–Neelsen (ZN) staining, as per Revised National Tuberculosis Program (RNTCP) guidelines. The presence of pink-colored acid-fast bacilli was recorded as smear-positive. [3]

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The second part was processed using the N-acetyl-L-cysteine-sodium hydroxide (NALC-Na OH) method and cultured in MGIT liquid media using the BACTEC 960 system. Positive tubes underwent ZN staining and 5% sheep blood agar culture to check for contamination. Tubes positive for acid-fast bacilli were tested for MPT 64 antigen using a rapid immunochromatography test (ICT). ICT-positive tubes were classified as *Mycobacterium tuberculosis* complex, while ICT-negative tubes were considered non-tuberculous mycobacteria (NTM).

Results were recorded in Microsoft Excel, and sensitivity and specificity were calculated using 2×2 tables, comparing smear microscopy, Xpert MTB/RIF, culture, DST, and FLLPA.

Statistical Analysis

Each diagnostic method was assessed for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Statistical significance was determined using appropriate tests, with a p-value of <0.05 considered significant. All analyses were conducted using IBM SPSS Statistics software, version 30.0.0.0.

Results

A total of 149 gastric aspirate (GA) samples were tested using the Xpert MTB/RIF assay on the GeneXpert system (Cepheid, USA) during the study period. Five follow-up samples were excluded, leaving 144 samples from 128 patients for evaluation. Of these, 89 (61.80%) samples were from males and 55 (38.19%) from females, while the 128 patients comprised 81 males (63.28%) and 47 females (36.72%), with a male-to-female ratio of 1.72:1. Among the 9 GA samples (6.25%) that tested positive for MTB, 6 (66.67%) were from females and 3 (33.33%) from males. [Figure 1] These samples belonged to 7 patients (5.47%), including 4 females (57.14%) and 3 males (42.85%), resulting in a male-to-female ratio of 0.75:1 among MTB-positive cases

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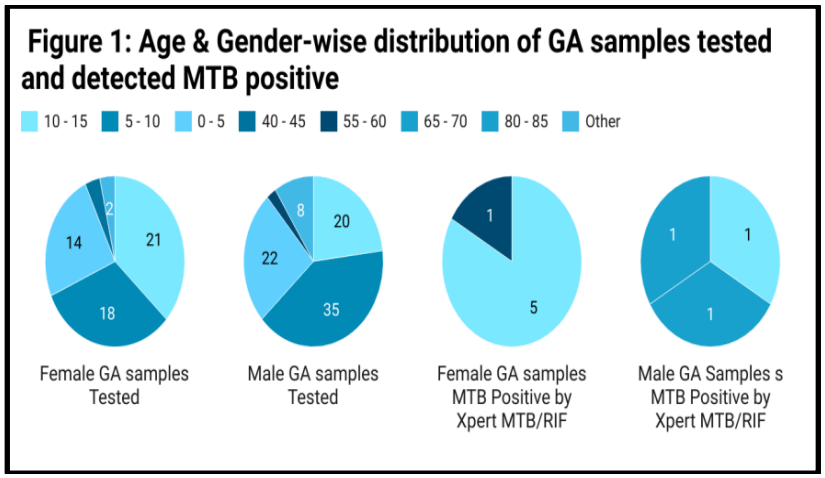

Out of the 144 GA samples, 130 (90.28%) were from children under 15 years of age, while 14 (9.72%) were from adults aged 30 years and above. The highest proportion of tested cases was in the 5–10 years age group (36.81%), followed by 10–15 years (28.47%). The mean patient age was 11.77 years, ranging from <1 month to 83 years. MTB-positive cases were predominantly in children aged 10–15 years (66.66%), while all MTB-positive adults were over 55 years old. [Table 1]

Table 1: Age and Gender-wise distribution of GA samples tested with Xpert MTB/RIF Assay and MTB positive cases

Age group	GA samples Tested			MTB Positive by Xpert MTB/RIF		
	Female	Male	Grand Total	Female	Male	Grand Total
0 – 5	14 (9.72%)	22 (15.28%)	36 (25%)	-	-	-
5 – 10	18 (12.5%)	35 (24.31%)	53 (36.81%)	-	-	-
10 - 15	21 (14.58%)	20 (13.89%)	41 (28.47%)	5 (83.33%)	1 (33.33%)	6 (66.66%)
30 - 35	-	1 (0.69%)	1 (0.69%)	-	-	-
35 - 40	-	2 (1.39%)	2 (1.39%)	-	-	-
40 - 45	2 (1.39%)	1 (0.69%)	3 (2.08%)	-	-	-
45 - 50	-	1 (0.69%)	1 (0.69%)	-	-	-
55 - 60	1 (0.69%)	2 (1.39%)	3 (2.08%)	1 (16.66%)	-	1 (16.66%)
60 - 65	1 (0.69%)	-	1 (0.69%)	-	-	-
65 - 70	-	1 (0.69%)	1 (0.69%)	-	1 (33.33%)	1 (16.66%)
70 - 75	-	1 (0.69%)	1 (0.69%)	-	-	-

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80 - 85	-	1 (0.69%)	1 (0.69%)	-	1 (33.33%)	1 (16.66%)
Total	57 (39.58%)	87 (60.42%)	144 (100%)	6 (100%)	3 (100%)	9 (100%)

Among the 144 GA samples tested, 9 (6.25%) were positive for MTB using the Xpert MTB/RIF assay, 5 of 77 samples (6.49%) were culture-positive, and 2 of 86 samples (2.32%) tested positive for acid-fast bacilli in smear microscopy. [Table 2]

Table 2: Results obtained by GeneXpert, ZN Staining & MGIT Liquid Culture in GA samples

Result	GeneXpert	ZN ᵞ Stain	MGIT[H] Culture
Positive	9 (6.25%)	2 (2.35%)	5 (6.49%)
Negative	135 (93.75%)	83 (97.65%)	72 (93.51%)
Total	144	85	77

ᵞ ZN=Ziehl- Neelsen, [H] MGIT=Mycobacteria Growth Indicator Tube

Of these 144 samples, 2(1.39%) yielded error results, and 1(0.69%) was invalid. Upon retesting , MTB was not detected in any of these three samples.

Value of Multiple GA Specimens

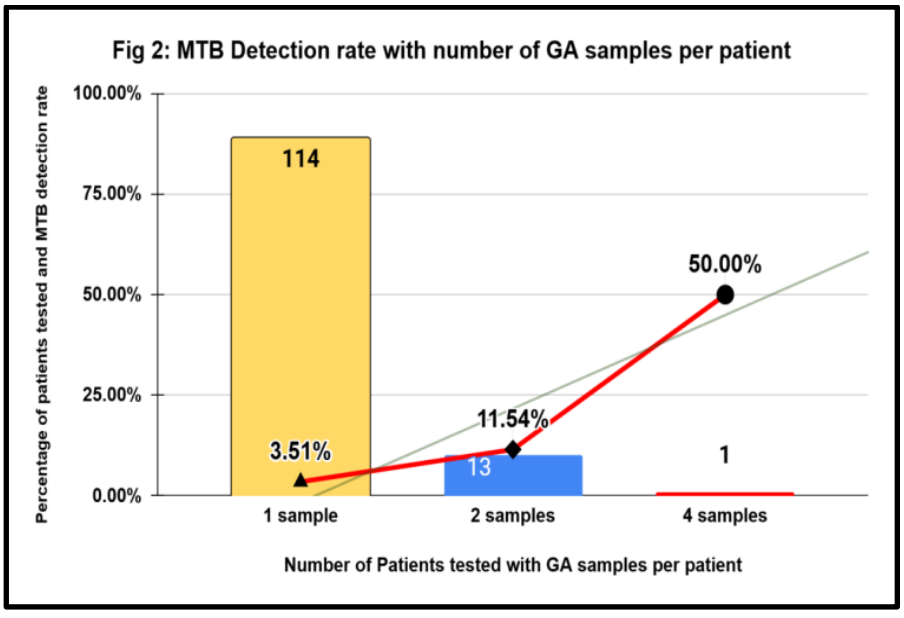
In our study population, the number of gastric aspirate (GA) samples obtained per patient varied from 1 to 2 and 4, with a mean of 1.12 and a median of 1. Among the 128 patients, 114 (89.06%) were tested with a single GA sample, 13 (10.16%) with two samples, and 1 (0.78%) with four samples, totaling 144 GA samples. Table 3 presents the MTB detection rate based on 1, 2, or 4 GA samples per patient.

Table 3: MTB detection rate of Xpert MTB/RIF assay with number of GA samples per patient

No. of GA GA samples/ per patient	Number of Patients	Total GA samples tested (n)	Xpert MTB/RIF assay	
			MTB Detected	MTB Not detected
1 sample	114 (89.06%)	114 (79.17%)	4 (3.51%)	110 (96.49%)
2 samples	13 (10.15%)	26 (18.06%)	3 (11.54%)	23 (88.46%)
4 samples	1 (0.78%)	4 (2.78%)	2 (50%)	2(50%)
Grand Total	128 (100%)	144 (100%)	9 (6.25%)	135 (93.75%)

The data was analyzed to determine the association between MTB detection rates and the number of GA samples per patient tested using the Xpert MTB/RIF assay. Since the dataset had three categories (1, 2, and 4 samples per patient) [Table 3], Fisher's Exact Test was not directly applicable in its standard form.

Figure 2 shows the increasing rate of detection with increase in the number of GA samples tested. Given the observed increasing trend in detection rates, a Chi-Square Test for Trend (Cochran-Armitage Trend Test) and a Chi-Square Test of Independence were performed.



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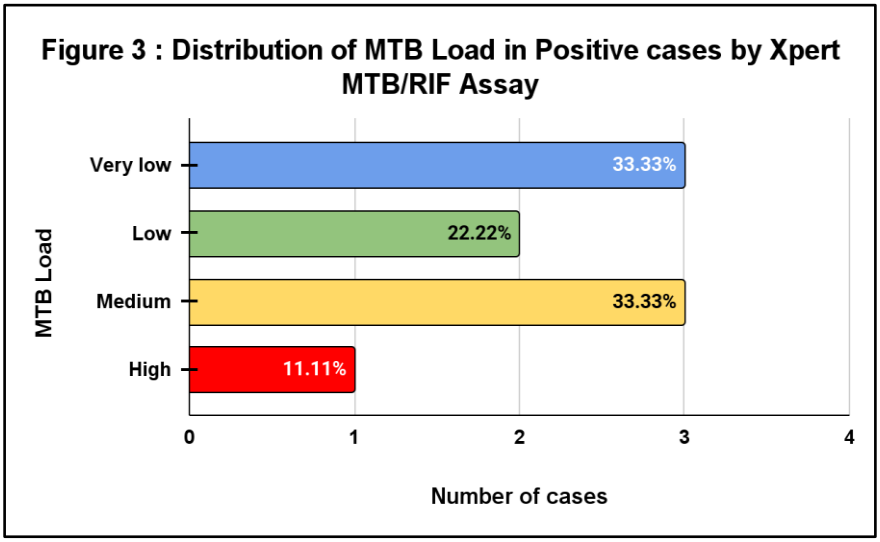
The null hypothesis (H_0) stated that there was no association between the number of GA samples per patient and MTB detection rate, while the alternative hypothesis (H_1) suggested a direct association, with detection rates increasing as the number of GA samples per patient increased.
The Chi-Square Test for Trend indicated an increasing trend, though it was not statistically significant. However, the Chi-Square Test of Independence yielded a p-value of 0.00038 (<0.05), indicating a highly significant association between the number of GA samples tested and MTB detection rates [Table 4].

Table 4: Association of Number Of GA samples per patient and MTB detection by Xpert MTB/RF assay

Test Name	Statistical test	p-value	Significance (α = 0.05)
Chi Square test of trend (Cochran-Armitage Trend Test)	Z = 1.63	0.0516	Not Significant
Chi-Square Test of Independence	χ^2 = 15.77	0.00038	**Significant**

MTB detection by Xpert MTB//RIF Assay, ZN Staining & Liquid culture (MGIT)
Among the 144 GA samples, the Xpert MTB/RIF assay detected MTB in 9 samples (6.25%). Of these, 5 (55.55%) were also culture-positive in liquid media, and 2 (22.22%) were smear-positive for acid-fast bacilli. [Table 2] The 5 culture-positive samples belonged to 4 patients, all (100%) of whom had *Mycobacterium tuberculosis* complex organisms.
Among the 9 Xpert MTB/RIF-positive samples, MTB load distribution was as follows: very low (3/9, 33.33%), low (2/9, 22.22%), medium (3/9, 33.33%), and high (1/9, 11.11%). [Figure 3]



The sensitivity and specificity of the ZN stain and Xpert MTB/RIF assay were calculated using culture as the gold standard. For GA samples, the Xpert MTB/RIF assay demonstrated a sensitivity of 100%, specificity of 94.44%, positive predictive value (PPV) of 55.56%, and negative predictive value (NPV) of 100%.
In comparison, the ZN stain showed a sensitivity of 40%, specificity of 100%, PPV of 100%, and NPV of 96%. [Table 5].

Table 5: Diagnostic Performance of Xpert MTB/RIF Assay and ZN Smear Microscopy using MTB Culture as Gold Standard

Smear Microscopy	MTB Culture Positive	MTB Culture Negative	Total	Xpert MTB/RIF	MTB Culture Positive	MTB Culture Negative	Total
AFB Positive	TP* = 2	FP** = 0	2	MTB detected	TP = 5	FP = 4	9
AFB Negative	FN# = 3	TN## = 72	75	MTB not detected	FN = 0	TN = 68	68
Total	**5**	**72**	**77**	Total	**5**	**72**	**77**
Metric	**Formula**	**Calculation**	**Value**	**Metric**	**Formula**	**Calculation**	**Value**
Sensitivity	TP /	2 /	**40%**	Sensitivity	TP /	5 /	**100%**

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	(TP + FN)	(2 + 3)			(TP + FN)	(5 + 0)	
Specificity	TN / (TN + FP)	72 / (72 + 0)	**100%**	Specificity	TN / (TN + FP)	68 / (68 + 4)	**94.44 %**
PPV[&]	TP / (TP + FP	2 / (2 + 0)	**100%**	PPV	TP / (TP + FP	5 / (5 + 4)	**55.56 %**
NPV[£]	TN / (TN + FN)	72 / (72 + 3)	**96%**	NPV	TN / (TN + FN)	68 / (68 + 0)	**100%**
Note : *TP- True Positive, **FP- False positive, [#]TN- True Negative, [##]FN- False Negative, [&]PPV- Positive Predictive Value, [£]NPV- Negative Predictive Value.							

Rifampicin Resistance

The Xpert MTB/RIF assay detects both *Mycobacterium tuberculosis* (MTB) and rifampicin resistance. Among the 9 MTB-positive samples identified by GeneXpert, rifampicin resistance was detected in 1 sample (11.11%), while 7 samples (77.78%) showed no resistance, and 1 sample (11.11%) had an indeterminate result. The rifampicin-resistant sample had a very low MTB load (n=1/3, 33.33%).

A comparison of rifampicin resistance detection between the Xpert MTB/RIF assay and Drug Susceptibility Testing (DST)/ First-Line Line Probe Assay (FLLPA) with liquid culture (n=5, 55.55%), was conducted. The results showed 1 true positive, 1 false negative, 3 true negatives, and no false positives.

Using liquid culture, DST, and FLLPA as the gold standard, the diagnostic performance of the Xpert MTB/RIF assay for rifampicin resistance detection in this study demonstrated: Sensitivity: 50%, Specificity: 100%, Positive Predictive Value (PPV): 100% and Negative Predictive Value (NPV): 75% .[Table 6]

Table 6: Diagnostic Performance of Xpert MTB/RIF Assay for Rifampicin resistance detection using MTB Culture & DST or FLLPA as Gold Standard

Xpert MTB/RIF Assay RIF Resistance	**Liquid Culture & DST[@]/ FLLPA[$] Rifampicin Resistant**	**Liquid Culture & DST/FLLPA Rifampicin Susceptible**	**Total**
Detected	TP* = 1	FP** = 0	1
Not detected	FN[##] = 1	TN[##] = 3	4
Total	2	3	5
Metric	**Formula**	**Value**	**Value**
Sensitivity	TP / (TP + FN)	1 / (1 + 1)	**50.00%**
Specificity	TN / (TN + FP)	3 / (3 + 0)	**100.00%**
Positive Predictive Value	TP / (TP + FP	1 / (1 + 0)	**100.00%**
Negative Predictive Value	TN / (TN + FN)	3 / (3 + 1)	**75.00%**
Note : [@]DST- Drug Susceptibility Test, [$]FLLPA- First Line Line Probe Assay, *TP- true Positive, **FP- False positive, [#]TN- True Negative, [##]FN- False Negative,			

Discussion

The Xpert MTB/RIF assay on gastric aspirate (GA) samples is an effective diagnostic tool for *Mycobacterium tuberculosis* (MTB), particularly in children and patients unable to produce sputum. This study supports upfront molecular testing and inclusion of GA samples in TB diagnostic algorithms. With its high sensitivity, specificity, and rapid turnaround time, GeneXpert serves as a valuable tool, especially in settings where invasive procedures are not feasible.

For bacteriological confirmation of TB, a bacterial load of 131 CFU/ml is required for GeneXpert and 10^4/ml bacilli for smear microscopy.[11,12] In this study, GeneXpert yielded a diagnostic rate nearly three times higher (6.25% [n=9/144]) compared to ZN smear microscopy (2.32% [n=2/86]) [Table 2].[13,14] The ability of GeneXpert to detect residual nucleic acids from dead bacilli post-antitubercular therapy explains why some MGIT liquid cultures were negative despite positive GeneXpert results, potentially indicating inactive infection.[15]

This study identified four cases where MTB was detected by GeneXpert but was negative in smear and MGIT culture. Two samples were positive across all three modalities, while three smear-negative samples were positive by Genexpert & culture. These findings align with previous studies.[13,14]

Most patients tested belonged to the 5–10 years age group, with positive cases primarily in the 10–15 years range. While more males were tested, females had a higher MTB positivity rate. MTB positive patient ages ranged from 12 to 83 years, and unlike other studies, adult patients exhibited a significantly higher diagnostic yield (21.43%) compared to pediatric cases (4.62%). [17]

This study supports multiple GA sample testing for MTB, especially in cases with a high index of suspicion but an initial negative test. The MTB detection rate increased from 3.51% (1 sample) to 11.54% (2 samples) and 50% (4 samples) per patient. A previous study by Elisabetta V et al. demonstrated a cumulative sensitivity of 34%, 40.4%, and 47.4% for

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1, 2, and 3 GA samples per patient, respectively. [25] In this study, the Chi-Square Test for Trend suggested an increasing detection rate, but without statistical significance, whereas the Chi-Square Test of Independence confirmed a significant association ($p = 0.00038$) between the number of GA samples tested and MTB detection by GeneXpert. These findings contribute to evidence-based laboratory protocols for improving diagnostic accuracy. ZN smear microscopy had comparable specificity (100% vs. 94.44%),

As some previous studies, using MTB culture as the gold standard, the sensitivity, specificity, PPV, and NPV of GeneXpert for GA samples were sensitivity 100%, specificity 94.44%, PPV 55.56% and NPV 100%. [16-21 In contrast, the ZN stain had sensitivity 40%, specificity: 100%, PPV 100% and NPV 96%. GeneXpert demonstrated a 250% higher sensitivity than ZN staining. These results outperformed a systematic review and meta-analysis, which reported 66% sensitivity and 98% specificity for MTB/RIF assay on GA samples.[24] ZN smear microscopy had comparable specificity (100% vs. 94.44%),. Previous studies show that GeneXpert increases TB detection rates by 23–60% among culture-confirmed cases, compared to 49.56% in this study.[16]

The prevalence of rifampicin resistance detected by Xpert MTB/RIF assay was 11.11%, consistent with previous studies.[11,12] The diagnostic performance of GeneXpert for rifampicin resistance, compared with Culture and DST, and/ FLLPA, showed sensitivity 50%, specificity 100%, PPV 100% and NPV 75%.

In this study, GeneXpert correctly identified all rifampicin-susceptible cases (no false positives) and was always correct when rifampicin resistance was detected. However, it has low sensitivity (50%) and NPV (75%) indicate a 25% chance of missing resistance.

This retrospective study had some limitations, namely, not all patients underwent all three diagnostic modalities and unequal distribution of patients with 1, 2, and 4 GA samples, potentially introducing bias. A well-designed prospective study addressing these limitations could further strengthen the evidence base.

Conclusion

The Xpert MTB/RIF assay on gastric aspirate (GA) samples has proven to be a valuable diagnostic tool for detecting *Mycobacterium tuberculosis* (MTB) and rifampicin resistance, particularly in children and patients unable to produce sputum. In our study, GeneXpert demonstrated superior sensitivity compared to smear microscopy, with a diagnostic yield nearly three times higher. The assay also showed high specificity and rapid turnaround time, making it an effective alternative for early TB detection in resource-limited settings.

Our findings suggest that testing multiple GA samples per patient increases MTB detection rates, reinforcing the need to revise diagnostic protocols to include repeated testing in cases with a high clinical suspicion of TB. The study also confirmed that while Xpert MTB/RIF assay reliably detects rifampicin resistance with high specificity, its sensitivity for resistance detection remains suboptimal, highlighting the continued need for confirmatory drug susceptibility testing.

Despite certain limitations, including retrospective study design and variations in sample numbers per patient, our results contribute to the growing evidence supporting the inclusion of GA samples in TB diagnostic algorithms. Further well-structured prospective studies are needed to refine testing strategies and optimize laboratory protocols for improved diagnostic accuracy.

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