ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Role of GeneXpert MTB/RIF-(CBNAAT), and LPA Genotype MTBDR*Plus* and MTBDR*sl* (Version 2.0) in the identification of Drug-Resistance in Smear-Negative Cases of Tuberculosis.

Gunjal PN[1]*, Iravane JA[2], Lamba A[3], Gunjal SP[4], Shanmugam S[5], and Dave M[6].

[1]Ph.D. Scholar (MUHS) at Dept. of Microbiology GMC, Chhatrapati Sambhaji Nagar, Assistant Professor Dept. of Microbiology, DVVPF's Medical College & Hospital, Ahilyanagar, Maharashtra, India.
[2]Prof. & Head Dept. of Microbiology, Government Medical College and Hospital, Chatrapati Sambhajinagar, Maharashtra, India.
[3]Prof. & Head Dept. of Respiratory Medicine, Government Medical College and Hospital, Chatrapati Sambhajinagar, Maharashtra, India.
[4]Associate Professor, Dept. of Microbiology, DVVPF's Medical College and Hospital, Ahilyanagar, Maharashtra, India.
[5]Head Dept. of Bacteriology, ICMR- National Institute of Research in Tuberculosis (NIRT), Chennai, Tamil Nadu, India.
[6]Microbiologist, Culture and Drug Susceptibility Testing Laboratory for *Mycobacterium tuberculosis*, National Accreditation Board of Laboratories Accredited (NABL) Laboratory ISO15189:2012, Dept. of Microbiology, Government Medical College and Hospital, Chatrapati Sambhajinagar, Maharashtra, India.

ABSTRACT

Tuberculosis (TB) is the second most lethal infectious agent. Drug-resistant tuberculosis (DR-TB) should be identified early for proper treatment, to avoid developing Multi-Drug Resistant (MDR) / Extensively-Drug Resistant (XDR) tuberculosis. Poor sensitivity to microscopy and delays in culture reports are the diagnostic limitations, making GeneXpet MTB/RIF (CBNAAT), GenoType MTBDR*Plus,* MTBDR*sl* (Version 2.0) Line Probe Assay (LPA) an effective substitute. Ziehl-Neelsen's (ZN) staining, CBNAAT, LPA, liquid Culture, Drug Susceptibility Testing (DST) by *Mycobacteria* Growth Indicator Tube 960 (MGIT), was performed on 236 TB specimens. Out of 236 specimens, 192 (81.35%) were Acid Fast Bacilli (AFB), and 42 (17.79%) were smear-negative. CBNAAT detected the *Mycobacterium tuberculosis* complex (MTB complex) in 20 (47.61%) of the 42-smear negative specimens. Out of these 20 specimen's 1st line, LPA detected Rifampicin (RIF) resistance in 5 (25%). Of these 5 specimens, 4 (80%) showed resistance to Rifampicin, and Isoniazid (INH) suggesting MDR. The results of CBNAAT and LPA were concordant. Second-line LPA identified 3 (60%) of the 5 specimens with fluoroquinolone resistance suggesting Pre-XDR, and 1 (20%) as XDR with fluoroquinolone and injectable anti-tubercular drug resistance. These results were well in agreement with the gold standard MGIT DST results, which detected 4 (80%) specimens as Pre-XDR, and 1 (20%) sensitive. CBNAAT & LPA exhibited superior performance to culture regarding early case detection, facilitating prompt treatment initiation.
Keywords: CBNAAT, Line Probe Assay, Liquid DST, *Mycobacterium tuberculosis*, Smear-negative.

https://doi.org/10.33887/rjpbcs/2024.15.6.36

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Tuberculosis persists as a formidable global health scourge, superseded only by HIV/AIDS among deadly infectious pathogens. Despite arduous mitigation efforts, TB exacts an immense annual mortality toll of 1.6 million and a morbidity burden of 10 million incident cases worldwide [1]. The COVID-19 pandemic, compounded by extant health crises, conflicts, food insecurity, and political volatility, portends an exacerbated global TB burden. This convergence of challenges mandates innovative, collaborative strategies to combat TB's impact amid COVID-19 [2]. India shoulders a disproportionate TB caseload, comprising approximately 80% of high-burden countries and 27% of global new diagnoses. Early, accurate diagnosis remains pivotal yet compromised in resource-limited settings reliant on smear microscopy - an affordable albeit modestly sensitive (~60%) frontline diagnostic prone to misinterpretation. Moreover, Extrapulmonary Tuberculosis (EPTB) frequently eludes microscopic detection, perpetuating diagnostic delays, treatment failures, and community transmission [3].

Overcoming these obstacles requires multi-pronged interventions encompassing diagnostic innovations, health system fortification, and sustained research investment to mitigate TB's scourge amid compounding pandemic pressures. The insidious proliferation of Drug-resistant Tuberculosis (DR-TB) strains in resource-constrained, low-income regions can attributed to suboptimal therapeutic regimens and inadequacies in available diagnostic modalities [4]. This convergence of factors has precipitated a perpetual under-detection of TB cases, enabled sustained transmission, and imposed an onerous burden upon overburdened healthcare infrastructures, thereby escalating treatment and management costs [4].

Despite the exigent need for robust diagnostic solutions, mycobacterial culture remains the contemporary gold standard for DR-TB detection, yet its elevated expenses, protracted turnaround times, and consequent diagnostic delays impede timely treatment initiation [4, 5]. Addressing these multifarious challenges is imperative to enhance DR-TB diagnosis and clinical management in resource-limited milieus. This article comprehensively explores these obstacles and elucidates potential strategies to mitigate the deleterious public health impact of DR-TB. Emergent molecular diagnostic modalities offer expedited analysis and reporting capabilities, enabling direct nucleic acid detection from patient specimens. These techniques are invaluable for the identification of MDR and XDR-TB cases. Both CBNAAT and LPA can diagnose drug resistance for critical anti-tubercular 1st line agents like Rifampicin (RIF), and Isoniazid (INH), garnering recommendations from the World Health Organization (WHO) [3, 5]. CBNAAT, through the deployment of three specific primers, not only detects the *Mycobacterium tuberculosis* complex (MTBC) but also confers mutational resistance to rifampicin and isoniazid. The CBNAAT, being a fully automated, cartridge-based Deoxyribose Nucleic Acid (DNA) detection technique, generates results within a 2-hour time limit, rendering it one of the most efficient and time-saving automated molecular diagnostic tools [3, 5].

Concurrently, the LPA endorsed by the WHO facilitates the diagnosis and unveiling of the resistance to rifampicin, and isoniazid, as well as drugs from the second-line group like fluoroquinolones, and injectables [3-7]. These innovative molecular diagnostic techniques are meticulously designed to discern mutational drug resistance with exceptional precision, applicable to clinically suspected cases, smear-negative specimens, and direct clinical samples. This study aims to compare the diagnostic accuracies of CBNAAT and LPA in clinically suspected smear-negative specimens for the early detection and diagnosis of MDR-TB.

MATERIALS AND METHODS

Sample size: 236.

Study Duration: April 2019 to Dec 2022.

Sampling Method: Total consecutive sampling method.

Study Type: Cross-Sectional.

Statement of Ethics

- The study was approved by the Institutional Ethics Committee.
- Written informed consent was obtained from the participants before specimen collection.
- Patient confidentiality was maintained.

Place of Study: Culture and DST Laboratory accredited by the National Accreditation Board of Laboratories (NABL Accredited Laboratory ISO15189:2012) Department of Microbiology, Government Medical College, Chattrapati Sambhajinagar, Maharashtra.

Methods

A total of 236 specimens of Pulmonary TB (PTB) and EPTB were collected. Sputum samples were collected in sterile wide-mouth containers as per the guidelines of the National Tuberculosis Elimination Program (NTEP) laboratory guidelines [8].

All the 236 specimens were subjected to decontamination. Specimen decontamination was done according to the standard guidelines of the NTEP Laboratory Manual by the N-acetyl-L-cysteine-sodium hydroxide solution (NALC- NaOH) method. 0.25g in 50 mL sodium hydroxide solution and equal volume of specimens was incubated at room temperature (RT) for 15 min. Subsequently, for the decontamination process, the solution was diluted in 15 mL phosphate buffer solution (PBS) (pH 6.8) and centrifuged for 15 min. at 8000 rotations per min (rpm). The sediments were then stored at -20^0 C, in Tris-EDTA (TE) buffer solution (pH 8.0), for further assays and procedures [3, 8, 9].

All the decontaminated specimens were subjected to ZN staining, smears were graded, and results were interpreted as per the NTEP manual's standard guidelines [3, 8].

All the smear-negative specimens were screened for MTBC and RIF drug resistance by CBNAAT. Briefly, the procedure incorporates treating decontaminated specimens with a 2:1 sample reagent diluent having isopropanol and sodium hydroxide (NaOH). Samples were homogenized inside a biological safety cabinet to prevent contamination and aerosol production. The closed specimen was then left at RT for 15 min. and subsequently shaken twice. This inactivated substance was then transferred to a 2 mL disposable test cartridge. The next steps were automated. *Mycobacterium tuberculosis* (MTB) genomic copies can be found in as few as 130–150 cfu/mL. Since each module operates on its own, batching is not necessary & separate tests can be launched at various times. Cartridges were disposed of under the hazardous material disposal requirements in the same manner as sputum containers [3, 10, 11]. Probes A-E, which are complementary to the whole 81 bp rpoβ core region, were used to detect *Mycobacterium tuberculosis.* At least two of the five probes must provide positive signals with a cycle threshold (CT) of d"38 cycles to diagnose MTB. CT is the number of DNA multiplications needed to detect MTB DNA. MTB-specific beacon CT differences between early and late CTs are used to determine RIF resistance.

RESULTS AND INTERPRETATION

1. Identification of *Mycobacterium tuberculosis* in specimen.
2. Detection of resistance or susceptibility to RIF.
3. RIF resistance is interpreted as High, Medium, Intermediate, Low, or Very Low.

a. RIF resistance indicates a significant RIF resistance in the bacterium. To confirm drug resistance to 1st and 2nd line Anti-Tubercular Drugs (ATD), rapid molecular testing is needed.
b. No resistance to RIF indicates susceptibility to RIF.
c. Specimens with Intermediate resistance to RIF should be retested for CBNNAT.
d. Specimens with invalid results must be repeated.
e. In case of Error or No Results, troubleshooting is to be done, and the specimen is retested [3, 10, 11] (Check the Bucket style is different For all superscript references)

All 236 specimens were subjected to the MGIT for culture and DST for 1st-line and 2nd-line drugs. MGIT consists of 7H9 liquid media tubes containing PANTA + growth enhancer factors. About 500 µl of the decontaminated specimen was added to MGIT. Cultures that flagged positive were observed for growth. ZN staining was performed on growth tubes to look for the presence of acid-fast bacilli. Specimens with MTB growth were subjected to standard identification methods for MTB [3, 12, 13 14]. DST was performed according to NTEP guidelines [3, 12].

ISSN: 0975-8585

INTERPRETATION OF RESULTS

The instrument growth chamber (GC) indicated a signal when the value of growth unit (GU) reached 400. These sets were then removed after scanning. DST reports were interpreted as “S” and “R” for sensitivity and resistance respectively. The test result was "susceptible" if the GC was 400 or more and the drug tube's GU value was less than 100, and "resistant" if it was 100 or higher. These growth values were recorded for each set of the DST tubes. If the GU value of the control does not reach 400 even after 21 days, the instrument indicates an error X200, indicating inadequate growth. On the contrary, if GU exceeded 400 before day 4, the device reported an error X400, suggesting contamination or over-inoculation [3, 12-14].

Quality control (QC) was conducted by incorporating the *Mycobacterium tuberculosis H37Rv* strain (ATCC27294) when setting up a batch of DST. All subsequent findings in that batch were deemed void if resistance in the QC strain was detected. Specimens with growth were subjected to standard identification methods for MTB [3, 12-14].

LPA was performed as per the guidelines of kit insert (Hain Lifescience, Nehren, Germany). It detected *Mycobacterium tuberculosis Complex* (MTBC) and 1st and 2nd line drug resistance analysis respectively [15, 16]. *Mycobacterium tuberculosis* GenoLyse’s Kit (Hain Lifescience) was used for DNA extraction. Polymerase Chain Reaction (PCR) was carried out with an amplification mixture (AM-A & AM-B) procured with the kit. Hybridization was performed using an automatic (GT Bolt) & then washing was done. Strips were dried and impregnated on paper. Interpretation was done according to the instruction manual [3, 15, 16].

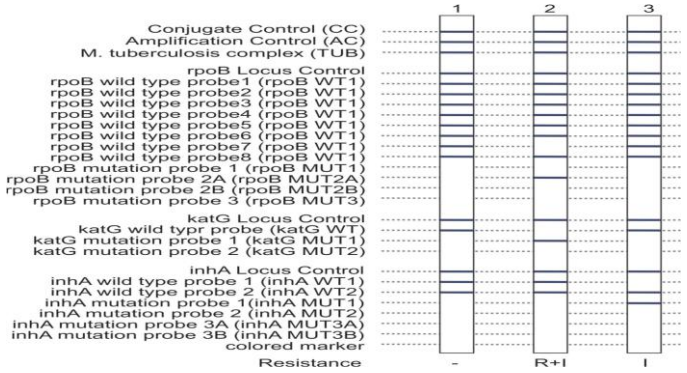


Figure 1: 1. Wild type MTB, 2. MTB resistant to INH and RIF, and 3. MTB is resistant to INH only. (Strips of LPA 1st line drugs) GenoType® (adapted from Hain Life Sciences). Available via license: CC BY-NC-ND 3.0 Content may be subject to copyright [11].

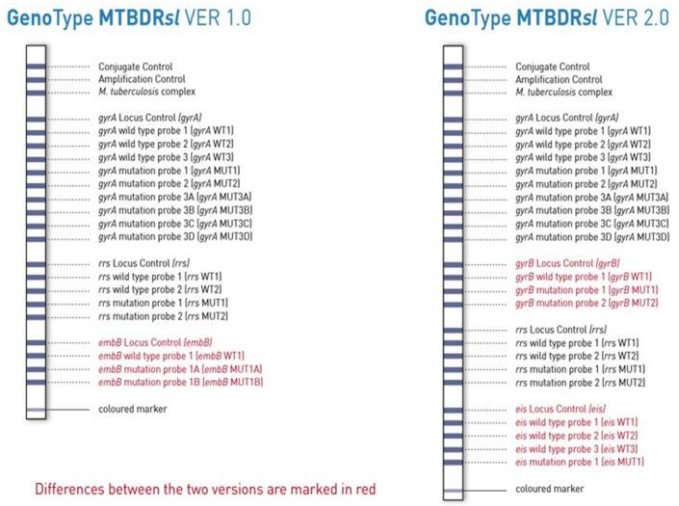
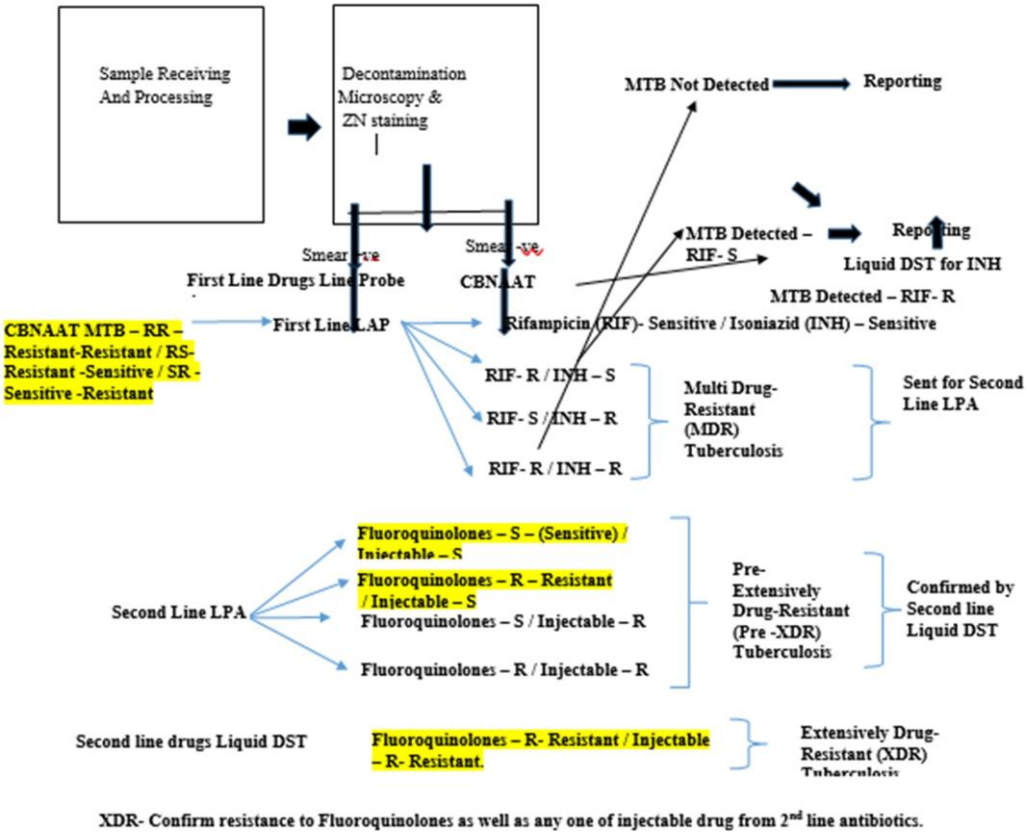


Figure 2: Differences between Genotype MTBDR*Plus* and MTBDR*sl* (adapted from Hain Life Sciences 2015). Available via license: CC BY-NC-ND 3.0 Content may be subject to copyright [15].

ISSN: 0975-8585

Figure 3: Diagrammatic Representation of Sample Processing Flow Chart.



RESULTS

A total of 236 specimens were included in the study, out of which 147 (62.28%) were males & 37.71% were females (Table no. 01).

Table 1: Distribution of study population (n=236).

Sr. No.	Gender	Total Number of patients (n=236)	Total Percentage (%)
1	Male	147	62.28
2	Female	89	37.71
3	**Total**	**236**	**100**

The age group of the study population ranged between 10 to 80 years. The maximum number of study participants was between the age group of 21–30 years (Table no. 02).

The chi-square test determined a significant association between gender and age. The maximum participants were males in the age group of 21-30 years & the minimum was 01 male in the age group of 1-10 years & 01 in the age group of 71-80 years. There were no female participants in the age group of 1-10 years (table no. 02).

Table 2: Gender & age-wise distribution of study population (n=236).

Sr. No.	Gender	Age Groups (Years)							
		01-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80
1	Male	01	19	39	**41**	21	15	09	02
2	Female	00	26	**33**	08	10	07	04	01
3	**Total**	**01**	**45**	**72**	**49**	**31**	**22**	**13**	**03**

Value of χ^2 =20.890, p=0.0039, significant.

ISSN: 0975-8585

Out of 236 cases, 139 (58.89%) were new cases, while 53 (23%) were known positive follow-up cases. Out of 236 specimens, 116 (49.15%) were smear-positive and 42 (17.79%) were smear-negative. Overall, out of 236 specimens, smear positivity was greater (82.20%) than smear negativity (17.79%). Smear positivity was higher in females (61.11) (Table no. 03).

The chi-square test determined a strong correlation between gender and smear-positive and smear-negative samples.

Table 3: Distribution of smear-positive and smear-negative specimens (n=236).

Sr. No	Gender	Smear Positive (%)	Follow-up sample (Known Cases) (%)	Total Smear Positive (n=236)	Total Smear Negative (n=236)
1	Male (n=146)	86 (58.90)	38 (26.53)	124 (52.54)	22 (14.96)
2	Female (n=90)	55 (61.11)	15 (17.97)	70 (29.66)	20 (22.47)
3	**Total**	**141 (59.74)**	**53 (23.00)**	**194 (82.20)**	**42 (17.79)**

Value of χ^2 =8.997, p=0.0014, significant

Out of 42 smear-negative specimens, 20 (47.61%) were positive by CBNAAT and also exhibited growth by MGIT, and 22 (52.38%) were negative by CBNAAT and exhibited no growth by MGIT. The results of CBNAAT & MGIT were 100% in agreement with each other (Table no. 04).

Table 4: Results of CBNAAT in smear-negative specimens (n=42).

Sr. No.	Positive (%)	Negative (%)	Total (n=42)
CBNAAT	20 (47.61)	22 (52.38)	42 (100)
BACEC MGIT 960 Liquid Culture	20 (47.61)	22 (52.38)	42 (100)

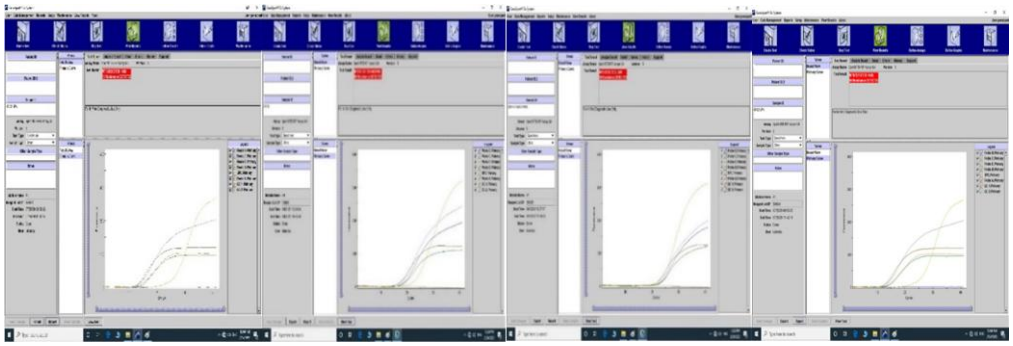


Figure 4. CBNAAT-GeneXpert *MTB/RIF*: The graph lines showed the Rifampin resistance from smear-negative specimens.

Further, out of the 20 specimens which were positive by CBNAAT, 5 (25%) isolates were detected with resistance to rifampicin (RR) and the remaining 15 (75%) were sensitive to rifampicin (SR) (Table no. 05).

Table 5: Rifampin sensitivity pattern of smear-negative CBNAAT positive specimens (n=20).

Sr. No.	CBNAAT Positive (n=20)	Rifampin Resistant (RR) (%)	Rifampin Sensitive (SR) (%)	Total (%)
1	20	05 (25%)	15 (75%)	20 (100%)

Rifampin-resistant isolates were subjected to 2nd-line LPA & 2nd line liquid DST. All the isolates were susceptible to an injectable group of drugs. The pattern of drug susceptibility is elaborated in Table No. 06.

ISSN: 0975-8585

Table 6: Pattern of 2nd Line – Line Probe Assay (GenoType MTBDR*sl*) and 2nd line – Liquid Drug Susceptibility Testing (DST) of Rifampicin-resistant isolates (n=5).

Sr. No.	Name of (ATD)	1st Sample		2nd Sample		3rd Sample		4th Sample		5th Sample	
		LPA 2nd Line	DST Liquid	LPA 2nd Line	DST Liquid	LPA 2nd Line	DST Liquid	LPA 2nd Line	DST Liquid	LPA 2nd Line	DST Liquid
Injectable group	Kanamycin	S	S	S	S	S	S	S	S	S	R
	Carpreomycin		S		S		S		S		S
	Amikacin		S		S		S		S		S
Fluoroqui-nolones group	Levofloxacin	S	S	R	R	R	R	R	R	R	R
	Moxifloxacin (0.5)		S		R		R		R		S
	Moxifloxacin (1.0)		S		R		R		R		S
	Moxifloxacin (2.0)		S		S		S		S		S
	Ofloxacin		S		R		R		S		S

LPA- Line Probe Assay, ATD- Anti-Tubercular Drugs, SS – Sensitive to Fluoroquinolones and Injectable 2nd line drugs for TB, RS- Resistant to Fluoroquinolones & sensitive to 2nd line injectable drugs for TB, Km- Kanamycin, Cm- Capreomycin, Am- Amikacin, Lf- Levofloxacin, Mfx (0.5)- Moxifloxacin (0.5 drug concentration) Mfx – Moxyfloxacin (2.0), Olfx – Ofloxacin, LZD- Linezolid, Mox- Moxifloxacin (1.0), DST- Drug susceptibility test.

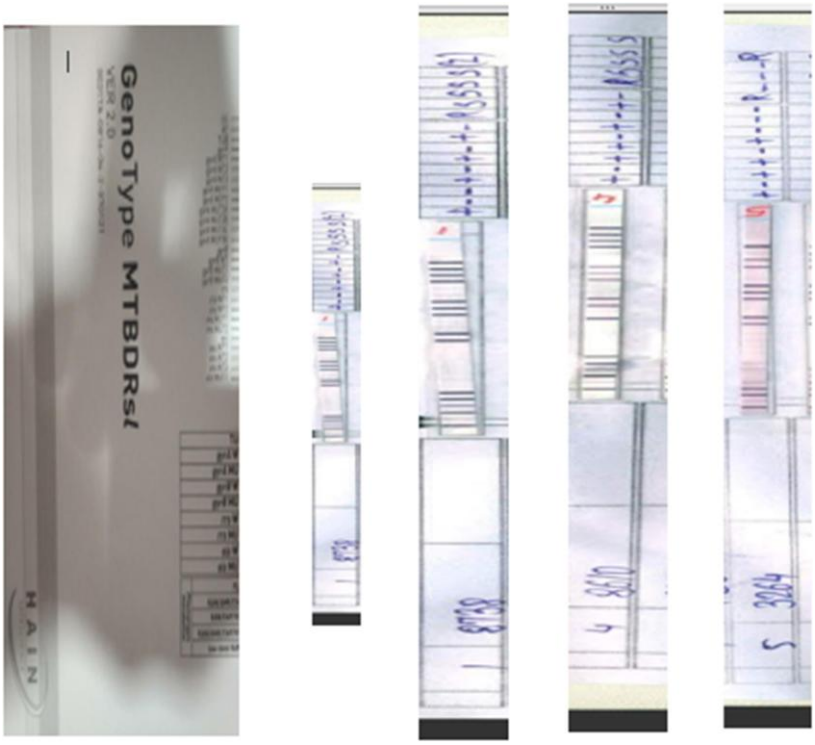


Figure 6: The Band Patterns of Genotype MTBDR*sl* (Ver. 2.0) 2nd Line Results of LPA – 2nd line (Bands showing Resistance to 2nd line Anti-Tb drugs Group Fluoroquinolones & Sensitive to 2nd line Injectable drugs).

ISSN: 0975-8585

DISCUSSION

The escalating incidence of tuberculosis infections has imperiled ongoing endeavors to curtail the dissemination of drug-resistant strains. Spontaneous mutational events within the *Mycobacterium tuberculosis* genome are the primary drivers of drug resistance acquisition [17]. On a global scale, resistance to the pivotal first-line agents' isoniazid (INH) and rifampicin (RIF) is proliferating, giving rise to multidrug-resistant tuberculosis (MDR-TB) [18]. Conventional techniques for the cultivation of MTB and ATD sensitivity analysis usually are protracted moreover intricate processes, necessitating sequential diagnosis. This delay propagates the mistreatment of patients, amplifying the transmission of drug-resistant strains and exacerbating resistance. Consequently, the early detection and identification of MDR-TB or extensively drug-resistant (XDR-TB) strains are imperative for global TB prevention and control strategies [15].

The present study cohort comprised 236 specimens, of which 147 (62.28%) were obtained from male participants, and 89 (37.71%) were from females, indicating a male preponderance. Among the total cases, 139 (58.89%) were newly diagnosed, while 53 (23%) represented known positive follow-up cases. Our findings align with the observations of Abyot Meaza et al., who reported a male predominance of 118 (65%) out of 182 cases, with 64 (35%) female patients [19]. Similarly, Vijay et al. documented a 68% male preponderance in their study involving 425 participants, with 32% female representation [3]. The study participants exhibited a range spanning from the age of 10 to 80yrs., amidst noticeable concentration from the age group of 21-30 yrs. The median age of our cohort was 41 years, which aligns with the reported median age of 40 years by Vijay et al. The age range within our cohort was 20-65 years (3). The Chi-square test demonstrated a statistically significant correlation between age and sex, as indicated by a $\chi 2$ value of 20.890, and a p-value of 0.0039, indicating statistical significance. Smear-positivity was noted in 116 (59.74%) new cases and 23% of follow-up cases while smear-negative were 42 (17.79%) specimens out of a total of 236 specimens.

Our results align with those that have been previously reported, by Vijay et al., who observed a smear positivity rate of 52%. However, the proportion of smear-negative specimens in their study was notably higher compared to our findings, at 48% [3]. Overall, our study identified smear positivity in 194 specimens (82.20%) and smear negativity in 42 specimens (17.79%), aligning well with previous research. Yadav RN et al. noted a smear positivity of 74.65% with smear negativity of 25.34% [20]. While our study recorded slightly higher smear positivity findings compared to Yadav RN et al., their study reported slightly higher smear negativity rates compared to ours [20]. The Chi-square test demonstrated a notable correlation between gender and smear status, with a $\chi 2$ value of 8.997 and a p-value of 0.0014, indicating statistical significance.

A total of 42 smear-negative samples, constituting 17.79% of the total 236 specimens, were subjected to inoculation on MGIT liquid culture, resulting in growth detection of Mycobacterium tuberculosis in 20 samples (47.61%). The results align with observations by Vijay et al., who noted a 43% culture positivity rate in smear-negative specimens [3]. Similarly, Agarwal M. et al. reported 14 positive specimens out of 170, using the CBNAAT and MGIT 320 liquid culture system [21].

In our investigation, 20 out of 42 smear-negative specimens (47.61%) were identified as positive by CBNAAT. This detection rate slightly exceeds the rates observed by Vijay et al [3], as well as by Dash et al [22], who reported CBNAAT positivity rates of 31.2%.

Among the CBNAAT-positive specimens, rifampicin resistance (RR) was observed in 25%. This finding aligns with the observations of Vijay et al [3], and Dash et al [22], who reported rifampicin resistance rates of 28.12% and 18 out of 64 (28.12%) specimens, respectively, using CBNAAT. Rifampin sensitivity (RS) was 22 (52.38%) out of 42 which is comparatively low with the findings of other investigators, Vijay et al have reported 71.87% sensitivity [3].

All the 5 (25%), RR specimens detected by CBNAAT were subjected to 1st-line LPA and detected resistance to rifampicin and isoniazid in total 4 (80%) indicating multi-drug-resistant strains. These results are in accordance with the results of CBNNAT results. 2nd line LPA had detected 3 (60%) of the 5 specimens as Pre-XDR with the resistance to fluoroquinolone group of drugs and 1 (20%) as XDR with resistance to fluoroquinolones and injectable group of anti-tubercular drugs. These results are well

agreement with the results of gold standard MGIT liquid DST which had detected 4 (80%) as Pre-XDR and 1 (20%) as sensitive.

CONCLUSION

In conclusion, this study underscores the potential of CBNAAT as a superior alternative to microscopy for detecting misdiagnosed smear-negative cases in primary healthcare settings. Additionally, LPA Genotype MTBDRPlus and MTBDRsl (Version 2.0) exhibited enhanced efficacy in identifying cases of MDR-TB and Pre-XDR-TB compared to MGIT 960 Liquid DST results. These molecular diagnostic methods offer rapid and precise detection, enabling timely initiation of targeted treatment for tuberculosis cases that may have been misdiagnosed or underdiagnosed. This highlights the importance of integrating molecular techniques into routine TB diagnostic protocols to improve patient outcomes and mitigate the spread of strains becoming Multi-Drug-Resistant or Extensively-Drug-Resistant.

REFERENCES

[1] https://cdn.who.int/media/docs/default-source/hq-tuberculosis/tb-report-2021/factsheet-global-tb-report-2021.pdf?sfvrsn=86011b1e_5&download=true

[2] https://cdn.who.int/media/docs/default-source/hq-tuberculosis/global-tuberculosis-report-2022/top-findings-and-messages-in-the-2022-report-eng.pdf?sfvrsn=91a184dc_18&download=true

[3] Vijay DD, Meharaj SHS, Jayanthi S, et al. A Comparison Study of CBNAAT, Gene Xpert and Line Probe Assay in the Diagnosis of Tuberculosis in smear-negative Specimens. J Pure Appl Microbiol 2022;16(3):1953-1963.

[4] Aricha SA, Kingwara L, Mwirigi NW, Chaba L, et al. Comparison of GeneXpert and line probe assay for detection of Mycobacterium tuberculosis and rifampicin-monoresistance at the National Tuberculosis Reference Laboratory, Kenya. BMC Infectious Diseases 2019; 19: 852.

[5] Wang H, Chunyan Zhao FL, Rapid identification of Mycobacterium tuberculosis Complex By a Novel Hybridization. Braz J Microbiol 2011; 42: 964-972.

[6] Isakova J, Sovkhozova N, Vinnikov D, et al. Mutations of rpoB, KatG, inhA, and ahp genes in rifampicin and isoniazid-resistant Mycobacterium tuberculosis in Kyrgyz Republic. BMC Microbiol 2018;18(1):22.

[7] https://fctc.who.int/publications/i/item/WHO-HTM-TB-2014.11.

[8] Revised National Tuberculosis Control Program Laboratory, Guidelines for Quality Assurance of smear microscopy for diagnosing tuberculosis. 2005.

[9] Potdar P, Thakur P. Development of sequence-based molecular diagnostic test to evaluate MDR and XDR in M. tuberculosis patients from Western India. American J of Infect Dis & Microbiol 2013; 1(3):50-58.

[10] Garg, Archana; Agarwal, Lakshmi1,; Mathur, Rishab2. Role of GeneXpert or CBNAAT in Diagnosing Tuberculosis: Present Scenario. Medical Journal of Dr. D.Y. Patil Vidyapeeth 2022;15(1): 14-19.

[11] Sarkar S. Recent Development in Molecular Diagnosis of Tuberculosis. Bengal Physician Journal 2018;5(2):10-16.

[12] Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Revised National TB Control Programme: Manual of Standard Operating Procedures (SOPs). Culture of Mycobacterium tuberculosis and drug susceptibility testing on solid medium (Internet) Version no. 01.01, dated 01/04/2009.

[13] Siddiqi S, Ahmed A, Asif S, Behera D, Javaid M, Jani J, Jyoti A, Mahatre R, Mahto D, Richter E, Rodrigues C, Visalakshi P, Rüsch-Gerdes S. Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the Bactec MGIT 960 system: a multicenter study. J Clin Microbiol 2012;50(2):435-40.

[14] Kandhakumari G, Stephen S, Kavitha K. Isolation of Diverse Mycobacterium Tuberculosis Strains Employing Automated and Conventional Culture from Lymphadenitis in a Tertiary Care Center, Pondicherry, India J Res Med Dent Sci 2019; 7 (3):164-168.

[15] https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/genotype-mtbdrplus.html

[16] Seifert M, Georghiou SB, Catanzaro D, Rodrigues C, Crudu V, Victor TC, Garfein RS, Catanzaro A, Rodwell TC. MTBDRplus and MTBDRsl Assays: Absence of Wild-Type Probe Hybridization and Implications for Detection of Drug-Resistant Tuberculosis. J Clin Microbiol 2016;54(4):912-8.

[17] Rai DK, Pandey S. A Hospital-Based Cross-sectional Study on Clinico-demographic Characteristic of Extrapulmonary Tuberculosis Cases Coming to a Tertiary Hospital of Bihar. Indian J Community Med 2018; 43(2):122-123.
[18] Seung KJ, Keshavjee S, Rich ML. Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harb Perspect Med 2015 27;5(9): a017863.
[19] Abyot M, Abebaw K, Zelalem Y, Zekarias D, Shewki M, Bazezew Y, et al Evaluation of genotype MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in smear positive and negative sputum samples. BMC Infect Dis 2017;17(1):280.
[20] Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis. J Clin Diagn Res 2016;10(5): DC09-12.
[21] Yadav RN, Singh BK, Sharma R, Chauby J, et al. Comparative Performance of Line Probe Assay (Version 2.0) and Xpert MTB/RIF Assay for Early Diagnosis of Rifampicin-Resistant Pulmonary Tuberculosis. Tuberc Respir Dis 2021; 84:237-244.
[22] Dash M, Samal S, Swain TR, Behera B, Sahu S, Mahapatra S. Diagnostic yield of cartridge-based nucleic acid amplification test in Mycobacterium tuberculosis in a tertiary care medical college and hospital of Southern Odisha, India. Int J Res Med Sci 2019;7(12):4460-4463.