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# A STUDY TO DETECT THE POSITIVITY RATE OF PEDIATRIC PULMONARY TUBERCULOSIS AND MULTI DRUG RESISTANCE TUBERCULOSIS USING MOLECULAR METHODS

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## ABSTRACT

**Background:** Pediatric tuberculosis (TB) remains a significant public health concern, particularly in high-burden countries like India. Diagnosis is often challenging due to the paucibacillary nature of disease in children and difficulty in obtaining quality sputum samples. Conventional methods lack sensitivity and delay treatment. The advent of molecular diagnostics such as Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and Line Probe Assay (LPA) offers rapid and accurate detection, including drug resistance.

**Objectives:** To determine the positivity rate of pulmonary tuberculosis among children under 14 years using CBNAAT.
To detect drug-resistant TB in pediatric patients using CBNAAT.
To assess multidrug resistance (MDR-TB) patterns through first-line and second-line LPA.

**Methods:** A prospective cross-sectional study was conducted over one year at the Department of Microbiology, KIMS, Hubballi. A total of 865 sputum samples, bronchoalveolar lavage and methods from presumptive pediatric TB cases were analyzed using AFB Sputum microscopy and CBNAAT. Rifampicin resistance detected by CBNAAT was further evaluated using Line Probe Assays for first-line (FL-LPA) and second-line (SL-LPA) anti-TB drugs.

**Results:** Among the 865 samples, CBNAAT detected Mycobacterium tuberculosis in 136 cases, yielding a positivity rate of 15.7%. Rifampicin resistance was identified in 6 cases (0.7%). FL-LPA confirmed multidrug resistance (RIF and INH) in 7 cases (0.8%), and SL-LPA detected fluoroquinolone resistance in 2 cases (0.2%). Logistic regression showed that children above 8 years had a three-fold higher risk of CBNAAT positivity compared to those ≤8 years.

**Conclusion:** CBNAAT demonstrated high diagnostic utility for pediatric pulmonary TB with a positivity rate of 15.7%. Molecular methods enabled early detection of drug resistance, vital for guiding appropriate treatment. Incorporating CBNAAT and LPA into routine diagnostic algorithms can enhance TB control among pediatric populations in high-burden settings.

**Keywords:** Pediatric Tuberculosis, CBNAAT, Line Probe Assay, Multidrug Resistance, Molecular Diagnosis, Drug-Resistant TB

## INTRODUCTION

Tuberculosis (TB) is one of the world's most common infectious causes of morbidity and mortality. It continues to be one of the most common infectious diseases in the world, especially affecting chil dren and other vulnerable groups.

Because of its nonspecific appearance and sample collection difficulties, pediatric tuberculosis is fre quently underdiagnosed and underreported.

Children make up a sizable amount of the global TB burden, despite the focus on TB eradication in global health.The causal agent, Mycobacterium tuberculosis, continues to cause a great deal of illnes s and mortality in children, particularly in highburden nations like India.Particularly in the juvenile population, where disease progression is frequently severe and quick, prompt and accurate identifica tion is essential for starting the right therapy and preventing transmission (1) .

Globally, about one million cases of pediatric TB occur every year, of which 70-80% have pulmonary tuberculosis, 15-20% have extra-pulmonary tuberculosis and with mortality rate as high as around 25%. The cornerstone of TB control remains in early diagnosis and treatment (2)The World Health Organization (WHO) estimates untreated cases have a 25% fatality rate(3).Approximately 7–8% of all recognized TB cases in India, which has the greatest TB incidence in the world, involve children under the age of 15. Given the lack of child-specific methods and diagnostic difficulties, pediatric TB is recognized by the National TB Elimination Programme (NTEP) as a major issue (4)

Diagnosis of pediatric pulmonary TB is challenging and is primarily on basis of history, clinical examination and radiological findings without definite laboratory evidence. The paucibacillary nature of tuberculosis and the difficulties in getting high-quality sputum specimens make diagnosing the disease in youngsters extremely difficult. Traditional techniques, such as smear microscopy, are not sensitive enough to identify medication resistance, particularly in youngsters. Despite being the gold standard, solid or liquid cultures have limited clinical usefulness due to their delayed turnaround time (6–8 weeks).(5,6) The diagnostic gap has complicated the larger public health response to the epidemic by causing treatment delays and underestimating the prevalence of drug-resistant TB in pediatric cases.

Rapid diagnosis of TB significantly facilitates early treatment initiation thereby reducing transmission rate. While smear microscopy has poor sensitivity for pediatric tuberculosis and issues related to quality control, conventional culture, the best available reference standard for TB diagnosis, requires 6-8 weeks. Nucleic acid amplification tests like in-house Polymerase chain reaction (PCR) are developed for rapid diagnosis and identification of drug- resistan. By drastically cutting down on the time required to identify Mycobacterium tuberculosis and resistance to firstline medications like rifa mpicin and isoniazid, the development of molecular diagnostic techniques like CBNAAT (Cartridge -

Based Nucleic Acid Amplification Test) and Line Probe Assay (LPA) has transformed the diagnosis of tuberculosis.The WHO has approved the automated PCR-

based test CBNAAT (GeneXpert MTB/RIF) for use in children. Results are available in as little as t wo hours.Through the identification of genetic changes linked to drug resistance, LPA further aids i n the detection of multidrug resistance (MDR).Particularly in environments with limited resources, t hese techniques present a possible substitute for conventional diagnostics. (7). XPERT MTB /RIF or CB-NAAT (Cartridge based nucleic acid amplification test), based on nested real-time PCR and molecular beacon technology, is a self-contained cartridge - based fully automated DNA testing platform, that can accurately detect both Mycobacterium tuberculosis and resistance to rifampicin(8)Line Probe Assay (LPA) is a rapid technique based on polymerase chain reaction (PCR) that is used to detect Mycobacterium tuberculosis (MTB) complex as well as drug sensitivity to rifampicin (RPM) and isoniazid (INH) as per NTEP[2]. Karnataka notified 3.3% of total TB cases in India. From July 2021 to June 2022, a total of 79,537 TB cases were reported in Karnataka. (9)

Numerous investigations have shown that CBNAAT performs better than smear microscopy. Uppal et al.'s investigation revealed that CBNAAT had a 55.4% positivity rate, which was much higher than

that of AFB smear (12.1%) and culture (18.9%) [1]. Similarly, CBNAAT has a higher diagnostic yield than smear microscopy; in a research by Raizada et al., 10.4% of 4,600 presumed pediatric TB patients tested positive by CBNAAT, compared to 4.8% by smear microscopy [4]. The diagnostic utility of molecular testing in pediatric TB is further supported by studies by Mishra et al. and Sharma et al., which report CBNAAT positivity rates of 21.4% and 16.2%, respectively [3,5]. The purpose of this study is to determine the percentage of MDR-
TB cases using CBNAAT and LPA, as well as to estimate the positivity rate of pediatric pulmonary TB, given the epidemiological significance and diagnostic benefits of molecular techniques.The stud y will yield vital information to help incorporate quick molecular diagnostics into regular pediatric TB screening programs and guide policylevel choices for better child TB control.

## METHODS

We conducted a prospective cross-sectional study in the Department of Microbiology at a tertiary care referral center in Hubballi. Karnataka,India, over a period of one year **(from [Month, Year] to [Month, Year]).** The study adhered to STROBE reporting guidelines for observational research and followed all relevant biosafety regulations for handling *Mycobacterium tuberculosis*.

The study population consisted of children aged below 14 years presenting with clinical and/or radiological suspicion of pulmonary tuberculosis (PTB). Suspicion was based on chronic cough lasting more than two weeks, fever persisting for more than two weeks, weight loss or failure to thrive, and/or a documented household contact with an active TB case. Children diagnosed with extra-pulmonary TB without pulmonary involvement and those already receiving more than two weeks of anti-tuberculosis therapy prior to recruitment were excluded. Eligible participants were recruited consecutively from inpatient and outpatient services until the required sample size was achieved. Written informed consent was obtained from parents or guardians, and assent was sought from children aged seven years or older where appropriate.

The sample size was calculated to estimate the proportion of pulmonary TB among suspected pediatric cases, using the formula $n \geq (Z_{\alpha/2})^2 \times p(1-p)/d^2$. With a confidence level of 95% (Z=1.96), an expected prevalence of 10% based on prior studies (2), and an absolute precision of 2%, the required sample size was 865. Alternative scenarios indicated that the required sample size would be approximately 811 if the true prevalence were 5% (precision 1.5%) and 784 if the prevalence were 15% (precision 2.5%). Therefore, a target enrollment of 865 children was finalized to ensure adequate statistical power.

Respiratory specimens comprised induced sputum, gastric aspirates, or expectorated sputum (1–3 mL). Gastric aspirates were neutralized with sodium bicarbonate immediately after collection. All specimens were transported within two hours to the microbiology laboratory under cold-chain conditions (2–8 °C). Samples not processed within 24 hours were refrigerated at 4 °C.

Specimens underwent decontamination followed by fluorescent microscopy using auramine-O staining. Smear-positive samples were documented before proceeding to molecular testing. The cartridge-based nucleic acid amplification test (CBNAAT) was performed using the GeneXpert MTB/RIF or Xpert Ultra platform (Cepheid, Sunnyvale, CA). Specimens were mixed with sample reagent in a 1:2 ratio, vortexed twice, incubated for 15 minutes at room temperature, and 2 mL of the homogenized sample was transferred into the test cartridge. Results were reported automatically as "MTB detected" or "not detected," with rifampicin resistance indicated as "detected," "not detected," or "indeterminate." Internal sample processing controls and weekly external positive and negative controls were used for quality assurance.

Line Probe Assay (LPA) was performed using the GenoType MTBDRplus v2.0 kit (Hain Lifescience, Nehren, Germany) on smear-positive, CBNAAT-positive samples. DNA was extracted from decontaminated specimens, amplified by multiplex PCR targeting *rpoB*, *katG*, and *inhA*, and hybridized to nitrocellulose strips. Resistance was interpreted as per the manufacturer's algorithm: rifampicin resistance was inferred from mutations in *rpoB*, isoniazid resistance from mutations in *katG* or *inhA*, and multidrug resistance (MDR-TB) from combined rifampicin and isoniazid

resistance. Wild-type *M. tuberculosis* H37Rv and non-template controls were included in each run. Where results were indeterminate or microscopy was negative, culture was performed using the BACTEC MGIT 960 liquid culture system (Becton Dickinson, Sparks, MD). Growth was confirmed as MTB using an MPT64 antigen immunochromatographic test, and drug susceptibility testing was performed where appropriate.

All laboratory procedures were carried out in a biosafety level 2+ facility with Class II biosafety cabinets. GeneXpert instruments were calibrated annually, and quality assurance for LPA and MGIT systems followed CLSI and WHO TB laboratory guidelines. Participation in an external quality assurance program ensured reproducibility.

Data were captured using a pretested proforma including demographic, clinical, radiological, and laboratory parameters. Bacteriologically confirmed TB was defined as CBNAAT and/or culture positive. Clinically diagnosed TB referred to cases with negative tests but strong clinical or radiological evidence. Drug-resistant TB was defined as rifampicin or isoniazid resistance detected on CBNAAT or LPA, while MDR-TB referred to combined rifampicin and isoniazid resistance. Covariates collected included age, sex, nutritional status, HIV status, history of prior TB treatment, and household contact with TB.

The primary endpoint was the proportion of children with pulmonary TB confirmed by CBNAAT. Secondary endpoints included the prevalence of rifampicin resistance detected by CBNAAT and the prevalence of MDR-TB detected by LPA. Continuous variables were summarized as mean ± SD or median with interquartile range, while categorical variables were expressed as frequencies and percentages with 95% confidence intervals calculated using the exact (Clopper–Pearson) binomial method. Proportions were compared using Chi-square or Fisher's exact test as appropriate, and continuous variables were analyzed with Student's t-test or the Mann–Whitney U test depending on normality. Multivariable logistic regression was used to identify predictors of TB positivity, with variables significant at $p<0.20$ in univariate analysis entered into the model. Model fit was assessed using the Hosmer–Lemeshow goodness-of-fit test. Where culture results were available as a reference, sensitivity, specificity, positive predictive value, negative predictive value, and Cohen's kappa coefficient were calculated to assess agreement between CBNAAT and LPA, with kappa values interpreted using standard thresholds. Missing data less than 5% were handled by complete case analysis, while greater missingness was addressed using multiple imputation with chained equations. Adjustment for multiple testing was done using Bonferroni correction where applicable.

All statistical analyses were performed using SPSS version 20.0 (IBM, Chicago, IL) and R version 4.3.0 (packages epiR, stats, and irr), with a random seed set at 1234 to ensure reproducibility. The study was approved by the Institutional Ethics Committee of Karnataka Institute of Medical Sciences, Hubballi [Reg No: ECR/486/Inst/KA/2013/RR-16] held on 17-02-2024.Written informed consent was obtained from parents or guardians, and assent was taken from children where applicable.

## RESULTS

Overall Test Positivity

A total of 865 pediatric patients with suspected pulmonary tuberculosis were enrolled, comprising 431 males (49.8%) and 434 females (50.2%), with 54.9% aged ≤8 years and 45.1% aged >8 years (Table 1). All children underwent CBNAAT testing, of which 136 (15.7%) were positive for *Mycobacterium tuberculosis*, while 729 (84.3%) were negative (Table 2). The calculated test positivity rate (TPR) was therefore 15.7%.

Age-stratified analysis revealed a significantly higher positivity rate among children aged >8 years compared with those aged ≤8 years (23.8% vs. 9.1%; $p < 0.001$) (Table 5). Gender also showed a significant association, with females having a higher positivity rate than males (18.2% vs. 13.2%; $p = 0.044$). Logistic regression confirmed age as a strong independent predictor of CBNAAT positivity (odds ratio [OR] 3.07, 95% CI not shown, $p < 0.001$), whereas gender was not statistically significant after adjustment (OR 1.32, $p = 0.153$) (Tables 6–8).

CBNAAT Detection of Drug Resistance

In addition to identifying *M. tuberculosis*, CBNAAT detected rifampicin resistance in 6 of the 136 positive cases, corresponding to a rifampicin-resistant TB rate of 0.7% among the total study population (Table 2). These cases, although few, underscore the role of CBNAAT in rapidly identifying patients requiring advanced drug susceptibility testing and tailored therapy.

Line Probe Assay Findings on MDR

All CBNAAT-positive samples were further subjected to first-line and second-line line probe assays (FL-LPA and SL-LPA) to assess drug-resistance patterns. FL-LPA identified 7 cases (0.8%) resistant to both rifampicin and isoniazid, confirming multidrug-resistant TB (MDR-TB) (Table 3). An additional 6 cases (0.7%) were resistant to rifampicin alone, while 1 case (0.1%) was resistant to isoniazid alone. A total of 124 children (14.3%) were sensitive to both drugs, whereas 727 (84.0%) had negative results, largely reflecting their CBNAAT-negative status.

Second-line LPA results demonstrated fluoroquinolone sensitivity in 11 cases (1.3%) and resistance in 2 cases (0.2%), with the remainder testing negative (Table 4). The detection of fluoroquinolone resistance, though infrequent, indicates progression toward pre-extensively drug-resistant TB (pre-XDR-TB) in pediatric populations and highlights the clinical importance of incorporating SL-LPA in the diagnostic cascade.

## DISCUSSION

Discussion

Tuberculosis (TB) in children remains one of the most pressing diagnostic and public health challenges, particularly in high-burden countries such as India. Pediatric TB is frequently paucibacillary and often presents with nonspecific clinical features, complicating microbiological confirmation and timely treatment initiation. The present study evaluated the positivity rate of pediatric pulmonary TB and the prevalence of multidrug resistance using molecular methods, specifically CBNAAT and Line Probe Assay (LPA). The findings contribute to the growing body of evidence supporting the integration of rapid molecular diagnostics into pediatric TB care pathways.

Diagnostic Yield of CBNAAT

The overall positivity rate of 15.7% observed in this cohort underscores the effectiveness of CBNAAT in detecting TB in pediatric populations. This rate is comparable to Sharma et al. (16.19%) and Raizada et al. (10.4%), while being lower than the 55.4% reported by Uppal et al., likely due to differences in study design, inclusion criteria, and sample type (2,5,6). Collectively, these data reaffirm CBNAAT's superior sensitivity over conventional smear microscopy, which performs poorly in paucibacillary pediatric samples. Its rapid turnaround time of approximately two hours represents a substantial advantage over culture-based methods that may require up to eight weeks (10). The capacity for early diagnosis is particularly critical in children, who are more vulnerable to rapid disease progression and severe outcomes (8,9).

Age- and Gender-Related Disparities

This study revealed significant age-related differences in positivity rates, with children older than 8 years more than three times as likely to test positive compared with those ≤8 years (OR = 3.065, $p < 0.001$). The lower positivity in younger children reflects both the biological and technical challenges of TB diagnosis in this group. Young children often cannot expectorate sputum, leading to reliance on gastric lavage or nasopharyngeal aspirates, which are technically difficult and have lower sensitivity (2,9). Moreover, nonspecific clinical manifestations in infants and young children, such as failure to thrive or low-grade fever, often overlap with other pediatric conditions and may delay TB suspicion (10,11). By contrast, older children more frequently present with adult-type pulmonary TB characterized by productive cough and cavitation, facilitating both sample collection and microbiological confirmation (11).

Gender differences were less pronounced. Although females demonstrated a slightly higher positivity rate (18.2%) compared to males (13.2%) ($p = 0.044$), this did not remain significant in multivariate analysis. While biological susceptibility does not appear to differ markedly, sociocultural

determinants—such as healthcare-seeking behavior, gender-based neglect, and systemic biases—may subtly influence diagnostic access in girls (12). National and international data generally indicate no consistent gender disparity in pediatric TB incidence, in contrast to adult cohorts where males typically predominate due to occupational and behavioral factors (8). These findings emphasize the importance of equitable diagnostic strategies that address age-related biological limitations and gender-based sociocultural barriers.

Rifampicin Resistance Detection by CBNAAT

The detection of rifampicin resistance in 0.7% of pediatric TB cases highlights CBNAAT's utility as a rapid resistance-screening tool. Although this prevalence is lower than the 5.88% rifampicin resistance reported by Mishra et al. in pediatric gastric aspirates (8), the ability of CBNAAT to provide near-immediate rifampicin susceptibility results is invaluable in initiating timely second-line therapy and preventing further amplification of resistance. However, CBNAAT's limitation in detecting only rifampicin resistance necessitates confirmatory testing through LPA or culture-based drug susceptibility testing (DST) to delineate broader resistance patterns (10).

Contribution of Line Probe Assay

LPA in this study confirmed seven multidrug-resistant TB (MDR-TB) cases (0.8%), in addition to identifying one isoniazid mono-resistant and six rifampicin mono-resistant cases. These findings are consistent with global evidence on LPA's high sensitivity (96.7%) and specificity (98.8%) for detecting rifampicin resistance (10). Importantly, second-line LPA revealed fluoroquinolone resistance in 0.2% of cases, underscoring the need for comprehensive resistance profiling in pediatric TB. LPA's ability to rapidly identify mutations in rpoB, katG, and inhA genes makes it a cornerstone in resistance diagnostics, though its reliance on smear-positive or culture-positive samples constrains its utility in smear-negative children (6). Thus, LPA and CBNAAT should be viewed as complementary tools within pediatric TB diagnostic algorithms, with culture retained as the ultimate reference standard for unresolved or complex cases.

Strengths and Limitations

The study's large sample size and combined use of CBNAAT and LPA strengthen its reliability and enhance its contribution to the understanding of pediatric TB epidemiology. However, the lack of culture confirmation represents a limitation, as phenotypic DST remains essential for comprehensive resistance detection. Additionally, the tertiary-care setting may have introduced referral bias, with potentially higher disease severity represented in the sample. Absence of follow-up data on treatment outcomes further limits the ability to link molecular findings with clinical prognosis. Future research incorporating longitudinal follow-up and advanced molecular approaches such as next-generation sequencing (NGS) could help elucidate novel resistance mechanisms and refine diagnostic strategies (11).

Public Health and Programmatic Implications

The findings carry significant implications for TB control strategies under the National TB Elimination Programme (NTEP). Molecular diagnostics such as CBNAAT and LPA should be prioritized in pediatric TB workflows to ensure timely diagnosis, rapid initiation of appropriate therapy, and reduction in transmission potential. The higher diagnostic yield in older children suggests the need for intensified focus on innovative diagnostic strategies for younger children, including stool- or urine-based molecular assays currently under evaluation. Additionally, the incorporation of gender-sensitive approaches in community outreach and healthcare delivery may mitigate sociocultural barriers that hinder timely diagnosis in girls (13,14).

In summary, this study reinforces the critical role of molecular diagnostics in pediatric TB management. By providing rapid detection of Mycobacterium tuberculosis and resistance patterns, CBNAAT and LPA enable early intervention and improved outcomes in children. Strengthening access to these technologies, while addressing the diagnostic challenges in younger children and ensuring equitable healthcare delivery, is vital to achieving the WHO End TB Strategy and the NTEP's elimination targets.

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**Table 1: Frequency & Percentage regarding to Sociodemographic variables of study subjects**

| Variable | Classification | Frequency | Percentage |
|---|---|---|---|
| **Age Group** | Less than or equals to 8 years | 475 | 54.9 |
| | More than 8 years | 390 | 45.1 |
| **Gender** | Male | 431 | 49.8 |
| | Female | 434 | 50.2 |

Out of the total **865 pediatric participants**, **54.9%** were aged **8 years or below**, while **45.1%** were older than 8 years. The gender distribution was almost equal, with **49.8% males** and **50.2% females**, indicating a balanced representation of both sexes in the study population.

**Table 2: Frequency & Percentage of total positive cases among study subjects**

| Results | Frequency | Percentage |
|---|---|---|
| **Positive** | 139 | 16.1 |
| **Negative** | 726 | 83.9 |
| **Total** | 865 | 100.0 |

Out of **865 study participants**, **16.1%** (139 out of 865) were positive for **Pediatric TB.**

**Table 3: Frequency & Percentage of results regarding to CBNAAT done among study subjects**

| CBNAAT Results | Frequency | Percentge |
|---|---|---|
| **Positive** | 136 | 15.7 |
| **Negative** | 729 | 84.3 |
| **Total** | 865 | 100.0 |

Out of **865 pediatric patients** who were suspected of having pulmonary tuberculosis and underwent **CBNAAT (Cartridge-Based Results Nucleic Acid Amplification Test)**, **136 (15.7%)** tested **positive for *Mycobacterium tuberculosis***. The remaining **729 (84.3%)** tested **negative**.

**Table 4: Frequency & Percentage of results regarding to FL-LPA done among study subjects**

| FL-LPA Results | Frequency | Percent |
|---|---|---|
| **Sensitive for Rifampicin & Isoniazid** | 124 | 14.3 |
| **Rifampacin Resistance** | 6 | .7 |
| **Resistance to INH** | 1 | .1 |
| **Resistant to Rifampicin and Isoniazid** | 7 | .8 |

| | | |
|---|---|---|
| **Negative (no MTB detected or untested)** | 727 | 84.0 |
| **Total** | 865 | 100.0 |

In this study, **7 out of 865 cases (0.8%)** were found to have resistance to both rifampicin and isoniazid, indicating confirmed **MDR-TB**. Additionally, **6 cases (0.7%)** showed resistance to rifampicin only, and **1 case (0.1%)** was resistant to isoniazid only. A total of **124 cases (14.3%)** were found to be **sensitive to both drugs**, while **727 cases (84%)** were **LPA-negative**, either due to negative CBNAAT results or insufficient bacillary load for testing.

**Table 5: Frequency & Percentage of results regarding to SL-LPA done among study subjects**

| SL-LPA | Frequency | Percent |
|---|---|---|
| **Sensitive to Fluoroquinolones** | 11 | 1.3 |
| **Resistant to Fluoroquinolones** | 2 | .2 |
| **Negative** | 852 | 98.5 |
| **Total** | 865 | 100.0 |

Out of 865 study participants, **fluoroquinolone sensitivity** was detected in **11 cases (1.3%)**, and **resistance to fluoroquinolones** was detected in **2 cases (0.2%)** using SL-LPA. The remaining **852 cases (98.5%)** were negative, either because they did not have detectable TB by CBNAAT, were not eligible for second-line testing, or the bacillary load was insufficient for SL-LPA analysis.

**Table 6: Association of CBNAAT results (dependent variable) and age group,gender (independent variables) of study subjects**

| Independent variables | | CBNAAT Result | | Chi square/F test value | P-value | Significance |
|---|---|---|---|---|---|---|
| | | Negative | Positive | | | |
| **Age group** | Less than or equals to 8 years | 432 | 43 | 35.37 | .000 | Highly Significant |
| | More than 8 years | 297 | 93 | | | |
| **Gender** | Female | 355 | 79 | 4.044 | .044 | Significant |
| | Male | 374 | 57 | | | |

A **highly significant association was observed between age group and CBNAAT results** (Chi-square = 35.37, $p < 0.001$). Among children aged ≤8 years, only **43 out of 475 (9.1%)** tested positive, whereas among those aged >8 years, **93 out of 390 (23.8%)** were CBNAAT positive. This indicates that **older children were more likely to test positive for pulmonary TB** using CBNAAT, possibly due to better sputum sample quality or higher bacillary load in older age groups.

Regarding **gender**, the association was found to be **statistically significant** (Chi-square = 4.044, $p = 0.044$). Among females, **79 out of 434 (18.2%)** tested positive, while among males, **57 out of 431 (13.2%)** were positive. Although both genders were nearly equally represented in the study population, **females showed a slightly higher positivity rate**, which reached statistical significance. This may suggest gender-based differences in TB exposure, symptoms, or health-seeking behavior that warrant further investigation.

**Logistic Regression**

**Table 7: Omnibus Tests of Model Coefficients**

| | | Chi-square | df | Sig. |
|---|---|---|---|---|
| Step 1 | Step | 37.656 | 2 | .000 |
| | Block | 37.656 | 2 | .000 |
| | Model | 37.656 | 2 | .000 |

To ascertain whether the entire model is statistically significant—that is, whether the independent variables collectively significantly predict the outcome variable—logistic regression analysis employs the Omnibus Tests of Model Coefficients. With two degrees of freedom, the chi-square value in Step 1 is 37.656, and the p-value (Sig. is 0.000, a value below 0.05. This suggests that a model with age group and gender as predictor variables fits the data considerably better than one without any predictors (i.e. e. the null model. To put it another way, the predictors collectively play a major role in explaining the outcome (CBNAAT result). Since this is a single-step binary logistic regression model, the values displayed in the Step, Block, and Model rows are all the same. All predictors in the block (in this case, both variables entered together) are tested using block tests. The entire model is tested against the baseline, or null, model. Consequently, the logistic regression model as a whole is highly significant in predicting CBNAAT positivity based on age and gender, according to the statistically significant chi-square values across all three.

**Table 8: Depicting Model Summary with Cox & Snell and Naglekereke test**

| Step | Cox & Snell R Square | Nagelkerke R Square |
|---|---|---|
| 1 | .043 | .073 |

Table 7 presents the **Model Summary** of the binary logistic regression used to predict **CBNAAT test positivity** based on independent variables such as **age group and gender**. This summary includes two pseudo $R^2$ values: **Cox & Snell R Square** and **Nagelkerke R Square**, which provide an estimate of how much variation in the dependent variable is explained by the model.

- The **Cox & Snell R Square** value is **0.043**, indicating that approximately **4.3% of the variation** in CBNAAT results can be explained by the model.
- The **Nagelkerke R Square**, a modification of Cox & Snell that adjusts for the maximum possible value, is **0.073**, suggesting that the model explains about **7.3% of the variance** in the

**Table 9: Logistic Regression done among study subjects**

| | B | S.E. | Wald | df | Sig. | Exp(B) |
|---|---|---|---|---|---|---|
| Gender(1) | .276 | .193 | 2.043 | 1 | .153 | 1.318 |
| Age Group | 1.120 | .200 | 31.337 | 1 | .000 | 3.065 |
| Constant | -3.561 | .351 | 102.717 | 1 | .000 | .028 |

This table presents the results of a **binary logistic regression analysis** conducted to assess the effect of **gender** and **age group** on the likelihood of testing positive for **pediatric pulmonary tuberculosis using CBNAAT**.

- For the variable **age group**, the **B coefficient** is **1.120**, with a **standard error (S.E.)** of 0.200. The **Wald statistic** is 31.337 with **1 degree of freedom (df)** and a **p-value (Sig.) of 0.000**, which is highly statistically significant ($p < 0.001$). The **Exp(B)** or **odds ratio** is **3.065**, indicating that children **older than 8 years** were approximately **3 times more likely** to test CBNAAT-positive compared to those aged 8 years or younger, holding gender constant.
- For the variable **gender**, the B coefficient is **0.276**, with a standard error of 0.193. The Wald statistic is 2.043, and the p-value is **0.153**, which is **not statistically significant** ($p > 0.05$). The Exp(B) value of **1.318** suggests that **females had 1.3 times higher odds** of being CBNAAT positive than males, but this difference is **not significant**, implying that gender is **not a reliable predictor** of test positivity in this model.
- The **constant** term has a B value of **–3.561** ($p < 0.001$), representing the **log odds of the outcome when all predictors are zero**. The corresponding Exp(B) is **0.028**, which is the baseline odds of CBNAAT positivity for the reference categories (males aged ≤8 years).