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# Comparative Evaluation of the Xpert MTB/RIF Assay and Ziehl–Neelsen Smear Microscopy for Pulmonary Tuberculosis Detection at Saint Monica's Hospital in Nigeria

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## ABSTRACT

**Introduction:** Tuberculosis (TB) remains a major public health problem, particularly in high-burden countries such as Nigeria, which is among the countries with a high burden of multidrug-resistant tuberculosis (MDR-TB). This study investigated the diagnostic performance of Ziehl–Neelsen smear microscopy (ZNSM) compared with the Xpert MTB/RIF assay as the reference molecular test for the detection of pulmonary tuberculosis (PTB) among patients attending Saint Monica's Hospital, Adikpo, Benue State, Nigeria. **Methods:** This hospital-based cross-sectional study compared ZNSM with the Xpert MTB/RIF assay for the detection of PTB at Saint Monica's Hospital from February to August 2024. A total of 163 adult patients with presumptive TB were recruited based on the calculated sample size. Sputum samples were analysed using both ZNSM and the Xpert MTB/RIF assay; the latter served as the reference molecular test. Rifampicin-resistant samples identified by the Xpert MTB/RIF assay underwent additional resistance profiling. Data were analysed using descriptive statistics, McNemar's test for paired proportions, and the chi-square test or Fisher's exact test for associations, with significance set at $P < 0.05$. **Results:** *Mycobacterium tuberculosis* was detected in 17 patients (10.43%) using the Xpert MTB/RIF assay, compared with 9 patients (5.52%) detected by ZNSM, and the difference was statistically significant (McNemar's test, $P = 0.008$). The Xpert MTB/RIF assay detected a higher proportion of TB cases across most sociodemographic subgroups, including those stratified by sex, age, number of spouses, number of children, marital status, educational status, occupation, and human immunodeficiency virus (HIV) status. The study also identified four rifampicin-resistant samples: three with isolated rifampicin resistance (SM4, SM89, and SM33; rifampicin-resistant TB [RR-TB]) and one with additional isoniazid resistance (SM51; MDR-TB) according to World Health Organization (WHO) definitions. ZNSM and the Xpert MTB/RIF assay showed substantial agreement ($\kappa = 0.67$; 95.1% overall agreement) for the detection of *M. tuberculosis*. **Conclusion:** The findings underscore the importance of rapid molecular diagnostics in improving TB detection and supporting timely clinical decision-making, particularly for the early identification of drug-resistant TB. These findings support strengthening TB diagnostic systems and expanding access to molecular assays for broader resistance profiling, highlighting the need for improved strategies to strengthen TB control in Nigeria.

## INTRODUCTION

Tuberculosis (TB) is an infectious disease that remains a major global public health threat. In 2023, TB caused an estimated 1.25 million deaths worldwide, including 161,000 deaths among people living with human immunodeficiency virus (HIV), and the World Health Organization (WHO) reported an estimated 10.8 million new TB cases globally, comprising 1.2 million children, 3.6 million women, and 6.0 million men [1]. The latest WHO Global Tuberculosis Report (2025, presenting 2024 estimates) indicates approximately 10.7 million incident TB cases and 1.23 million TB deaths worldwide, confirming that the burden remains above 10 million cases and 1 million deaths annually [2].

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Tuberculosis continues to pose a significant health challenge in Nigeria, which is one of the 30 high TB burden countries [3]. According to the WHO Global Tuberculosis Report 2024 (2023 data), Nigeria had an estimated TB incidence of 219 per 100,000 population (approximately 467,000 new cases) and an estimated 154,000 TB-related deaths [1]. The 2025 WHO reporting cycle similarly continues to rank Nigeria among the 30 high TB burden countries, with a high estimated incidence and mortality burden [2, 3].

Drug-resistant tuberculosis (DR-TB), including multidrug-resistant TB (MDR-TB), presents an ongoing public health crisis and a threat to global health security; an estimated 400,000 new cases of multidrug-resistant/rifampicin-resistant TB (MDR/RR-TB) occurred globally in 2023, with an estimated 150,000 deaths attributed to these resistant strains during the same period [1]. Nigeria also carries a high TB/HIV co-infection burden, contributing substantially to the global TB burden [1, 3]. Reports from other African countries, including Tanzania, Zambia, and Kenya, have similarly indicated that a notable percentage of *Mycobacterium tuberculosis* strains are resistant to at least one first-line anti-TB drug [4–6]. However, the true prevalence of MDR-TB may be underestimated owing to limited access to diagnostic tools [1].

Timely diagnosis and effective treatment of TB, particularly DR-TB, are essential in reducing transmission and improving patient outcomes [7, 8]. To curb the burden of DR-TB, WHO advocates for global access to drug susceptibility testing, enabling clinicians to administer appropriate therapies without delay [1]. Nigeria has witnessed notable improvements in TB control strategies, focusing on modernizing diagnostic and treatment approaches [9].

While smear microscopy fails to detect drug resistance, the Xpert MTB/RIF assay can reliably identify rifampicin resistance, which is a surrogate marker for MDR-TB [10]. The Xpert MTB/RIF assay provides superior sensitivity compared with smear microscopy and is recommended by WHO as the initial diagnostic test for patients with presumptive TB [11]. However, there are limited published data on the routine performance of the Xpert MTB/RIF assay in Benue State. Saint Monica's Hospital, Adikpo, Kwande Local Government Area, Benue State, Nigeria, recently adopted the assay, and few local data exist on its comparative diagnostic yield relative to Ziehl–Neelsen smear microscopy (ZNSM). Similar comparisons have been reported in other settings [12, 13], but to the best of our knowledge, no previous study has directly compared the Xpert MTB/RIF assay with ZNSM in this rural hospital setting in Nigeria. This study therefore assesses the diagnostic performance of ZNSM compared with the Xpert MTB/RIF assay for the detection of pulmonary tuberculosis (PTB) among patients at Saint Monica's Hospital in this setting to generate context-specific evidence for TB diagnosis in rural Benue State.

## MATERIAL AND METHODS

**Study design and setting.** This hospital-based cross-sectional study was conducted from February to August 2024 at Saint Monica's Hospital, Adikpo, Kwande Local Government Area, Benue State, North-Central Nigeria. Sputum samples were processed and analysed in the hospital's Microbiology Laboratory.

**Study area.** Adikpo and neighbouring communities are predominantly rural, with agriculture as the main occupation [14]. Adikpo is situated between approximately 6°30′N and 7°00′N latitude and 9°00′E and 9°30′E longitude [15]. The area has a tropical climate with two distinct seasons: a wet season from April to October and a dry season from November to March. Mean annual temperatures range from 20°C to 35°C, and annual rainfall is approximately 1,200–2,000 mm [15]. Benue State has an estimated population of more than 5 million people [16], and Saint Monica's Hospital provides secondary healthcare, including TB screening, diagnosis, and treatment, to residents of Adikpo and surrounding communities. This setting was therefore appropriate for evaluating TB diagnostic methods under routine rural healthcare conditions.

**Study population.** The study population comprised adults aged ≥18 years with presumptive TB who attended the TB clinic of Saint Monica's Hospital, Adikpo.

**Inclusion criteria.** Participants were eligible if they presented with one or more symptoms suggestive of TB, including cough lasting ≥2 weeks, weight loss, persistent low-grade fever, night sweats, loss of appetite, fatigue, chest pain, shortness of breath, or haemoptysis.

**Exclusion criteria.** Patients with confirmed TB who had received anti-TB treatment within the previous six months and those who were critically ill were excluded.

**Sample size determination.** The minimum sample size was calculated using Araoye's formula [17]:

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where n is the minimum required sample size, Z is the standard normal deviate at a 95% confidence level, p is the estimated prevalence from a previous study, q is 1 − p, and d is the margin of error. Using Z = 1.96, p = 0.121, q = 0.879, and d = 0.05, the sample size was calculated as follows:

$n = (1.96^2 \times 0.121 \times 0.879) / 0.05^2 = 163.4 \approx 163$

**Cohen's kappa.** Agreement between ZNSM and the Xpert MTB/RIF assay results was assessed using Cohen's kappa (κ) statistic [18]. The analysis was based on the 2 × 2 cross-tabulation of both diagnostic methods. κ was calculated as follows:

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$\kappa = (P_o - P_e) / (1 - P_e)$

Where $P_o$ is the observed proportion of agreement and $P_e$ is the expected proportion of agreement due to chance. Values of κ were interpreted according to standard criteria as follows: $\kappa < 0$ = poor agreement; 0.00–0.20 = slight agreement; 0.21–0.40 = fair agreement; 0.41–0.60 = moderate agreement; 0.61–0.80 = substantial agreement; and 0.81–1.00 = almost perfect agreement [19].

**Data collection.** Data were collected from patients with presumptive PTB who presented to the TB clinic at Saint Monica's Hospital and met the inclusion criteria. An interviewer-administered structured questionnaire was used to obtain participants' sociodemographic data. Participants' HIV status was retrieved from their medical records.

**Sample collection.** Sputum samples were collected from 163 eligible participants in labelled sterile, leak-proof sputum containers. Each sputum sample was divided into two portions: one portion was used for the Xpert MTB/RIF assay, and the other was used for ZNSM. Samples were transported promptly to the hospital's Microbiology Laboratory for analysis.

**Laboratory methods.** Sputum samples were analysed using the Xpert MTB/RIF assay and ZNSM. For ZNSM, direct sputum smears were prepared on glass slides, air-dried, heat-fixed, and stained according to the method described by Hussey and Zayaitz [20]. For the Xpert MTB/RIF assay, sputum samples were mixed with Xpert sample reagent (SR) at a ratio of 2:1 (sample reagent to sputum), homogenised thoroughly, and incubated at room temperature for 15 minutes. Subsequently, 2 mL of the processed sample was transferred into the Xpert MTB/RIF cartridge and analysed using the GeneXpert platform according to the manufacturer's instructions [21]. Results were generated in approximately 90 minutes.

**Drug susceptibility testing.** Participants whose sputum samples were positive for rifampicin-resistant *M. tuberculosis* underwent further drug susceptibility testing. The Xpert MTB/RIF assay was retained as the rifampicin-resistance screening test, and the Xpert MTB/XDR assay was used to detect additional resistance to isoniazid (INH), fluoroquinolones (FQ), amikacin (AMK), kanamycin (KAN), and capreomycin (CAP), whereas ethambutol (EMB) resistance was determined using a GenoType MTBDRsl line probe assay (LPA; Hain Lifescience, Nehren, Germany) at the North Central TB Reference Laboratory, Jos. The Xpert MTB/RIF assay targets the *rpoB* gene to detect rifampicin resistance, whereas the Xpert MTB/XDR assay targets resistance-conferring mutations primarily in the *katG* gene and the *inhA* promoter region (INH), the *gyrA* and *gyrB* genes (FQ), and the *rrs* gene (AMK, KAN, and CAP).

**Statistical analysis.** Data were analysed using descriptive statistics (frequencies and percentages). McNemar's test was used to compare paired proportions of TB positivity between ZNSM and the Xpert MTB/RIF assay. Associations between sociodemographic variables and TB positivity by each diagnostic method were assessed using the chi-square test or Fisher's exact test, as appropriate for expected cell counts. Statistical analyses were performed using IBM SPSS Statistics version 26.0, and $P < 0.05$ was considered statistically significant.

## RESULTS

**Sociodemographic distribution of *M. tuberculosis* positivity.** Overall, the Xpert MTB/RIF assay detected a higher proportion of TB cases than ZNSM, with positivity rates of 10.43% and 5.52%, respectively (Table 1; Figure 1). Among the participants, 64.42% were HIV-negative and 35.58% were HIV-positive. TB positivity was higher among HIV-positive participants than among HIV-negative participants by both methods (ZNSM: 13.79% *vs.* 0.95%; Xpert MTB/RIF: 22.41% *vs.* 3.81%).

By the number of spouses, TB positivity was highest among participants with no more than one spouse (ZNSM: 7.82%; Xpert MTB/RIF: 13.04%) and lowest among those with four spouses, in whom no TB cases were detected. By the number of children, participants with 0–5 children had positivity rates of 9.00% by ZNSM and 10.42% by Xpert MTB/RIF, whereas those with 11–15 children had rates of 0.00% and 25.00%, respectively.

Male participants had higher TB positivity than female participants (ZNSM: 6.67% *vs.* 3.45%; Xpert MTB/RIF: 9.52% *vs.* 7.95%). Participants aged ≥ 60 years had the lowest positivity rates (ZNSM: 4.55%; Xpert MTB/RIF: 7.95%), whereas those aged 20–39 years had the highest rates (11.11% by both methods). Separated participants had the highest positivity rates (ZNSM: 50.00%; Xpert MTB/RIF: 100.00%), followed by married participants (ZNSM: 7.50%; Xpert MTB/RIF: 15.00%) and single participants (ZNSM: 1.27%; Xpert MTB/RIF: 1.27%).

TB positivity generally decreased with increasing educational level: no formal education (ZNSM: 8.33%; Xpert MTB/RIF: 14.58%), primary education (ZNSM: 6.00%; Xpert MTB/RIF: 10.00%), secondary education (ZNSM: 4.65%; Xpert MTB/RIF: 6.98%), and tertiary education (ZNSM: 0.00%; Xpert MTB/RIF: 9.09%). Across occupational groups, positivity was 8.33% by ZNSM and 9.72% by Xpert MTB/RIF among farmers, 0.00% and 7.50% among students, 3.57% and 3.57% among working-class participants, and 8.70% and 26.09% among non-working participants.

Using the Xpert MTB/RIF assay, TB positivity was significantly associated with sex ($P$=0.031), age group ($P$=0.020), number of spouses ($P$=0.046), and

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number of children ($P$=0.041). Significant associations were also observed with marital status ($P$=0.001), HIV status ($P$=0.002), educational status ($P$<0.001), and occupation ($P$<0.001), based on the chi-square test or Fisher's exact test, as appropriate (Figure 1). Overall, the Xpert MTB/RIF assay detected a higher proportion of TB-positive cases than ZN smear microscopy (ZNSM) across most sociodemographic subgroups. Notably, TB positivity was detected by Xpert MTB/RIF in certain subgroups, including students and participants with tertiary education, in which no ZNSM-positive cases were observed. However, because some subgroups contained relatively few participants, these findings should be interpreted with caution.

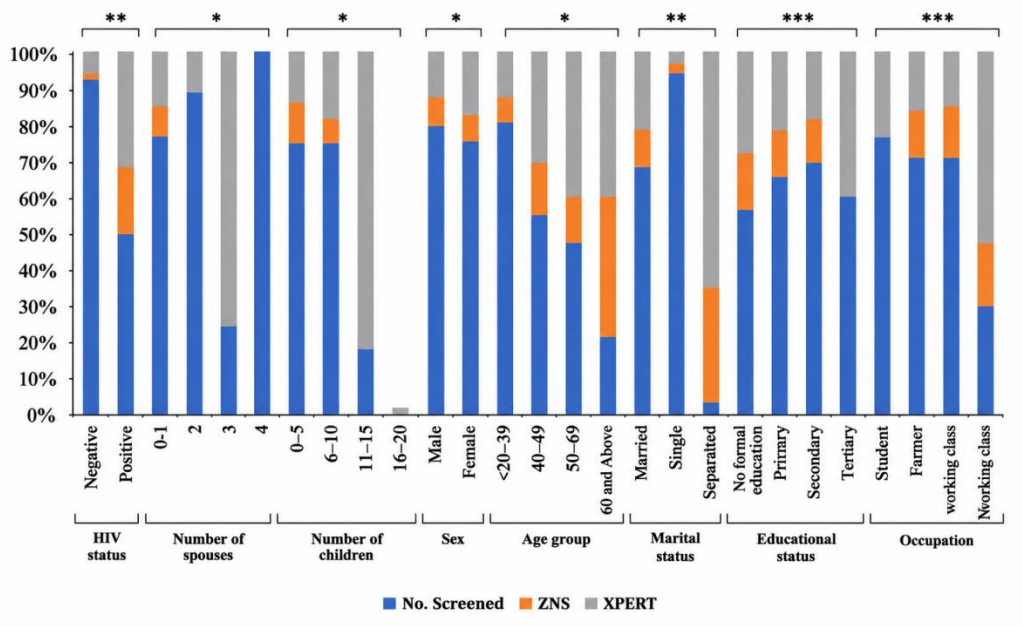



**Fig. 1.** Distribution of the study population according to TB screening and detection results using ZN smear microscopy (ZNSM) and the Xpert MTB/RIF assay, stratified by sociodemographic characteristics at Saint Monica's Hospital, Adikpo, Kwande Local Government Area, Nigeria. $\chi^2$, chi-square test; ZNSM, Ziehl–Neelsen smear microscopy. $P$<0.05, statistically significant; P<0.01, statistically significant; $P$<0.001, highly statistically significant.

**Detection of *M. tuberculosis* by ZNSM and the Xpert MTB/RIF assay.** Table 1 compares the results of ZNSM and the Xpert MTB/RIF assay for the sputum samples obtained from patients attending Saint Monica's Hospital, Adikpo, Kwande Local Government Area. A total of 163 sputum samples were analysed. By the Xpert MTB/RIF assay, 17 (10.43%) samples were positive for *M. tuberculosis*, whereas 146 (89.57%) were negative. The semiquantitative distribution of the Xpert-positive samples was as follows: very low, 2 (1.23%); low, 4 (2.45%); medium, 3 (1.84%); and high, 8 (4.91%). By ZNSM, 9 (5.52%) samples were positive for acid-fast bacilli (AFB), whereas 154 (94.48%) were negative. Among the smear-positive samples, 3 (1.84%) were graded as 1+, 2 (1.23%) as 2+, and 4 (2.45%) as 3+ (Table 1). The difference in positivity between the two methods was statistically significant (McNemar's test, $P$ = 0.008).

**Table 1.** Detection of *M. tuberculosis* by Xpert MTB/RIF assay and Ziehl–Neelsen smear microscopy in sputum samples from patients attending Saint Monica's Hospital, Adikpo, Kwande Local Government Area

| Test / result category | n | % |
|---|---|---|
| **Xpert MTB/RIF assay** | | |
| Positive | 17 | 10.43 |
| Very low | 2 | 1.23 |
| Low | 4 | 2.45 |
| Medium | 3 | 1.84 |
| High | 8 | 4.91 |
| Negative | 146 | 89.57 |
| **Ziehl–Neelsen smear microscopy** | | |
| Positive | 9 | 5.52 |
| 1+ | 3 | 1.84 |
| 2+ | 2 | 1.23 |
| 3+ | 4 | 2.45 |
| Negative | 154 | 94.48 |
| **Total** | **163** | **100** |

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Table 2 shows the concordance between ZNSM and the Xpert MTB/RIF assay. All 9 ZNSM-positive samples were also positive by the Xpert MTB/RIF assay. In addition, the Xpert MTB/RIF assay detected 8 positive samples among the 154 ZNSM-negative samples. Overall, 146 samples were negative by both methods (Table 2).

**Table 2.** Concordance between Ziehl–Neelsen smear microscopy (ZNSM) and Xpert MTB/RIF assay results for *M. tuberculosis* detection

| | Xpert positive | Xpert negative | Total |
|---|---|---|---|
| ZNSM positive | 9 | 0 | 9 |
| ZNSM negative | 8 | 146 | 154 |
| **Total** | **17** | **146** | **163** |

**Cohen's kappa agreement analysis.** Cohen's kappa agreement analysis showed that the observed proportion of agreement ($P_o$) between ZNSM and the Xpert MTB/RIF assay was 0.951 (95.1%), while the expected proportion of agreement due to chance ($P_e$) was 0.852 (85.2%). The resulting Cohen's kappa coefficient was $\kappa = 0.67$, indicating substantial agreement between ZNSM and the Xpert MTB/RIF assay in detecting *M. tuberculosis*.

**Drug susceptibility profile of *M. tuberculosis*-positive samples.** Table 3 presents the drug susceptibility profile of *M. tuberculosis* among the Xpert MTB/RIF-positive patients attending Saint Monica's Hospital, Adikpo. Thirteen positive samples were susceptible to rifampicin, whereas four samples (SM4, SM89, SM33, and SM51) were resistant to rifampicin. Of the rifampicin-resistant samples, SM4, SM89, and SM33 had isolated rifampicin resistance (RR-TB) and remained susceptible to INH, FQ, AMK, KAN, CAP, and EMB, whereas SM51 was additionally resistant to INH and was classified as MDR-TB.

**Table 3.** Drug susceptibility profile of rifampicin-resistant *M. tuberculosis*-positive sputum samples obtained from patients attending Saint Monica's Hospital, Adikpo, Kwande Local Government Area

| Sample ID | Detected organism | RIF | INH | FQ | AMK | KAN | CAP | EMB |
|---|---|---|---|---|---|---|---|---|
| SM4 | *M. tuberculosis* | + | − | − | − | − | − | − |
| SM89 | *M. tuberculosis* | + | − | − | − | − | − | − |
| SM33 | *M. tuberculosis* | + | − | − | − | − | − | − |
| SM51 | *M. tuberculosis* | + | + | − | − | − | − | − |

Abbreviations: RIF, rifampicin; INH, isoniazid; FQ, fluoroquinolones; AMK, amikacin; KAN, kanamycin; CAP, capreomycin; EMB, ethambutol. +, resistant; −, susceptible.

## DISCUSSION

Tuberculosis remains one of the leading causes of death globally, particularly in low- and middle-income countries [1, 22]. Early detection and prompt initiation of treatment are critical to reducing TB-related mortality. However, the emergence of DR-TB, together with limited access to rapid and accurate diagnostic tools, continues to hinder global TB control efforts [23].

In the present study, *M. tuberculosis* was detected in 10.43% (17/163) of samples using the Xpert MTB/RIF assay, compared with 5.52% (9/163) detected by ZNSM. This finding demonstrates a higher detection rate with the Xpert MTB/RIF assay than with ZNSM, which is consistent with previous studies [12, 13]. Munir *et al*. [12] reported smear positivity of 67.5% compared with 77.4% using the Xpert MTB/RIF assay, although their study population and inclusion criteria differed substantially from those of the present study. Similarly, Umair *et al*. [13] reported positivity rates of 23.73% by smear microscopy and 34.24% by Xpert MTB/RIF assay. Therefore, although increased TB detection with the Xpert MTB/RIF assay is a consistent finding, the absolute positivity rates are not directly comparable because of differences in study populations, inclusion criteria, case definitions, and diagnostic algorithms.

The relatively low positivity rate observed in the present study may be attributable to differences in patient selection, low bacillary load, early-stage disease, HIV co-infection, which is often associated with paucibacillary disease, poor sputum quality, and age-related variations in immune response. In addition, ZNSM is operator-dependent, and its sensitivity may vary across laboratories and geographical settings [24]. In contrast, nucleic acid amplification–based methods, such as the Xpert MTB/RIF assay, generally provide more consistent detection because they identify *M. tuberculosis* DNA directly. Although ZNSM remains useful for assessing infectiousness and transmission risk [25], its relatively high detection limit of approximately 5,000–10,000 bacilli/mL of sputum means that many paucibacillary cases may be missed [24, 26].

Although culture remains the gold standard for the diagnosis of tuberculosis, it is time-consuming, resource-intensive, and requires specialized laboratory infrastructure [27]. In contrast, AFB smear microscopy is rapid and inexpensive but has variable sensitivity (20–80%) and cannot differentiate *M. tuberculosis* from non-tuberculous mycobacteria [26]. To overcome these limitations, WHO recommended the automated Xpert MTB/RIF assay as a rapid and reliable tool for detecting



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tuberculosis and rifampicin resistance [11]. The Xpert MTB/RIF assay offers several advantages, including a short turnaround time, high sensitivity, and simultaneous detection of *M. tuberculosis* and rifampicin resistance [28], and it has demonstrated added diagnostic value over smear microscopy in routine practice [29].

In the present study, a TB positivity rate of 22.41% was recorded among HIV-positive participants using the Xpert MTB/RIF assay, a finding that is consistent with the high TB/HIV co-infection burden documented in Nigeria [1, 30]. Similarly, among HIV-positive populations, studies from other parts of the country have reported TB positivity rates of 29.0% in Ogun State [31], 25.0% in Lagos [32], and 34.5% in Lafia [33]. In contrast, lower rates have been documented in Kano (11.2%) [34], among pregnant women in southwest Nigeria (5.1%) [35], and in Agbor, Delta State (10.6%) [36]. These differences may be explained by variations in study design, sample size, antiretroviral therapy (ART) use, diagnostic methods, population characteristics, and access to healthcare services.

In the present study, the TB positivity rate was 9.52% among males and 7.95% among females, with a statistically significant association between sex and TB positivity ($P = 0.031$). This finding is consistent with the report by Herzmann *et al*. [37], who observed a female-to-male ratio of 1:1.3 for tuberculosis in the German population. Similar patterns have also been reported in Niger State [38] and Nasarawa State [39]. The higher TB positivity observed among males may be related to gender-associated stigma, behavioural risk factors such as smoking and alcohol use, and greater social or occupational exposure to infection [40]. Differences in health-seeking behaviour and access to healthcare may also contribute to variations in case detection [41]. In addition, biological differences between the sexes may contribute to differential susceptibility to tuberculosis [42]. However, a higher burden of tuberculosis among females has been reported in Pakistan [43], consistent with WHO findings that tuberculosis remains one of the leading causes of death among women globally [1].

In the present study, the highest TB positivity rate was observed among individuals aged 20–39 years (11.11%). This finding is consistent with reports from Delta State [44] and Jigawa State [45], and is further supported by national age-disaggregated data from Kenya [3]. Although tuberculosis is often perceived as more common among older adults, the present findings suggest that younger, economically active individuals may also be substantially affected [1]. This pattern may be partly explained by increased exposure to occupational and social risk factors within this age group.

Marital status was also associated with TB positivity in the present study. Similar findings have been reported in Kano [34], southwest Nigeria [35], and Ethiopia [46], where higher TB positivity rates were observed among married individuals. This pattern may be related to shared living environments, prolonged household exposure, and the potential for transmission between spouses. However, Kolade *et al*. [31] reported contrasting findings regarding the association between marital status and TB positivity. Moreover, several of these studies were conducted among HIV-positive or pregnant populations, which limits direct comparison with the present findings.

Educational level was strongly associated with TB positivity in the present study, with the highest rate observed among respondents with no formal education (14.58%). This finding is consistent with previous reports from Nigeria [47, 48]. Lower educational attainment may be linked to reduced health literacy, delayed healthcare-seeking, and poor awareness of TB transmission and symptoms [47–49], contributing to treatment interruption and poorer treatment outcomes [1, 50, 51].

Occupation was also associated with TB positivity, with positivity highest among non-working participants (26.09%), followed by farmers (9.72%). This observation is consistent with earlier studies that reported a higher TB burden among individuals engaged in farming and other manual occupations as well as among socioeconomically disadvantaged groups [52, 53]. The increased positivity among these groups may reflect a combination of socioeconomic vulnerability, limited access to healthcare, delayed diagnosis, poor living conditions, and possible occupational or environmental exposures.

In addition, four samples were identified as rifampicin-resistant using the Xpert MTB/RIF assay; three had isolated rifampicin resistance, and one sample had additional isoniazid resistance, indicating MDR-TB. This finding highlights the potential emergence of DR-TB strains in the study area and underscores the need for continuous surveillance, prompt drug susceptibility testing, and improved access to molecular diagnostic methods.

Molecular diagnostic tools, including LPAs and the Xpert MTB/RIF assay, have significantly improved tuberculosis diagnosis by enabling rapid detection of *M. tuberculosis* and identification of drug-resistant strains [11, 54, 55]. These methods support early treatment initiation, appropriate patient management, and improved clinical outcomes, including in resource-limited settings where conventional diagnostic approaches may be limited.

In the present study, no ZNSM-positive/Xpert MTB/RIF-negative result was observed, as the Xpert MTB/RIF assay detected all smear-positive samples. This finding supports the high diagnostic sensitivity of the Xpert MTB/RIF assay for smear-positive tuberculosis, consistent with previous reports [56]. The possibility of false-positive Xpert MTB/RIF results due

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to detection of DNA from nonviable bacilli was reduced by excluding previously treated patients [57]. However, false-negative Xpert MTB/RIF results may occur in specimens with low bacillary load or in the presence of polymerase chain reaction (PCR) inhibitors, particularly among smear-negative cases. Therefore, although the findings support the utility of the Xpert MTB/RIF assay for TB diagnosis, especially among HIV-infected individuals, negative results should be interpreted alongside clinical findings and other diagnostic evidence where available [58].

Although patients who had received anti-tuberculosis treatment within the previous six months were excluded to reduce the likelihood of false-positive Xpert MTB/RIF results due to detection of DNA from nonviable bacilli, mycobacterial DNA may persist for longer than six months in some cases. Therefore, this exclusion criterion may not have completely eliminated the possibility of false-positive results. Furthermore, the study was conducted at a single facility, culture was not used as a confirmatory reference standard, and several sociodemographic subgroups contained small numbers, limiting the precision of subgroup estimates. Moreover, the Xpert MTB/RIF assay provides information on rifampicin resistance only, and newer iterations, such as the Xpert MTB/RIF Ultra assay, may offer improved diagnostic sensitivity, particularly in paucibacillary specimens.

The Xpert MTB/RIF assay showed a higher detection rate for *M. tuberculosis* than ZNSM, detecting additional cases, including those with very low bacillary loads. The assay's rapid turnaround time, ease of use, and minimal labour requirements highlight its potential utility for routine tuberculosis diagnosis in clinical laboratories, especially in high-burden settings such as Nigeria. The substantial agreement observed between ZNSM and the Xpert MTB/RIF assay, as indicated by Cohen's kappa coefficient of 0.67 and 95.1% overall agreement, further supports the diagnostic value of the Xpert MTB/RIF assay.

In conclusion, national tuberculosis diagnostic and management systems in Nigeria should be further strengthened, particularly for the detection and management of drug-resistant tuberculosis. Expanded access to advanced molecular diagnostic technologies that identify resistance beyond rifampicin is strongly recommended. Such tools include LPAs, notably GenoType MTBDRplus and GenoType MTBDRsl, which detect resistance to first- and second-line anti-tuberculosis drugs, and whole-genome sequencing, which provides comprehensive resistance profiling [54, 55]. Exclusive reliance on rifampicin resistance may fail to identify other clinically significant forms of drug resistance.

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## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

## FUNDING

This research received no external funding.

## AI DISCLOSURE

Generative artificial-intelligence tools (ChatGPT, version GPT-4, OpenAI, San Francisco, CA, USA) were used only to improve language and formatting during manuscript preparation. The authors reviewed and edited all AI-assisted content and take full responsibility for the integrity and accuracy of the work.

## DATA AVAILABILITY

The de-identified datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request, subject to ethical and data-protection approvals.

## AUTHORS' CONTRIBUTIONS

ICA: Conceptualization, methodology, investigation, formal analysis, software, visualization, and writing—original draft. OJE: Conceptualization, data curation, formal analysis, software, visualization, and writing—review and editing. HZ: Supervision, validation, and writing—review and editing. YAA: Supervision, validation, and writing—review and editing. ACK: Software, data curation, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

## ETHICS STATEMENT

Ethical approval for the study was obtained from the Health Research Ethics Committee of Saint Monica's Hospital, Adikpo, Benue State, Nigeria, on 11 July 2023, with approval number SMH/HREC/60/V.I/85. Written informed consent was obtained from all participants before sample collection and enrolment in the study.

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