



(RESEARCH ARTICLE)



MOLECULAR DETECTION OF FIRST-LINE ANTI-TUBERCULOSIS DRUG RESISTANCE GENES IN *MYCOBACTERIUM TUBERCULOSIS* USING PCR AND AGAROSE GEL ELECTROPHORESIS

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ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a major global health threat and is becoming more serious with the emergence of drug-resistant strains. In this study, polymerase chain reaction (PCR) and agarose gel electrophoresis were used to investigate the molecular detection of first line anti-tuberculosis drug resistance genes (*rpoB*, *katG*, *inhA* and *embB*). A total of 110 newly diagnosed pulmonary TB patients were recruited from selected health facilities in Bayelsa State, Nigeria. Sputum samples were collected and decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) and processed for Ziehl-Neelsen microscopy and GeneXpert MTB/RIF assay for initial confirmation. Genomic DNA was isolated using a commercial kit and the target resistance genes were amplified using gene specific primers. PCR products were resolved on a 1% agarose gel and visualised under a blue-light transilluminator. The results indicated that all the target genes were successfully amplified with the expected size of the fragments of 400 bp (*rpoB*), 200 bp (*katG*), 500 bp (*inhA*) and 300 bp (*embB*). Multiple isolates of the *rpoB* gene consistently showed the presence of rifampicin resistance-associated mutations. The *katG* gene was also frequently amplified, indicating widespread isoniazid resistance via high-level resistance mechanisms. Conversely, the majority of isolates showed no or weak *inhA* amplification suggesting a lower prevalence of promoter-mediated isoniazid resistance. Several isolates harboured the *embB* gene, suggesting the circulation of ethambutol resistance-associated mutations. Resistance-associated genes were more dominant in males and in individuals between 25 and 46 years of age, according to the analysis of gender and age distribution. This study showed that PCR and agarose gel electrophoresis can be used for the rapid detection of genes associated with TB drug resistance. The results indicate the dominance of *rpoB* and *katG* mutations in the study population and the importance of molecular diagnostics in improving tuberculosis control strategies and providing suitable therapeutic interventions in Nigeria.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Drug Resistance, PCR, *RpoB*, *KatG*, *InhA*, *EmbB*, Nigeria

1 INTRODUCTION

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB) which is one of the leading causes of morbidity and mortality from infectious diseases worldwide. In Nigeria, like in many other low- and middle-income countries, TB remains a major public health challenge despite significant advances in diagnosis and treatment. In 2022, the World

Health Organization estimated that 10.6 million people developed TB and recorded approximately 1.3 million deaths worldwide, highlighting the persistent burden of the disease (Lee et al., 2024). The situation is complicated by the emergence and spread of drug resistant strains of *M. tuberculosis* which threaten global TB control efforts.

Drug resistant TB, especially MDR-TB (multidrug-resistant TB) is defined as resistance to at least rifampicin and isoniazid, the two most effective first-line anti-TB drugs. Rifampicin-resistant TB (RR-TB) is often used as a proxy for MDR-TB, as there is a strong association with concurrent resistance to isoniazid (Bisimwa et al., 2021). The increasing burden of RR-TB has been associated with a combination of microbial, clinical and programmatic determinants such as spontaneous genetic alterations, poor treatment compliance, delayed diagnosis and poor healthcare infrastructure (Kévelaitiené et al., 2025).

The molecular basis of resistance to first-line anti-TB drugs is primarily mediated by mutations in specific genes of *M. tuberculosis*. Resistance to rifampicin is due to mutations in the *rpoB* gene (encoding the beta subunit of RNA polymerase) that change the drug-binding site. Resistance to isoniazid, in contrast, is mainly due to mutations in the *katG* gene encoding the catalase-peroxidase that activates the drug and the *inhA* gene involved in mycolic acid synthesis. Mutations in the *embB* gene, which plays a role in cell wall arabinogalactan biosynthesis, have also been associated with ethambutol resistance (Hafeez et al., 2021; Xiang et al., 2021). These genetic alterations confer drug resistance, allowing persistent infection, prolonged transmission and poor treatment outcomes.

Timely and accurate detection of these resistance conferring mutations is crucial for effective management of TB. Traditional phenotypic drug susceptibility testing methods are time consuming and may delay initiation of appropriate therapy. Molecular methods such as polymerase chain reaction (PCR) and agarose gel electrophoresis can rapidly, sensitively and specifically detect the genes responsible for resistance. PCR amplification allows the detection of specific gene fragments and agarose gel analysis allows visualization of amplified DNA based on molecular size, thus confirming the presence of target resistance genes (Khehra et al., 2025).

Nigeria still carries a heavy burden of drug-resistant TB with increasing reports of resistance to rifampicin and isoniazid in different parts of the country (Oladimeji et al., 2022). However, molecular epidemiological data on the distribution of specific resistance genes is still missing, especially in the Niger Delta region. Information on the genetics of drug resistance in local populations is required to guide treatment decisions, improve diagnostic strategies and public health interventions.

Therefore, this study was designed to investigate the molecular detection of first-line anti-tuberculosis drug resistance genes *rpoB*, *katG*, *inhA* and *embB* in *Mycobacterium tuberculosis* isolates by PCR and agarose gel electrophoresis, respectively. The study characterizes the presence and distribution of these resistance-associated genes to contribute to the growing body of evidence required to strengthen TB control programs and combat the spread of drug-resistant TB in Nigeria.

2 MATERIALS AND METHODS

2.1 Chemicals/Reagents

All chemicals and reagents used in the present study were of analytical grade.

2.2 Study Region

This study was carried out in Bayelsa State, situated in the South-South geopolitical zone of Nigeria in the Niger Delta region. It is bounded by the Delta State in the North, Rivers State in the East and the Atlantic Ocean in the West and South. It consists of a landmass of approximately 21,110 km² and is predominantly riverine with approximately 75% of its terrain being water bodies. Many communities are only accessible by water, and only a few local government headquarters are reached by road.

The study was conducted at major tuberculosis (TB) diagnostic and treatment centers which include National Tuberculosis and Leprosy Training Center (NTBLTC), Federal Medical Center (FMC) Yenagoa, Niger Delta University Teaching Hospital (NDUTH) Okolobiri, Bayelsa State Medical University Diagnostic Center and Tobis Clinic & Consulting, Yenagoa. These facilities are important referral centers for diagnosis & management of TB in the state.

2.3 Ethical Approval

Ethical approval The study received approval from the Bayelsa State Ministry of Health and the participating healthcare institutions. The study was performed in accordance with the ethical principles of the Declaration of Helsinki for

research involving human subjects. All participants gave written informed consent prior to enrolment. In case of illiterate participants, thumbprints were used for taking consent after detailed verbal explanation of the study.

2.4 Study Design, Population, and Sample Size

A hospital-based cross-sectional study design was employed. A total of 110 newly diagnosed pulmonary tuberculosis patients aged ≥ 14 years were recruited from selected healthcare facilities.

The sample size was determined using the Cochran formula (1977): $n = \frac{t^2 \times P(1-P)}{m^2}$

Where $t = 1.96$ (95% confidence level), p = estimated TB prevalence (0.218), and $m = 0.05$. This yielded a minimum sample size of approximately 70 participants. However, 110 eligible participants who completed the study were included to improve statistical power. Only isolates with detected resistance genes ($n = 40$) were included.

2.5 Inclusion and Exclusion Criteria

Newly diagnosed patients of either gender with *Mycobacterium tuberculosis* infection age ≥ 14 years confirmed by GeneXpert MTB/RIF assay and smear microscopy and who consented to participate.

Patients were excluded if they had previously been treated for TB (relapse or retreatment cases), had comorbidities such as diabetes mellitus or hypertension, had renal or hepatic impairment, or were being treated for leprosy.

2.6 Sample Processing & Collection

Spot and early morning sputum samples were collected from consenting participants. Patients were trained in the appropriate technique for producing deep-cough sputum to assure specimen quality. Samples smaller than 2 mL were excluded, as were salivary samples.

Specimens were stored at 2–8°C and transported to the laboratory. Each sample was decontaminated by the N-acetyl-L-cysteine–sodium hydroxide (NALC–NaOH) method, centrifuged at 3000 g for 15 min, and the supernatant was discarded. Smears were prepared for microscopy and pellets for further molecular analysis.

2.7 Ziehl-Neelsen smear microscopy

Smears were stained by Ziehl–Neelsen (ZN) technique. Slides were flooded with carbol fuchsin and heated until steaming and left for 5 minutes. Decolorization was carried out with acid-alcohol and counterstaining with malachite green. Slides were examined for acid-fast bacilli (AFB) by light microscopy.

2.8 GeneXpert MTB/RIF Test

All sputum samples were tested by GeneXpert MTB/RIF assay as per manufacturer instructions. The assay detects *M. tuberculosis* DNA and rifampicin resistance by detection of mutations in the *rpoB* gene. The results were obtained in about 2 hours.

2.9 DNA Isolation and Quantification

Genomic DNA was isolated using Quick-DNA Miniprep Kit (Zymo Research) according to the manufacturer's protocol. DNA purity and concentration were measured by NanoDrop 1000 spectrophotometer.

2.10 Amplification of Resistance Genes by PCR

The resistance associated genes *rpoB*, *katG*, *inhA* and *embB* were amplified with gene-specific primers. PCR reactions were carried out using DreamTaq Master Mix with optimized cycling conditions for 35 cycles in a 30 μ L reaction volume. The amplification parameters were 94°C for initial denaturation, gene specific annealing temperatures (52–60°C) and 72°C for final extension.

2.11 Agarose Gel Electrophoresis

PCR products were separated on 1% agarose gel in TBE buffer and stained with EZ-vision dye. Each amplicon along with a 100 bp molecular weight ladder was loaded in 10 microliters. Electrophoresis was carried out at 120 V for 30 minutes and DNA bands were visualized on a blue-light transilluminator.

Expected amplicon sizes were:

- *rpoB*: 400 bp

- *katG*: 200 bp
- *inhA* : 500 bp
- *embB*: 300 bp

2.11.1 Statistical Analysis

Data were analyzed using SPSS version 21.0 (IBM Corp., USA). Descriptive statistics were used to summarize data. Associations between categorical variables were tested using Chi-square and Student's t-test where appropriate. Statistical significance was set at $p < 0.05$.

3 RESULTS

Figure s 1, 2, 3 and 4 show the Visualization of *rpoB*, *katG*, *inhA* and *embB* Genes by Agarose Gel Electrophoresis. The amplification of *rpoB*, *katG*, *inhA* and *embB* genes using specific primers and resolution on 1% agarose gel electrophoresis with a 100 bp ladder revealed gene bands sizes of 400bp, 200bp, 500bp and 300bp respectively (Figure 1, 2, 3 and 4). Figure 1 shows that for the *rpoB* gene, a clear amplification product was observed at approximately 400 bp across multiple lanes (1–11), indicating successful detection of the gene region associated with rifampicin resistance. The uniformity of band size across samples suggests conserved primer binding and successful amplification of the target region without significant non-specific products. This confirms the presence of *M. tuberculosis* isolates carrying the *rpoB* gene region targeted in the assay, which is critical for identifying rifampicin resistance-associated mutations.

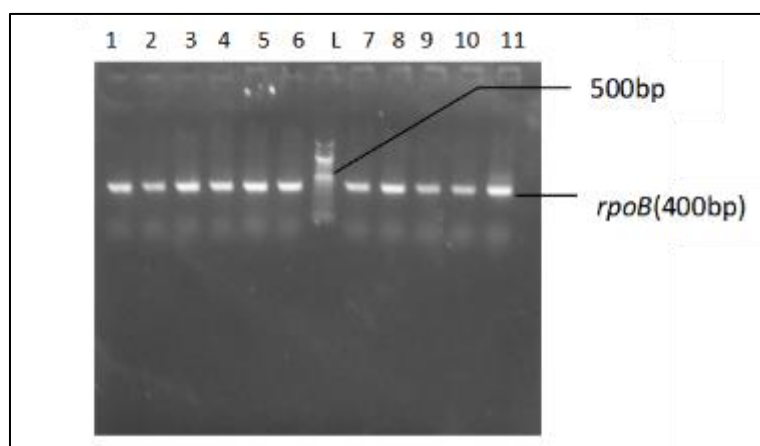


Figure 1: Gel electrophoresis of amplified *rpoB* gene bands 400bp. Lanes 1-11 are the *rpoB* gene bands and lane L is the 100 bp molecular ladder.

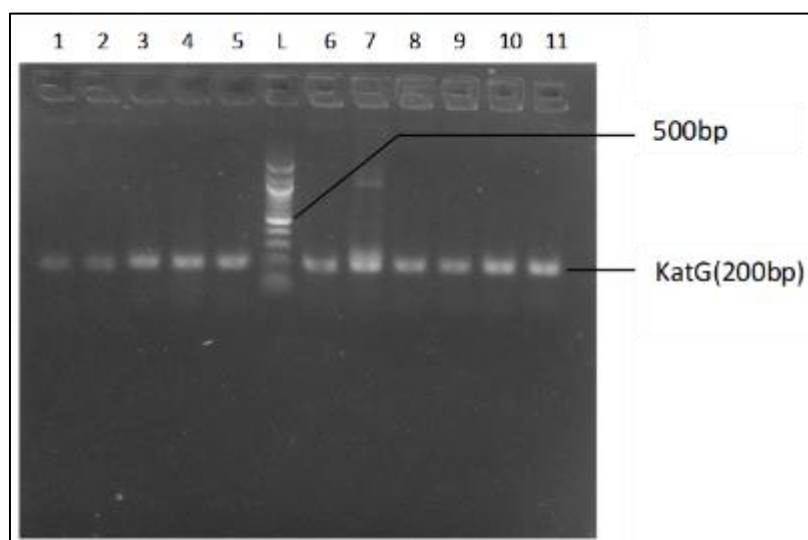


Figure 2: Agarose gel electrophoresis showing the amplified *KatG* gene bands at 200bp. Lanes 1-11 represent the *KatG* gene bands while lane L represents the 100 bp molecular ladder.

Figure 2 shows a clear band at about 200 bp for the *katG* gene confirming amplification of the gene segment associated with high level isoniazid resistance. Most lanes show discrete single bands indicating good primer specificity and successful amplification of *katG* gene regions from several isolates. This supports the presence of genetic determinants associated with isoniazid resistance in the studied *M. tuberculosis* population.

Figure 3 shows that for the *inhA* gene, amplification was detected at approximately 500 bp, although fewer or no consistent bands were observed across all samples compared to *rpoB* and *katG*. This pattern suggests that mutations or absence of amplification in some isolates may reflect lower prevalence of *inhA*-mediated isoniazid resistance mechanisms in the study population. The absence or weak amplification in some lanes may also indicate sequence variability or reduced target abundance affecting primer binding efficiency.

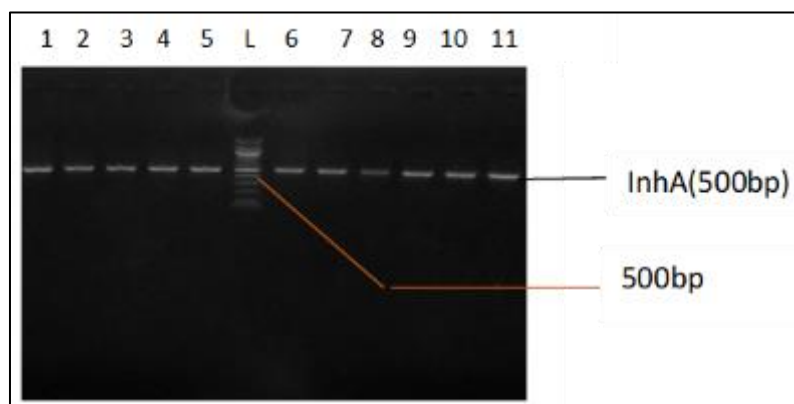


Figure 3: Agarose gel analysis of *InhA* gene bands (500bp). Lanes 1-11 show the *InhA* gene bands and lane L shows 100 bp molecular ladder.

Figure 4 shows the *embB* gene was associated with a specific band of approximately 300 bp, thus confirming the successful detection of genetic markers associated with ethambutol resistance. The presence of *embB* amplicons in multiple lanes suggests the existence of circulating mutations associated with ethambutol resistance, which may exist in a subset of *M. tuberculosis* isolates studied. The differences in band intensity between lanes may be indicative of differences in clinical samples in terms of bacterial load or gene copy abundance.

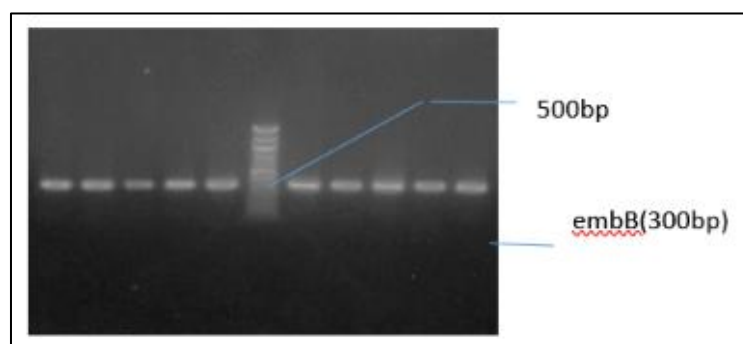


Figure 4: Amplified *embB* gene bands at 500bp by agarose gel electrophoresis method. Lane L represents the 100 bp molecular ladder and lanes 1-10 represent the *embB* gene bands.

Table 1 Shows the Distribution of Mutant Genes Mediating Resistance to the first-line anti TB Drug-Therapy by Gender. The distribution of mutant genes mediating resistance to the first-line anti TB Drug-Therapy by gender showed that of the 15 patients *rpoB* putative resistance-associated gene detection. 9(60.0%) were from the males while 6(40.0%) were from their female counterparts. The prevalence of *rpoB* mutants was significantly higher in males than in females ($P < 0.05$). *katG* mutant genes were 14 out of which 6(42.8%) and 8(57.2%) were from males and females respectively. Of the 11 patients *embB* mutant genes, 7(63.6%) and 4(32.4%) were from males and females respectively. The distributions of *katG* and *embB* resistance markers showed no statistical difference by gender ($P > 0.05$). On the other hand, no mutation was detected in the *inhA* genes.

Table 1: Distribution of *rpoB*, *katG*, *inhA* and *embB* mutant genes mediating resistance to the first-line anti TB Drug-Therapy by gender

GENE	MALE n (%)	FEMALE n (%)	TOTAL n (%)
<i>rpoB</i>	9(60.0)	6(40.0)	15 (100)
<i>katG</i>	6(42.8)	8(57.2)	14 (100)
<i>inhA</i>	-	-	-
<i>embB</i>	7(63.6)	4(36.4)	11(100)

Table 2 shows the distribution of Rifampicin, *rpoB*, Isoniazid, *katG*, *inhA* and Ethambutol *embB* Mutant Genes by Age. The distribution of *rif*, *katG*, *inhA* and *embB* mutant genes by age revealed that subjects in the age group studied recorded 15 *RIF* resistance, 8(53.3%) were detected in subjects within the age bracket 25-35, 36-46 years. The prevalence of *RIF* resistance was statistically higher in the above age groups when compared to subjects in other groups. *katG* prevalence was also statistically higher in the subjects of age group 14-24, 25-35 and 36 to 46 years who all recorded 3(21.4%), 3(21.4%) and 5(35.7%) respectively.

Table 2: Distribution of *rpoB*, *katG*, *inhA* and *embB* mutant genes mediating resistance to the first-line anti TB Drug-Therapy by age

AGE	<i>rpoB</i> n (%)	<i>katG</i> n (%)	<i>inhA</i> n (%)	<i>embB</i> n (%)	TOTAL (n)
14-24	3 (20.0)	3 (21.4)	0 (0.0)	1 (9.1)	7
25-35	4 (26.7)	3 (21.4)	0 (0.0)	1 (9.1)	8
36-46	4 (26.7)	5 (35.7)	0 (0.0)	7 (63.6)	16
47-57	2 (13.3)	1 (7.1)	0 (0.0)	1 (9.1)	4
58-68	1 (6.7)	1 (7.1)	0 (0.0)	1 (9.1)	3
69-79	1 (6.7)	1 (7.1)	0 (0.0)	0 (0.0)	2
TOTAL	15 (100)	14(100)	0 (0.0)	11 (100)	40

4 DISCUSSION

The present study demonstrated successful amplification and visualisation of important first-line anti-tuberculosis (TB) drug resistance genes, *rpoB*, *katG*, *inhA* and *embB* by PCR and agarose gel electrophoresis confirming the applicability of molecular approaches for rapid detection of drug resistant *Mycobacterium tuberculosis*. The amplicon sizes observed (400 bp for *rpoB*, 200 bp for *katG*, 500 bp for *inhA* and 300 bp for *embB*) were in accordance with the expected gene fragment sizes indicating the high specificity of the primers and reliability of the assay. These findings corroborate recent molecular diagnostic studies validating the sensitivity and specificity of PCR-based detection for identifying TB drug resistance-associated genes (Tawfick et al., 2023; Xiong et al., 2024).

The consistent amplification of the *rpoB* gene in a number of isolates indicates that gene regions linked to resistance are widespread in the study population. This finding is consistent with recent reports showing that *rpoB* mutations remain the most common genetic mechanism of rifampicin resistance worldwide, especially in the rifampicin resistance-determining region (RRDR) (Amusengeri et al., 2022). Additionally, studies from Nigeria and other sub-Saharan African countries have reported a high burden of rifampicin-resistant TB, highlighting the significance of *rpoB* as a major molecular marker in the surveillance of multidrug-resistant TB (MDR-TB) (Noorizhab et al., 2023; Mtetwa, et al., 2023). The uniformity of *rpoB* bands in this study further supports the conserved nature of primer binding regions although mutations may still exist within the amplified segments.

Amplification of *katG* gene in a significant number of isolates suggests its role as mediator of high level isoniazid resistance. Mutations in *katG*, especially at codon 315, that impair activation of the prodrug are widely reported to be the predominant mechanism of isoniazid resistance (Valafar 2021; Hsu et al., 2020). These findings are consistent with recent molecular epidemiological studies indicating that *katG* mutations are more common than *inhA* mutations in many high-burden settings (Bollela et al., 2016). The sharp and discrete amplification bands indicate that the primers used are specific and confirm the presence of *katG*-mediated resistance in the population under study.

This study did not find any mutation or amplification of the *inhA* gene. This suggests a low prevalence or possible absence of *inhA*-mediated isoniazid-resistance mechanisms in the tested isolates. This is in line with recent findings showing that *inhA* promoter mutations explain a smaller proportion of isoniazid resistance cases than *katG*, often associated with low-level resistance and cross-resistance to ethionamide (Nono et al., 2025; Ranjan et al., 2023). However, the absence of *inhA* detection could also be due to the limitation of primer binding efficiency or genetic variability within the target region, as previously described in PCR based studies (Valafar, 2021). Thus, sequencing-based methods might be required for a complete exclusion of *inhA* mutations.

Amplification of the *embB* gene in a number of isolates suggests the presence of ethambutol resistance associated gene regions. Mutations in *embB*, especially at codon 306, are strongly associated with ethambutol resistance and are frequently observed in MDR-TB strains (Li et al., 2025). Variability in band intensity was observed in the present study which might suggest differences in bacterial load, DNA quality or gene expression levels as reported in similar studies (Arslan et al., 2021; Wittmeier et al., 2020). The occurrence of *embB* mutations together with *rpoB* and *katG* mutations also suggests the possibility of co-resistance patterns, which is a growing concern in TB management.

The analysis of gender distribution showed that *rpoB* and *embB* mutations were more frequent in males, and *katG* mutations were slightly more frequent in females, but only *rpoB* was statistically significant. These results are in line with epidemiological studies showing higher rates of TB burden and drug resistance in males often linked to increased exposure risks, occupational factors, and healthcare-seeking behaviours (Feldman et al., 2024). However, the absence of strong sex differences for *KatG* and *embB* implies that genetic resistance mechanisms are not significantly sex-linked, but are instead driven by transmission dynamics and treatment factors.

Distribution by age showed a higher prevalence of resistance-associated genes, particularly *rpoB* and *katG*, among people aged 25–46 years. This is in line with recent reports that the most affected demographic group for drug-resistant TB are young and middle-aged adults, probably because they are more socially active, economically active, and mobile, which facilitates transmission (Cui et al., 2025). The higher prevalence in these age groups also raises concern of productivity loss and socioeconomic burden.

5 CONCLUSION

Findings from this study are consistent with recent worldwide and regional evidence showing predominance of *rpoB* and *katG* mutations mediating resistance to rifampicin and isoniazid, respectively, but less common mutations in *inhA*. Successful use of PCR and agarose gel analysis further emphasises the importance of molecular diagnostic tools for rapid detection and surveillance of drug resistant TB. In comparison with conventional methods, these techniques have shorter turnaround times and increased sensitivity, which is crucial to the timely commencement of appropriate therapy and control of resistant strains.

Importantly, however, PCR-based detection identifies the presence of gene regions but does not directly confirm specific mutations without sequencing. Thus, integration of sequencing and advanced molecular methods such as line probe assays or whole genome sequencing would give a more complete picture of resistance patterns.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors affirm that there is no conflict of interest.

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