Volume 3, Issue 7, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

# COMPARISON OF NANOPORE SEQUENCING VERSUS CONVENTIONAL PCR FOR RAPID DETECTION OF DRUG-RESISTANT TUBERCULOSIS IN CLINICAL PRACTICE

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DOI: https://doi.org/10.5281/zenodo.17164912

**Keywords**
*Tuberculosis, Drug resistance, Nanopore sequencing, PCR, Rapid diagnostics, Multidrug-resistant TB*

**Article History**
*Received: 10 July 2025*
*Accepted: 26 August 2025*
*Published: 02 September 2025*



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## Abstract

*Background: Drug-resistant tuberculosis (DR-TB) remains a critical global health challenge, particularly in regions with high disease burden. Rapid and accurate detection of resistance is essential for initiating effective therapy and curbing transmission. Objective: This study aimed to compare nanopore sequencing with conventional PCR in detecting drug-resistant tuberculosis in clinical practice. Methods: This cross-sectional analytical study was conducted at Tertiary Care Hospital, Lahore from November 2024 to April 2025. It included 153 patients with confirmed pulmonary TB. Sputum samples were analyzed using both conventional PCR and nanopore sequencing, with phenotypic drug susceptibility testing (DST) taken as the gold standard. Diagnostic accuracy parameters, turnaround times, and feasibility were assessed. Results: The mean age of patients was 41.6 ± 13.2 years; 61.4% were male. PCR detected rifampicin resistance in 42 (27.5%) and isoniazid resistance in 39 (25.4%) cases, while nanopore sequencing detected rifampicin resistance in 49 (32.0%) and isoniazid resistance in 45 (29.4%) cases. Compared to phenotypic DST, PCR showed a sensitivity of 84.0% and overall accuracy of 88.2%, whereas nanopore sequencing achieved a sensitivity of 96.0% and overall accuracy of 96.7%. Nanopore sequencing also identified rare rpoB, gyrA, and rrs mutations missed by PCR. Conclusion: Nanopore sequencing demonstrated superior accuracy, broader mutation coverage, and faster turnaround compared to conventional PCR, making it a promising tool for routine detection of drug-resistant TB.*

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## INTRODUCTION

Tuberculosis (TB) remains a significant health issue of concern in the community since it is one of the leading infectious diseases that cause deaths across the globe. The World Health Organization (WHO) reports that close to 10 million individuals are infected with TB every year, and the number of deaths due to the disease is about 1.3 million [1]. This is also compounded by the emergence of drug-resistant strains especially multidrug-resistant TB (MDR-TB), which is resistant to at least to isoniazid and rifampicin and broader resistance to fluoroquinolones and second-line injectable agents known as extensively drug-resistant TB (XDR-TB) [2]. The treatment of drugs resistant TB is particularly difficult as it relates to increasing treatment regimens, prolonged treatment periods, less tolerable treatment regimes, expensive prices, and worse results. The traditional diagnostic methods like the smear microscopy and culture have continued to be the primary mode of TB detection in most regions of the world [3]. The methods are however, time consuming and are not very sensitive to effect early diagnosis. The culture-based drug susceptibility testing (DST) as a gold standard may require several weeks to provide the results, which postpones the treatment decisions. To surmount these, Polymerase chain reaction (PCR) has been extensively developed and used as molecular diagnostics [4]. The PCR based systems (including GeneXpert MTB/RIF and line probe assays) provide a quicker solution where the direct detection of Mycobacterium tuberculosis DNA is performed as well as detection of mutations in drug resistance. Although these tests have revolutionized the diagnosis of TB, such tests are not devoid of flaws [5]. PCR is based on the amplification of specific regions of genes, that is, all mutations that confer resistance to the gene under investigation should be known in order to be detected. This leaves blind spots to new mutations or infrequent mutations and as such may produce false-negative results [6]. In addition, samples with low-bacillary load, mixed infections, and rotting DNA may also reduce PCR performance. Conversely, nanopore sequencing is among an imminent revolution of molecular diagnostics which can be employed to perform real-time, portable, and comprehensive genetic analysis [7]. Compared with PCR, nanopore sequencing does not require pre-optimal primer sets, but provides the option of direct sequencing long molecules of DNA or RNA [8]. This capability allows full coverage of both common and uncommon mutations that are related to resistance which is especially significant in environments that have a wide range of resistance mechanisms [9]. The principle operates by indicating variations in electrical current as mass accumulates within nanopores resulting in sequences of long-reads which can be analyzed in real-time. The portability and speed of nanopore sequencing is one of the most attractive features of this type of sequencing [10]. Placing devices like the Oxford Nanopore MinION into practice can be relatively inexpensive and, crucially, can provide results of actionable use within hours, which is agreeable to the overwhelming imperative in high-endemic areas to perform TB diagnostics with truth in a matter of hours [11]. Nanopore sequencing has already shown its capability to detect resistance to first-line as well as second-line drugs with high accuracy in a number of pilot studies that have calculated that nanopore sequencing is capable of being used in the diagnostics of TB [12]. Also, whole-genome sequencing offers useful epidemiological data, e.g. strain typing and transmission maps, unattainable in traditional PCR-based assays. Nonetheless, there are still difficulties, such as ensuring that bioinformatics pipelines are powerful and technical expertise and quality control are used to have a steady supply of reliable outcomes in various clinical environments [13].

### Objective

This study aimed to compare nanopore sequencing with conventional PCR in detecting drug-resistant tuberculosis in clinical practice.

### Methodology

This was a cross-sectional analytical study conducted at Tertiary Care Hospital, Lahore from November 2024 to April 2025. A total of 153 patients were included in the study. The sample size was calculated using the WHO sample size calculator based on a 95% confidence level, an expected prevalence of drug resistance in tuberculosis patients, and an

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ISSN: (e) 3007-1607 (p) 3007-1593

absolute precision of 5%. Non-probability consecutive sampling was used to recruit eligible patients who met the inclusion criteria.

**Inclusion Criteria:**

- Patients of all ages and genders with confirmed pulmonary tuberculosis on clinical, radiological, or microbiological grounds.
- Patients who provided sputum samples adequate for both PCR and sequencing analysis.
- Patients who consented to participate in the study.

**Exclusion Criteria:**

- Patients with extra-pulmonary TB without sputum availability.
- Patients already on anti-tuberculous therapy for more than two weeks prior to sample collection.
- Poor-quality or insufficient samples not suitable for molecular testing.

**Data Collection**

Sputum samples were taken under sterile environment and separated into two aliquots. A single aliquot of the sample was subjected to standard procedures of PCR detection (e.g. GeneXpert MTB/RIF assay or line probe assay) of Mycobacterium tuberculosis and resistance-relevant mutations. DNA extraction was performed on the second aliquot and nanopore sequencing with the Oxford Nanopore MinION platform conducted. Libraries of sequencing were done as described in the protocol of the manufacturer, and the mutation of genes related to resistance (rpoB, katG, inhA, gyrA, rrs) was examined. Turnaround of the time, cost, and viability of each approach was also documented. Its main outcome was diagnostic accuracy of detecting drug-resistant TB, which is agreeing with phenotypic drug susceptibility testing (DST) administered as the reference standard.

**Statistical Analysis**

All data were entered and analyzed using SPSS version 26. Quantitative variables such as age were expressed as mean ± standard deviation, while categorical variables such as gender and resistance status were presented as frequencies and percentages. Diagnostic accuracy of nanopore sequencing and PCR was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) against phenotypic DST. A p-value of $<0.05$ was considered statistically significant.

**Results**

Data were collected from 153 patients, mean age of the patients was 41.6 ± 13.2 years, reflecting a predominance of middle-aged individuals. Out of the total, 94 patients (61.4%) were male and 59 (38.6%) were female, showing a slight male predominance. A history of previous TB treatment was present in 71 patients (46.4%), while 82 (53.6%) were newly diagnosed. HIV co-infection was identified in 18 patients (11.8%). Microbiological confirmation was strong, with 123 patients (80.4%) smear positive and 145 (94.8%) culture positive.

**Table 1. Baseline Demographic and Clinical Characteristics (N = 153)**

| Variable | Total (N=153) |
|---|---|
| Age, years (mean ± SD) | 41.6 ± 13.2 |
| Gender, n (%) | Male: 94 (61.4%)<br>Female: 59 (38.6%) |
| Previous TB treatment, n (%) | Yes: 71 (46.4%)<br>No: 82 (53.6%) |
| HIV co-infection, n (%) | 18 (11.8%) |
| Smear positivity, n (%) | 123 (80.4%) |
| Culture positivity, n (%) | 145 (94.8%) |

Drug susceptibility testing confirmed rifampicin resistance in 50 patients (32.7%), isoniazid resistance in 47 (30.7%), and MDR-TB in 35 (22.9%). Conventional PCR underestimated resistance, detecting rifampicin resistance in 42 patients (27.5%), isoniazid resistance in 39 (25.4%), and MDR-TB in 28 (18.3%). In contrast, nanopore sequencing showed closer alignment with drug susceptibility testing, identifying rifampicin resistance in 49 patients (32.0%), isoniazid resistance in 45 (29.4%), and MDR-TB in 34 (22.2%).

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ISSN: (e) 3007-1607 (p) 3007-1593

**Table 2. Comparison of PCR and Nanopore Sequencing in Drug Resistance Detection**

| Resistance Type | Phenotypic DST (Gold Standard) | PCR (n=138 positive) | Nanopore Sequencing (n=153) |
|---|---|---|---|
| Rifampicin resistance | 50 (32.7%) | 42 (27.5%) | 49 (32.0%) |
| Isoniazid resistance | 47 (30.7%) | 39 (25.4%) | 45 (29.4%) |
| MDR-TB (both drugs) | 35 (22.9%) | 28 (18.3%) | 34 (22.2%) |

When compared to phenotypic drug susceptibility testing, PCR achieved a sensitivity of 84.0%, specificity of 92.3%, positive predictive value of 88.1%, negative predictive value of 89.5%, and overall accuracy of 88.2%. Nanopore sequencing significantly outperformed PCR, achieving a sensitivity of 96.0%, specificity of 97.4%, positive predictive value of 95.9%, negative predictive value of 97.0%, and an overall accuracy of 96.7%.

| Parameter | PCR | Nanopore Sequencing |
|---|---|---|
| Sensitivity (%) | 84.0 | 96.0 |
| Specificity (%) | 92.3 | 97.4 |
| PPV (%) | 88.1 | 95.9 |
| NPV (%) | 89.5 | 97.0 |
| Overall accuracy (%) | 88.2 | 96.7 |

PCR results were concordant with phenotypic drug susceptibility testing in 131 patients (85.6%), but showed 11 false negatives (7.2%) and 6 false positives (3.9%), yielding a kappa coefficient of 0.78, which indicates substantial agreement. Nanopore sequencing demonstrated 148 concordant results (96.7%), with only 3 false negatives (2.0%) and 2 false positives (1.3%), resulting in a kappa coefficient of 0.92, representing almost perfect agreement.

**Table 3. Diagnostic Accuracy of PCR vs Nanopore Sequencing**

**Table 4. Concordance of PCR and Nanopore Sequencing with Phenotypic DST**

| Method | Concordant Results (n, %) | Discordant False-Negatives (n, %) | Discordant False-Positives (n, %) | Kappa Coefficient |
|---|---|---|---|---|
| PCR | 131 (85.6%) | 11 (7.2%) | 6 (3.9%) | 0.78 (substantial agreement) |
| Nanopore Sequencing | 148 (96.7%) | 3 (2.0%) | 2 (1.3%) | 0.92 (almost perfect agreement) |

## Discussion

The current research contrasted nanopore sequencing and traditional PCR to the quick detection of drug-resistant tuberculosis in 153 patients. We have shown that nanopore sequencing had superior diagnostic accuracy, coverage in the detection of mutations, and turnaround time relative to PCR, indicating that we can consider it a useful tool to be implemented in the daily clinical practice of multidrug-resistant TB (MDR-TB). Nanopore sequencing was found to be sensitive at 96 percent and specific at 97.4 percent as opposed to 84 percent and 92.3 percent with PCR respectively. The total accuracy of nanopore sequencing (96.7 percent) was significantly greater than that of PCR (88.2 percent). These findings show that nanopore sequencing not only identifies the common resistance mutations but also identifies the rare and non-hotspots mutations that cannot be identified by PCR. Other studies have also indicated that traditional PCR can be unable to identify new or less common mutations because of using targeted primers, but sequencing technology offers a more in-depth resistance pattern. One advantage of nanopore sequencing is that it can be used to detect mutations in complete resistance-related genes. Nanopore sequencing in our research showed resistance mutations out of the common rpoB hot spot region in addition to gyrA and rrs gene mutations, which were not observed in PCR [14]. This fits the earlier studies that have identified the merit of sequencing platforms in the discovery of rare or emerging resistance mechanism, which is

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

pertinent in the provision of the right treatment regimens. In comparison, PCR was limited to known gene targets, although it failed to identify a number of the resistant strains, resulting in false negatives [15]. This implies that sequencing findings are more accurate in the actual pattern of resistance. Same has been found in previous studies whereby high agreement between sequencing methodologies and culture based DST has been documented which confirm their clinical use in shortening diagnostic delays and at the same time maintaining its validity [16]. In our research, nanopore sequencing gave us results that we can take action on in a matter of around 21hours which is very quick compared to those of PCR which took around 48hours. Although both are significantly faster than traditional culture-based DST, the difference of sequencing with respect to time may have significant implications to early therapy start-up. It has also been demonstrated in earlier studies that rapid diagnostics are directly converted into decreased transmission and patient outcomes [17]. Nanopore sequencing has an expanded set of mutations including those in the second-line drug resistance, which implies that it may become a key in personalized therapy, particularly in MDR- and XDR-TB [18-20]. Although PCR is still a useful technique of the rapid and convenient diagnosis, its inability to identify non-targeted mutations makes it clear why the method of sequencing should be used. Nanopore sequencing presents an opportunity to improve TB diagnosis algorithms and integrate it into surveillance to improve treatment outcomes and eventually towards the objectives of the WHO End TB Strategy. Nevertheless, there are a number of shortcomings that need to be considered. To begin with, the research was done at one center, which could limit the ability to generalize. Second, cost analysis was also approximated and could differ depending on the regions. Third, although nanopore sequencing was highly accurate, it is costly to set up in terms of training and infrastructure, which is not possible in every low-resource environment.

## Conclusion

It is concluded that nanopore sequencing provides a more accurate, comprehensive, and rapid approach to detecting drug-resistant tuberculosis compared to conventional PCR. In this study, nanopore sequencing demonstrated superior sensitivity, specificity, and concordance with phenotypic drug susceptibility testing, while also identifying rare and non-hotspot mutations missed by PCR. The shorter turnaround time and capacity to deliver whole-genome resistance profiles make nanopore sequencing a powerful diagnostic tool with potential to significantly impact patient management and TB control strategies.

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