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Rifampicin-resistant *Mycobacterium tuberculosis and* unsuccessful results from Xpert® MTB/Rif-Ultra assay in Amhara Region, Ethiopia

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A R T I C L E I N F O

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MDR-TB
Unsuccessful results
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A B S T R A C T

Background: Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis (Mtb),* causes 10 million new infections and 1.3 million deaths annually. The treatment of TB is hampered by the increasing incidence rate of drug resistance associated with TB. To diagnose TB and identify drug-resistant TB cases, rapid molecular technologies such as Xpert MTB/RIF, Truenat MTB, MTB Plus, and MTB-RIF Dx tests are recommended by the World Health Organization (WHO) and rolled out globally. Xpert MTB/RIF-Ultra assay is the most widely used in developing countries like Ethiopia. However, this rapid technology has inherent limitations, such as error reports, invalid results, and no results collectively reported as unsuccessful tuberculosis results. The purpose of this study was to retrospectively evaluate the trend of rifampicin resistance and unsuccessful results in the Xpert MTB/RIF-Ultra assay facility of Northwest Ethiopia.

Methods: Retrospective data archived in the Amhara Public Health Institute (APHI) TB laboratory from 2019 to 2024 were reviewed. Xpert MTB/RIF-Ultra software data were retrieved and transferred to Microsoft Excel. Then, it was checked for completeness, cleaned manually, and imported to Statistical Package for the Social Sciences (SPSS) version 25 software. The rate of *mycobacterium tuberculosis (Mtb.)* positives, multi-drug resistance tuberculosis (MDR-TB), and Unsuccessful results were analyzed from the total and year-wise. The final results were depicted using tables and different charts.

Results: From June 30, 2019, to June 30, 2024, a total of 587,128 sputum samples were obtained from presumptive TB patients in 111 GeneXpert sites in the Amhara Region. Of these samples analyzed using Xpert MTB/RIF-Ultra, 6.17 % (36,212/587,128) were Mtb positive. Furthermore, the overall proportion of rifampicin resistance (RR) among Mtb-confirmed cases decreased to 3.03 % (1,096/36,212) and showed a downward trend from 4.62 % (184/3979) in 2020 to 2 % (176/8806) in 2024. The overall unsuccessful results (error, invalid & no result) were 6.48 %. The rate of unsuccessful results remained above the national target of < 5 % throughout the study periods.

Conclusion and recommendation: The rate of *Mtb* and MDR-TB showed a decreasing trend in the last six years in Northwest Ethiopia. However, unsuccessful results remained above the national target. The cause of unsuccessful results should be investigated, and the Xpert MTB/RIF-Ultra-related quality assurance system must be enhanced to reduce the rate of Xpert MTB/RIF-Ultra unsuccessful results.

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1. Background

Tuberculosis (TB) is a chronic and complex infectious disease caused by *Mycobacterium tuberculosis (M. tuberculosis,* Mtb*)*, causing over 10 million new infections and 1.3 million deaths annually at a global scale [1]. Tuberculosis mostly affects adults in their most productive years [2]. While the lung is the primary site of TB disease initiation, TB can affect anybody outside the lung parenchyma, such as lymph nodes, kidneys, brain, spine, and skin [3,4]. There are countries globally where MDR-TB continues to emerge and is still rising in certain areas, threatening the TB control program [5,6].

Even though WHO declared that Ethiopia is out of the 30 high MDR-TB burden countries [7]; an upsurge in MDR was documented in several parts of Ethiopia and made a great impact on tuberculosis treatment. The pooled rate of MDR-TB was 7.24% between 1997 and 2017 [8], and increased by 12.86% from 1990 to 2015, and by 1.3% from 2016 to 2019 in Ethiopia [9] respectively. The prevalence of MDR-TB was also 15.8 % in Central and 7.42% in West Ethiopia; [10] and [11] respectively.

Tuberculosis diagnosis relied on Microscopy (ZN/FM microscopy), rapid molecular diagnostics (Xpert MTB RIF, line probe assay, TB-LAMP), culture, immunological techniques (TB-LAM) and clinically with the assistance of X-ray, ultrasound, histopathology and biochemical analysis [12]. Currently, due to high sensitivity, short turnaround time and ability to simultaneously detect Mtb and DR-TB, Xpert MTB/RIF-Ultra is used as a primary bacteriological method for diagnosis of TB in developing countries [13,14].

Despite being preventable, treatable, and curable, Tuberculosis continues to be a current global health threat including sequelae, and higher cost and complexity [15]. This ancient disease continues to kill more people each year than HIV and malaria combined and remains the leading causative agent of morbidity and mortality in low- and middle-income countries [4,9,16,17].

What is the supplementary bad of tuberculosis, in recent years, the rate of multidrug-resistant tuberculosis has been increasing at an alarming rate and is a major public health concern in the whole world, and it remains higher in several countries [16,18–21]. Moreover, studies noted an upward trend in the prevalence of Multi-Drug Resistance Tuberculosis [22–24]. Even, a 0.6% per year increase in resistant tuberculosis was observed after the implementation of the GeneXpert® MTB/RIF-Ultra Test [25].

Acid Fast Bacilli Microscopy and Tuberculosis Culture played the lion's share in diagnosing and treating tuberculosis. However, sensitivity and prolonged time are the crucial constraints of the microscopy and TB culture respectively in the era of molecular techniques such as GeneXpert MTB/RIF assay technology relief both the issue of sensitivity of the AFB microscope and the prolonged time of TB culture. Nevertheless, unsuccessful results such as error, invalid and no results are the other challenges facing the GeneXpert MTB/RIF assay technology. As studies noted, the rate of unsuccessful rate remains high in several countries and hurts diagnosis and early treatment of TB. The overall rate of unsuccessful results was 5.7% in India [26], 11.0% in Nigeria [27], and 9.6% in other part of Ethiopia [28] respectively

Data about the rate of GeneXpert MTB/RIF assay unsuccessful results and DR-TB in the Amhara Region is inadequate, calling current epidemiological data in the Region. Additionally, continuous surveillance and regular monitoring of TB and DR-TB and unsuccessful results are critical for effective intervention plans and control methods. Despite reports on DR-TB in Ethiopia, data on the prevalence and trend of DR-TB as well as unsuccessful results in the study area is still limited. As a result, the purpose of this study was to determine the trend of DR-TB and unsuccessful results in the Amhara Region, Ethiopia.

2. Materials and methods

2.1. Study design, area, period, and setting

A retrospective study was conducted using data archived in GeneXpert sites in Amhara Regional State, Ethiopia between June 30, 2019, to June 3024. Amhara region has about 111 GeneXpert sites serving more than 23 million people. Amhara National Regional State had 99 General Hospitals and 917 Health Centers serving more than 25 million people. The number of facilities offering the Xpert MTB/RIF-Ultra test has increased from three in 2014 to 111 in 2024 in the Region. The data collection period was from June 2019 to June 2024.

2.2. Study population and variables

This study used Xpert® MTB/RIF-Ultra assay data obtained from TB presumptive cases in the Amhara regional state. The study used all GeneXpert results archived in the study period in the Amhara region. Data from GeneXpert software was extracted and transferred to an Excel sheet and then imported into SPSS version 20. Xpert® MTB/RIF-Ultra assay results, which contain either successful or unsuccessful results, were included.

2.3. Operational definitions

Utilization rate: The utilization rate of the machine was calculated based on a 2-h turnaround time of the machine, assuming an average of 6-h working period per day within the laboratory (i.e., for a four-module machine, it should run 12 tests per day) [29];

Utilization rate = Total tests performed by GeneXpert machine in the given period X 100

GeneXpert capacity expected to test in the given period

Unsuccessful results: GeneXpert results including error, invalid, and no result

Invalid: a result by the GeneXpert machine when the Sample Processing Control (SPC) did not meet the acceptance criteria

Error: A result by the GeneXpert system when Probe Check Control failed due to many factors, and the assay was aborted

No Result: the machine fails to produce a result due to power failure or is stopped by the operator before the test completion by the machine [30].

2.4. Data quality assurance, analysis, and interpretation

The data were obtained from facilities where external quality assurance (EQA) is in place. To maintain the quality of the data, manual entry was avoided. Data from Xpert MTB/RIF-Ultra software was retrieved and transferred to Microsoft Excel, checked for completeness, cleaned manually, and imported to SPSS version 25 software. The rates of *M. tuberculosis*, MDR-TB, and unsuccessful results were computed and illustrated using tables and graphs.

2.5. Ethical consideration

The study was approved by the Ethical Review Committee (ERC) of the Health Research Development Directorate of Amhara regional state, Ethiopia with reference number NoH/R/T/T/D/07/88. Then support was obtained from ERC and submitted to each data collection site before endorsing the study.

2.6. Result dissemination

The final article will be presented in the APHI FoRSe Seminar Participation. The research findings will be disseminated by publishing in a trustworthy international journal and presented at scientific conferences/meetings.

3. Results

The number of Xpert MTB/RIF-Ultra assay facilities has increased from 25 in 2019 to 111 in 2024 in the region. Correspondingly, the utilization of Xpert MTB/RIF machines showed an upward trend from 2019 to 2023. However, the utilization rate of the machine fell from 88 % in 2023 to 59 % in 2024 (Fig. 1).

3.1. Expert® MTB/RIF assay results

From June 30, 2019, to June 30, 2024, a total of 587,128 samples from presumptive TB patients were examined for Mtb in the GeneXpert sites in the Amhara Region. The absolute number of tests conducted each year from 2019 to 2024 has more than doubled, from 62,065 to 166,349. Of these samples analyzed, the highest rate of successful results was documented in 2019 (94.57 %), followed by 2023(93.69 %). On the contrary, the highest rate of unsuccessful results was reported in 2022 (7.66 %) followed by 2024(6.53 %). Even though the rate of Mtb positivity rate declined from 8.66 % in 2019 to 5.29 in 2024, the actual number of Mtb confirmed cases were increased from 5,375 in 2019 to 8, 806 in 2024. The overall positivity rate of *M. Tuberculosis* in the Amhara region was 6.17 % (36,212/587,128), and the absolute number of rifampicin-sensitive Mtb rose from 5,114 in 2019 to 8,630 in 2024. Then, the overall rate of Rifampicin resistance (RR) among Mtb confirmed cases was gauged at 3.03 % (1,096/36,212) (Table 1).

The trend of unsuccessful result rate was analyzed by years along the National targets in the Amhara Region, and miserably, the rate of unsuccessful results remained above the national target (<5%) in the last six years. In contrast, the rate of invalid results in the Region fell under the National target (<1) over the previous six years. In addition, the GeneXpert error rate of the region falls under the national target (<3) in the previous two years (Fig. 2).

The overall unsuccessful results of Xpert MTB/RIF ultra were 6.48 % with error, invalid, and no result rates of 3.09 %, 0.46 %, and 2.93 %, respectively. The trend of unsuccessful results over the six years was almost constant (Table 1), and the rate of Mtb and RR showed declined trend over the six years (Fig. 3).

3.2. Discussions

This study aimed to analyze the trends of *M. tuberculosis*, MDR-TB detection, utilization rate, and the rate of unsuccessful results by Xpert MTB/RIF-Ultra in Amhara Reginal State. Substantial year-to-year GeneXpert machine distribution was increased in the region due to the national scale-up program (from 25 in 2019 to 111 in 2024). Consequently, in the last six years (2019 – 2024), more than half a million (587,128) samples from presumptive TB patients were examined for MTB in the region, with an upward utilization rate of 40 % to 88 % in 2019 to 2023. However, the utilization rate of the Xpert MTB/RIF-Ultra fell from 88 % in 2023 to 59 % in 2024 because of the shocking civil war in the Amhara region, and the continuous road lockdown triggered chaos in hospitals and primary care services as well as sample referral systems.

The absolute number of Mtb in the study period rose from 5,375 in 2019 to 8,806 in 2024. Consistent with our data, the year trend of Mtb cases rose from 25 Mtb in 2015 to 415 Mtb cases in 2019 in southern Nigeria [31]. The upsurge of Mtb in number was also seen from 2018 to 2021; 124 and 371 confirmed Mtb cases, respectively in Gondar, Ethiopia [11]. The actual number of Mtb showed an increment in Addis Ababa, Ethiopia, from 503 in 2016 to 518 in 2018 [32]. It was also the number of Mtb also increased from 61 in 2018 to 89 in 2023 in Tigray, Ethiopia [33]. However, the rate of Mtb in our data showed a year-to-year decline, which is supported by global reports of tuberculosis incidence from 1990 to 2019 [34]. This may be the investment outcome of the WHO End TB Strategy, which sets targets for 2030, with an 80 % decrease in TB incidence compared to 2015 [35]. The other possible reason may be that the absolute number of tests conducted each year from 2019 to 2024 has more than doubled from 62,065 to 166,349 in our result, which may affect the rate of Mtb.

In this study, the overall positivity rate of *M. tuberculosis* detection among TB-suspected patients was 6.17 % in the study periods, which was comparable with previous studies from Addis Ababa, Ethiopia 6.5 % [36] and 7.9 % in selected zones of Tigray, Northern Ethiopia [37].

However, it was lower compared to other studies in Tigray, Ethiopia 9.7 %, 11.7 %, and 24.3 % [33,38,39] respectively, 11.8 %, 27.3 %, and 30.5 % [40–42] in the Southern part of Ethiopia individually, 20.0 % in Gambella Regional State, Southwest Ethiopia [43], 24.5 % in the Afar Region, the North East part of Ethiopia [44] 17.0 % in Addis Ababa, Ethiopia [45]. The possible reason is the large sample size used in our study, while the corresponding studies in Ethiopia have lower sample sizes, which may lead to higher TB prevalence.

Studies from other countries also showed a higher rate of TB detection; corresponding to 12.9 % of southwest Nigeria [46], 15.5 % in Kebbi state, Nigeria [47], 16.9 % in Benue State, Nigeria [48], 11 % in eastern Democratic Republic of the Congo [49], 21.2 % in Central Africa Gabon [50], 9.4 % in South Africa [51], and 14 % in Swaziland [52]. On the other hand, the observed rate of TB detection in the present study was higher than the 4.9 % obtained from Port Harcourt, Nigeria [53], and 3.5 % in Uganda [54]. This variation may be due to differences in study design, timelines, and study durations between the present assessment and the previous studies, with most former studies covering shorter years.

In our data, the rate of MDR-TB showed a year-to-year decline in the region, which may be because viral load suppression rates increased as viral load testing coverage significantly improved over time from 2015

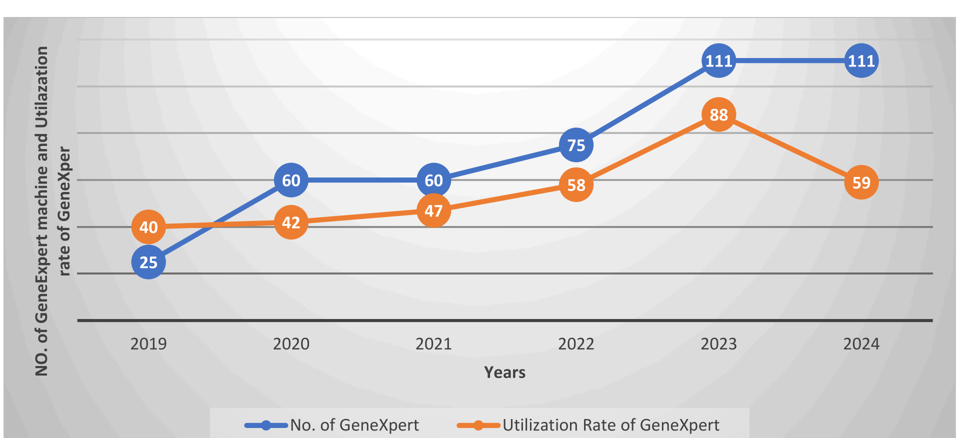

Fig. 1. Trend of Regional GeneXpert Expansion and Utilization Rate in Amhara Reginal State (2019–2024).

Table 1
Frequency and rate of Xpert MTB/RIF assay results in the Amhara Region (2019 – 2024).

Years	Total Tests	SR N (%)	OUN N (%)	E N (%)	I N (%)	NR N (%)	T N (%)	RR* N (%)	PR N (%)
2019	62,065	58,692(94.57)	3,374(5.43)	1,749(2.82)	248(0.40)	1,376(2.22)	5,114(8.24)	198(3.68)	5,375(8.66)
2020	55,769	52,229(93.65)	3,540(6.35)	1,933(3.47)	208(0.37)	1,399(2.51)	3,740(6.71)	184(4.62)	3,979(7.13)
2021	66,269	62,004(93.56)	4,265(6.44)	2,581(3.89)	203(0.31)	1,481(2.23)	4,638(7.00)	187(3.76)	4,971(7.50)
2022	78,824	72,783(92.34)	6,041(7.66)	2,636(3.34)	336(0.43)	3,069(3.89)	5,074(6.44)	181(3.35)	5,406(6.86)
2023	157,852	147,889(93.69)	9,963(6.31)	4,326(2.74)	1,031(0.65)	4,606(2.92)	7,505(4.75)	170(2.21)	7,675(4.86)
2024	166,349	155,485(93.47)	10,864(6.53)	4,910(2.95)	695(0.42)	5,259(3.16)	8,630(5.19)	176(2.00)	8,806(5.29)
Overall	**587,128**	**549,082(93.52)**	**38,046(6.48)**	**18,135(3.09)**	**2,721(0.46)**	**17,190(2.93)**	**34,701(5.91)**	**1,096(3.03)**	**36,212(6.17)**

Keys: SR: successful results, OUN: overall unsuccessful results, RR: rifampicin resistant Mtb, T: rifampicin sensitive Mtb, E: error, I: invalid, NR: no result, TI: Mtb positive Rifampicin resistance indeterminate, PR: positivity rate, N: frequency.
* **Rate of RR**= number RR cases in the given period X100/ total number of confirmed TB cases in the given period ⇒ % RR = N of RR X100/N of P.
NB: Rate of SR, OUT, E, I, NR, T, and PR were calculated from total tests performed in their respective given years.

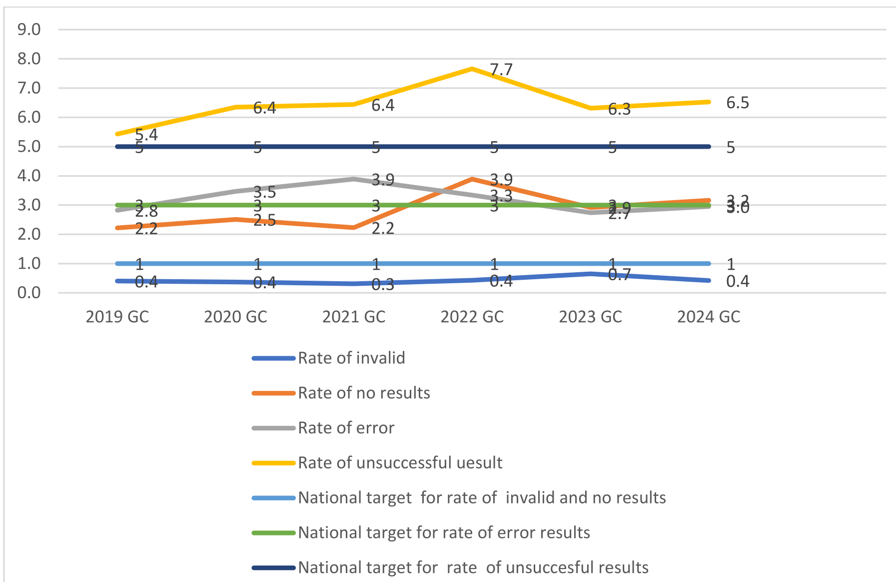

Fig. 2. Trend of GeneXpert's unsuccessful results in the Amhara Region (2019– 2024).

to 2021 in the Amhara region [55] as the success of TB treatment depends on factors such as HIV [56]. Even though great caution and profound research are needed to interpret the relation between viral load and MDR-TB, we believe that viral load suppression may improve immune response, leading to better treatment success, as it helps fight the *Mtb* bacteria. Supporting our explanation, a research noted that Viral load suppression in people living with HIV is strongly associated with improved TB treatment outcomes [57]. Scholars also found that a detectable viral load at MDR-TB treatment initiation was more likely to experience treatment failure [58] and MDR-TB and HIV coinfected patients were less likely to be successfully treated than HIV negative MDR-TB patients [59].

Additionally, earlier in our study period, Ethiopia had adopted the ENDTB 90-(90)-90 2020 targets and made the five-year strategic plan to ensure 90 % of all people with tuberculosis are diagnosed and treated, ensure 90 % of the key populations in the country are diagnosed for TB, ensure 90 % of people diagnosed complete treatment may also the reason to decline the MDR-TB in the region [60].

Consistent findings were noted in a study in the Oromia region, Ethiopia [61], in Adis Ababa, Ethiopia [45], and a *meta*-analysis in Sub-Saharan Africa [62], the global report from 2000 to 2018) [63]. In contrast, the South African Tuberculosis Drug-Resistant TB survey noted an increase in MDR-TB as compared to our findings [64]; this discrepancy might be due to the significant geographical variation of TB lineage distribution as studies showed that the Central African and East African-Indian lineages were confined to East Africa while the East Asian lineage was predominantly found in Southern Africa [65,66]. More meaningfully, *Mycobacterium tuberculosis* Sub-Lineage 4.2.2/ SIT149 was found as dominant Drug-Resistant Clade in our study area which supports our hypothesis to the differences of the results [67].

The overall rate of Rifampicin resistance *M. tuberculosis* among MTB positives was 3.03 % in our study. Comparable results were reported in Southwest Ethiopia (4.2 %) [41], in Ethiopia (4.8 %) [68], in Nigeria (3.3 %)[53], WHO estimates of MDR-TB in new cases in the Eastern Mediterranean region(3.4 %) [69] and 3.4 % of new TB cases globally [70].

Particularly, the observed MDR-TB detection in the present study was lower than the Nigerian study (7.5 %) [46], 9 % in Central Africa Gabon [50], a study from Gedeo Zone, Southern Ethiopia (5.1 %) [40], Central Tigray, Ethiopia(8.1 %) [33], in selected zones of Tigray(8.7 %) [37], in Northwest Ethiopia 7.42 % [11], in Addis Ababa (9.9 %) [45], in Botswana (8 %) [71], and Swaziland(12 %) [52].

In contrast, a lower rate of MDR-TB was reported in southern Ethiopia (1.24 %) [42], in Tigray, Northern Ethiopia (0.7 %) [38], in the Eastern zone of Tigray, North Ethiopia(2.2 %) [39], in sub-Saharan Africa (2.1 %) [62], 0.54 % in South Africa [51], among new cases in Brazil(1.2 %) [72].

This variation may be due to the difference in the study population, timelines, and sample size. Moreover, variation in study participants with relapse, HIV status, return after lost follow-up, and treatment

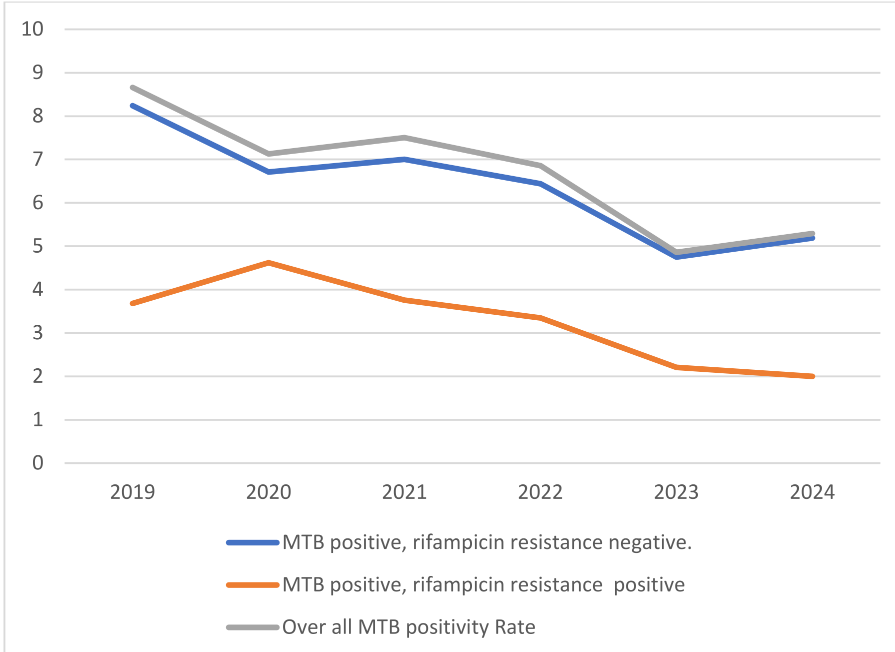

Fig. 3. Trends of GeneXpert positivity, Rifampicin Resistance, and sensitive *M. Tuberculosis* rate by years in the Amhara Region (2020–2024).

failure are among the determinants of multidrug-resistant tuberculosis that affect the prevalence of MDR-TB among the population [73].

Having excellent sensitivity and short turnaround time for Tuberculosis tests, unsuccessful results such as error, invalid, and no results are the challenges facing the GeneXpert MTB/RIF-Ultra assay technology. In the present study, the trend of unsuccessful result rate was analyzed by years along the National targets in the Amhara Region, and miserably, the rate of unsuccessful results remained above the national target (<5%) [74] in the last six years. Moreover, the findings showed an upward trend of unsuccessful results during 2019 and 2022. This rise in unsuccessful results may be due to the devastating impact of COVID-19 19 followed by the destructive Civil war in the Amhara region and the continuous road lockdown that triggered chaos in hospitals, primary care services, and sample referral systems. These two disasters tackle mentoring, onsite evaluation for continual improvement, training, and capacity building to laboratory technicians and health centers by regional reference laboratory experts.

The rate of inclusive unsuccessful results in our data was 6.48 % during the study periods. Comparable results (6.3 %) were reported by Ardizzoni, Fajardo et al. [75], results in three countries(Pakistan(5.9 %), Moldova(5.9 %), and Bangladesh (6.9 %) respectively) [76], 7 % in Swaziland [52], 5.7 % [26] and 7.2 % in India [77], and 9.6 % in Ethiopia [68] correspondingly.

In contrast, a lower rate of unsuccessful results was documented in South Africa 1.9 % [51], in Shanghai, in China 2.37 % [78]. On the other hand, a higher rate of unsuccessful results as compared to our data was also reported in Botswana (15 %) [71], 11.0 % in Nigeria [27], and 9.6 % in other parts of Ethiopia [28] respectively.

These variations may be due to the differences in underlying causes of unsuccessful tests such as sample processing, power supply, cartridge/module related, temperature-related, environmental dust-related, or other GeneXpert-related supported by some studies [71,79,80].

3.3. Limitations of the study

As we use archived secondary data, sociodemographic and clinical factors are not included to analyze the statistical association between dependent and independent variables.

3.4. Conclusion and Recommendations

The rate of *M. tuberculosis* and multidrug-resistant tuberculosis has declined in the last six years in the Amhara region. However, unsuccessful results remain above the national target. The cause of unsuccessful results should be assessed, and continuous improvement on GeneXpert sites is required.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

All the data sets analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of competing interestS

The authors declare that they have no competing interests.

FUNDING.

There is no specific fund received for this study.

CONTRIBUTION OF AUTHORS.

GB, HG, TB, and YG contributed to conceiving the research idea, data collection, and data analysis. GB, AT, MG, TM, and MT contributed to the conception of the research idea, method rationalization, data analysis, results interpretation, scientific content evaluation, and manuscript preparation. DM and AT also reviewed and edited the manuscript. All authors have read and approved the final manuscript for submission.

Ethical consideration

The study was approved by the Ethical Review Committee (ERC) of the Health Research Development Directorate of Amhara regional state, Ethiopia, with reference number NoH/R/T/T/D/07/88. Then, support was obtained from the ERC,Ethiopia and submitted to each data collection site before endorsing the study.

CRediT authorship contribution statement

Gizeaddis Belay: Investigation. **Hailu Getachew:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tigist Birku:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Aimro Tadese:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Yosef Gashaw:** Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Michael Getie:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Tazeb Molla:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Molalign Tarekegn:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Daniel Mekonnen:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Alemayehu Abate:** Writing – review & editing, Supervision, Software, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] WHO. Global tuberculosis report. Geneva Switzerland; 2023.
[2] Dlodlo R, Brigden G, Heldal E, Allwood B, Chiang C, Fujiwara P, et al. Management of Tuberculosis: a Guide to Essential Practice. Paris, France: International Union Against Tuberculosis and Lung Disease, 2019. theunion org/sites/default/files/ 2020-08/TheUnion_Orange_2019 pdf. 2021.
[3] Alemie GA, Gebreselassie F. Common types of tuberculosis and co-infection with HIV at private health institutions in Ethiopia: a cross sectional study. BMC Public Health 2014;14(1):1–5.
[4] Organization WH. Tuberculosis in the WHO African Region: 2023 progress update. Tuberculosis in the WHO African Region: 2023 progress update2023.
[5] Dheda K, Mirzayev F, Cirillo DM, Udwadia Z, Dooley KE, Chang K-C, et al. Multidrug-resistant tuberculosis. Nat Rev Disease Primers 2024;10(1):22.
[6] Lv H, Zhang X, Zhang X, Bai J, You S, Li X, et al. Global prevalence and burden of multidrug-resistant tuberculosis from 1990 to 2019. BMC Infectious Diseases 2024; 24(1):243.
[7] Organization WH. WHO releases new global lists of high-burden countries for TB, HIV-associated TB and drug-resistant TB. Geneva, Switzerland: WHO. 2021.
[8] Girum T, Muktar E, Lentiro K, Wondiye H, Shewangizaw M. Epidemiology of multidrug-resistant tuberculosis (MDR-TB) in Ethiopia: a systematic review and meta-analysis of the prevalence, determinants and treatment outcome. Trop Diseases Travel Med Vacc 2018;4:1–12.
[9] Arja A, Tadesse S, Agachew M, Getnet F, Beksisa J, Mohammed S, et al. The burden of tuberculosis across regions in Ethiopia: a systematic subnational analysis for the global burden of disease study 2019. Ethiopian J Health Development 2023;37(2).
[10] Selfegna S, Alelign A. Detection of mycobacterium tuberculosis and rifampicin resistance using GeneXpert MTB/RIF assay at Enat Hospital, Central Ethiopia. Tuberculosis Research and Treatment. 2022;2022.
[11] Biset S, Teferi M, Alamirew H, Birhanu B, Dessie A, Aschale A, et al. Trends of Mycobacterium tuberculosis and Rifampicin resistance in Northwest Ethiopia: Xpert® MTB/RIF assay results from 2015 to 2021. BMC Infectious Diseases 2024; 24(1):238.
[12] MoH-Ethiopia. National Comprehensive Tuberculosis, Leprosy and TB/HIV Training Manual for Health Care Workers. Addis Ababa: Ministry of Health; 2018.
[13] Andama A, Jaganath D, Crowder R, Asege L, Nakaye M, Katumba D, et al. The transition to Xpert MTB/RIF ultra: diagnostic accuracy for pulmonary tuberculosis in Kampala, Uganda. BMC Infect Diseases 2021;21:1–7.
[14] Williams V, Calnan M, Edem B, Onwuchekwa C, Okoro C, Candari C, et al. GeneXpert rollout in three high-burden tuberculosis countries in Africa: A review of pulmonary tuberculosis diagnosis and outcomes from 2001 to 2019. African Journal of Laboratory Medicine 2022;11(1):1–8.
[15] Tiberi S, Utjesanovic N, Galvin J, Centis R, D'Ambrosio L, van den Boom M, et al. Drug resistant TB–latest developments in epidemiology, diagnostics and management. Int J Infect Dis 2022;124:S20–5.
[16] Bagcchi S. WHO's global tuberculosis report 2022. The Lancet Microbe 2023;4(1): e20.
[17] Arinaminpathy N, Mukadi YD, Bloom A, Vincent C, Ahmedov S. Meeting the 2030 End TB goals in the wake of COVID-19: a modelling study of countries in the USAID TB portfolio. PLOS Global Public Health 2023;3(10):e0001271.
[18] Mohammed KA, Khudhair GS, Al-Rabeai DB. Prevalence and drug resistance pattern of isolated from tuberculosis patients in Basra. Iraq Polish Journal of Microbiology 2022;71(2):205–15.
[19] Min S, Puxuan L, Weijun F, Yuanyuan H, Ruiyun L. The global tuberculosis report 2022: key data analysis for China and the global world. Electron J Emerg Infect Diseases 2023;8(1):87.
[20] Chunrong L, Hongxia F, Puxuan L, Lecai J. The global tuberculosis report 2021: key data analysis for China and the global world. Electron J Emerg Infect Diseases 2021;6(4):368.
[21] Salari N, Kanjoori AH, Hosseinian-Far A, Hasheminezhad R, Mansouri K, Mohammadi M. Global prevalence of drug-resistant tuberculosis: a systematic review and meta-analysis. Infectious Diseases of Poverty 2023;12(1):57.
[22] Oladimeji O, Othman Y, Oladimeji KE, Atiba BP, Adepoju VA, Odugbemi BA. Patterns of presentation of drug-resistant tuberculosis in nigeria: A retrospective file review. Microbiol Res 2022;13(3):609–19.
[23] Tao NingNing TN, He XiaoChun HX, Zhang XianXin ZX, Liu Yao LY, Yu ChunBao YC, Li HuaiChen LH. Trends and characteristics of drug-resistant tuberculosis in rural Shandong. China 2017.
[24] Tao N-n, He X-c, Zhang X-x, Liu Y, Yu C-b, Li H-c. Trends and characteristics of drug-resistant tuberculosis in rural Shandong, China. Int J Infect Diseases. 2017;65: 8-14.
[25] Berra TZ, Bruce ATI, Alves YM, Ramos ACV, Giacomet CL, Arcêncio RA. Impact of the GeneXpert® MTB/RIF rapid molecular test on tuberculosis detection: temporal trends and vulnerable territories. Rev Lat Am Enfermagem 2021;29:e3441.
[26] Reddy R, Alvarez-Uria G. Molecular epidemiology of rifampicin resistance in Mycobacterium tuberculosis using the GeneXpert MTB/RIF assay from a rural setting in India. J Pathogens 2017;2017(1):6738095.
[27] Gidado M, Nwokoye N, Nwadike P, Ajiboye P, Eneogu R, Useni S, et al. Unsuccessful Xpert® MTB/RIF results: the Nigerian experience. Public Healthaction 2018;8(1):2–6.
[28] Kebede A, Beyene D, Yenew B, Diriba G, Mehamd Z, Alemu A, et al. Monitoring quality indicators for the Xpert MTB/RIF molecular assay in Ethiopia. PLoS One 2019;14(11):e0225205.
[29] Gidado M, Nwokoye N, Ogbudebe C, Nsa B, Nwadike P, Ajiboye P, et al. Assessment of GeneXpert MTB/RIF performance by type and level of health-care facilities in Nigeria. Nigerian Medical Journal 2019;60(1):33–9.
[30] GXMTB-ULTRA-MII R. Xpert® MTB/RIF Ultra. 2019.
[31] Alao MA, Ibrahim OR, Ogunbosi BO. Trends of Xpert MTB/RIF in the diagnosis of Mycobacterium tuberculosis and rifampicin resistance in Southwest Nigeria: A 4-year retrospective study. J Pan African Thoracic Soc 2022;4(1):31–41.
[32] Araya S, Negesso AE, Tamir Z. Rifampicin-resistant Mycobacterium tuberculosis among patients with presumptive tuberculosis in Addis Ababa. Ethiopia Infect Drug Resist 2020:3451–9.
[33] Gebremariam G, Kiros M, Hagos S, Hadush H, Gebremichael A, Gebrekirstos G, et al. Trend of pulmonary tuberculosis and rifampicin-resistance among tuberculosis presumptive patients in Central Tigray, Ethiopia; 2018-2023: a six-year retrospective study. Trop Diseases Travel Med Vaccines 2024;10(1):15.
[34] Dodd PJ, Yuen CM, Jayasooriya SM, van der Zalm MM, Seddon JA. Quantifying the global number of tuberculosis survivors: a modelling study. The Lancet Infectious Diseases 2021;21(7):984–92.
[35] Bai W, Ameyaw EK. Global, regional and national trends in tuberculosis incidence and main risk factors: a study using data from 2000 to 2021. BMC Public Health 2024;24(1):12.
[36] Sinshaw W, Kebede A, Bitew A, Tesfaye E, Tadesse M, Mehamed Z, et al. Prevalence of tuberculosis, multidrug resistant tuberculosis and associated risk factors among smear negative presumptive pulmonary tuberculosis patients in Addis Ababa, Ethiopia. BMC infectious diseases 2019;19:1–15.
[37] Dejene TA, Hailu GG, Kahsay AG, Wasihun AG. Mycobacterium tuberculosis and rifampicin-resistant tuberculosis among tuberculosis presumptive patients in selected zones of Tigray, Northern Ethiopia, 2016–2019. Heliyon. 2024;10(13).
[38] Wasihun AG, Dejene TA, Hailu GG. Frequency of MTB and rifampicin resistance MTB using Xpert-MTB/RIF assay among adult presumptive tuberculosis patients in Tigray, Northern Ethiopia: a cross sectional study. PLoS One 2020;15(11): e0240361.
[39] Abay G, Hailay B. Trends of Mycobacterium Tuberculosis and Rifampicin resistance in Adigrat General Hospital, Eastern zone, Tigray Region, Northern Ethiopia. 2020.
[40] Diriba K, Awulachew E, Churiso G. The magnitude of MTB and rifampicin resistance MTB using Xpert-MTB/RIF assay among tuberculosis suspected patients in Gedeo Zone, Southern Ethiopia. Infect Drug Resist. 2021:3961–9.
[41] Haile A, Haile K, Shemsu S. Trend and associated factors of Mycobacterium tuberculosis and rifampicin resistance in southwest Ethiopia: institutional based retrospective study. HealthSci J 2021;15(9):1–6.
[42] Andarge DB, Anticho TL, Jara GM, Ali MM. Prevalence of Mycobacterium tuberculosis infection and rifampicin resistance among presumptive tuberculosis cases visiting tuberculosis clinic of Adare General Hospital. Southern Ethiopia SAGE open medicine 2021;9:20503121211045541.
[43] Ejeta E, Beyene G, Bonsa Z, Abebe G. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and Rifampicin resistance in high human immunodeficiency virus setting in gambella regional state, southwest ethiopia. J Clin Tuberculosis Other Mycobacterial Diseases 2018;12:14–20.
[44] Gebremedhn Bizayen Gebrehiwet GBG, Atsebaha Gebrekidan Kahsay AGK, Letemichael Negash Welekidan LNW, Amlsha Kahsay Hagos AKH, Getahun Kahsay Abay GKA, Dawit Gebreegziabiher Hagos DGH. Rifampicin resistant tuberculosis in presumptive pulmonary tuberculosis cases in Dubti Hospital, Afar, Ethiopia. 2019.
[45] Diriba G, Alemu A, Tola HH, Eshetu K, Yenew B, Amare M, et al. Detection of Mycobacterium tuberculosis and rifampicin resistance by Xpert® MTB/RIF assay among presumptive tuberculosis patients in Addis Ababa, Ethiopia from 2014 to 2021. IJID regions 2022;5:97–103.
[46] Alao MA, Ibrahim OR, Ogunbosi BO. Trends of Xpert MTB/RIF in the diagnosis of Mycobacterium tuberculosis and rifampicin resistance in Southwest Nigeria: A 4-year retrospective study. J Pan African Thoracic Soc 2023;4(1):31–41.
[47] Danlami MB, Aliyu B, Samuel G. Incidence of rifampicin-resistance presumptive M. Tuberculosis cases among outpatients in Kebbi State, Nigeria. African. J Infect Diseases 2021;15(1):47–52.
[48] Ejeh FE, Undiandeye A, Okon K, Moshood KH. Prevalence of rifampicin resistance tuberculosis among HIV/TB coinfected patients in Benue State Nigeria. Pan African Med J 2021;38(1).
[49] Bulabula AN, Nelson JA, Musafiri EM, Machekano R, Sam-Agudu NA, Diacon AH, et al. Prevalence, predictors, and successful treatment outcomes of xpert MTB/RIF–identified rifampicin-resistant tuberculosis in post-conflict eastern democratic

republic of the Congo, 2012–2017: a retrospective province-wide cohort study. Clin Infect Dis 2019;69(8):1278–87.
[50] Abdul JBPAA, Adegbite BR, Ndanga MED, Edoa JR, Mevyann RC, Mfoumbi GRAI, et al. Resistance patterns among drug-resistant tuberculosis patients and trends-over-time analysis of national surveillance data in Gabon, Central Africa. Infection 2023;51(3):697–704.
[51] da Silva MP, Cassim N, Ndlovu S, Marokane PS, Radebe M, Shapiro A, et al. More than a decade of GeneXpert® mycobacterium tuberculosis/rifampicin (ultra) testing in South Africa: laboratory insights from twenty-three million Tests. Diagnostics 2023;13(20):3253.
[52] Sikhondze W, Dlamini T, Khumalo D, Maphalala G, Dlamini S, Zikalala T, et al. Countrywide roll-out of Xpert® MTB/RIF in Swaziland: the first three years of implementation. Public Health Action 2015;5(2):140–6.
[53] Otokunefor K, Otokunefor TV, Omakwele G. Multi-drug resistant Mycobacterium tuberculosis in port harcourt, Nigeria. Afr J Laborat Med 2018;7(2):1–4.
[54] Mboowa G, Namaganda C, Ssengooba W. Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: a retrospective study. BMC Infectious Diseases 2014;14:1–5.
[55] Addisu T, Tilahun M, Wedajo S, Sharew B. Trends Analysis of HIV Infection and Antiretroviral Treatment Outcome in Amhara Regional from 2015 to 2021, Northeast Ethiopia. HIV/AIDS-Research and Palliative Care. 2023:399-410.
[56] Chaves Torres NM, Quijano Rodríguez JJ, Porras Andrade PS, Arriaga MB, Netto EM. Factors predictive of the success of tuberculosis treatment: A systematic review with meta-analysis. PLoS One 2019;14(12):e0226507.
[57] Olajide OS, Okonkwo P, Ajayi O, Adetoye D, Ogunsola OO, Ogundele O, et al. Predictors of tuberculosis treatment outcomes among people living with HIV in some States in Nigeria. Pan Afr Med J 2024;47:149.
[58] Geiger K, Patil A, Budhathoki C, Dooley KE, Lowensen K, Ndjeka N, et al. Relationship between HIV viral suppression and multidrug resistant tuberculosis treatment outcomes. PLOS Global Public Health 2024;4(5):e0002714.
[59] Van Hout MC, Hope V. Treatment outcomes and antiretroviral uptake in multidrug-resistant tuberculosis and HIV co-infected patients in Sub Saharan Africa: a systematic review and meta-analysis. BMC Infectious Diseases 2019;19:1–8.
[60] Federal Democratic Republic of Ethiopia MoH. GUIDELINES FOR MANAGEMENT OF TB, DR-TB AND LEPROSY IN ETHIOPIA, Addis Ababa Federal Democratic Republic of Ethiopia, Ministry of Health. 6th, November, 2017.
[61] Bedaso MH, Kalil FS. Trends of drug resistance tuberculosis from 2014 to 2018, Bale zone, Oromia region, Ethiopia. Infection and Drug Resistance. 2021:2073-8.
[62] Musa BM, Adamu AL, Galadanci NA, Zubayr B, Odoh CN, Aliyu MH. Trends in prevalence of multi drug resistant tuberculosis in sub-Saharan Africa: a systematic review and meta-analysis. PLoS One 2017;12(9):e0185105.
[63] Organization WH. Global tuberculosis report 2021: supplementary material: World Health. Organization 2022.
[64] Ismail NA, Mvusi L, Nanoo A, Dreyer A, Omar SV, Babatunde S, et al. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. The Lancet Infectious Diseases 2018;18(7):779–87.
[65] Chihota VN, Niehaus A, Streicher EM, Wang X, Sampson SL, Mason P, et al. Geospatial distribution of Mycobacterium tuberculosis genotypes in Africa. PLoS One 2018;13(8):e0200632.
[66] Mbugi EV, Katale BZ, Streicher EM, Keyyu JD, Kendall SL, Dockrell HM, et al. Mapping of Mycobacterium tuberculosis complex genetic diversity profiles in Tanzania and other African countries. PLoS One 2016;11(5):e0154571.
[67] Mekonnen D, Munshea A, Nibret E, Adnew B, Getachew H, Kebede A, et al. Mycobacterium tuberculosis Sub-Lineage 4.2. 2/SIT149 as dominant drug-resistant clade in northwest Ethiopia 2020–2022: In-silico whole-genome sequence analysis. Infection and Drug Resistance 2023:6859–70.
[68] Abebaw Kebede AK, Dereje Beyene DB, Bazezew Yenew BY, Getu Diriba GD, Zemedu Mehamd ZM, Ayinalem Alemu AA, et al. Monitoring quality indicators for the Xpert MTB/RIF molecular assay in Ethiopia. 2019.
[69] Zignol M, Gemert Wv, Falzon D, Sismanidis C, Glaziou P, Floyd K, Raviglione M. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007-2010. Bulletin of the world Health Organization. 2012;90:111-9.
[70] Mirzayev F, Viney K, Linh NN, Gonzalez-Angulo L, Gegia M, Jaramillo E, et al. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. Eur Respir J 2021;57(6).
[71] Agizew T, Boyd R, Ndwapi N, Auld A, Basotli J, Nyirenda S, et al. Peripheral clinic versus centralized laboratory-based Xpert MTB/RIF performance: Experience gained from a pragmatic, stepped-wedge trial in Botswana. PLoS One 2017;12(8): e0183237.
[72] Iem V, Dean A, Zignol M, Vongvichit P, Inthavong D, Siphanthong S, et al. Low prevalence of MDR-TB in Lao PDR: results from the first national anti-tuberculosis drug resistance survey. Trop Med Int Health 2019;24(4):421–31.
[73] Zereabruk K, Kahsay T, Teklemichael H, Aberhe W, Hailay A, Mebrahtom G, et al. Determinants of multidrug-resistant tuberculosis among adults undergoing treatment for tuberculosis in Tigray Region, Ethiopia: a case–control study. BMJ OpenRespir Res 2024;11(1):e001999.
[74] Ababa A. Implementation Guideline for GeneXpert MTB/RIF Assay in Ethiopia. 2014.
[75] Ardizzoni E, Fajardo E, Saranchuk P, Casenghi M, Page A-L, Varaine F, et al. Implementing the Xpert® MTB/RIF diagnostic test for tuberculosis and rifampicin resistance: outcomes and lessons learned in 18 countries. PLoS One 2015;10(12): e0144656.
[76] Creswell J, Codlin AJ, Andre E, Micek MA, Bedru A, Carter EJ, et al. Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries. BMC Infectious Diseases 2014;14:1–12.
[77] Raizada N, Sachdeva K, Sreenivas A, Vadera B, Gupta R, Parmar M, et al. Feasibility of decentralised deployment of Xpert MTB/RIF test at lower level of health system in India. PLoS One 2014;9(2):e89301.
[78] Wei J-H, Qian X-Q, Wan Y-M, Zhao X-K, Zhang C-Y, Guo W, et al. Analysis of unsuccessful tests and the effect of prolonged clinical sample preprocessing in the GeneXpert MTB/RIF assay. BMC Infectious Diseases 2024;24(1):770.
[79] Bono G, Caputo F, Marconi M, Musella I. Insights into the Cepheid distance scale. Astrophys J 2010;715(1):277.
[80] Organization WH. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. World Health Organization; 2014. Report No.: 9241506709.