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Emergence of second line drug resistant TB in Bhutan, report from annual drug resistance surveillance 2010-2023

Principal Investigator: Lila Maya Adhikari (Royal Centers for Disease Control, Ministry of Health)
Co-Investigator(s): Lila Maya Adhikari (Royal Center for Disease Control), Pavitra Bhujel (Royal Center for Disease Control), Karchung Tshering (Royal Center for Disease Control), Rada Dukpa (National TB control program, Ministry of Health), Jit Bdr Darnal (Royal Center for Disease Control), Sangay Zangmo (Royal Centre for dDisease Control), Tsheten Tsheten (Australian National University)

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Disclaimer

Emergence of Second-line Anti-TB Drug Resistance in Bhutan: Report from Annual Drug Resistance Surveillance 2010-2023

Lila Adhikari[1], Pavitra Bhujel[1], Karchung Tshering[1], Jit Bdr. Darnal[1], Rada Dukpa[2], Sangay Zangmo[1], Tsheten[3]

[1]Royal Centers for Disease Control, Ministry of Health, Bhutan

[2]National TB Control Program, DoPH, Ministry of Health, Bhutan

[3]Australian National University, Canberra, Australia

Keywords: Drug Resistance; Second-line TB drugs; Tuberculosis; Bhutan

Abstract

Drug-resistant tuberculosis (DR-TB) is a public health challenge in Bhutan. While there are studies identifying the burden of first-line anti-TB drug (FLD) resistance in Bhutan, currently there are no reports specifically highlighting the occurrence second-line anti-TB drug (SLD) resistance. SLD resistance pose a greater threat with higher mortality rates and prolonged medication. This study utilized laboratory-based nationwide drug resistance surveillance data collected by the National TB Reference Laboratory (NTRL) from the inception in 2010 until 2023. The laboratory diagnostic algorithm for the surveillance varied across the years. New diagnostic facilities like liquid culture and drug susceptibility test (DST) and molecular tests were gradually rolled out in Bhutan. At present, drug resistant test for SLDs is conducted for fluroquinolones and injectable drugs using Line Probe Assay (LPA). The overall completion rate for SLD screening for TB in Bhutan was 60.1% (n=423). The proportion of SLD resistance among multi-drug resistant TB/rifampicin resistant (MDR-TB/RR-TB) strains was 8.0% (95% CI 5.7-11.2, n=34). High proportion of pre-extensively drug resistant TB (pre-XDR TB) with fluoroquinolone resistance was seen in 6.9% of the cases. The highest incidence rate of SLD resistance was seen in 2019 (0.8 per 100,000 population). Only one case of XDR-TB has been detected so far. The highest proportion of SLD resistance was seen in 2012 (75%, n=2/5), while not a single case was detected in 2018. SLD resistance detection was higher using the phenotypic method compared to molecular testing using LPA (adjusted odds ratio [AOR] 2.8, p value 0.01). Male patients were at higher risk compared to females (AOR 2.1, p value 0.06) for SLD resistance. With the introduction of second-line LPA in 2018, the overall duration for second-line testing decreased from a median of 322 days (interquartile range [IQR]:255-348) in 2010 to 27 days (IQR: 13-36) in 2023.There is a growing need to further test and investigate the burden of SLD resistant TB in the country. Facilities for screening of all TB drugs particularly the newer drugs need to be established urgently to address the threat of developing further resistance.

Introduction

The emergence of second line-drug resistant TB further aggravates the challenges in controlling and ending TB worldwide. In 2006, the US Center for Disease Control and Prevention and the WHO first analyzed the emergence of SLD resistant TB, mainly XDR-TB which was then defined as *Mycobacterium Tuberculosis* (MTB) strains that is resistant to both fluroquinolones and second-line injectables in addition for the first line drugs rifampicin and isoniazid. It was reported that 2% of the 17,960 isolates of MTB from international networks of laboratories were XDR-TB (1)**.**

In 2023, 10.8 million incident cases of TB were reported, of which 4.3 million cases were bacteriologically confirmed. Among the bacteriologically confirmed pulmonary TB cases, a total of 28,982 cases of SLD resistant TB were detected (2). Compared to the drug sensitive TB or first line drug resistant TB, patients with second line drug resistance have lower treatment success rate, higher case fatality rates, worse adverse drug events and high treatment cost. The treatment success rate of 44.2% for XDR-TB and 63.3% for pre-XDR TB is found to be much lower than the MDR-TB's treatment success rate of 68.0% (3) .Furthermore, the outcomes are worse for people with HIV co-infection, with studies reporting treatment failures of up to 18.1% and mortality rates as high as 63.9% (4). The expansion of resistance beyond MDR-TB signals urgent threat and challenge towards incurable tuberculosis with limited effective drugs (5).

The WHO recommends drug susceptibility testing (DST) for SLDs for patients with rifampicin resistant TB (6). For developing effective regimen to improve treatment outcomes, reduce transmission and acquisition of further resistance, second-line DST is imperative and has cost saving benefits in the long run. Although rapid & accurate DST using molecular techniques are available for fluoroquinolones, not all patients have access to SLD testing. For example, in 2022,

only 50% of the patients underwent fluoroquinolone testing worldwide (7). For other SLDs, the availability of only well-established phenotypic methods that takes weeks or months for completion and require specialized laboratory facilities limit the access to testing (8).

Bhutan is a landlocked country sharing borders with high TB-burden countries like China in the north and India in the south, west and east, which accounted for 26% and 6.8% global TB cases respectively in 2024(2). Limited research is available for drug resistant TB in Bhutan. According to the national annual drug resistant surveillance report, the number of MDR-TB has been increasing over the years (9). In the last decade, National TB Control Program (NTCP) has been steadfast in its effort to control MDR-TB and has maintained a treatment success rate of 96.0%(10) (unpublished report). However, there are no published reports presenting the onset of SLD resistance. Given the country's small population of just over 700,000, a single case of XDR-TB would be a cause of concern. This report aims to establish the emergence and burden of SLD resistant TB in the country in the last 14 years since the initiation of drug resistant surveillance in the country. We also look at the factors associated with the detection of SLD resistance and assess the diagnostic facilities available in Bhutan.

Methodology

Study setting

This study included data of all the MDR-TB/RR patient samples received at National TB Reference Laboratory (NTRL) in Bhutan from 2010 to 2023. The NTRL established its laboratory-based nationwide annual drug resistance surveillance for TB in 2010.

Sample collection and testing

Sputum and extra-pulmonary smear positive samples from all the microscopy centers across the country were transported to NTRL to perform culture and DST. From 2017 onwards, in addition to the above, samples testing positive by Xpert MTB/RIF Assay (T/RR) were also subjected to culture and DST. The microscopy centers are in the primary health care centers and district hospitals while the GeneXpert facilities are in the regional referral hospitals, national referral hospital and high burden districts for TB. There were 32 microscopy centers in 2010 and gradually increased to 46 microscopy centers at present. In 2017 four GeneXpert machines were introduced and at present it has been expanded to 17 sites with 20 machines in the country (**Figure 1).**

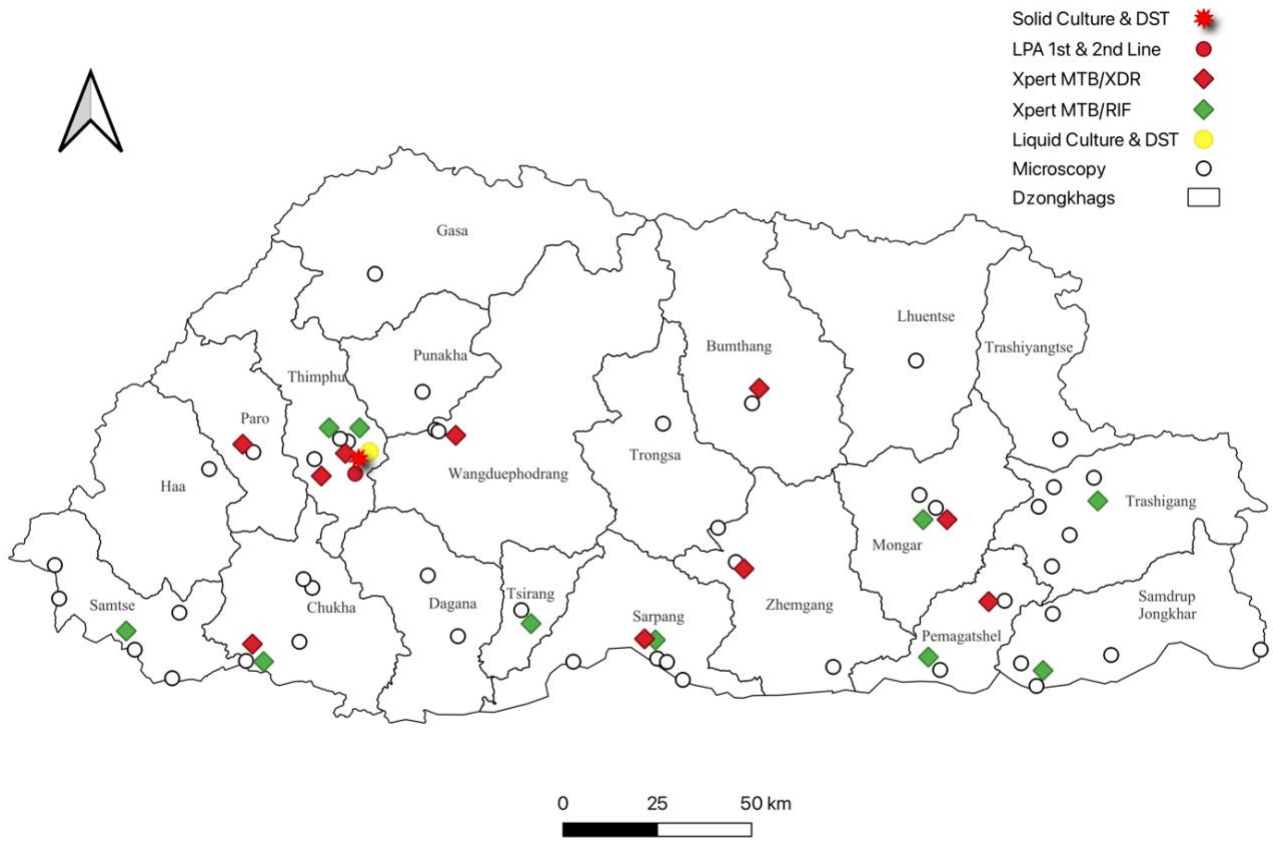
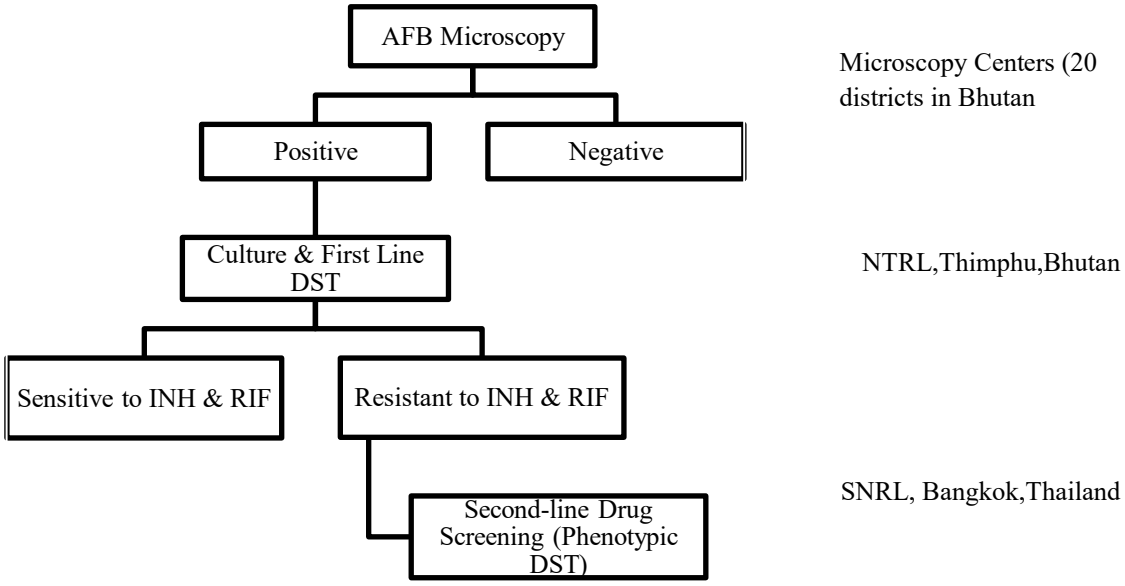
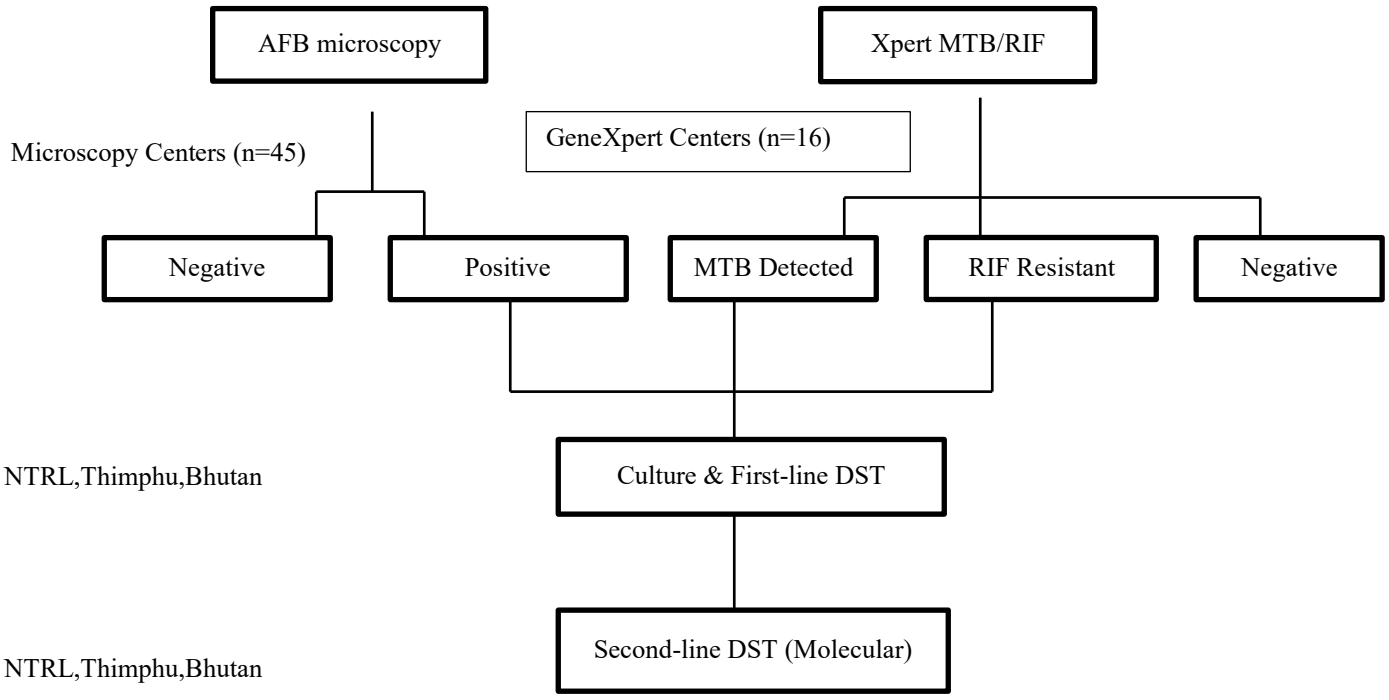


Figure 1. The diagnostic facilities distribution across various districts (known as dzongkhags) in Bhutan in 2024

In 2010, positive sputum smear microscopy was followed by solid culture, conventional identification and solid DST for all samples. In 2012, liquid culture and DST was introduced at NTRL. In 2014, LPA or rapid detection of MDR-TB first line drug was established at NTRL. In 2017, Xpert MTB/RIF was introduced in four hospitals- the national referral hospital, two regional referral hospitals and one high burden district. This has gradually been expanded to 12 other high burden districts. Until 2017, all second-line DST was performed at the Supra National Reference Laboratory (SNRL) in Thailand. In 2018, LPA for second-line line anti-TB drugs was established at NTRL and in 2022, Xpert MTB/XDR was introduced in NTRL and Gidakom Hospital, a hospital specialised and dedicated to the treatment of TB cases **(Figure 2).** Quality control for district microscopy centers is performed by NTRL**.** The quality control for NTRL is conducted by SNRL in Thailand for culture and DST and from SAARC TB & HIV center in Nepal for microscopy.



a. Laboratory diagnostic algorithm for second-line drug resistance screening in 2010



b. Laboratory diagnostic algorithm for second-line drug resistance screening in 2023

AFB: Acid Fast Bacilli, MTB: *Mycobacterium tuberculosis*, DST: Drug Sensitivity Test, INH: Isoniazid, RIF: Rifampicin, NTRL: National TB Reference Laboratory, SNRL: Supra National Reference Laboratory

Figure 2: Laboratory diagnostic algorithm for second-line drug resistance screening in 2010 and 2023

Operational definitions

The following standard definitions of RR/MDR-TB, Pre-XDR TB and XDR-TB have been used based on the diagnostic facilities available in the country (1, (11).

MDR-TB/RR: MTB strains resistant to isoniazid and rifampicin or rifampicin only (for cases diagnosed using GeneXpert for first line anti-TB drugs (XPERT MTB/RIF)

Pre-XDR TB: MDR-TB strain resistant to either fluoroquinolones or second line injectables

XDR-TB: MDR-TB strain resistant to both fluoroquinolones and second line injectables

Data collection

From 2010 to 2013, the patient details including demographic, clinical and district laboratory reports were filled in the sample shipment form. These details were entered in NTRL's register alongside the culture and DST reports. From 2014 onwards, the details were entered in the web-based system for TB surveillance called the Tuberculosis Information & Surveillance System (TbISS). The data for this study was extracted as per the study's objectives from manual registers and TbISS. The total Bhutanese population was extracted from the world bank data (source: https://data.worldbank.org/indicator/SP.POP.TOTL?locations=BT)

Data analysis

Frequencies and percentages were used to describe the annual cases and pattern of second-line drug resistant TB among the MDR-TB cases. Loess smoothened curve was fitted to understand the trend across years for incidence of SLD resistant TB cases. Odds ratios (OR) and 95% confidence intervals were used to present the magnitude of association between demographic, clinical and laboratory variables that included age, sex, year of diagnosis, history of previous treatment, disease site, culture result, microscopy result and second-line diagnosis method and the outcome variable of resistance to any SLD . Chi square test was used for categorical variables and $p<0.05$ was considered statistically significant. Logistic regression model was fitted for multivariable analysis. The duration required for sample shipment to NTRL, for completion of

DST for first & second-line diagnosis were compared across years. All statistical analysis were performed using R software version 4.3.3.

Ethical approval

The study was approved by Research Ethics Board of Health, Ministry of Health, Bhutan (Reference number Ref. No. /PO/RL/2024.12.NW). Since this study only used de-identified dataset collected from patient records, the ethics board granted waiver for patient informed consent.

Result

A total of 704 MDR-TB cases were registered at NTRL from January 2010 until December 2023. SLD screening was available for 60.1%(n=423) of all MDR-TB cases. The completion of second-line DST in 2010 was 90.5%(n=19) while that in 2023 was 66.1%(n=37). In 2016, no second-line DST was available and in 2019, the completion was highest with 94.4% (n=68) **(Figure 1).**

Among the cases with DST completion, the overall proportion of any second-line drug resistance was 8.0% (95% CI 5.7-11.2, n=34). Among SLD screened cases, the proportion of fluoroquinolone resistance was 6.9% (95%CI 4.7-9.8, n=29) and that of injectables was 0.2% (95%CI, 0.0-1.5; n=1). Resistance to both fluoroquinolone and injectable was seen in 0.2% (95%CI, 0.0-1.5, n=1) of cases. Other resistance that excluded resistance to fluoroquinolone or second-line injectable was seen in 0.7% (95%CI 0.2-2.2; n=3) cases which included resistance to ethionamide (ETH) and para-Aminosalicylic acid (PAS).

Year-wise, the highest proportion of second-line drug resistance was seen in 2012 (75%, n=2/5) and the lowest was seen in 2018 with zero cases detected. A case of resistance to both fluoroquinolones and injectables (XDR-TB) was detected in 2019 **(Figure 3).**

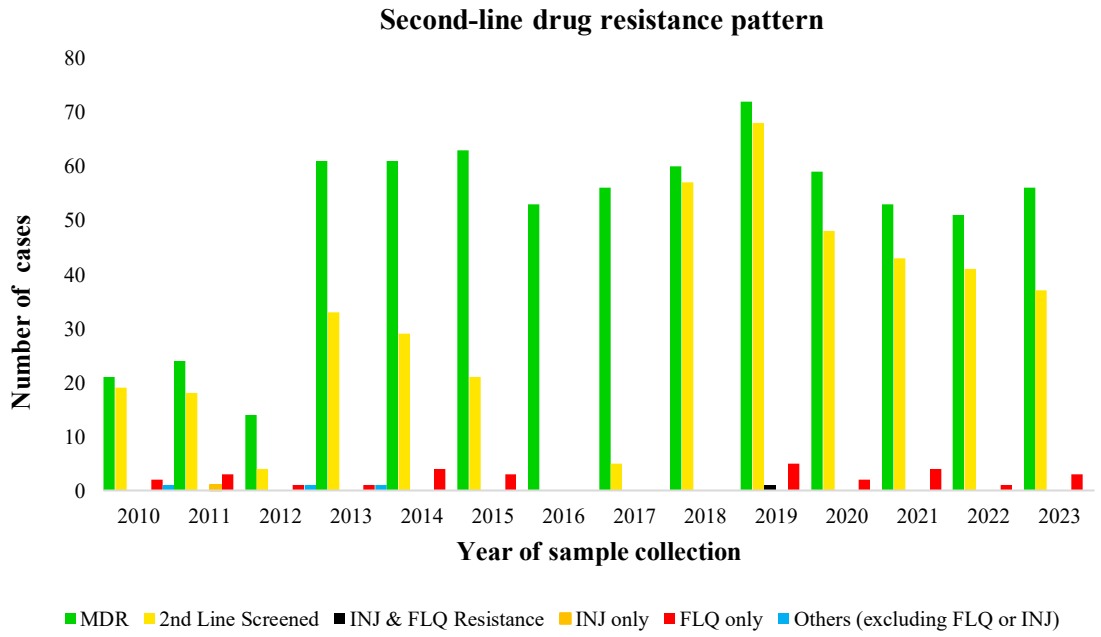


Figure 3: Number of second-line drug resistance screening by year and the pattern of second-line drug resistance

The first case of second-line drug resistance was detected in a 56-year-old male. The sample was collected on 22nd of January 2010 from Gidakom Hospital. The results for the first-line drugs were available on 9th July 2011 using solid culture and DST. The samples were shipped to SNRL in Thailand and results for the second-line drugs were available after 381 days from the date of collection on 7th of March 2011. Similarly, two other cases of second-line drug resistance were seen in the same year. From the year 2010 onwards, at least one case of second-line drug resistance was detected every year except in 2016 when the second-line DST was not performed and in 2018 where no cases were detected **(Figure 4).**

The test for linear trend showed no statistical association (p value 0.66). Loess smoothening curve was fitted for the annual incidence data. While there were some declines in 2014 and 2015, no cases were detected in the next three years mainly due to lack of diagnostic capacity. There was a small increase around 2019 but the incidences are on decline and has remained low through the recent years. Thus the trend suggests a non-linear pattern with no consistent upward or downward overall annual incidences.

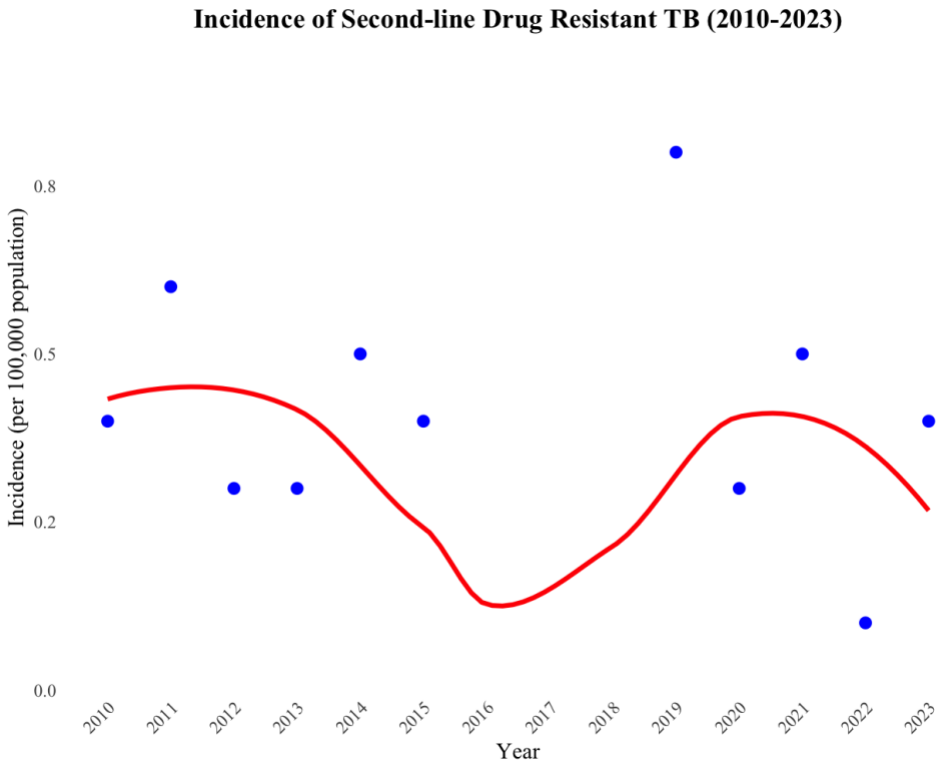


Figure 4: Incidence of second-line drug resistance based on the date of first sample collection

Characteristics of second-line drug resistance cases

The median age of MDR-TB drug resistance was 25 (IQR 21-34) years while that of SLD resistance was 27 (IQR 22-43) years. Male sex and having a previous history of TB treatment were associated with having SLD resistance on univariable analysis (OR 2.5, 95% CI 1.2-5.4 & OR 2.5, 95% CI 1.5-5.4 respectively) but did not show significant association after adjusting with other variables including age, disease site, year, culture result, microscopy result and second-line diagnosis method. Similarly, the year 2018 to 2023 category showed lower odds of detecting resistance in crude analysis (unadjusted OR 0.3, 95% CI 0.2-0.78) compared to the year 2010-2013. The odds of detecting second-line drug resistance were higher among samples that had growth in solid media (unadjusted OR=2.8, 95% CI 1.2-6.7). On adjustment with all the demographic, clinical and other laboratory variables, the detection of second-line drug resistance using phenotypic method remained strongly associated (adjusted OR 2.8, 95% CI 1.2-6.3). No statistical association was seen with microscopy result, disease site or age of the patient

Table 1: Factors associated with detection of second-line drug resistant TB

		% (number)							
SN	Variables	SLD resistant	SLD sensitive	Crude OR	95%CI	p value	AOR	95% CI	p value
1	Age, n=423								
	Less than 18 years	3.7(1)	96.3(26)	Referent	NA	NA	Referent	NA	NA
	18-39 year	7.4(23)	92.6(286)	1.9	0.3-45.1	0.47	2	0.4-37.7	0.50
	40-59 year	12.3(8)	87.7(57)	3.2	0.5-84.6	0.21	2.8	0.5-54.8	0.35
	>=60 year	9.1(2)	90.9(20)	2.4	0.2-79.4	0.43	3.1	0.3-72.2	0.37
2	Sex, n=423								
	F	5.1(12)	94.9(225)	Referent	NA	NA	Referent	NA	NA
	M	11.8(22)	88.2(164)	2.5	1.2-5.4	0.01	2.1	1.0-4.6	0.06
3	History of treatment, n=423								
	New	6.7(21)	93.3(293)	Referent	NA	NA	Referent	NA	NA
	Previously treated	15.4(12)	84.6(66)	2.5	1.2-5.4	0.01	1.4	0.1-3.4	0.41
	Unknown	3.2(1)	96.8(30)	0.5	0.0-2.7	0.45	0.4	0.0-2.0	0.34
4	Disease site, n=423								
	Extra-pulmonary	6.9(2)	93.1(27)	Referent	NA	NA			
	Pulmonary	8.1(32)	91.9(362)	1.1	0.3-7.8	0.81			
5	Year, n=423								
	2010-2013	14.9(11)	85.1(63)	Referent	NA	NA			
	2014-2017	12.7(7)	87.3(48)	0.8	0.3-2.3	0.73			
	2018-2023	5.4(16)	94.6(278)	0.3	0.2-0.8	0.01			
6	Culture Result, n=423								
	Solid & Liquid Growth	6.2(16)	93.8(244)	Referent	NA	NA			
	Liquid growth	7.4(6)	92.6(75)	1.2	0.3-3.4	0.69			

	Solid growth	15.6(12)	84.4(65)	2.8	1.5-6.7	0.01			
	No Growth	0.0(0)	100.0(5)	0	0.0-18.0	0.57			
7	Microscopy Result, n=366								
	Positive	7.1(21)	92.9(275)	Referent	NA	NA			
	Negative	5.7(4)	94.3(66)	0.8	0.2-2.3	0.68			
8	Second line diagnostic method, n=423								
	Genotypic	15.1(18)	84.9(101)	Referent	NA	NA	Referent	NA	NA
	Phenotypic	5.3(16)	94.7(288)	3.2	1.6-6.6	<0.01	2.8	1.2-6.3	0.01

*Note: Adjustment done with sex, history of TB treatment and second-line diagnostic method. Culture result was removed from the final model despite statistical significance as one of the variable did not have data (no growth=0) and year variable was excluded due to multicollinearity.

Note: Variables included in the adjusted analysis for final model were age, sex, history of TB treatment and second-line DST method

Duration

With the introduction of genotypic method in 2018, the overall duration for second-line test decreased from a median of 322 days (IQR:255 -348) in 2010 to 27 days (IQR: 13-36) in 2023. Similarly, for first-line DST the duration varied from 69 days (IQR 48-80) in 2010 to 21 days (IQR13-33) in 2023. For the first-line DST significant improvements were seen in 2014 onwards after the introduction of LPA for first line drugs and Xpert MTB/RIF in 2017. The duration of shipment of sputum samples from microscopy centers to NTRL did not have signification variation over the years **(Figure 5).**

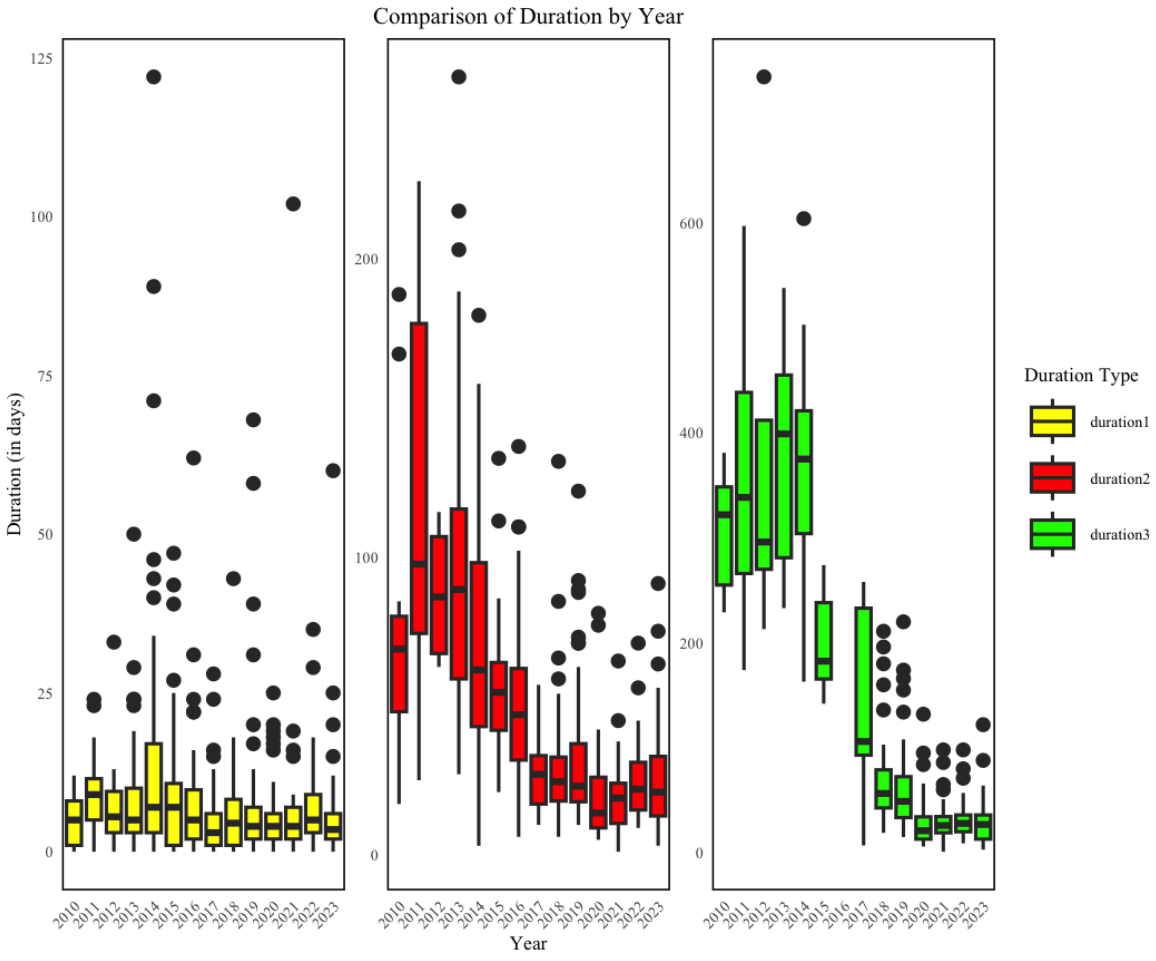


Figure 5: Comparison of different durations according by year (duration1 is the duration of sample shipment from microscopy centers to NTRL in Bhutan, duration2 is the duration for the first-line DST from the date of sample collection and duration3 is the duration for second line DST from the date of sample collection)

Discussion

This study highlights the detection of MTB strains resistant to SLD in Bhutan since the commencement of nationwide annual drug resistance surveillance in 2010. However, due to the unavailability of reports prior to 2010, the exact time of the emergence could not be established. Possibility of emergence before 2010 can also be linked to the worldwide circulation of XDR-TB

strains in samples from 2000-2004 according to the first assessment of second-line drug resistance from 14 participating SNRLs (1)..

The overall DST completion of 60.1% for SLDs indicates that the incidence of resistance to SLDs might be an underestimation. The WHO's projection of 9.9% XDR-TB among MDR-TB further substantiates the underestimation. Additionally, Bhutan's MDR-TB of 10.6% in 2018 to 2021 is one of the highest in the region compared to India's overall estimate of 6.7% in 2016-2022 (12)(13) and Thailand's Multidrug-resistant TB (MDR-TB) accounted for 0.8% (95% CI: 0.5–1.4) of new TB cases and 13.0% (95% CI: 6.5–24.4) of previously treated TB cases (14) compared to Bhutan's proportion of 14.4% in new and 27.1% in previously treated cases. The most frequent SLD resistance was detected in fluoroquinolones with overall resistance of 6.9%. Similar trends were noted in other countries like Kuwait and China which indicates further challenges in the treatment using newer drugs (15, 16). Although no published findings of molecular characterization of MTB strains are available from Bhutan, the detection of fluroquinolone resistance among Bhutanese TB patients might be attributed to presence of Bejing strain which are associated with fluroquinolone resistance. The strain is commonly found in neighboring districts of India in Sikkim (62.4%) and in Assam (35.5%) or Bangladesh with which Bhutan shares porous borders (17,18,19). Fluoroquinolone resistance, like other antimicrobial resistance has been attributed to weak healthcare system and the implications include poor treatment outcomes including XDR acquisition in 17.0% of patients with baseline FQ resistance (20). Moreover, only slight variation in incidence over time showing no linear trends needs further confirmation and assessment of TB control measures in addition to the diagnostic capacity in the country.

Higher odds of second-line drug resistance were seen in males compared to female. Higher prevalence, particularly of XDR-TB has been attributed to more engagement of males in

employment settings and Bhutan's workforce participation of females is lower than males (73.4% males vs 53.5% females in 2022) (21). However, further investigation with other factors such as alcohol and tobacco use, co-morbidities such as HIV or diabetes needs to be investigated (22). Previously treated cases showed strong association with second-line drug resistance but adjustment with other variables decreased its statistical power (p value 0.41). It might be due to smaller sample size as this association has been consistently highlighted in similar studies with greater sample sizes (22, 23, 24). Lower odds of detection were noted in the year 2018 to 2023 compared to the baseline year of 2010 to 2013 on crude analysis but did not show association in multivariable analysis. The phenotypic diagnostic test performed at SNRL from year 2010 until 2015 included drugs like ethionamide, cycloserine, *p*-aminosalicyclic acid, kanamycin, ofloxacin, capreomycin and levofloxacin while with genotypic method (LPA) only fluroquinolones and second-line injectables were screened from 2018 onwards. Lack of facilities for phenotypic DST at NTRL means three is still lack of DST facilities for newer SLDs including linezolid, bedaquiline and delamanid. The decrease in proportion of second-line drug resistance after the establishment of molecular method needs parallel comparison with phenotypic method to ensure that the variation is not due to sensitivity and specificity of the diagnostic methods. Similar approach of conducting parallel comparison with whole genome sequencing method showed 85% sensitivity compared to phenotypic DST in China (23).

From 2018 onwards, 100% of the samples received at NTRL were subjected to culture but 16.2%(n=57) did not have second-line DST reports. This might be due to sample shipment delays (average 7.4 days) and issues with cold chain maintenance. Therefore, it is important to further assess the quality of sample collected including the proportion of mucopurulent and saliva samples. Healthcare facilities need to ensure that samples are collected prior to treatment initiation.

Possibilities of obtaining alternative samples such as bronchoscopy specimen can be explored in patients who are unable to produce good quality sputum (24). This is indicated by higher odds of detecting SLDs resistance among culture positive samples in solid growth (Unadjusted OR 2.8) in univariable analysis. However, since there was no correlation with liquid medium further investigation is required to understand the association.

The surveillance for TB in Bhutan is also used as a diagnostic network as no other facilities has set up for culture and DST using phenotypic methods. However, molecular methods using GeneXpert are available at 17 sites (Figure 1). The delay is shipping samples to NTRL, RCDC & SNRL resulted in long duration between the patients first visit to treatment initiation. Although no evidence is available directly for the treatment initiation delays, our study found that the median duration between sample collection and second-line DST took up to more than a year (median 399 days in 2013), which was the highest prior to 2018. After the establishment of second-line LPA at NTRL, the maximum duration for second-line DST has reduced to a median of 56.5 days in 2018.

This study analyzed a nationally representative data collated through a centralized system of drug resistance surveillance in Bhutan. However, due to the use of retrospective study method, missing data could not be retrieved, and data collected could not be verified particularly for the history of previous treatment. However, for data after 2014, with the rolling out of TBISS, the system captured patient's repeated visits and treatment history. In addition, with limited variables, it did not adequately capture risk factors including co-morbidities, socioeconomic status or alcohol and tobacco use.

Conclusion

The emergence of second-line drug resistant TB imposes further challenges to the country's end TB goal by 2035. This report highlights the growing cases of fluoroquinolone resistance in Bhutan and the urgent need to establish screening methods for newer drugs as the treatment has been already rolled out. To improve the coverage of second-line DST, expansion of culture facilities to district hospitals might help in getting a greater yield. Inclusion of second-line drug resistant TB on the list of national notifiable disease might help in the response. Comparison between phenotypic and genotypic test can be conducted so compare the sensitivity, specificity, PPV and NPV of the samples that has been shipped from microscopy centers. Further investigation needs to be conducted to understand the burden, transmission pattern, impact and outcome of the second-line drug resistant TB in Bhutan.

Declaration of competing interests

The authors have no competing interests to declare.

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References

1. Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martín-Casabona N, et al. Worldwide Emergence of Extensively Drug-resistant Tuberculosis. Emerg Infect Dis [Internet]. 2007 Mar [cited 2025 Mar 19];13(3):380–7. Available from: http://wwwnc.cdc.gov/eid/article/13/3/06-1400_article.htm

2. Global Tuberculosis Report 2024. 1st ed. Geneva: World Health Organization; 2024. 1 p.

3. Pedersen OS, Holmgaard FB, Mikkelsen MKD, Lange C, Sotgiu G, Lillebaek T, et al. Global treatment outcomes of extensively drug-resistant tuberculosis in adults: A systematic review and meta-analysis. J Infect [Internet]. 2023 Sep [cited 2025 Mar 19];87(3):177–89. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0163445323003377

4. Kvasnovsky CL, Cegielski JP, Van Der Walt ML. Treatment Outcomes for Patients with Extensively Drug-Resistant Tuberculosis, KwaZulu-Natal and Eastern Cape Provinces, South Africa. Emerg Infect Dis [Internet]. 2016 Sep [cited 2025 Mar 20];22(9). Available from: http://wwwnc.cdc.gov/eid/article/22/9/16-0084_article.htm

5. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med [Internet]. 2017 Apr [cited 2025 Mar 19];5(4):291–360. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213260017300796

6. 2022 - WHO Consolidated Guidelines on Tuberculosis. Modul.pdf.

7. Global Tuberculosis Report 2022. 1st ed. Geneva: World Health Organization; 2022. 1 p.

8. Kendall EA, Cohen T, Mitnick CD, Dowdy DW. Second line drug susceptibility testing to inform the treatment of rifampin-resistant tuberculosis: a quantitative perspective. Int J Infect Dis [Internet]. 2017 Mar [cited 2025 Mar 19];56:185–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1201971216316538

9.Royal Centre for Disease Control. Annual Scientific Report 2022 [Internet]. Thimphu: Royal Centre for Disease Control; 2022 [cited 2025 May 18]. Available from: https://www.rcdc.gov.bt/web/wp-content/uploads/2023/06/Annual-Scientific-Report_2022_RCDC-1.pdf

10.World Health Organization. Tuberculosis country profiles: Bhutan [Internet]. Geneva: World Health Organization; [cited 2025 May 18]. Available from: https://worldhealthorg.shinyapps.io/tb_profiles/?_inputs_&tab=%22tables%22&lan=%22EN%22&iso2=%22BT%22&entity_type=%22country%22

11.WHO Consolidated Guidelines on Tuberculosis. Module 4: Treatment - Drug-Resistant Tuberculosis Treatment, 2022 Update. 1st ed. Geneva: World Health Organization; 2022. 1 p.

12.Dorji T, Tshering K, Adhikari L, Jamtsho T, Bhujel P, Lhaden P, et al. High incidence of multidrug-resistant tuberculosis in Bhutan: A cohort study based on national TB surveillance data. IJID Reg [Internet]. 2024 Dec [cited 2025 Mar 22];13:100471. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2772707624001413

13. Atre S, Padalkar D, Srivastava T, Rathod H, Chaugule H, Patvekar M, et al. Prevalence of Multidrug-Resistant Tuberculosis (MDR-TB) in India: A Mini Systematic Review and Meta-Analysis (2016–2022). Med J Dr Patil Vidyapeeth [Internet]. 2023 Jul [cited 2025 Mar 19];16(4):500–4. Available from: https://journals.lww.com/10.4103/mjdrdypu.mjdrdypu_18_23

14. Kamolwat P, Nateniyom S, Chaiprasert A, Disratthakit A, Mahasirimongkol S, Yamada N, et al. Prevalence and associated risk factors of drug-resistant tuberculosis in Thailand: results from the fifth national anti-tuberculosis drug resistance survey. Trop Med Int Health [Internet]. 2021 Jan [cited 2025 Mar 22];26(1):45–53. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tmi.13502

15. Al-Mutairi NM, Ahmad S, Mokaddas E. Increasing prevalence of resistance to second-line drugs among multidrug-resistant Mycobacterium tuberculosis isolates in Kuwait. Sci Rep [Internet]. 2021 Apr 8 [cited 2025 Mar 19];11(1):7765. Available from: https://www.nature.com/articles/s41598-021-87516-0

16. Li S, Tan Y, Deng Y, Bai G, Huang M, Shang Y, et al. The emerging threat of fluroquinolone-, bedaquiline-, and linezolid-resistant Mycobacterium tuberculosis in China: Observations on surveillance data. J Infect Public Health [Internet]. 2024 Jan [cited 2025 Mar 19];17(1):137–42. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1876034123004057

17. Devi KR, Pradhan J, Bhutia R, Dadul P, Sarkar A, Gohain N, et al. Molecular diversity of Mycobacterium tuberculosis complex in Sikkim, India and prediction of dominant spoligotypes using artificial intelligence. Sci Rep [Internet]. 2021 Apr 1 [cited 2025 Mar 19];11(1):7365. Available from: https://www.nature.com/articles/s41598-021-86626-z

18. Devi KR, Bhutia R, Bhowmick S, Mukherjee K, Mahanta J, Narain K. Genetic Diversity of Mycobacterium tuberculosis Isolates from Assam, India: Dominance of Beijing Family and Discovery of Two New Clades Related to CAS1_Delhi and EAI Family Based on Spoligotyping and MIRU-VNTR Typing. Mokrousov I, editor. PLOS ONE [Internet]. 2015 Dec 23 [cited 2025 Mar 19];10(12):e0145860. Available from: https://dx.plos.org/10.1371/journal.pone.0145860

19. Tasnim T, Tarafder S, Alam FM, Sattar H, Mostofa Kamal SM. Pre-Extensively Drug Resistant Tuberculosis (Pre-XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) Patients in Bangladesh. J Tuberc Res [Internet]. 2018 [cited 2025 Mar 19];06(03):199–206. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/jtr.2018.63018

20. Jabeen K, Shakoor S, Hasan R. Fluoroquinolone-resistant tuberculosis: implications in settings with weak healthcare systems. Int J Infect Dis [Internet]. 2015 Mar [cited 2024 Apr 22];32:118–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1201971215000119

21. National Statistics Bureau. Statistical Yearbook of Bhutan 2023 [Internet]. Thimphu: National Statistics Bureau; 2023 [cited 2025 May 18]. Available from: https://www.nsb.gov.bt/publications/statistical-yearbook/

22. Khan AH, Nagoba BS, Shiromwar SS. A Critical Review of Risk Factors Influencing the Prevalence of Extensive Drug-Resistant Tuberculosis in India. Int J Mycobacteriology [Internet]. 2023 Oct [cited 2025 Mar 19];12(4):372–9. Available from: https://journals.lww.com/10.4103/ijmy.ijmy_143_23

23. Liu D, Huang F, Zhang G, He W, Ou X, He P, et al. Whole-genome sequencing for surveillance of tuberculosis drug resistance and determination of resistance level in China. Clin Microbiol Infect [Internet]. 2022 May [cited 2025 Mar 22];28(5):731.e9-731.e15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X21005346

24. Kanade S, Mohammed Z, Kulkarni A, Nataraj G. Comparison of xpert MTB/RIF assay, line probe assay, and culture in diagnosis of pulmonary tuberculosis on bronchoscopic specimen. Int J Mycobacteriology [Internet]. 2023 [cited 2025 Mar 19];12(2):151. Available from: https://journals.lww.com/10.4103/ijmy.ijmy_86_23