Report on Fourth Nationwide Anti-Tuberculosis Drug Resistance Survey in Myanmar 2020-2021

National Tuberculosis Program
Department of Public Health
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List of Acronyms

ART – Anti Retroviral Therapy

AHRN – Asian Harm Reduction Network

DMU – Data Management Unit

DS-TB – Drug susceptible tuberculosis

DR-TB – Drug resistant tuberculosis

DRS – Anti-Tuberculosis drugs resistance survey/ Drug Resistance Survey

DST – Drug Susceptibility Testing

GCP – Good Clinical Practice

Hr- Isoniazid mono-resistance

LJ – Löwenstein–Jensen media

LPA – Line Probe Assay

MDR-TB – Multi Drug Resistant Tuberculosis

MGIT – Mycobacterium Growth Indicator Tube

MMA – Myanmar Medical Association

MTB – Mycobacterium tuberculosis complex

NGO – Non-governmental organization

NTM – Non-tuberculosis mycobacterium

NTP – National Tuberculosis Program

NTRL – National Tuberculosis Reference Laboratory

OHP – Other healthcare partners

PPM – Private-public mixed healthcare providers

PPS – Probability Proportional to Size Sampling

PMDT – Programmatic Management of Drug-Resistance Tuberculosis

PSI – Population Services International, Myanmar

RR – Rifampicin resistant

RS – Rifampicin susceptible

SOP – Standard Operating Procedure

SRL – Supranational Reference Laboratory

STR – Shorter Treatment Regimen

TAT – Turnaround time

TB – Tuberculosis

TOR – Terms of reference

TS – Township (geographical unit)

TSTBC – Township Tuberculosis center (main public tuberculosis diagnostic, treatment and reporting unit within a township)

WGS – Whole genome sequencing

1.Executive summary

The fourth National anti-tuberculosis Drug Resistance Survey in Myanmar was conducted by the National Tuberculosis Program in 2020–2021. The survey aimed to estimate the prevalence of Drug Resistance tuberculosis at the national level.

In addition, the survey sought to estimate the prevalence of other Drug Resistant Tuberculosis at the national level. Moreover, it could be done to compare this survey result with the result of the third National anti-tuberculosis Drug Resistance survey in 2012-2013. The salient difference in fourth National anti-tuberculosis Drug Resistance survey was that bacteriologically confirmed (by smear microscopy and/or Xpert MTB/RIF test) pulmonary tuberculosis cases was included and undergo liquid culture & Line Probe Assay. Moreover, new technology Whole Genome Sequencing was used in this survey to detect the genotypic mutations of first and second line anti-tuberculosis drugs and newer anti-tuberculosis drugs.

The final sample size to determine new bacteriologically confirmed pulmonary tuberculosis cases was 1877 but was rounded to 2,000 according to WHO's Guidelines for Surveillance of Drug Resistance in tuberculosis (2015) using the formula for sample size calculation[17].

The survey sampling frame consisted of 305 townships because 8 non-reporting townships and 17 townships with less than 10 cases were removed. In all, 50 data collection points (survey clusters) were chosen by probability proportional to size sampling (PPS). Of these, 12 were in the states, 23 in regions other than Yangon, and 15 in the Yangon region.

Because the sample size of the study was 2000, 40 new bacteriologically confirmed tuberculosis patients were recruited per cluster and all previously treated patients simultaneously during the survey period. At the same time, tuberculosis Patients enrolled from Myanmar Medical Association and Population Services International Myanmar clinics were consecutively collected. Pre-survey visit to Drug Resistance Survey cluster sites were done by Regional/State tuberculosis officers.

Regarding the steps of Drug Resistance Survey, the survey protocol and detailed procedures were carried out after approval from Institutional Ethical Review Board. Pre-survey training to respective Township Medical Officers, tuberculosis Coordinators & Laboratory technicians at Nay Pyi Taw on 28th January 2020. Sample collection period last from February 2020 to August 2020.

At the end of data collection period i.e. August, 2020, (1901/2000) 95% of sample target was obtained so that the representative sample size was achieved in this study.

The enrollment of tuberculosis patients by townships showed that 36 clusters/townships had more than 40 samples. Among 14 remaining townships, two townships had very low tuberculosis patients' enrolment. Most of the clusters reached the target but 9 clusters could not have the required sample size. Those clusters were as follows: Sintgaing, Lemyetnyar, Loilem, Kamaryut, Sanchaung, Oktwin, Palaw, Paukkhaung and Ngaputaw.

Out of 50 clusters, sputum samples from 35 clusters were sent to National Tuberculosis Reference Laboratory and sputum samples from 15 clusters were sent to Upper Myanmar Tuberculosis Center, Mandalay since February 2020. The following tests were done at both laboratories: Xpert MTB/RIF, Liquid culture, Line Probe Assay for first line and second line anti-tuberculosis drugs (1st and 2nd line LPA) and DNA extraction. After DNA extraction from both laboratories, DNAs were gathered at National Tuberculosis Reference Laboratory and from National Tuberculosis Reference Laboratory, they were sent to Otago University, New Zealand for Whole Genome Sequencing.

Data entry was completed in the month of December, 2020 at National Tuberculosis Reference Laboratory, Upper Myanmar and Central Data Unit. Data validation was implemented to assure data quality

assurance from January 2021 to September 2021. Epi Info version 3.5.4 was used for the Drug Resistance Survey. The survey data was stored in Access format.

Data Analysis was done from October 2021 to November 2022. for electronic capture of data, cross-validation checks and reporting.

Steering committee meeting could be conducted for 2 times; one on (3-12-19) and another on (28-12-20) at Nay Pyi Taw. Monthly progress meeting was regularly performed from May 2020 to December 2020.

During survey period, Senior consultant microbiologist from National Tuberculosis Reference Laboratory, Drug Resistance Survey central coordinator, upper and lower Myanmar coordinator together with WHO Drug Resistance Survey focal person and WHO national consultant for National anti-tuberculosis Drug Resistance Survey supervised and monitored the Drug Resistance Survey activities. Due to travel restriction Myawaddy, Tachileik, Palaw and Dawei couldn't be monitored by central team.

Country Rifampicin Resistant Tuberculosis, **Multi-Drug-Resistant** Tuberculosis and Isoniazid mono-resistance were estimated by two different analyses. Based on available Whole Genome Sequencing results (1409), two methods like *complete case analysis method* and **Multiple Imputation Model analysis (MI model analysis)** method were done. They showed that the prevalence of Rifampicin Resistant Tuberculosis among new tuberculosis patients was 2.9% and 3.3% by complete analysis and MI model analysis respectively. Thus, final agreed prevalence of Rifampicin Resistant Tuberculosis among new was 3.3% by MI model analysis. (95% Confidence Interval 2.4% - 4.5%).

On the other hand, the prevalence of **Multi-Drug-Resistant** Tuberculosis among new was 2.7% and 3.0% by complete case analysis and MI model analysis respectively. Final agreed prevalence of Rifampicin Resistant Tuberculosis among new was 3.0% by MI model analysis (95% Confidence Interval 2.1% - 4.1%)

Moreover, the prevalence of any Isoniazid resistant tuberculosis was 10.3% and 10.2% by complete case analysis and MI model analysis respectively size included in analysis. Final agreed prevalence of Isoniazid mono-resistant tuberculosis was 10.2% (95% Confidence Interval 8.7% -12%).

Regarding the previously treated tuberculosis patients, based on available Whole Genome Sequencing results (134) and complete case analysis showed the prevalence of Rifampicin Resistant Tuberculosis among those patients was 13.9% and 13.1% by MI model analysis. Final agreed prevalence of Rifampicin Resistant Tuberculosis was 13.1% by MI model analysis. (95% Confidence Interval 7.8% - 21%)

On the other hand, the prevalence of **Multi-Drug-Resistant** Tuberculosis among previously treated Tuberculosis patients was 12.6% and 11.8% by complete case analysis and by MI model analysis respectively. Final agreed prevalence of **Multi-Drug-Resistant** Tuberculosis among those patients was 11.8% by MI model analysis. (95% Confidence Interval 6.8% -19.6%)

Moreover, the prevalence of Isoniazid mono-resistant Tuberculosis was 20.9% and 20.8% by complete case analysis and by MI model analysis respectively. Final agreed prevalence of Isoniazid mono-resistant Tuberculosis was 20.8% by MI model analysis. (95% Confidence Interval 14.8% -28.5%).

The only one risk factors associated with Rifampicin Resistant Tuberculosis was the previous Tuberculosis treatment history which was statistically significant with p value= 0.000165.

Comparing the previous three national anti-tuberculosis drug resistance surveys, the decline of **Multi-Drug-Resistant** Tuberculosis among new and previously treated tuberculosis patients were prominently found in the fourth National anti-tuberculosis Drug Resistance Survey. The fall of **Multi-Drug-Resistant** Tuberculosis prevalence among new Tuberculosis patients was from 4% in first National anti-tuberculosis Drug Resistance

Survey to 3 % in 4th National anti-tuberculosis Drug Resistance Survey and among previously treated Tuberculosis patients was from 15.5% to 11.8%.

The predominate genotype was “Indo-Oceanic and EAI “which was followed by “East Asian (Beijing)”, Euro-American and Central Asian (CAS)/Delhi in order of frequency. By regions/states, “Indo-Oceanic and EAI “genotype was the commonest. However, Beijing genotype was more common than EAI in Kachin and 3 Shan States. Beijing genotype was commonly associated with **Multi-Drug-Resistant** Tuberculosis which was the consistent with the finding from other country surveys.

The other relevant findings of overall agreement and positive agreement of Rifampicin results between two tests, Line Probe Assay versus Whole Genome Sequencing was the highest out of Xpert versus Line Probe Assay and Xpert versus Whole Genome Sequencing.

Apart from that, the proportions of Anti-tuberculosis Drug Resistance among Rifampicin Resistant Tuberculosis patients were crucial for National Tuberculosis Program. It was found among Rifampicin Resistance Tuberculosis cases (Whole Genome Sequencing), Isoniazid and Ethambutol Resistance constituted 75% of Rifampicin Resistant Tuberculosis cases. Thus, those resistance were crucial factor because first diagnosis of Rifampicin Resistant Tuberculosis was currently identified by using initial X pert MTB/RIF assay, prediction could be made 3 drugs (Rifampicin, Isoniazid and Ethambutol) were already resistant in three fourth (3/4) of Rifampicin Resistant Tuberculosis cases.

Additionally, the considerable proportion of resistance to levofloxacin/moxifloxacin among Rifampicin Resistant Tuberculosis was 20.3%. That result gave rise to use the Line Probe Assay second line drugs or X pert MTB-XDR must be necessary before giving fluoroquinolone containing regimen.

In contrast to this, the proportion of resistance to injection Amikacin among Rifampicin Resistant Tuberculosis was 1.1%. That very small proportion of injection Amikacin would be fortunately spare drugs for individualized regimen. If those patients could not fit for oral longer/shorter regimen, injection could be included to construct the effective regimen for them.

Line Probe Assay also revealed the same proportions of fluoroquinolone and injection Amikacin Resistance among Rifampicin Resistant Tuberculosis cases which was discovered by Whole Genome Sequencing. 18.2% and 1.1% accordingly. It was concluded that the results from Line Probe Assay and Whole Genome Sequencing were comparable.

The very interesting finding was that Xpert MTB/RIF assay showed that the prevalence of Rifampicin Resistant Tuberculosis was higher than that of Whole Genome Sequencing (approximately 1%). Whole Genome Sequencing also detected rifampicin resistance outside the rpoB gene (I491F mutation) which could not be detected by routine Xpert MTB/RIF testing.

The National Drug Resistance Tuberculosis Survey 2020–2021 was completed successfully, with a high participation rate and fewer missing values. The survey showed that the **Multi-Drug-Resistant** Tuberculosis burden in Myanmar has declined significantly, due to the huge efforts of the National Tuberculosis Program and its partners. It could provide the sound estimation of resistant profile of all Tuberculosis cases in a population. By knowing the resistant profile of anti-tuberculosis drugs, National Tuberculosis Program could update and revise the Drug Resistant Tuberculosis management guideline to improve treatment outcomes of multi drug resistant Tuberculosis patients & avert the transmission of resistance strains. Drug Resistant Tuberculosis guidelines would also be necessary to be revised because high proportions of any resistance to isoniazid and fluoroquinolone were identified by Line Probe Assay as well as Whole Genome Sequencing. Fortunately, the resistance to newer anti-tuberculosis drugs (Bedaquiline, Linezolid, Delamanid) were not detected so those drugs could be used in Shorter Treatment Regimen. On the other hand, it was recommended that the rapid diagnostic tests (genotypic Drug Susceptibility Testing) with higher sensitivity

should be widely used in Myanmar for early detection of Drug Resistant tuberculosis patients. Though the Tuberculosis burden has been declining significantly, the absolute number of tuberculosis patients who could be identified with new technologies could be higher. There is a need to lay greater emphasis on multisectoral approaches, decentralization and the integration of basic Tuberculosis services into primary care facilities.

Finally, this survey could give rise to strong evidence-based documentation to coming Myanmar tuberculosis National Strategic Plan (2026-2030).

2. Introduction

2.1 Current Tuberculosis Epidemiological Situation, including Burden of tuberculosis, Tuberculosis/HIV and Drug Resistant Tuberculosis

Tuberculosis is one of the top 10 causes of death, and the leading cause from a single infectious agent (above HIV/AIDS) worldwide: millions of people continue to suffer from the disease each year. At the same time, drug resistant tuberculosis becomes a significant public health problem for almost all countries over the world. In 2018, tuberculosis caused an estimated 1.2 million deaths among HIV negative people, and there were an additional 251,000 deaths (range 223,000 – 281,000) from tuberculosis among HIV positive people. tuberculosis affects all countries and all age groups. The severity of National epidemics varies widely. In 2018, the disease burden varies from fewer than five to more than 500 new cases per 100,000 population per year among countries. Almost 90% of cases each year are in 30 high burden countries including Myanmar. Drug resistant tuberculosis continues to be a public health threat. Globally in 2018, there were about half a million (range, 417,000 – 556,000) new cases of Rifampicin Resistant Tuberculosis (of which 78% had multi drug resistant tuberculosis). Globally, 3.4% of new tuberculosis cases and 18% of previously treated tuberculosis cases had **Multi-Drug-Resistant** Tuberculosis/Rifampicin Resistant Tuberculosis.

Myanmar is one of the world's 30 high tuberculosis burden countries according to WHO Global TB Report 2018[16]. Myanmar in 2018, the incidence of all forms of tuberculosis (includes HIV + tuberculosis) was 338 (range, 222 – 477) per 100,000 population. Among them, 44% was bacteriologically confirmed among pulmonary TB cases. Mortality rate was 39 (range, 23 – 58) per 100,000 population excludes HIV + tuberculosis. In new and relapse tuberculosis patients, 9% were HIV positive cases and ART coverage was 71%. The incidence of **Multi-Drug-Resistant** Tuberculosis/Rifampicin Resistant Tuberculosis is 21 (range, 14 – 30) per 100,000 population. Estimated proportion of tuberculosis cases with **Multi-Drug-Resistant** Tuberculosis/Rifampicin Resistant Tuberculosis in 2018 are 4.9% in new tuberculosis cases and 20% in previously treated tuberculosis cases. From the laboratory aspect, laboratory confirmed cases of **Multi-Drug-Resistant** Tuberculosis/Rifampicin Resistant Tuberculosis were 3479 while 35 cases were extensively drug-resistant tuberculosis.

The third national Anti-tuberculosis drug resistance survey (2012 – 2013) showed **Multi-Drug-Resistant** Tuberculosis rate of 5% among new cases and 27.1% among previously treated cases. There were three nationwide drug resistance surveys done in Myanmar. These surveys revealed that the proportion of **Multi-Drug-Resistant** Tuberculosis among new and previously treated tuberculosis patients was 4% and 15.5% in 2002-2003, 4.2% and 10% in 2007 – 2008, 5% and 27.1% in 2012 – 2013 respectively.

The third nationwide drug resistance survey revealed relatively high **Multi-Drug-Resistant** tuberculosis rates. Non-adherence to treatment, transmission in congregate settings, living in Drug Resistant Tuberculosis crisis regions are major risk factors for the development of Drug Resistant Tuberculosis.

Therefore, National Tuberculosis Program is scaling up the efforts to diagnose, treat and prevent the spread of **Multi-Drug-Resistant** Tuberculosis particularly in Yangon and Mandalay regions and the Fourth National Anti-tuberculosis Drug Resistant Survey was therefore conducted in 2019 – 2020.

In previous three Anti-tuberculosis drug resistant surveys, only smear positive pulmonary patients were included and solid culture were done. Resistant patterns were known to First line Anti-tuberculosis drugs only in these previous surveys[8].

In Fourth National anti-tuberculosis Drug Resistance Survey, difference from previous three surveys, bacteriologically confirmed (by smear microscopy and/or Xpert MTB/RIF test) pulmonary tuberculosis cases were included and underwent liquid culture. Moreover, new technology Whole Genome Sequencing was used in this survey to detect resistant pattern to first line anti-tuberculosis drugs, second line anti-tuberculosis drugs and newer anti-tuberculosis drugs.

2.2 National Tuberculosis Program and Relevant Healthcare Providers

Majority of notified tuberculosis cases were registered and treated under National tuberculosis control program. Population Services International Myanmar and Myanmar Medical Association act as the major contributors to National Tuberculosis Program by implementing Public-private mix activities in community. Other implementing partners (OHP) such as AHRN, MSF-H, MSFCH, MAM, MDM and SMRU also contribute certain number to National Tuberculosis Program. Apart from National program, Public-public mix Hospitals, Population Services International, Myanmar and Myanmar Medical Association were major contributors for tuberculosis control[9]. (Figure 1)

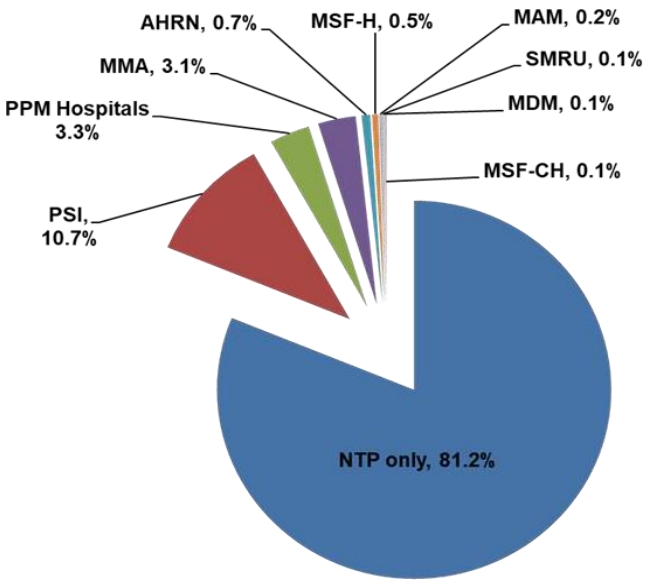


Figure 1. Contribution of National Tuberculosis Program and relevant healthcare providers

2.3 Programmatic Management of Drug Resistant Tuberculosis

Programmatic management of drug-resistant tuberculosis (PMDT) is one of the integral parts of the National Strategic Plan to control Tuberculosis (2015-2020). The National Drug Resistant tuberculosis committee was formed in 2006. National PMDT Guideline was first published in 2013 and updated in 2017. Shorter treatment regimen (STR) was started to be given in fourth quarter, 2017 to eligible patients to improve management of patients with **Multi-Drug-Resistant** Tuberculosis. Line Probe Assay for Second line anti-tuberculosis drugs can be done for all new **Multi-Drug-Resistant** Tuberculosis patients who are eligible for Shorter Treatment Regimen. For the newly diagnosed Rifampicin Resistant Tuberculosis/Multi-Drug-Resistant Tuberculosis patients who are eligible for Shorter Treatment Regimen, undergo preliminary counselling for Shorter treatment regimen and Line Probe Assay for Second line anti-tuberculosis drugs. If there is no resistance to second line injectable and fluoroquinolones, undergo patient education and counselling. If the patient gives consent, the patient will be enrolled in shorter treatment regimen. For the patient who is not eligible for Shorter Treatment Regimen and who does not give consent for Shorter Treatment Regimen,

he/she will be enrolled in conventional **Multi-Drug-Resistant** Tuberculosis/Rifampicin Resistant Tuberculosis-TB regimen. If there is resistance pattern in Line Probe Assay for Second line anti-tuberculosis drugs result, he will be enrolled in Pre-extensively drug resistant tuberculosis/extensively drug resistant tuberculosis category and treatment will be given accordingly. Total 721 patients enrolled in Shorter Treatment Regimen from 2018 to third quarter of 2019[10].

In July 2009, DOTS-Plus pilot project was started in 10 townships in Yangon and Mandalay regions, in close collaboration with WHO and MSF-H. DOTS-Plus pilot project enrolled 275 **Multi-Drug-Resistant** Tuberculosis patients on a standardized second-line anti-tuberculosis drug regimen during a two-year period. Only retreatment failures with Drug Susceptibility Testing results confirming **Multi-Drug-Resistant** Tuberculosis were included in this project and treatment outcomes were 71.2% cured, 16.8% died, 10.9% defaulted, 1.1% failed.

In 2015, 108 townships were expanded for treating **Multi-Drug-Resistant** Tuberculosis patients and entire Yangon region has been covered by **Multi-Drug-Resistant** Tuberculosis management in 2015. All 330 townships became **Multi-Drug-Resistant** Tuberculosis townships since first quarter, 2016. Gene Xpert expansion was scaling up and total 79 Xpert MTB/RIF sites were installed in 2018. By the end of 2018, 2802 **Multi-Drug-Resistant** Tuberculosis cases were enrolled for second-line anti-tuberculosis treatment. Among them, 313 patients were enrolled in Shorter Treatment Regimen. Thirty-five extensively drug resistant tuberculosis (XDR-TB) cases were notified and among them, 29 patients were initiated treatment in 2018.[9]

3. Justification

Until the capacity for a continuous surveillance system is established, WHO recommends conducting anti-tuberculosis (TB) drug resistance surveys every five years. Anti-Tuberculosis drugs resistance surveys provide a sound estimation of the resistance profile of all tuberculosis cases in a population and can consequently be used to inform treatment guidelines to improve treatment outcomes and avert transmission of resistant strains, whilst at the same time contributing to strengthening in-country laboratory capacity, specimen transport and referral systems.

A genomics (sequencing) -based anti-tuberculosis drug resistance survey (DRS) is being proposed in Myanmar to minimize potential bias in producing resistance estimates. This is upon consideration of previous studies, which suggest that sputum smear positive patients in the country are predominantly associated with tuberculosis Beijing genotype that is in turn more likely to be resistant to in-use anti-tuberculosis drugs. Next-generation sequencing (NGS) overcomes many of the significant challenges associated with conventional phenotypic testing as well as the limitations of other less comprehensive molecular tests by providing rapid, detailed sequence information for multiple gene regions or whole genomes of interest. Moreover, a comprehensive review of the recent data combined with a multi-country, population-based study of over 7,000 tuberculosis patients, suggests sequencing to have better accuracy for the prediction of resistance to anti-tuberculosis drugs in comparison to phenotypic drug susceptibility testing (DST), and sufficient sensitivity to estimate the true prevalence of drug resistance for tuberculosis surveillance. Hence, the fourth National anti-tuberculosis Drug Resistance Survey gave rise to Xpert MTB/RIF molecular confirmation of pulmonary tuberculosis plus sequencing approaches for genotype characterization and detection of mutations conferring resistance to anti-tuberculosis drugs.

4. Overarching Goal and Objectives

4.1 Overarching Goal

• Characterize the resistance profile to anti-tuberculosis drugs to inform treatment guidelines, improve treatment outcomes and help avert transmission of resistant tuberculosis genotypes

4.2 Primary Objective

• Estimate the prevalence of Drug Resistant Tuberculosis

4.3 Secondary Objectives

1. To determine the proportion of patients with resistance to first- and second-line anti-tuberculosis drugs;

2. To characterize the genotypic mutations conferring putative resistance to rifampicin and other anti-tuberculosis drugs;

3. To describe socio-demographic and clinical characteristics of the target tuberculosis population;

4. To investigate possible risk factors for Rifampicin Resistant Tuberculosis/ **Multi-Drug-Resistant** Tuberculosis;

5. To characterize the tuberculosis genotypes and their resistance profiles among the target population, in relation to socio-demographic and clinical population traits;

5. Survey Design

The study was a facility based cross sectional analytical study which could be considered an add on to routine diagnostic and treatment procedures.

The target population were all new bacteriologically confirmed pulmonary tuberculosis cases by either sputum smear microscopy and/or Xpert MTB/RIF in the country.

The sampling frame for the study comprised 305/330 townships (geographical units) in the country with 2018 tuberculosis notification data. The 305 Townships s were distributed across 17 states and collectively notified 44,707 new bacteriologically confirmed pulmonary tuberculosis cases by sputum smear microscopy and/or Xpert MTB/RIF in 2018. The main public (NTP-led) tuberculosis diagnostic, treatment and reporting unit in each Township is the so-called “Township tuberculosis center (TSTBC)”, albeit other public tuberculosis diagnostic and/or treatment facilities may also be present.

Other healthcare partners (OHPs) and mixed private-public healthcare providers (PPMs) also contributed to the diagnosis, treatment and reporting of tuberculosis in many Myanmar Townships s. Overall, 82% of bacteriologically confirmed new pulmonary tuberculosis notifications in 2018 in Myanmar were from Township Tuberculosis Centers, 16% from Other Healthcare partners and 2% from Public-public mix. Among the Other Healthcare partners, 90% of tuberculosis notifications were from the Population Services International Myanmar and Myanmar Medical Association delivering care through private general practitioners. Considering data from the three main healthcare providers in Myanmar (i.e. public sector [Township tuberculosis centers], Population Services International Myanmar and Myanmar Medical Association), which collectively reported 96% of new bacteriologically confirmed pulmonary tuberculosis notifications in 2018, 8/330 TSs notified zero cases, and the remaining 322/330 Townships, notified between 1 and 1592 cases (median = 90). Among 322 TSs, 17 townships notified < 10 cases.

5.1. Definitions

A "new case" was defined as a newly registered episode of tuberculosis in a patient who, in response to direct questioning, reports never having been treated for tuberculosis or reports having taken anti-tuberculosis drugs for less than one month; or, where adequate documentation was available, for whom there was no evidence of having taken anti-tuberculosis drugs for one month or more.

A "previously treated case" was defined as a newly registered episode of tuberculosis in a patient who, in response to direct questioning, reports having received one month or more of anti-tuberculosis drugs in the past; or, where adequate documentation was available, there is evidence of having received one month or more of anti-tuberculosis drugs.

A "bacteriologically confirmed case" was defined as a patient with Mycobacterium tuberculosis complex identified from a clinical specimen, by one of the smear microscopies and/or Xpert MTB/RIF testing.

5.2. Inclusion and Exclusion Criteria

The inclusion and exclusion criteria were as follows:

1) Inclusion

- All newly registered (new and previously treated) pulmonary tuberculosis patients bacteriologically confirmed by sputum smear microscopy and/or Xpert MTB/RIF, above 8 years of age and regardless HIV status.

2) Exclusion

- Extra-pulmonary tuberculosis patients;
- Sputum smears negative patients that are also "MTB not detected" by Xpert MTB/RIF
  Patients who had already received more than seven days of treatment in their current treatment course.
- Prisoners

5.3. Sample Size

The following formula was used to determine the sample size for new bacteriologically confirmed pulmonary tuberculosis cases, as defined in WHO's Guidelines for Surveillance of Drug Resistance in tuberculosis (2015)[17]:

$$n = \frac{N * z^2 * p * (1 - p)}{d^2 * (N - 1) + z^2 * p * (1 - p)}$$

Where:

- N = total number of pulmonary tuberculosis patients registered in 2018 who were bacteriologically confirmed by smear microscopy and/or Xpert MTB/RIF = 44,707
- z = z-value (from the standard normal distribution) that corresponds to the desired confidence level for a 95% confidence interval = 1.96;
- d = absolute precision = 0.015;
- p = expected proportion of Rifampicin Resistant Tuberculosis/ **Multi-Drug-Resistant** Tuberculosis among new cases in 2019 = 0.049

Given the large number of tuberculosis diagnostic facilities in Myanmar (519 Microscopy Centers, 79 Gene Xpert Sites) Townships were chosen as the sampling unit and a cluster-based design was chosen over a 100% sampling design. To account for the cluster-based design (and to consider the correlation between individuals within a cluster) a design effect of '2' was assumed, thus doubling the sample size. The sample size was also inflated by 20% to account for anticipated losses of eligible patients.

The final sample size for new bacteriologically confirmed pulmonary tuberculosis patients was 1877 but was rounded to 2,000. Previously treated patients were enrolled simultaneously during the study period until the sample size for new patients was reached in each cluster and no specific sample size was calculated for previously treated cases. Based on 2018 notification data, where 12% of tuberculosis notifications corresponded to retreatment cases, we expected that ~ 240 newly registered previously treated patients were enrolled into the study in addition to the 2,000 new cases (2000/ [1-0.12]). It was hence estimated that the overall number of patients enrolled into the Drug Resistance Survey was ~ 2,240.

5.4. Cluster Sampling

Due to logistic and feasibility considerations, sample Townships (i.e. sampling unit) were chosen and then patients were enrolled in all tuberculosis diagnostic centers in selected Townships. Public-public mix, Other Healthcare Providers (OHPs) other than Population Services International Myanmar or Myanmar Medical Association. Those from prison/military health centers, which collectively represented 4.3% of the target population based on notification data, were excluded from the survey. In addition, 25 Townships reporting ≤ 10 new bacteriologically confirmed pulmonary tuberculosis cases in 2018 from the combined public plus Population Services International Myanmar and/or Myanmar Medical Association were also excluded.

The approach resulted in the overall exclusion of 4.6% of the total target population (2065/44707) and was hence acceptable (i.e. To draw a representative sample of Townships, a sampling frame was assembled which comprised the list of eligible Townships in the country (> 10 new bacteriologically confirmed cases notified from eligible sectors) and the corresponding number of notified new bacteriologically confirmed pulmonary tuberculosis cases by either sputum smear microscopy or Xpert MTB/RIF in 2018. Notification data from excluded reporting units (above) was not considered in the sampling frame. To attain the desired sample (n=2,000 newly registered new cases), 50 clusters were selected across all Townships using probability proportional to size sampling (PPS).

The cluster size (defined as the number of new patients to be enrolled by each cluster [n=40]), was obtained by dividing the sample size (n=2,000) by the number of clusters (n=50). All eligible previously treated patients were then to be enrolled in each cluster until the target cluster size for new cases was reached. If the selected cluster could not get the required sample size within 8 months, the needed samples were taken from nominated adjacent township according to PPS sampling method. Following this approach, 50 clusters were selected in 53 townships across the country. One cluster in each 50 townships and the additional 3 townships (Myothit in Magway Region, and Nam Sam in Shan South State, and Taze in Sagaing Region) were selected to reach the required sample size in time (Figure 2). For each cluster, the number of patients to be enrolled from the public sector, Myanmar Medical Association or Population Services International Myanmar, was proportional to the percentage notifications reported in the corresponding township from each sector. The proportional sampling was supported by data suggesting that the tuberculosis population profile may differ across sectors.

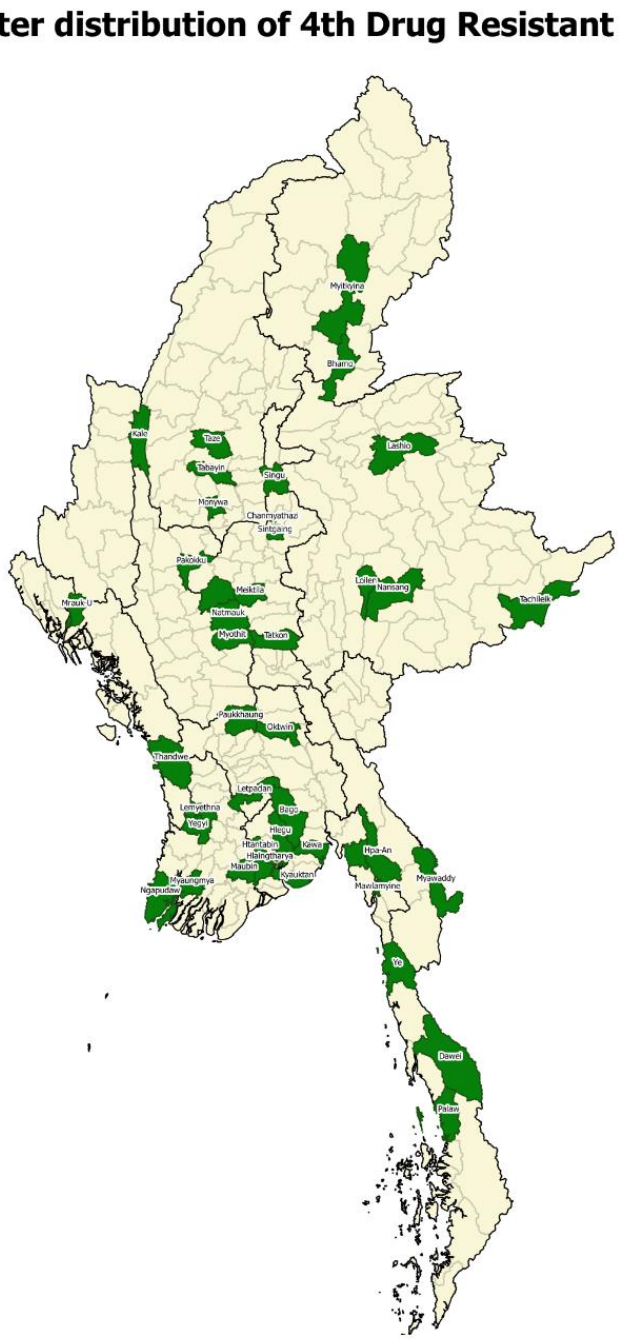


Figure 2: Cluster distribution in the Fourth National Anti-Tuberculosis Drug Resistance Survey

5.5. Staggered Design

If all townships were to begin patient enrolment at the same time, there would be overloaded in two big laboratories.

To (a) simplify survey monitoring and logistics, (b) ensure that manageable numbers of samples are processed at central laboratories each day, and (c) progressively increase numbers of enrolled patients so that laboratories were well trained before they must handle significant numbers of specimens, Townships were asked to start patient enrolment at different times (i.e. staggered design). Townships were divided in two zones and asked to ship samples to one assigned central laboratory (i.e. either Yangon National Tuberculosis Reference Laboratory or Upper Myanmar Tuberculosis Laboratory) for the duration of the study period, based on geographical location and logistic arrangements (Annex Table 2). Following the proposed staggered design, central laboratories are expected to process ≤ of 27 samples/day at the peak of the Drug Resistance Survey.

6. Collection and Laboratory Processing of Drug Resistance Survey Specimens

6.1. Overview of the Laboratory

Briefly, the three sputum samples were collected from eligible newly registered (new and previously treated) pulmonary tuberculosis patients that were bacteriologically confirmed at peripheral tuberculosis diagnostic sites by either sputum smear microscopy and/or Xpert MTB/RIF. If smear was positive, three early morning sputum samples (3 – 5 ml/sample, minimum 3ml) were directly sent to culture facility without testing Xpert MTB/RIF assay in township/district facility in order to avoid any delay for sputum collection and transportation. If smear was negative with any CXR abnormalities, three early morning sputum were asked from the patient, one for Xpert MTB/RIF testing in township/district facility and 2 were sent to culture facility if Xpert MTB/RIF result revealed any detection of TB. If Xpert MTB/RIF result was negative the rest 2 samples were discarded, and this patient were not entitled to include in the survey. Samples must be collected from patients within three days of his/her first visit to township tuberculosis center. Samples were then shipped to one of two culture laboratories depending on distance and logistic considerations (i.e. either the National Tuberculosis Reference Laboratory in Yangon or the Upper Myanmar Tuberculosis Laboratory in Mandalay) preferably within four days of sample collection.

At the central laboratories, one sample was tested by Xpert MTB/RIF test and both samples were done for culture in MGIT immediately on arrival. From each culture, one aliquot was stored in glycerol at -80°C and DNA will be extracted from the remaining growth (following subculturing in Löwenstein–Jensen (LJ) solid media) and stored at -80°C. Line Probe Assay for both first and second line anti-tuberculosis drugs were tested by MTB DRplus kit and MTB DRsl kit. The extracted DNA was kept at -80°C and was sent to Otago University, New Zealand in every quarter for Whole genome sequencing (WGS). Whole genome sequencing using Illumina technology was conducted on one DNA aliquot from all confirmed MTB specimens (i.e. one sample per patient). tuberculosis genotyping and characterization of mutations conferring resistance to first- and second-line anti-tuberculosis drugs were analysed by Whole genome Sequencing.

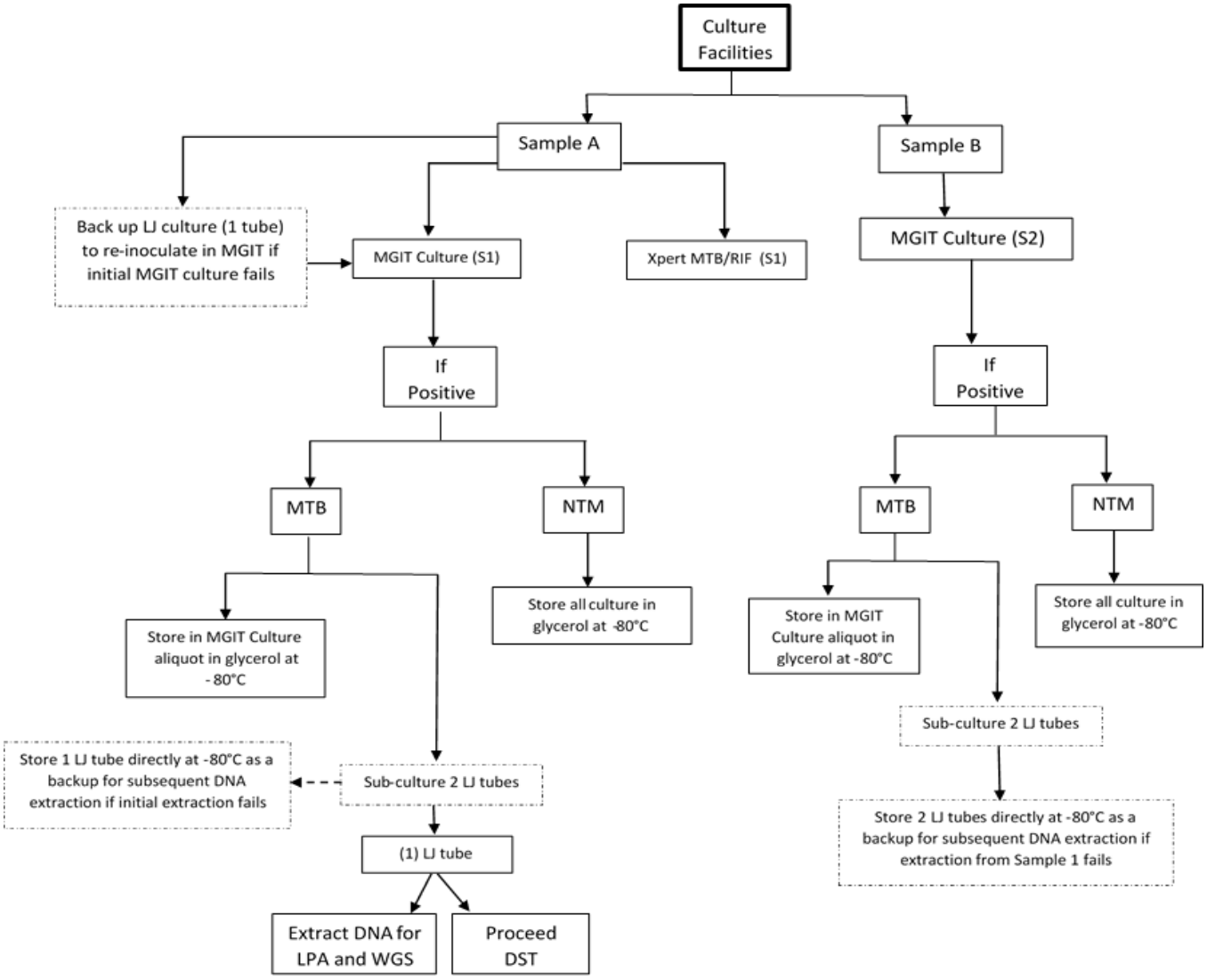


Figure 3. Drug Resistance Survey Laboratory Workflow at the Culture Facilities

6.2. Patient enrolment and Drug Resistance Survey specimen collection at township TB centers

According to the routine national tuberculosis diagnostic algorithm in Myanmar, two sputum samples were routinely collected from all presumptive pulmonary tuberculosis patients (one spot plus one early morning sample) for sputum smear microscopy examination (using ZN stain or Auramine stain depending on the facility). Sputum smear positive patients were immediately referred for treatment following collection of a third specimen which was preferred to be early morning sample for Xpert MTB/RIF testing.

Chest X-ray examination was conducted on those patients in facilities where X-ray was available. In the case of sputum smear negative patients, patients were referred for Chest X-ray examination. Patients were then asked to produce a third sputum for Xpert MTB/RIF testing and were immediately registered for treatment if chest X-ray was suggestive of tuberculosis. High risk/critically ill patients that were sputum smear negative may be tested for Xpert MTB/RIF and registered for treatment immediately if deemed necessary. Where feasible, Xpert MTB/RIF may be conducted on surplus microscopy specimen by-passing the need for collection of fresh specimens. Patients were frequently referred to neighbouring facilities for X-ray examination, and Xpert MTB/RIF samples were sent to one of 79 peripheral Xpert laboratories where Xpert testing was not available on site.

Townships invariable comprise one Township tuberculosis Center (with or without X-ray and Xpert MTB/RIF facilities, depending on the township), which is in turn the reporting unit for public sector township tuberculosis notifications based on treatment registrations. Within each township, other tuberculosis diagnostic and/or treatment units from the public, Public-public mix or Other Healthcare providers (OHP) sectors are often present. There is patient referral system between diagnostic facilities from the various sectors and proper linkage is present between those sectors. Therefore, there may be less chance to miss the eligible patients during the study period. Briefly, public sector health centers with microscopy facilities other than the Township tuberculosis center, as well private practitioners from Population Services International Myanmar and Myanmar Medical Association referred all presumptive pulmonary tuberculosis patients to the Township tuberculosis center for complete diagnosis of tuberculosis (i.e. sputum smear examination; X-ray; Xpert MTB/RIF testing). Patients that were sputum smear positive, were approached for enrolment as soon as the first sample was positive by smear examination, before treatment initiation and presumptive pulmonary tuberculosis sputum sample collection: (spot plus early morning sample) Patients that were negative by sputum smear microscopy, were enrolled as soon as Xpert MTB/RIF confirmation of tuberculosis was available.

Where possible, treatment initiation in sputum smear negative patients should be delayed until the Xpert MTB/RIF result is available to prevent collection of Drug Resistance Survey specimens after treatment initiation. In these instances, township tuberculosis center should submit the sample for Xpert MTB/RIF testing on the day of the X-ray examination and aimed to receive the result within 24 hours. Proper treatment was started according to National Drug susceptible tuberculosis or DR-TB Guidelines immediately after getting the Xpert MTB/RIF result to prevent treatment delay. Appropriate pretreatment counselling was conducted at least two times before the initiation of Drug susceptible tuberculosis or DR-TB treatment.

At each township tuberculosis center, the tuberculosis coordinator (i.e. nurse) oversaw all Drug Resistance Survey procedures and was the main responsible for patient enrolment. He/she enrolled sputum smear positive patients together with the tuberculosis laboratory technician immediately after the initial microscopy examination. The patient may either initiate appropriate treatment on site or be referred back to Population Services International, Myanmar, Myanmar Medical Association or other facility for treatment and follow-up.

Enrolment into the Drug Resistance Survey will entail obtaining the patient's written informed consent to partake in the study if eligible. Three sputum samples (preferred early morning 3 – 5 ml volume each) were then taken following routine standard procedures.

The tuberculosis coordinator then proceeded to interview the patient and cross-checked the medical records to complete the 'Clinical Information Form' (Annex 01), which contained a set of questions to allow classification of patients into new or previously treated. The enrolled patient was assigned a Drug Resistance Survey ID which was then noted in the Township tuberculosis Laboratory Register (See Annex 09). The laboratory technician, in coordination with the TB coordinator, arranged the shipment of Drug Resistance Survey specimens and forms to the relevant culture facility (i.e. either at Yangon or at Mandalay) as soon as possible, and completed a shipment form for the record in the process. Xpert MTB/RIF and sputum smear microscopy test results were noted in the "Clinical Information Form" for easy reference. Sputum smear microscopy results were noted using the "standardized WHO grading for reporting of smear positive results" (i.e. negative, scanty, 1+, 2+, 3+). Sample shipment should not be held up by the HIV test. Drug Resistance Survey data entry clerks in the central operational team did follow-up on missing test results by phone, once the Clinical Information Form had been received centrally.

6.3. Laboratory processing of samples at the National Tuberculosis Reference Laboratory

Three sputum samples were received at National Tuberculosis Reference Laboratory from each enrolled patient in assigned township tuberculosis centers. From these samples, one sample per patient was tested by Xpert MTB/RIF as soon as possible (≤ 24 hours from arrival and no later than 7 days from the time of sample collection). Two samples were inoculated in MGIT culture immediately on arrival, and within ≤3 days of sputum sample collection. For each sample, one Lowenstein-Jensen tube will be inoculated in parallel to

MGIT as a back-up, kept at 37°C and the sputum sample was discarded thereafter, to ensure that re-culturing in MGIT was possible if the initial MGIT culture failed. For each Drug Resistance Survey specimen, and for each positive MGIT culture that was confirmed as MTB using the Capilia TB ICT test kit, at least aliquot of viable culture was cryopreserved in glycerol at -80°C and the remaining culture tube was sub-cultured onto 2 Lowenstein-Jensen tubes. One positive sub-cultured Lowenstein-Jensen tube was used for DNA extraction for Line Probe Assay and Whole Genome Sequencing.

, Drug Susceptibility Testing was also done from this tube and the other tube was stored at -80°C as a back-up, in case further DNA extractions were required. The first and second-line Line Probe Assay were proceeded from extracted DNA of confirmed mycobacterium tuberculosis. DNA extractions for Whole Genome Sequencing was conducted using the DNeasy UltraClean Microbial Kit (QIAGEN) as per the manufacturer's instructions and extracted DNA was kept at -20°C before sending to Otago University for Whole Genome Sequencing.

For each Drug Resistance Survey specimen, and for each MGIT positive culture that was not confirmed as mycobacterium tuberculosis (i.e. Non-tuberculosis mycobacterium) using the Capilia test, the whole viable MGIT culture was cryopreserved in glycerol at -80°C until one year after the end of the study period for potential future investigations related to current study, but no DNA was extracted from that growth.

Viable cryopreserved cultures and surplus DNA were remained store until one year after the end of the analysis phase of the Drug Resistance Survey. This would allow undertaking further phenotypic and/or genotypic anti-tuberculosis drug susceptibility tests if/where appropriate.

6.4. Laboratory processing of samples at the Upper Myanmar Laboratory, Mandalay

The laboratory processing of samples at Upper Myanmar Laboratory were as per at the National Tuberculosis Reference Laboratory in Yangon. DNA extraction of mycobacterium tuberculosis confirmed MGIT cultures following Lowenstein-Jensen sub-culturing was conducted at the Upper Myanmar tuberculosis laboratory and the extracted DNA were then sent to National Tuberculosis Reference Laboratory for further process. National Tuberculosis Reference Laboratory received the extracted DNA sent from Upper Myanmar tuberculosis Laboratory and sent to Otago University for Whole Genome Sequencing.

6.5. Technical assistance by Chennai Supra-National Reference Laboratory in India and Otago University in New Zealand

Technical assistance from Chennai Supra-National Reference Laboratory deployed in-country proficiency testing of culture, Drug Susceptibility Testing and Line Probe Assay. A DNA sample from each patient with confirmed MTB culture was then be sent to Otago University, New Zealand for library preparation using the Nextera kit and subsequent Whole Genome Sequencing using the Illumina MiSeq sequencer, as per the manufacturer's instructions.

Otago University proceeded Whole Genome Sequencing from extracted DNA which were sent from National Tuberculosis Reference Laboratory, Yangon, Myanmar. Otago University contributed to the development of local laboratory Standard Operation Procedures (SOPs) at each laboratory.

Chennai Supra National Reference Laboratory in India provided the proficiency panels for culture and Line Probe Assay.

Samples were stored and batched to undertake Whole Genome Sequencing. Sequencing library was prepared using the NexteraTM XT DNA Kit and was subjected to LabChip® DNA High Sensitivity Assay on the LabChip GX Touch HT platform (Perkin Elmer, Waltham, MA) for the quality control check. The resulting library was then subjected to sequencing using paired-end 250-bp reads on an Illumina MiSeq using according to the manufacturer's instructions (Illumina Inc., Hayward, CA) The resulting sequencing reads was mapped to the H37Rv reference genome using in-house pipeline based on a combination of bcf and sam tools. The known genes associated with resistance to drugs that are commonly used to treat drug-susceptible and **Multi-Drug-Resistant** Tuberculosis cases in Myanmar was identified. Online analysis pipelines PhyResSE and tuberculosis Profiler was used to confirm and crosscheck all Whole Genome Sequencing results. PhyResSE and tuberculosis Profiler was used for strain/lineage classification. The sequencing data (FastQ) and bioinformatics analysis

data was stored on the University of Otago's High-Capacity Central File Storage (HCS) server. Data was password protected and made available to named investigators.

7. Data Capture Forms and Sample Tracking

7.1. Township tuberculosis Centers

The process of enrolling patients and shipping samples for the Drug Resistance Survey, a series of forms and registers and identifying patients and samples were kept and checked appropriately as follows:

- Clinical Information Form (Annex 01):

The main objective of the clinical information form is to correctly identify any past tuberculosis treatment of the patient. The form consisted of five categories of information as follows:

- identification of the patient and the facility; patient history, including age, sex, or other relevant information;
- identification of the sputum samples for the Drug Resistance Survey and smear microscopy results for each sample following WHO reporting standards;
- the initial screening Xpert MTB/RIF test result;
- documented data on history of previous treatment for tuberculosis;
- a final decision on history of previous treatment for tuberculosis.

The clinical information form must be signed by the interviewer (Medical Officer or tuberculosis Coordinator). The clinical information form consisted of an original copy and a carbonated copy. HIV status in original copy (keep record of HIV status in original cluster) was removed and shipped alongside Drug Resistance Survey samples to the assigned central laboratory. Carbonated copies were kept in a folder within a secured/locked cabinet within the facility until the end of the study period and destroyed thereafter.

- Informed Written Consent/Assent Form (Annex 02):

The form contained the patient's Drug Resistance Survey ID as noted in the Drug Resistance Survey register, patient details (name; surname; Township tuberculosis center, etc.) and a space for signature or fingerprint of the consented patient or the parent/guardian where applicable. The first part was given to the patient and the second part of consent/assent form was carbonized. The original copy was kept at Township tuberculosis center and the carbonized copy was given to the patient.

- Biological Consent Form (Annex 04):

The form contained the patient's name, surname, Township tuberculosis center) and a space for signature or fingerprint of the consented patient or the parent/guardian where applicable. The first part was given to the patient and the second part of this consent form was carbonized. The original copy was kept at Township tuberculosis center and the carbonized copy was given to the patient.

- Drug Resistance Survey Register at the facility (Annex 06):

  Each Township tuberculosis center kept a Drug Resistance Survey register to facilitate tracking whether and what patients were eligible for enrolment and had ultimately been enrolled into the Drug Resistance Survey. To this end, the register comprised the complete list of patients undergoing new (diagnostic) sputum smear examination (i.e. excluding follow-up cases), and a series of columns designed to collate the information needed to decide on the patient's eligibility, namely a separate column for the sputum smear result, the X-ray examination (i.e. suggestive or not suggestive of pulmonary tuberculosis) and the Xpert MTB/RIF result, plus a column to note the patient's original healthcare provider (i.e. either public sector, Myanmar Medical Association or Population Services International Myanmar). The last column should be used to note the unique Drug Resistance Survey ID assigned to each enrolled patient. In cases where a patient was not enrolled, the reasons for no

enrolment should be noted in this column instead (e.g. “not eligible based on tests results”; “eligible but patient did not consent”; “eligible but patient was lost-to-follow-up”; etc.). The register contained additional columns to note the date of enrolment and the dates of Drug Resistance Survey specimen collection where applicable. The unique Drug Resistance Survey ID assigned to each enrolled patient comprised a unique 2 digit code for each township tuberculosis center, a two digit serial number to uniquely identify each patient within each Township tuberculosis Center plus a two digit code to note the original healthcare sector provider (i.e. either National Tuberculosis Program, Myanmar Medical Association or Population Services International, Myanmar), plus. Sputum samples for each patient enrolled in the Drug Resistance Survey was identified with the patient’s Drug Resistance Survey Identification (ID), and either ‘A’ or ‘B’ or ‘C’.

- Shipment Form (Annex 07):
  Routine requisition form (TB 05) was used as a shipment form of National anti-tuberculosis Drug Resistance Survey. HIV status in this form was not recorded in this survey. Results were filled in the second page of TB 05.

- Township tuberculosis Center Shipment Register/Sputum dispatch record (Annex 08):
  A register was kept at the facility to track the shipment of Drug Resistance Survey samples and forms to the assigned central laboratory. The register consisted of an original copy (to be shipped along with samples and other forms), and a carbonated copy (to remain in the register of the township tuberculosis center until the end of the study period). One page of the register was completed for each shipment. The information recorded in the register comprised (but may not be limited to): the name of the person in charge of preparing the samples for shipment, the date of the shipment, the ID of the samples shipped, the date when samples were first collected, and tick boxes to check whether/what relevant forms had been checked for accuracy and completeness and included in the shipment to the assigned central laboratory. The name and phone number of the person in charge of transport (i.e. collecting the samples for shipment) may also be noted. It was also used as sputum dispatch record for transportation of sputum from townships to respective Xpert MTB/RIF Site or Culture facilities. As soon as Drug Resistance Survey Xpert MTB/RIF results were received from the assigned central laboratory, these (along with the date when results were first received) were achieved with the medical patient files as per routine practice. Copies of Drug Resistance Survey forms containing patient identifiers (i.e. Clinical Information Form), were destroyed at the end of the data entry to adhere to confidentiality principles.

- Township Laboratory Register (Annex 09):
  The “master list” of patients potentially eligible for enrolment into the Drug Resistance Survey was the list of patients undergoing sputum smear microscopy at the Township tuberculosis Center. The tuberculosis coordinator in coordination with the laboratory technician and the medical officer must follow-up all patients noted in the tuberculosis laboratory register and proceeded to enrol these where appropriate. For the case of sputum smear positive patients, enrolment would take place immediately upon confirmation of a positive smear, and without waiting for the Xpert MTB/RIF test result (albeit the Xpert MTB/RIF test result should ultimately be noted in the Drug Resistance Survey register and the Clinical Information form for the record). For the case of sputum smear negative cases, patients should be followed-up until the Xpert MTB/RIF test result was available and enrolled if bacteriologically confirmed as tuberculosis.

7.2. National tuberculosis Reference Laboratory, Yangon and Upper Myanmar tuberculosis Laboratory, Mandalay

Culture facilities used the laboratory worksheets containing Xpert MTB/RIF, culture, Line Probe Assay and Whole Genome Sequencing results. Laboratory register (Annex 20) for culture facilities in Drug Resistance

Survey was developed to track the Xpert MTB/RIF, culture and Line Probe Assay results of the Drug Resistance Survey specimens properly. National Tuberculosis Reference Laboratory filled the final results in laboratory register from laboratory worksheets and also Whole Genome Sequencing results when received back from Otago University.

8. Transport of Specimens and Study Logistics

Transport of Drug Resistance Survey Specimens from Township tuberculosis Centers to Yangon National Tuberculosis Reference Laboratory or the Upper Myanmar Tuberculosis Laboratory at Mandalay

All township tuberculosis centers partaking in the study were provided with packaging material for sputum sample transportation. The two Drug Resistance Survey specimens from each enrolled patient were transported in cool boxes with ice packs to the assigned central laboratory using triple packaging. For triple packaging, each sputum cup was wrapped individually in cotton wool or other similar material (e.g. absorbent paper) and placed in an individual zip lock plastic bag; specimens from the same patient were then placed together in a second zip lock bag, before placing the package/s in a cool box ready for shipment. Samples were transported by road to the assigned central laboratory using a contracted courier and ensuring that the timing between sample collection and MGIT culture is ≤ 4 days.

To ensure that turnaround time (TATs) from sample collection to processing at central laboratories are ≤ 3 days, sample shipments were organized at least three times weekly or as soon as samples were available. The following forms were shipped to the central laboratories alongside the corresponding samples:

1. Clinical Information form (Original copy [carbonated copy to remain at township tuberculosis centers]) –
2. Shipment form – Annex 07
3. Township shipment register/Sputum dispatch record (Original copy [carbonated copy to remain at township tuberculosis centers]) – (Annex 08) Drug Resistance Survey forms should be placed in their own plastic envelope inside the cool box envelope, ensuring adequate protection from sample leakage. Persons who were responsible for transportation of specimens must be informed about the ways of transmission of tuberculosis and the infection control measures for tuberculosis. The sputum samples for the survey were transported in separate transportation boxes with cold chain provided by National Tuberculosis Program. Rapid communication between township tuberculosis centers, the National Tuberculosis Program (NTP) and the assigned central laboratories were assured to facilitate timely sample transport, data clarifications, and feedback of results by coordinating units.

There were 3 coordinating units; Central, Upper Myanmar and Lower Myanmar coordinating units. Upper Myanmar and Lower Myanmar coordinating units will supervise the overall working process of respective clusters, also managed regarding logistics of sample collections and laboratories commodities and communicated with Central coordinating unit to promote the timely transport of samples from clusters to respective laboratories and feedback of results to each cluster.

9. Survey Monitoring and Quality Assurance Considerations

9.1. Preparatory and pre-survey visit

The ½ - 1 day per cluster pre-survey visit to each selected cluster by the study team was an essential step for a successful implementation of the survey. The main objective of the pre-visit was to explain the objectives and methods of the survey, review the township tuberculosis laboratory (premises, equipment, supplies, procedures and human resource) and identify requirements prior to the training session. The pre-visit team identified persons who were responsible for the data collection locally and developed a list of the staff invited for the Drug Resistance Survey training session. The pre-visit team established a detailed and diagnostic laboratory-specific plan for collection and transportation of sputum specimens from the township tuberculosis centers to the corresponding laboratory. The diagnostic facility (township tuberculosis centers) including

facility laboratory was assessed using a pre-defined checklist for personnel, equipment, workload and infrastructure (Annex 11). If any deficiency was identified during pre-visit assessment, the pre-visit team reported the findings to the survey coordinator thus appropriate corrective actions were taken as required and recorded for further references.

9.2. Supervisory visits during the survey period

Supervisory visits were performed for quality assurance of enrollment procedures. Those visits were conducted monthly by monitoring team that included representatives from the central operational team and relevant regional focal point. During those visits the monitoring team verified that all eligible patients were included in the survey register, availability of supplies, monitoring completeness and quality of Clinical Information Forms (CIFs), and re-interview a few enrolled patients to double check quality of clinical information forms of enrolled patients. Reinterviews were conducted for a random sample of patients that come for DOT on the day of the visit. Re-interview data were entered on a separate similar form (i.e. the original forms must not be updated) using the patient's identifiers for comparison in the analysis stage. A short-standardized monitoring report was prepared; identified problems was addressed on the spot or later by Drug Resistance Survey team at central office. For all visits, a standard checklist was used to also serve for reporting and for comparison of findings between clusters (Annex 12). Central survey laboratories in Yangon and Mandalay were visited regularly; a standard checklist was used for assessment (Annex 13 and 14).

9.3. Survey coordination

During the patient enrolment period, the survey data managers regularly produced survey progress reports based on the collected data. The research coordination team met weekly to discuss the progress of the survey and preliminary findings. Coordination team had a midterm review of the survey progress and produced a report to be presented at steering committees. Prior to the midterm review steering committee and coordination team conducted supportive supervision to a selected number of clusters so as to review actual implementation on the ground.

A monitoring visit form/checklist was used during monitoring field visits to systematically guide monitoring activities and ensure adequate reporting of monitoring outcomes during Drug Resistance Survey monthly meetings (Annex 12 and 16). During field monitoring visits, all rifampicin-resistant pulmonary tuberculosis patients identified by Xpert MTB/RIF at the central laboratories, was reinterviewed to ensure that the township tuberculosis centers have correctly classified patients into new or previously treated.

The WHO monitoring visit took place close to the start date of the survey. The central operational team visited almost all township tuberculosis centers every other week to follow-up on enrolment progress and discuss/identify any issues that arise. This monitoring was conducted by phone and a phone-based assessment form was used (Annex 17). A systematic monitoring schedule was developed prior to the start of the survey. Survey monitoring (field visits; phone-assessment) was supported/strengthened by continued communication between township tuberculosis centers, National Tuberculosis Program and central laboratories. Monitoring of Drug Resistance Survey quality and progress involved regular cross-tabulation of key indicators from the electronic database (Annex 18). Regarding the Quality assurance of laboratory procedures, SRL Chennai which took the responsibility for quality assurance of culture, drug susceptibility testing and Line Probe Assay and sent the proficiency testing samples to both National Tuberculosis Reference Laboratory and Upper Myanmar Tuberculosis Laboratory, Mandalay. Quality assurance of Whole Genome Sequencing took the responsibility from Otago University, New Zealand.

10. Data Management and Analysis

National Tuberculosis Program will identify the software that will be used for electronic capture of data, cross-validation check and reporting, in agreement with local expertise. It is anticipated that Epi Info version 3.5.4 will be used for the Drug Resistance Survey. The survey data will be stored in Access format. Access to the patient data will be limited through restricting access to National tuberculosis Program registers

to health workers and selected member of the study team and data entry screens and surveys data files will also be password-protected and strictly controlled under the supervision of the data managers. Any electronic data transfer will be encrypted. The datasets and associated electronic files used in the survey will be held securely with appropriate access controls in place to ensure that only authorized survey staff can view, edit or delete them. Survey data managers should be responsible for ensuring that data files are held securely and for authorizing appropriate access to the data files. The Drug Resistance Survey database will not contain patient identifiers other than the Drug Resistance Survey ID. At the end of the Drug Resistance Survey, the pseudonymised database will Page 39 of 112 be fully anonymised by destroying the link between the unique Drug Resistance Survey ID and any patient identifiers. The survey implemented a combination of paper and electronic data entry.

Data was collected at the facilities on paper first, using the survey Clinical Information Form (CIF), then entered in the database at one of two survey data management units (DMU) located at the assigned central laboratories (Upper Myanmar laboratory in Mandalay and National Tuberculosis Reference Laboratory in Yangon). Central laboratory test results (i.e. culture (MGIT) and identification) were entered on the paper Central Laboratory Results Form (Annex 07) and captured electronically at each Data Management Unit as soon as results became available. Xpert MTB/RIF results and final Whole Genome Sequencing test results were quarterly imported directly into the Drug Resistance Survey database in Excel format and linked to the central survey database by Drug Resistance Survey ID. The results from patient re-interviews during monitoring visits, was also entered in the Drug Resistance Survey database. Re-interview data was not overwritten the original results but was kept in a separate database table for quality assurance purposes.

The survey database included data entry module, build-in data validation module including data compare, automatically generated reports and ad-hoc analysis module. Entry of sample tracking information in the database facilitated the monitoring of sample transport and storage processes. Several levels of data validation were implemented to assure data quality assurance.

1. Survey data manager reviewed the filled forms to ensure completeness and then submitted the completed forms to data entry team.
2. Data was double entered at each Data Management Unit to reduce errors in transcription from paper to electronic records: the same records were entered separately by two different data entry clerks and the records was compared to ensure they were identical. Discrepancies were resolved using the original paper forms and local Drug Resistance Survey registers; all identified discrepancies were documented.
3. Built-in data validation checks (check-codes), including skip patterns, ranges, and others were added to data entry screens to prevent errors during data entry. Auto-search for entry of duplicated records were also available.
4. Survey database included data validation module to examine data after entry. The module run automatically by clicking on a button. The module included codes to look for duplicates, missing and inconsistent values. Data validation reports will be produced by data managers on regular base; the identified issues were addressed accordingly. Completed and cleaned databases were merged at the end of the study into a master database at the Naypyitaw Data Management Unit. The Upper Myanmar Data Management Unit kept all study forms received from assigned township tuberculosis centers until the end of the study period, and forms were then centralized at Naypyitaw Data Management Unit once enrolment was completed. The project identified 2 database managers for each data management unit and 4 part-time data entry clerks (2 per each Data Management Unit) to proceed with double-data entry. The data managers were responsible for designing the database, data validation and reporting. They coordinated double-data entry and conducted data compare. A database dictionary was prepared in advance to inform the database design. The database was kept up to date, and records were entered as soon as available.

The database was backed-up regularly in more than one secured computer/drive. In addition, back-up copies will not overwrite previous copies - a system of version control with storage of old copies was used instead.

During the Drug Resistance Survey enrolment period the database managers at both Data Management Units run cross validation checks regularly (e.g. at least once a week) and produced data

validation reports to facilitate data cleaning (e.g. by contacting township tuberculosis centers to retrieve correct information as appropriate). Both database managers regularly produced survey progress reports of cross tabulated data to monitor the quality and progress of the Drug Resistance Survey and inform Drug Resistance Survey coordinator and the survey team in regular meetings. At the end of the study, the final analysis was conducted with technical assistance from WHO. Briefly, percentage resistance was estimated among new and previously treated patients using statistical models (Multiple imputation method) that account for the cluster design. If needed, multiple imputation of missing values was performed, and sampling weights might be applied to account for under- or over-enrolment in each cluster.

11. Publication

Findings from this study was disseminated to Tuberculosis-Technical Strategic Group (TB-TSG) members and responsible persons from Department of Public Health and implementing partners including National and International Non-government Organizations. The study results and outcomes were compiled and would be published in international journals and also in a study report that would be shared with relevant stakeholders in Myanmar.

12. Pre-survey Training

Training of the survey staff National Tuberculosis Program developed the Standard Operation Procedure (SOP) for Fourth National anti-tuberculosis Drug Resistance Survey and pre-survey training session(s) to respective Township Medical Officer (TMO), tuberculosis Coordinators & Laboratory technicians for field teams was conducted at Nay Pyi Taw on 28th January 2020 before the start of the survey by using the developed standardized training materials (Annex 15).

The training lasted two days. The participants were informed on the rationale for the study, the protocol, and the data capture forms. The participants were engaged in a practical exercise on interview-taking and form-filling and good clinical practice (GCP). The training package involved enrolment procedures, sputum processing, recording, reporting, packaging, storage and shipment. It included sensitization on sputum transport and (safety issues) infection control to persons involved in transportation of sputum samples. Role-play exercise followed the training. Laboratory technologists were trained on the registration of Drug Resistance Survey specimens, recording of the results on the Drug Resistance Survey laboratory forms including how to fill the results of Xpert MTB/RIF tests, liquid culture, line probe assays and DNA extraction. The database was installed on computers of Drug Resistance Survey data management team members; survey data manager and data entry clerks were trained on data entry, data checking and cleaning and producing the reports. Harmonization and standardization of two culture laboratories were conducted in addition to above trainings.

13. Ethical Considerations

The survey protocol and detailed procedures were carried out after approval from Institutional Ethical Review Board. The study had no direct or indirect physical, emotional, mental and social impact on those who chose or did not choose to participate in it. There would be no harm to individuals, and the environment resulting from the study. The patients were informed that all participation is voluntary, no compensation was provided for participation and no services were withheld if they chose not to participate in the study. The privacy of the study participants was maintained by limiting access to patient identifiers to only those individuals directly involved in providing diagnostic, treatment and care services to the participants. No patient identifier was included in any study materials that were published or made public.

14. Drug Resistance Survey Management and Governance

For the implementation of Fourth National Anti-tuberculosis Drug Resistance Survey, the steering committee was set up. A survey steering committee (SC) was given the final responsibility for the protocol, funding, data collection, data management and analysis, and dissemination of results. The overall implementation of National anti-tuberculosis Drug Resistance Survey will be managed by Central National Tuberculosis Program. There was coordinating units for Central, Lower Myanmar and Upper Myanmar. Terms of reference for each person who were involved in implementation of Fourth National anti-tuberculosis Drug Resistance Survey were set. The responsible persons of following committee and groups were mentioned (annex)

- Steering committee
- Technical advisory group
- Survey preparation working group
- Central and Lower/Upper Myanmar coordinating units

15. Study Timelines

Activity	2019						2020												2021-22	
	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12		
Steering committee meeting and Procurement																				
Finalization of Protocol and Budget																				
Recruitment of personnel																				
Development of Standard Operation Procedures (SOPs) and supporting materials																				
Procurement of consumables																				
Township tuberculosis centers readiness assessment																				
Training of staff																				
Patient enrollment																				
laboratory tests																				
Whole Genome Sequencing																				
Data analysis																				
Report writing																				
Dissemination of results																				

16. Findings

16.1. Survey Flow and its results

The survey flowchart of presumptive tuberculosis patients from 50 townships and their sputum smear microscopy results, Drug Susceptibility Testing results by Xpert MTB/RIF assay of new and previously treated tuberculosis patients, MGIT culture results, Line Probe Assay and Whole Genome Sequencing results available status were described in figure (4)

Sputum smear microscopy

Two sputum specimens were collected from each patient and Xpert MTB/RIF assay was tested for sputum smear positive as well as sputum smear negative specimens. For sputum smear negative patients with suggestive of tuberculosis in chest X'ray were included for testing the Xpert MTB/RIF assay.

A total number of 2129 patients among them, 1901 new tuberculosis patients and 228 previously treated tuberculosis patients who gave informed consent were enrolled in the study. Among 1901 new tuberculosis patients, 1579 sputum smear positive patients and 322 sputum smear negative patients were tested for Xpert MTB/RIF assay.

X pert MTB/RIF results of new tuberculosis patients

Among the studied sputum smear positive new tuberculosis patients (1579), 1461 were detected as mycobacterium tuberculosis positive, while Rifampicin Resistant Tuberculosis was found in 60 patients and intermediate result was found in 5 patients, 39 patients were found negative results, 4 invalid results, 9 errors and 1 missing result were recorded. Of 322 sputum smear negative patients, 295 were detected as mycobacterium tuberculosis positive, while Rifampicin Resistant Tuberculosis was found in 15 patients and intermediate result was found in 12 patients.

X pert MTB/RIF results of previously treated tuberculosis patients

Among 179 of 228 previously treated tuberculosis patients, mycobacterium tuberculosis was detected in 138 patients, while 20 patients were found to be Rifampicin Resistance (RR), 2 were intermediate results, 17 patients were found negative results, 1 invalid result and 1 error result were recorded. Of 49 sputum smear negative patients, 44 patients were detected as mycobacterium tuberculosis positive, while Rifampicin Resistant Tuberculosis was found in 3 patients and intermediate result was found in 2 patients.

Undergoing Mycobacterium Growth Indicator Tube (MGIT) Culture

Of 1430 of 1577 sputum smear positive new tuberculosis patients, 242 of 321 smear negative new tuberculosis cases, 143 of 179 sputum smear positive previously treated tuberculosis patients and 24 out of 49 sputum smear negative previously treated tuberculosis patients were documented as MGIT culture positive. The MGIT culture negative results of new tuberculosis patients were noted in 107 cultures, non-tuberculous mycobacteria were found in 28 cultures and 12 were contaminated. The MGIT culture negative results of previously treated tuberculosis patients were noted in 30 cultures, non-tuberculous mycobacteria were found in 5 cultures and 1 was contaminated.

Line Probe Assay

Among 1672 total MGIT culture positive of new tuberculosis patients including sputum smear positive (1430) as well as sputum smear negative (242), Line Probe Assay results were available in 1663 patients. Of 1663 patients, 173 were Isoniazid resistance, 53 were Rifampicin resistance, 3 were Amikacin/Capreomycin/Kanamycin resistance and 25 were Fluoroquinolone resistance. Of 55 available Line Probe Assay results in culture negative sputum smear positive new tuberculosis patients, 8 were Isoniazid resistance, 3 were Rifampicin resistance. Amikacin/Capreomycin resistance and Fluoroquinolone resistance were 1 and 3 out of 50 patients. Rifampicin resistance was found in 1 out of 2 culture negative sputum smear negative new tuberculosis patients.

Among 167 total culture positive results of previously treated tuberculosis patients of both sputum smear status, 165 were Line Probe Assay results available. Isoniazid (INH) and Rifampicin resistance were found in 34 and 22 of 164 Line Probe Assay available results respectively. Amikacin/Capreomycin, Kanamycin and Fluoroquinolone resistance were found in 1 out of 163, 2 of 163 and 8 of 163 Line Probe Assay available results respectively. Rifampicin resistance was detected in 2 out of 16, Isoniazid resistance was 1 out of 16 among culture negative sputum smear positive previously treated tuberculosis patients. Amikacin/Capreomycin/ Fluoroquinolone resistance were not detected in this category of patients. Only 1 Rifampicin resistance was detected in 2 Line Probe Assay results available from sputum smear negative, culture negative new tuberculosis patients. No Line Probe Assay result was available from sputum smear negative culture negative patients.

Whole Genome Sequencing (WGS)

Regarding new tuberculosis patients, the whole genome sequencing results were available in 1409 out of 1672 total MGIT culture positive results. The resistance to Streptomycin (S), Isoniazid (H), Rifampicin (R), Ethambutol (E), Pyrazinamide (Z), Amikacin (Am), Levofloxacin (Lfx), Moxifloxacin (Mfx) and Ethionamide (Eto) were found in 141, 140, 43, 25, 14, 1, 21, 21 and 38 patients respectively. None were resistant to Para aminosalicylic acid (PAS), Linezolid (Lzd), Bedaquiline (Bdq), Cycloserine (Cs), Clofazimine (Cfz) and Delamanid (Dlm). The whole genome sequencing results were available in 134 previously treated tuberculosis patients out of 167 total MGIT culture positive results. The resistance to Streptomycin (S), Isoniazid (H), Rifampicin (R), Ethambutol (E), Pyrazinamide (Z), Amikacin (Am), Levofloxacin (Lfx), Moxifloxacin (Mfx) and Ethionamide (Eto)were found in 26,27,18,12,9,1,8,8 and 3 patients respectively. None are resistant to Para aminosalicylic acid (PAS), Linezolid (Lzd), Bedaquiline (Bdq), Cycloserine (Cs), Clofazimine (Cfz) and Delamanid (Dlm).

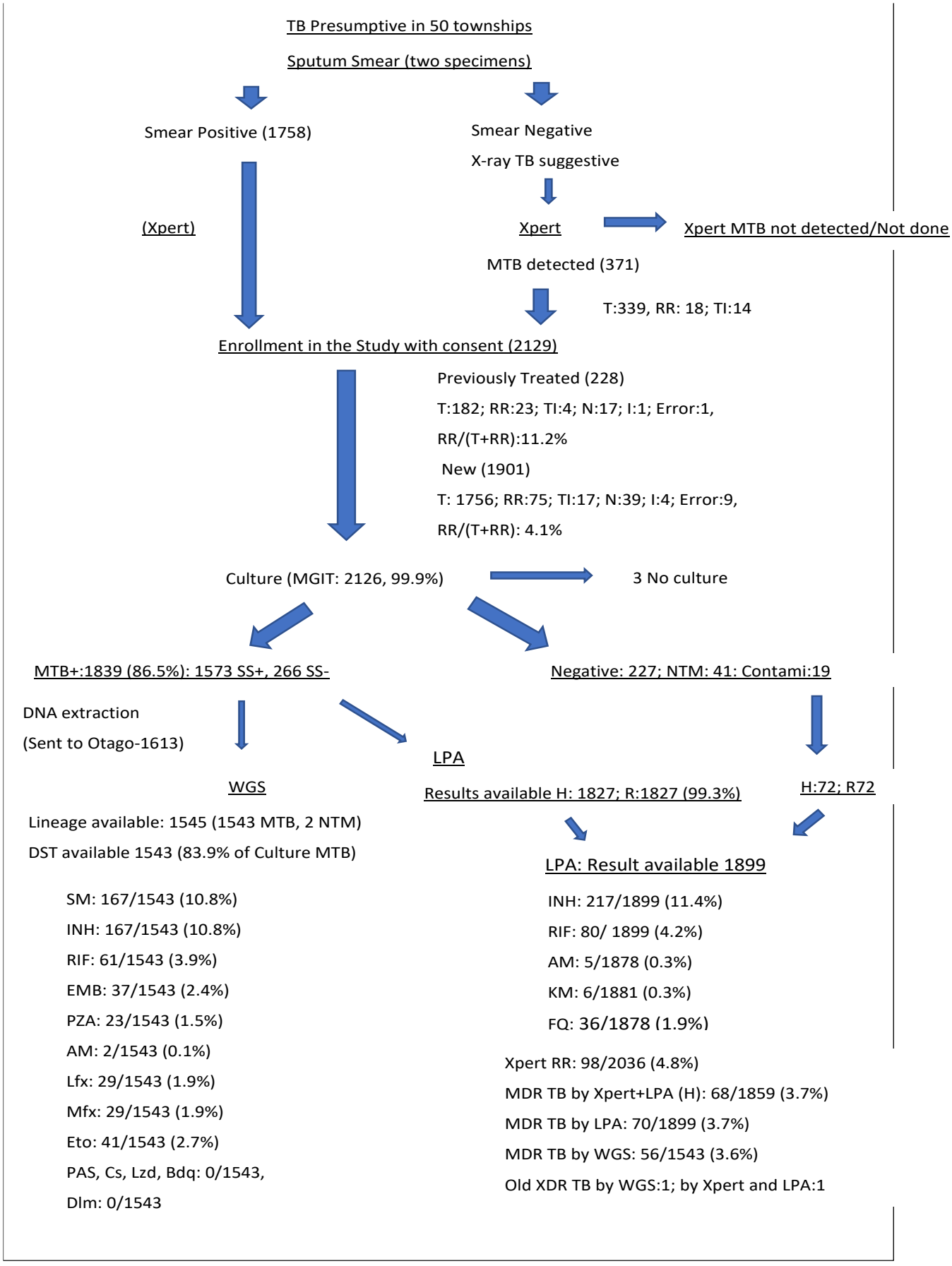


Figure (5) Survey Flow and its results

16.2. Primary Objective: Estimate the prevalence of Drug Resistant tuberculosis (DR-TB)

Prevalence of Rifampicin Resistant tuberculosis (RR-TB), Multi-Drug-Resistant tuberculosis (MDR-TB) and Isoniazid resistant tuberculosis and their respective 95% confidence interval (CI)

By Multiple Imputation Model Analysis, prevalence of Rifampicin Resistant Tuberculosis, **Multi-Drug-Resistant** Tuberculosis and Isoniazid Resistant tuberculosis were estimated in new and previously treated tuberculosis patients.

The point prevalence of Rifampicin Resistant Tuberculosis was 3.3% (95% CI 2.4% - 4.5%) in new tuberculosis patients and 13.1% (95% CI 7.8% - 21%) in previously treated tuberculosis patients.

It also showed that point prevalence of **Multi-Drug-Resistant** Tuberculosis was 3.0% (95% CI 2.1% - 4.1%) in new tuberculosis patients and 11.8% (95% CI 6.8% - 19.6%) in previously treated tuberculosis patients.

It was also noted that point prevalence of any Isoniazid resistant tuberculosis was 10.2% (95% CI 8.7% - 12%) in new tuberculosis patients and 20.9% (95% CI 14.8% - 19.6%) in previously treated tuberculosis patients. (Table 1)

Table 1: Point Prevalence and 95% Confidence Interval of Rifampicin Resistant Tuberculosis, Multi-Drug-Resistant Tuberculosis and any Isoniazid resistant tuberculosis by Multiple Imputation Model Analysis

Drug Resistance Pattern	Type of Patients	Point Prevalence	95% Confidence Interval
Rifampicin Resistant Tuberculosis	New	3.3%	2.4% - 4.5%
	Previously treated	13.1%	7.8% - 21%
Multi-Drug-Resistant Tuberculosis	New	3%	2.1% - 4.1%
	Previously treated	11.8%	6.8% -19.6%
Any Isoniazid resistant tuberculosis	New	10.2%	8.7% -12%
	Previously treated	20.8%	14.8% -28.5%

The point prevalence of Rifampicin Resistant Tuberculosis was 3.3% (95% CI 2.4% - 4.5%) in new tuberculosis patients and 13.1% (95% CI 7.8% - 21%) in previously treated tuberculosis patients.

It also showed that point prevalence of **Multi-Drug-Resistant** Tuberculosis was 3.0% (95% CI 2.1% - 4.1%) in new tuberculosis patients and 11.8% (95% CI 6.8% - 19.6%) in previously treated tuberculosis patients.

It was also noted that point prevalence of any Isoniazid resistant tuberculosis was 10.2% (95% CI 8.7% - 12%) in new tuberculosis patients and 20.9% (95% CI 14.8% - 19.6%) in previously treated tuberculosis patients.

16.3. Secondary Objectives 1: Determine the proportion of patients with resistance to first- and second-line anti-tuberculosis drugs

(1) Proportion of patients with Rifampicin Resistance by Xpert MTB/RIF and Whole Genome Sequencing

By using the different testing, Rifampicin Resistance proportion was different. Therefore, the proportions of Rifampicin Resistant tuberculosis cases among new tuberculosis cases were 4.1% (75/1831) and 3.1%

(43/1409) by X pert MTB/RIF and Line Probe Assay accordingly. The similar finding was noted among previously treated tuberculosis patients. The proportions of Rifampicin Resistant tuberculosis cases among previously treated tuberculosis patients were 11.2% (23/205) and 10.8% by Xpert MTB/RIF and Whole Genome Sequencing test accordingly. By Xpert MTB/RIF testing revealed that 98 (4.8%) out of 2036 all tuberculosis patients were Rifampicin resistance tuberculosis. (Table 2)

Table 2: Proportion of patients with Rifampicin Resistant tuberculosis by Xpert MTB/RIF and Whole Genome Sequencing

Test	New tuberculosis cases		Previously treated tuberculosis case		Total tuberculosis cases	
	Rifampicin Resistant Tuberculosis cases (%)	Total tested	Rifampicin Resistant Tuberculosis cases (%)	Total tested	Rifampicin Resistant Tuberculosis cases (%)	Total tested
Xpert MTB/RIF	75 (4.1%)	1831	23 (11.2%)	205	98 (4.8%)	2036
Whole Genome Sequencing	43 (3.1%)	1409	18 (10.8%)	167	61 (3.9%)	1576

(2) Proportion of patients with Multi-Drug-Resistant Tuberculosis by Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing

By using the different testing procedures, proportion of **Multi-Drug-Resistant** Tuberculosis was different. The proportions of **Multi-Drug-Resistant** Tuberculosis cases among new tuberculosis patients were 3.02% (51/1686), 2.97% (51/1719) and 2.84% (40/1409) by Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing accordingly. The similar finding was noted among previously treated tuberculosis patients. The proportions of **Multi-Drug-Resistant** Tuberculosis cases among previously treated tuberculosis patients were 9.83% (17/173), 10.56% (19/180) and 11.94% (16/134) by Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing accordingly. (Table 3)

Table 3: Proportion of patients with Multi-Drug-Resistant Tuberculosis by Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing

Test	New tuberculosis cases		Previously treated tuberculosis cases		Total	
	Multi-drug Resistant Tuberculosis cases (%)	Total tested	Multi-drug Resistant Tuberculosis cases (%)	Total tested	Multi-drug Resistant Tuberculosis cases (%)	Total tested
Xpert MTB/RIF & Line Probe Assay	51 (3.02%)	1686	17(9.83%)	173	68 (3.66%)	1859
Line Probe Assay	51 (2.97%)	1719	19 (10.56%)	180	70 (3.69%)	1899

Whole Genome Sequencing	40 (2.84%)	1409	16 (11.94%)	134	56 (3.63%)	1593

(3) Proportion of patients with Pre-extensively drug resistant tuberculosis (pre XDR-TB) by Xpert MTB/RIF and Line Probe Assay

By using the different testing, proportion of Pre extensively drug resistant tuberculosis was different. Therefore, the proportions of Pre-extensively drug resistant tuberculosis and extensively drug resistant tuberculosis (XDR-TB) cases among new tuberculosis cases were 0.65% and 0.76% by (Rifampicin Resistance by Xpert MTB/RIF + Fluoroquinolone resistance by Line Probe Assay) and (Rifampicin Resistance + Fluoroquinolone resistance by Line Probe Assay) accordingly. The similar finding was noted among previously treated tuberculosis. The proportions of **Multi-Drug-Resistant** tuberculosis cases among previously treated tuberculosis cases were 0.86% and 0.97% by (Rifampicin Resistance by Xpert MTB/RIF+ Fluoroquinolone resistance by Line Probe Assay) and (Rifampicin Resistance + Fluoroquinolone resistance by Line Probe Assay) accordingly. (Table 4)

Table 4: Proportion of patients with pre-extensively drug-resistant tuberculosis by Xpert MTB/RIF and Line Probe Assay

Test	New tuberculosis cases		Previously treated tuberculosis cases		Total	
	Pre XDR-TB cases (%)	Total tested	Pre XDR-TB cases (%)	Total tested	Pre XDR-TB cases (%)	Total tested
(Rifampicin Resistance by Xpert MTB/RIF + Fluoroquinolone resistance by Line Probe Assay)	11 (0.65%)	1686	5 (2.89%)	173	16 (0.86%)	1859
(Rifampicin Resistance + Fluoroquinolone resistance by Line Probe Assay)	13 (0.76%)	1702	5 (3.07%)	163	18 (0.97%)	1865

(4) Proportion of patients with extensively drug resistant tuberculosis (Pre XDR TB + resistance to Bedaquiline and/or Linezolid)

There was no patient with extensively drug resistant tuberculosis [Pre XDR-TB + resistance to Bedaquiline (Bdq) and/or Linezolid (Lzd)] detected among both new as well as previously treated tuberculosis cases.

Proportion of patients with Isoniazid-mono Resistant tuberculosis, Rifampicin Resistant tuberculosis and Fluoroquinolone Resistant tuberculosis by Line Probe Assay

Isoniazid mono-resistant tuberculosis was elaborated after Line Probe Assay testing. Therefore, 7.38% (127/1720) of new and 9.39% (17/181) of previously treated tuberculosis patients were Isoniazid mono-resistant.

Rifampicin mono-resistant tuberculosis was also detected after Line Probe Assay testing so that 0.29% (5/1720) of new and 2.21% (4/181) of previously treated tuberculosis patients were Rifampicin mono-resistant.

Fluoroquinolone Resistant tuberculosis only was also obtained after Line Probe Assay testing, so that 0.82% (14/1702) of new and 1.84% (3/163) of previously treated tuberculosis patients were Fluoroquinolone resistance.

It depicted that 0.18% (3/1702) of new cases which were resistant to Injectables (Amikacin/Capreomycin/Kanamycin) was detected only but there was no case in previously treated tuberculosis patients. (Table 5)

Table 5: Proportion of patients with Isoniazid mono-resistant tuberculosis, Rifampicin Resistant tuberculosis, Fluoroquinolone Resistant tuberculosis and resistance to injectables (Amikacin/Capreomycin/Kanamycin) by Line Probe Assay

Resistance pattern	New tuberculosis cases		Previously treated tuberculosis cases		Total	
	Resistance cases (%)	Total test	Resistance cases (%)	Total test	Resistance cases (%)	Total test
Isoniazid mono-resistant tuberculosis	127 (7.38%)	1720	17(9.39%)	181	217(11.42%)	1901
Rifampicin mono-Resistant tuberculosis	5 (0.29%)	1720	4(2.21%)	181	9 (4.7%)	1901
Fluoroquinolone resistant tuberculosis	14(0.82%)	1702	3(1.84%)	163	17(9.1%)	1865
Injectables (Am/Cm/Km) Resistance only	3(0.18%)	1702	0	163	3 (1.6%)	1865

Regarding the katG and inhA mutations of isoniazid resistance by Line Probe Assay, among Rifampicin sensitive cases, overall Isoniazid mono-resistant tuberculosis was 8.08% out of which 5.2%, 2.5% and 0.4% were katG, inhA and both katG and inhA resistance respectively.

Among Rifampicin resistant tuberculosis cases, overall Isoniazid mono-resistant tuberculosis was 87.5% out of which 81.3%, 5% and 1.25% were katG, inhA and both katG and inhA resistance respectively.

Total 1899 cases, overall Isoniazid mono-resistant tuberculosis was 11.4% out of which katG, inhA and both katG and inhA resistance proportions were 83.7%, 2.58%, 0.4% accordingly. (Table 6)

Table 6. Rifampicin resistance status and Isoniazid mutations by Line Probe Assay results

LPA	**Line Probe Assay**				**Total**
	Sensitive	Resistant			
		katG	inhA	Both	
Rifampicin Sensitive	1672	94	45	8	1819
Rifampicin Resistant	10	65	4	1	80
Total	1682	159	49	9	1899

16.4. Secondary Objectives 2: Characterize the genotypic mutations conferring putative resistance to rifampicin and other anti-tuberculosis drugs;

Genotypic Mutations of Rifampicin by Whole Genome Sequencing

Concerning the rifampicin mutation by Whole Genome Sequencing, the distribution of mutations was expressed in Table (7).

The different types of Genotypic Rifampicin mutations were found in 11 subgroups. Among those subgroups, the highest proportion was found in S450L (45, 2.92%). The second highest proportion was found in L430P and

I49IF (3 each, 0.19%). The remaining 8 groups contributed as a very proportion. The highlighted subgroup was I491F which was missed by Xpert MTB/RIF test could be detected by Whole Genome Sequencing.

Table 7: Genotypic Mutations of Rifampicin by Whole Genome Sequencing

Genotypic Mutation Pattern	Number	Proportion
Sensitive	1482	96.05%
S450L	45	2.92%
L430P	3	0.19%
I491F	3	0.19%
H445N	2	0.13%
H445D	2	0.13%
H445Y	1	0.06%
Q432L/K446Q	1	0.06%
L452P	1	0.06%
D435G	1	0.06%
D435V	1	0.06%
H445R	1	0.06%
Total	1543	100.00%

Characterization of genotypic mutations conferring putative resistance to rifampicin and other anti-tuberculosis drugs

The commonest mutations in Streptomycin (S), Isoniazid (H), Rifampicin (R), Ethambutol (E), Pyrazinamide (Z), Amikacin (Am), Fluoroquinolones and Ethionamide (Eto) resistance were K43R, S315T, S450L, M306V, G132D/P45L, C1402Trrs/g-10a eis, A90V and C15T. (Table 8)

Table 8. Common Mutations Detected by Whole Genome Sequencing

Name of anti-tuberculosis Drugs	**Streptomycin (S)**	**Isoniazid (H)**	**Rifampicin (R)**	**Ethambutol (E)**	**Pyrazinamide (Z)**	**Amikacin (Am)**	**Fluoroquinolones**	**Ethionamide (Eto)**
Common mutation	K43R	S315T	S450L	M306V	G132D/ P45L	C1402T rrs/g-10a eis	A90V	C15T

16.5. Secondary Objectives 3. Socio-demographic and clinical characteristics of the survey tuberculosis population;

A total of 2129 tuberculosis patients were enrolled in the study.
Of the 2129 study participants, 1901 (89.3%) were new and 228 (10.7%) were previously treated tuberculosis patients.
Among new tuberculosis patients, 1234 were males (65%) and 667 were females (35%). Male preponderance was found in the study.
Among previously treated tuberculosis patients, 165 were males (72%) and 63were females (28%)
Regarding the age group, the most contributed age group was 34-45 year age group. There were 439 (23%) in new tuberculosis patients and 46 (20%) in previously treated tuberculosis patients.
Regarding notification source, public sector was more pronounced than private sector 1696 (89%) and 197 (86%) in new and previously treated tuberculosis patient respectively.
Distribution of tuberculosis patients by Upper Myanmar and Lower Myanmar showed that majority of cases were from Lower Myanmar. Out of new tuberculosis patients, 1326 (70%) were enrolled from Lower Myanmar and out of previously treated tuberculosis patients, 73% were enrolled from Lower Myanmar.
Regarding the regions and states, the highest number was found in regions other than Yangon region. There were 844 (44%) and 92 (40%) among new and previously treated tuberculosis patients. Among all enrolled patients, 591 (31%) and 86 (32%) of new and previously treated tuberculosis patients lived in Yangon Region
In terms of smoking status, 988 (52%) of the new tuberculosis patients and 113 (50%) of previously treated tuberculosis patients were non-smokers. The proportion of study patients with smoking was the lowest and 22% and 21% among new and previously treated tuberculosis patients accordingly.
In terms of Diabetes Mellitus, 266 (14%) and 33 (15%) out of new and previously treated tuberculosis patients had diabetes mellitus. (Table 9)

Table 9. Socio-demographic and clinical characteristics of the survey tuberculosis population

Socio-demographic and clinical characteristics	New (1901)	Previously treated (228)	Total (2129)
Gender			
Male	1234, 65%	165, 72%	1399, 66%
Female	667, 35%	63, 28%	730, 34%
Age group			
<15	10,1%	2,1%	12,1%
15-24	266,14%	18,8%	284, 13%
25-34	336,18%	41,18%	377,18%
35-44	439, 23%	46,20%	485, 23%
45-54	360,19%	44, 19%	404, 19%
55-64	276, 15%	45, 20%	321, 15%
>= 65	214,11%	32, 14%	246, 12%
Notification source			
Public	1696, 89%	197, 86%	1893, 89%
Private	205, 11%	31, 14%	236, 11%
Enrollment by Upper and Lower Myanmar			
Upper Myanmar	575, 30%	62, 27%	637, 30%
Lower Myanmar	1326, 70%	166, 73%	1492, 70%
Region/State			
Yangon	591, 31%	86, 38%	677, 32%
Regions other than Yangon	844,44%	92, 40%	936, 44%
States	466, 25%	50, 22%	516, 24%
Smoking Status			
Smokers	410, 22%	47, 21%	457, 21%

Non-smokers	988, 52%	113, 50%	1101, 52%
Ex-smokers	503, 26%	68, 30%	571, 27%
Diabetes Mellitus status			
Yes	266, 14%	33, 15%	299, 14%
No	1355, 71%	165, 72%	1520, 71%
Unknown	280, 15%	30, 13%	310, 15%

16.6. Secondary objective 4. Investigate possible risk factors for Rifampicin Resistant Tuberculosis/ Multi-Drug-Resistant Tuberculosis

In sub-group analysis, the number and the proportion of Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) among new and previously treated tuberculosis patients were 75 (77%) and 23 (23%). Therefore, the proportion of Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) among new tuberculosis cases were 3.3 times greater than that among previously treated tuberculosis patients. Odds Ratio (OR) and 95% Confidence Interval (CI) were 2.73 and 1.68-4.45 with p value was 0.000165. It was found that there was a significant association between Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) and previous anti-tuberculosis treatment history.

The proportions of male and female Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) were 59% and 41%.

The greatest number (24) and the proportion (25%) of Rifampicin Resistant tuberculosis and Multi-Drug-Resistant Tuberculosis (MDRTB) cases was detected among the age group of 15-to-24-year age group.

Most of the Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) patients were detected in Yangon Region. Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) cases were 1.7 times and 3 times higher in Yangon Region than states and other regions.

Regarding the Diabetes Mellitus status, Rifampicin Resistant tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) was found in 13%, 76% and 11% among patients who had Diabetes Mellitus, no Diabetes Mellitus and unknown status. The proportion of Rifampicin Resistant tuberculosis and Multidrug-resistant tuberculosis (MDRTB) occurred in those who did not have Diabetes Mellitus was 5.7 times higher than in those who patients who had Diabetes Mellitus.

Regarding the smoking status, 19 %, 62% and 19% of those Rifampicin Resistant Tuberculosis and **Multi-Drug-Resistant** Tuberculosis (MDRTB) had a smoking status, non-smoking status and ex-smokers. It was pointed out that the proportion of Rifampicin Resistant Tuberculosis and **Multi-Drug-Resistant** tuberculosis (MDRTB) was 3.2 times greater among non-smokers than that of smokers. (Table 10)

Table 10. Risk factors for the development of Rifampicin Resistant Tuberculosis (Xpert MTB/RIF) among survey tuberculosis population

Variable	levels	Rifampicin Resistant Tuberculosis		Unadjusted		
		N	%	OR	95% CI	P Value
Treatment history	New	75	77%	1		
	Previously treated	23	23%	2.73	1.68-4.45	0.000165
Gender	Male	58	59%	1		
	Female	40	41%	1.34	0.89 – 2.03	0.190696
Age group	<15	1	1%	1		
	15-24	24	25%	1.02	0.13 – 8.20	1.000000
	25-34	23	24%	0.71	0.09 – 5.78	0.539559
	35-44	16	16%	0.38	0.05 – 3.09	0.344545
	45-54	11	11%	0.31	0.37 – 2.59	0.299560
	55-64	16	16%	0.58	0.07 – 4.75	0.472563

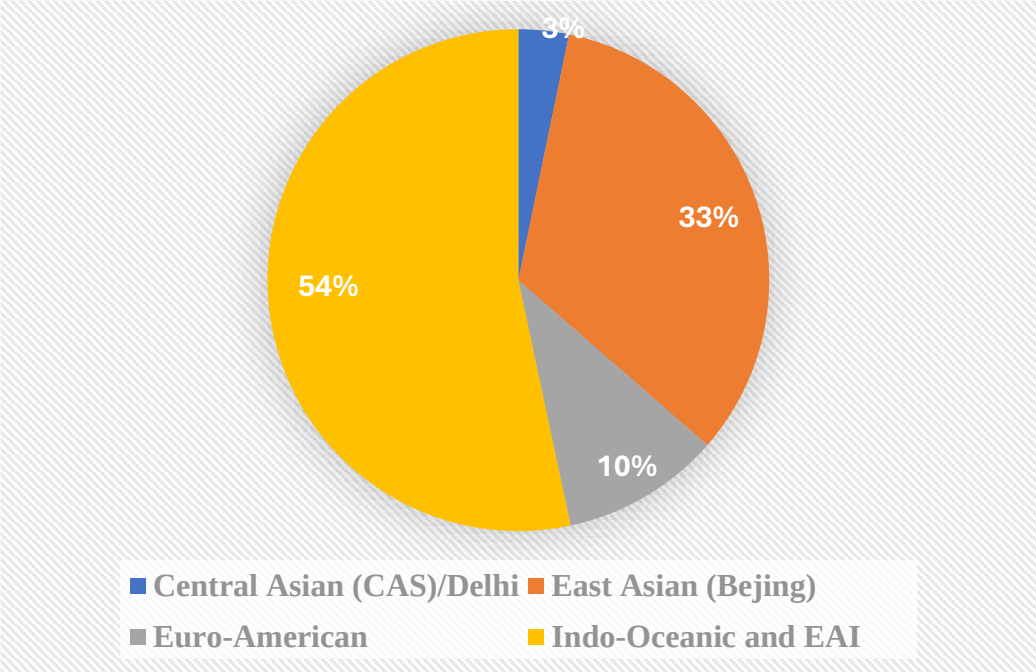
	>= 65	7	7%	0.32	0.04 – 2.85	0.32051
Region	Yangon	51	52%	1.74	0.99 – 3.06	0.051147
	Regions other than Yangon	29	30%	0.81	0.44 – 1.49	0.521319
	States	17	18%	1		
Diabetes Mellitus status	Yes	13	13%	0.89	0.49 – 1.62	0.76890
	No	74	76%	1		
	Unknown	11	11%	0.72	0.38 – 1.37	0.375259
Smoking status	Smokers	19	19%	0.74	0.44 – 1.25	0.313185
	Non-smokers	61	62%	1		
	Ex-smokers	18	19%	0.55	0.32 – 0.95	0.02888

16.7. Objective 5: Characterize the Tuberculosis genotypes and their resistance profiles among the target population, in relation to socio-demographic and clinical population traits;

16.7.1. Tuberculosis genotypes and their resistance profiles among the target population

Indo-Oceanic and EAI (823,54%) was most common genotype followed by East Asian (Beijing) (512,33%), Euro-American (158,10%) and Central Asian (CAS)/Delhi (50,3%). (figure 6)

Figure (6) Genotype distribution



Regarding both first and second line anti-tuberculosis drug resistance, the most common genotype was East Asian (Beijing). (Table 11)

Table 11: Tuberculosis genotypes and their resistance profiles

Genotype	Resistance to

	Streptomycin(S)	Isoniazid (H)	Rifampicin (R)	Ethambutol (E)	Pyrazinamide (Z)	Amikacin (Am)	Levofloxacin (Lfx)	Moxifloxacin (Mfx)	Ethionamide (Eto)	Para aminosalicylic acid/ Cycloserine/ Bedaquiline/ Linezolid/Clofazimine/ Delamanid
Indo-Oceanic and EAI	41	54	5	2	5	1	5	5	25	0
East Asian (Beijing)	115	90	49	31	17	1	20	20	7	0
Central Asian (CAS)/Delhi	6	8	3	0	1	0	0	0	3	0
Euro-American	5	15	4	4	0	0	4	4	6	0
Total	167	167	61	37	23	2	29	29	41	0

16.7.2. Genotype distribution by Regions and States

Indo-Oceanic and EAI was the most common genotype in all regions, Mon, Ayeyarwaddy, Bago, Kayin, Magway, Mandalay, Rakhine, Sagaing, Tanintharyi, Yangon except Kachin, Shan East, Shan North and Shan South where East Asian (Beijing) was dominant. (Table 12)

Table 12: Genotype distribution by Regions and States

Region/State	Indo-Oceanic and EAI	East Asian (Beijing)	Euro-American	Central Asian (CAS)/Delhi	Total
Mon	37(4.5%)	12(2.34%)	11(7%)	2 (4%)	50(3.2%)
Ayeyarwaddy	99(12%)	31(6.1%)	7(4.43%)	0	137(8.9%)
Bago	77(9.4%)	26(5.1%)	16(10.1%)	10(20%)	129(8.4%)
Kachin	17(2.1%)	40(7.8%)	6(3.8%)	2(4%)	65(4.2%)
Kayin	48(5.8%)	20(3.9%)	6(3.8%)	1(2%)	75(4.9%)
Magway	33(4.0%)	28(5.5%)	8(5.1%)	3(6%)	72(4.7%)
Mandalay	85(10.3%)	69(13.5%)	27(17.1%)	8(16%)	189(12.2%)
Rakhine	51(6.2%)	4(0.8%)	4(2.5%)	0	59(3.8%)
Sagaing	60(7.3%)	46(8.9%)	12(7.6%)	6(12%)	124(8%)
Shan East	9(1.1%)	12(2.34%)	7(4.43%)	1(2%)	29(1.9%)
Shan North	4(0.49%)	20(3.9%)	8(5.1%)	1(2%)	33(2.1%)

Shan South	2(0.24%)	20(3.9%)	1(0.6%)	0	23(1.5%)
Tanintharyi	44(5.35%)	25(4.9%)	8(5.1%)	2(4%)	79(5.1%)
Yangon	257(31.2%)	159(31.1%)	37(23.4%)	14(28%)	467(30.3%)
Grand Total	823 (53%)	512 (33%)	158 (10%)	50 (3%)	1543

16.7.3. Prevalence of Rifampicin Resistant tuberculosis and Multi-Drug-Resistant tuberculosis among new tuberculosis patients in relation to socio-demographic characteristics based on Whole Genome Sequencing Results

During the study period, a total number of 1672 new tuberculosis cases were tested for Whole Genome Sequencing. Out of them, Whole Genome Sequencing results were available in 1409 and 263 cases were missing. Rifampicin Resistant Tuberculosis was detected in 43 (2.9%), **Multi-Drug-Resistant** Tuberculosis was detected in 40 (2.7%) and Isoniazid Resistance +/- other drug resistance was found in 139 (10.2%) cases.

(1)Age group

The number of new tuberculosis patients aged 35-44 years (388) was the greatest which was followed by 45-54 years (322), 25-34 years (300), 15-24 years (237) ,55-64 years (236) and >=65 years (181). The less age group was <15 years (8). Rifampicin Resistant Tuberculosis detected in age groups in decrease sequence order were 15-24 years (17), 25-34 years (9), 45-54 years (7), 55-64 years (6),35-44 years (3), >=65 years (1) respectively. Rifampicin Resistant Tuberculosis was not found in <15 years. Multidrug-resistant tuberculosis was detected most in 15-24 years (16), 25-34 years (9), 45-54 years (7), 55-64 years (5),35-44 years (2), >=65 years (1) respectively. Rifampicin Resistant Tuberculosis was not found in <15 years. The 45-54 years (28) was most common in Isoniazid resistance +/- other drug resistance followed by 35-44 years (27) ,55-64 years (26), 15-24 years (24), 25-34 years (23),>=65 years (10) and <15 years (1).

(2)Gender

Out of 1672 total new tuberculosis cases, male was 1082 and female was 590. Whole Genome Sequencing results were available in 907 males and 502 females. The missing result in male was 175, female was 88. The frequency distribution among Rifampicin Resistant tuberculosis cases in male was 24 and 19 was female while 21 male and 19 female in **Multi-Drug-Resistant** Tuberculosis cases. Isoniazid Resistance +/- other drug resistance was found 83 cases in male and 56 in female.

(3)Region/ State

A total number of tuberculosis cases in Yangon region was 498 and in other Region and States was 1174. Whole Genome Sequencing results were available 414 in Yangon region and 995 in others. Based on Whole Genome Sequencing results among new tuberculosis cases, the frequency of Rifampicin Resistance was 18 in Yangon region, 25 in other regions, 17 **Multi-Drug-Resistant**

Tuberculosis cases in Yangon region, 23 in other regions, 53 Isoniazid Resistance +/- other drug resistance in Yangon region and 86 in other regions.

Table 13: Prevalence of Rifampicin Resistant Tuberculosis and Multi-Drug-Resistant Tuberculosis among New Tuberculosis patients in relation to socio-demographic characteristics based on Whole Genome Sequencing Results

Variable		Total No. MTB cases	No. WGS Results	No. Results missing	Complete Case Analysis			MI Model Analysis	Complete Case Analysis			MI Model Analysis	Complete Case Analysis			MI Model Analysis
					Rifampicin Resistant Tuberculosis	Crude (*)	% (**)	%(***)	Multi-Drug-Resistant Tuberculosis	Crude (*)	%(**)	%(***)	Isoniazid	Crude (*)	% (**)	% (***)
Total	-	1672	1409	263	43	3.1%	2.9%	3.3%	40	2.8%	2.7%	3.0%	139	9.9%	10.3%	10.2%
Gender	Male	1082	907	175	24	2.6%			21	2.3%	-	-	83	9.2%		
	Female	590	502	88	19	3.8%			19	3.8%	-	-	56	11.2%		
Age group	<15	8	7	1	0	0.0%			0	0.0%	-	-	1	14.3%		
	15-24	237	205	32	17	8.3%			16	7.8%	-	-	24	11.7%		
	25-34	300	251	49	9	3.6%			9	3.6%	-	-	23	9.2%		
	35-44	388	327	61	3	0.9%			2	0.6%	-	-	27	8.3%		
	45-54	322	267	55	7	2.6%			7	2.6%	-	-	28	10.5%		
	55-64	236	203	33	6	3.0%			5	2.5%	-	-	26	12.8%		
	>= 65	181	149	32	1	0.7%			1	0.7%	-	-	10	6.7%		
Region	Yangon	498	414	84	18	4.3%			17	4.1%	-	-	53	12.8%		
	Others	1174	995	179	25	2.5%			23	2.3%	-	-	86	8.6%		

***No. Resistant cases / No. cases with Results available**

**** Complete case analysis with adjustment for cluster size included in analysis**

***** With adjustment for cluster size included in analysis**

16.7.4. Prevalence of Rifampicin Resistant Tuberculosis and Multi-Drug-Resistant Tuberculosis among previously treated tuberculosis patients in relation to socio-demographic characteristics based on Whole Genome Sequencing Results

During the study period, a total number of 167 previously treated tuberculosis cases were tested for Whole Genome Sequencing. Out of them, 134 cases recognized Whole Genome Sequencing results available and 33 cases had missing results. Rifampicin Resistance was detected in 18 (13.9%), **Multi-Drug-Resistant** tuberculosis was detected in 16 (12.6 %) and Isoniazid Resistance +/- other drug resistance was found in 27 (20.9 %) cases.

(1)Age group

The number of previously treated tuberculosis patients aged 35-44 years was (35) followed by 55-64 years (32), 25-34 years and 45-54 years were (31each), and >= 65 years (21) 15-24 years (15) respectively. The smaller age group was <15 years (2). The highest to lowest number of Rifampicin Resistant Tuberculosis cases were 25-34 years (7), 35-44 years (4), 15-24 years (3), 55-64 years (2), 45-54 years and >= 65 years (1) each respectively. Rifampicin Resistant Tuberculosis was not found in age <15 years. The most common age group in Multidrug-resistant tuberculosis was 25-34 years (6), 35-44 years (4), 15-24 years (2), 55-64 years (2), >=65 years (1), 45-54 years (1). None of <15 years was **Multi-Drug-Resistant** Tuberculosis cases. The 25-34 years (8) was most common in Isoniazid resistance +/- other drug resistance followed by 35-44 years (6), 55-64 years (4), 15-24 years (4), >= 65 (3) and 45-54 years (2). None of <15 years group was detected Isoniazid resistance +/- other drug resistance.

(2)Gender

The number of Whole Genome Sequencing result available in male was 101 and female was 33. The missing result in male was 23, female was 10. The frequency distribution among Rifampicin Resistant Tuberculosis cases in male was 12 and 6 in female while 10 males and 6 females in **Multi-Drug-Resistant** Tuberculosis cases. Isoniazid Resistance +/- other drug resistance was found in 17 cases male and 10 cases in female. Out of 167 previously treated cases, male was 124 and female was 43. The number of Whole Genome Sequencing result available in male was 101 and female was 33. The missing result in male was 23, female was 10. The frequency distribution among Rifampicin Resistant Tuberculosis cases in male was 12 and 6 in female while 10 in male and 6 in female among **Multi-Drug-Resistant** Tuberculosis cases. Isoniazid Resistance +/- other drug resistance was found 17 cases in male and 10 cases in female.

(3)Regions/States

A total number of tuberculosis cases in Yangon region was 68 and others was 99. According to region wide prevalence of Whole Genome Sequencing results available was 54 in Yangon region and 80 in others. In Yangon region, 14 cases are missed and 19 are missed in other region. Rifampicin Resistant Tuberculosis cases detected in Yangon region were 12, 6 in others while 10 in Yangon region and 6 in others for **Multi-Drug-Resistant** Tuberculosis cases. Isoniazid Resistance +/- other drug resistance in Yangon region was 17 and 10 in others.

Table 14. Prevalence of Rifampicin Resistant Tuberculosis and Multi-Drug-Resistant Tuberculosis among previously treated MTB Positive in relation to socio-demographic characteristics based on Whole Genome Sequencing Results

Variable	Value	Total No. MTB cases	No. WGS Results	No. Results missing	Complete Case Analysis			MI Model Analysis	CompleteCaseAnalysis			MI Model Analysis	Complete Case Analysis			MI Model Analysis
					Rifampicin Resistant Tuberculosis	Crude (*)	% (**)	%(***)	Multi-Drug-Resistant Tuberculosis	Crude (*)	%(**)	%(***)	Isoniazid	Crude (*)	% (**)	% (***)
Total	-	167	134	33	18	13.4%	13.9%	13.1%	16	11.9%	12.6%	11.8%	27	20.1%	20.9%	20.8%
Gender	Male	124	101	23	12	11.9%			10	9.9%	-	-	17	16.8%		
	Female	43	33	10	6	18.2%			6	18.2%	-	-	10	30.3%		
Age group	<15	2	1	1	0	0.0%			0	0.0%	-	-	0	0.0%		
	15-24	15	11	4	3	27.3%			2	18.2%	-	-	4	36.4%		
	25-34	31	26	5	7	26.9%			6	23.1%	-	-	8	30.8%		
	35-44	35	31	4	4	12.9%			4	12.9%	-	-	6	19.4%		
	45-54	31	20	11	1	5.0%			1	5.0%	-	-	2	10.0%		
	55-64	32	29	3	2	6.9%			2	6.9%	-	-	4	13.8%		
	>= 65	21	16	5	1	6.3%			1	6.3%	-	-	3	18.8%		
Region	Yangon	68	54	14	12	22.2%			10	18.5%	-	-	17	31.5%		
	Others	99	80	19	6	7.5%			6	7.5%	-	-	10	12.5%		

***No. Resistant cases / No. cases with Results available**

**** Complete case analysis with adjustment for cluster size included in analysis**

***** With adjustment for cluster size included in analysis**

16.8. Comparison of Rifampicin results between Xpert MTB/RIF and Line Probe Assay

The rifampicin results of Line Probe Assay and Xpert MTB/RIF assay were compared. Among total 1859 results, agreed results were found as 1764 in Rifampicin sensitive and 75 in Rifampicin resistance. Therefore, overall agreement was 99%. Positive agreement was 79%. (Table 15)

Table 15. **Comparison of Rifampicin results between Xpert MTB/RIF and Line Probe Assay**

Xpert MTB/RIF	Line Probe Assay		Total
	Rifampicin Sensitive	Rifampicin Resistant	
Rifampicin Sensitive	1764	5	1769
Rifampicin Resistant	15	75	90
Total	1779	80	1859

Comparison of Rifampicin result between Xpert MTB/RIF and Whole Genome Sequencing

Congruent rifampicin sensitive results between testing performed by Xpert and Whole Genome Sequencing were obtained as 1440 in Rifampicin sensitive results and 50 in Rifampicin resistance results out of total 1521 samples. Therefore, overall agreement was 98% excluding Xpert MTB/RIF indeterminate result. Positive agreement was 68%. (Table 16)

Table 16. Rifampicin testing results by Xpert MTB/RIF and Whole Genome Sequencing

Xpert MTB/RIF	Whole Genome Sequencing		Total
	Rifampicin Sensitive	Rifampicin Resistant	
Rifampicin Sensitive	1440	10	1450
Rifampicin Resistant	14	50	64
MTB detected, RR indeterminate	6	1	7
Total	1460	61	1521

Comparison of Rifampicin result between Line Probe Assay and Whole Genome Sequencing

The rifampicin testing results of Line Probe Assay and Whole Genome Sequencing were compared. Among total 1543, Rifampicin sensitive 1478 and Rifampicin resistance 53 results were detected in both Line Probe Assay and Whole Genome Sequencing. Overall agreement was 99% and positive agreement was 82%. (Table 17)

Table 17. Rifampicin testing results by Line Probe Assay and Whole Genome Sequencing

Line Probe Assay	Whole Genome Sequencing		Total
	Rifampicin Sensitive	Rifampicin Resistant	
Rifampicin Sensitive	1478	8	1486
Rifampicin Resistant	4	53	57
Total	1482	61	1543

Summary comparison of overall agreement and positive agreement of Rifampicin results by Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing

The summary table showed that overall agreement of Rifampicin results was the highest in Line Probe Assay Versus Whole Genome Sequencing and the lowest agreement was detected in Xpert Versus Whole Genome Sequencing.

Therefore, the lowest positive agreement between Xpert Versus Whole Genome Sequencing may give rise to occurrence of false positive (Rifampicin resistance results) by Xpert MTB/RIF tests. (Table 18)

Table 18: Summary comparison of overall agreement and positive agreement of Rifampicin results by X pert MTB/RIF, Line Probe Assay and Whole Genome Sequencing

Comparison between two tests	Overall agreement	Positive agreement	Remark
Xpert Vs Line Probe Assay	99%	79%	medium
Xpert Vs Whole Genome Sequencing	98%	68%	lowest
Line Probe Assay Vs Whole Genome Sequencing	99%	82%	Highest

16.9. Anti-tuberculosis drug Resistance proportion among Rifampicin Resistant tuberculosis patients

Isoniazid and Ethambutol Resistance were detected in 48 each out of 64 Rifampicin Resistant tuberculosis cases (75%) by Whole Genome Sequencing. The lowest proportion of resistance was found in injection Amikacin/capreomycin. Luckily, there was no resistance to newer Drugs such as Bedaquiline, Cycloserine, Clofazimine, Para aminosalicylic acid, Linezolid and Delamanid. (Table 19)

Table 19: Anti-tuberculosis Drug Resistance among Rifampicin Resistant tuberculosis cases (by Whole Genome Sequencing)

Resistance	Denominator (available test)	Resistance among Rifampicin Resistant tuberculosis cases	
		n	%
Isoniazid Resistance	64	48	75.0%
Pyrazinamide Resistance	64	17	26.6%
Ethambutol Resistance	64	48	75.0%
Streptomycin	64	38	59.4 %
Amikacin/Capreomycin Resistance	89	1	1.1%
Kanamycin Resistance	88	2	3.1%
Fluoroquinolone Resistance	88	16	18.2%
Levofloxacin/moxifloxacin Resistance	64	13	20.3%
Ethionamide Resistance	64	3	4.7%
Bedaquiline, Cycloserine, Clofazimine, Para aminosalicylic acid, Linezolid, Delamanid	64	0	

It was highlighted that same proportions were detected in injection Amikacin/Capreomycin and fluoroquinolone resistance among Rifampicin Resistant tuberculosis cases by Line Probe Assay testing. (Table 20)

Table 20: Anti-tuberculosis Drug Resistance among Rifampicin Resistant tuberculosis cases (by Line Probe Assay)

Resistance	Denominator (available test)	Resistance among Rifampicin Resistant tuberculosis cases	
		n	%
Amikacin/Capreomycin Resistance	89	1	1.1%
Kanamycin Resistance	90	2	2.2%
Fluoroquinolone Resistance	88	16	18.2%

16.10. katG and inhA Isoniazid mutation by Line Probe Assay

It was demonstrated that Line Probe Assay testing would provide the two different types of Isoniazid mutation among Rifampicin Resistant tuberculosis and Rifampicin sensitive tuberculosis. The most common mutation was katG mutation which was found in both Rifampicin Resistant tuberculosis and Rifampicin sensitive tuberculosis. It was higher than inhA mutation.

The proportions of katG mutation were 63.9% and 92.9% in Rifampicin sensitive TB and Rifampicin Resistant tuberculosis cases respectively.

Because of presence of katG mutation, Isoniazid would be unlikely to be effective even at high doses in those cases. (figure 6)

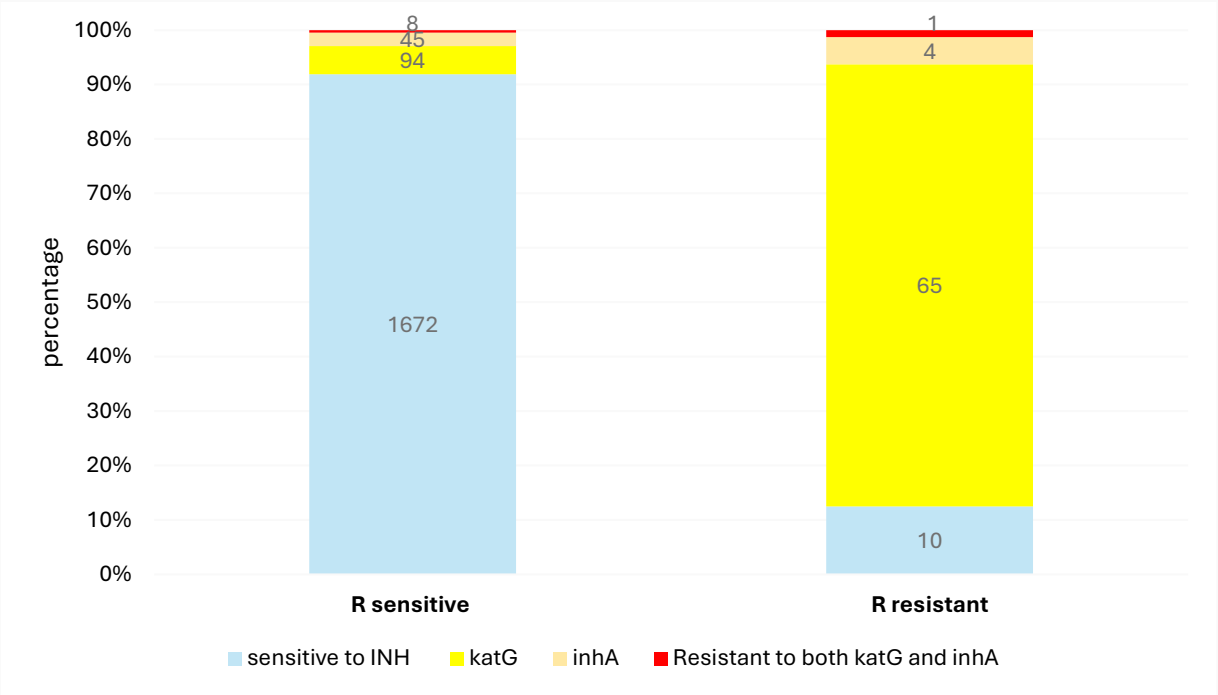


Figure 6 Proportion of katG and inhA Isoniazid mutation among Rifampicin sensitive and Rifampicin Resistant tuberculosis cases by Line Probe Assay

16.11. Comparison of Multi-Drug-Resistant Tuberculosis prevalence between four National anti-tuberculosis drug Resistance surveys in Myanmar

Comparing the previous three national anti-tuberculosis drug resistance Surveys, the decline of **Multi-Drug-Resistant** Tuberculosis among new and previously treated patients were prominently found in the fourth National anti-tuberculosis Drug Resistance Survey. The fall of **Multi-Drug-Resistant** Tuberculosis prevalence among new tuberculosis patients was from 4% in first National anti-tuberculosis Drug Resistance Survey to 3% in 4th National anti-tuberculosis Drug Resistance Survey and among previously treated tuberculosis patients was from 15.5% to 11.8%.[8] (Figure 7)

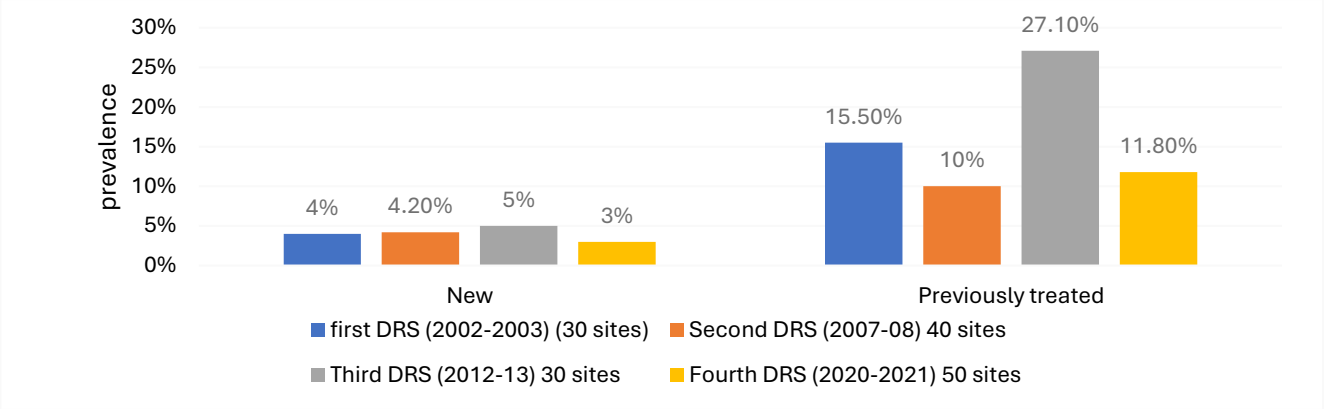


Figure 7 Comparison of Multi-Drug-Resistant Tuberculosis prevalence between four National anti-tuberculosis drug Resistance surveys in Myanmar

16.12. Comparison of Multi-Drug-Resistant Tuberculosis prevalence between four National anti-tuberculosis Drug Resistance Surveys in Asian Countries

The 3 Asian countries conducted National anti-tuberculosis Drug Resistance Surveys and their **Multi-Drug-Resistant** Tuberculosis prevalence were shown in Figure 8.

It showed that the prevalence of **Multi-Drug-Resistant** Tuberculosis among new patients from Myanmar was the highest among 4 Asian countries[4], however, the prevalence of previously treated tuberculosis patients of India was the highest.

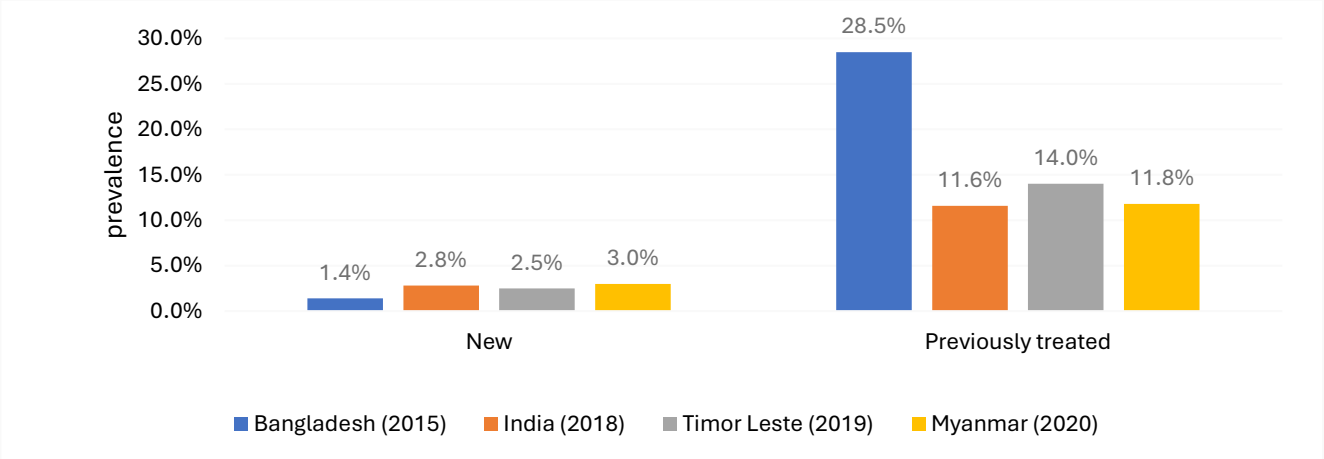


Figure 8 Comparison of Multi-Drug-Resistant Tuberculosis prevalence between four National anti-tuberculosis Drug Resistance Surveys in Asian Countries

17. Discussions

17.1 Quality management of the survey

The fourth National anti-tuberculosis Drug Resistance Survey in Myanmar had a unique design. Estimates of prevalence of Rifampicin Resistant tuberculosis, **Multi-Drug-Resistant** Tuberculosis and Isoniazid mono-resistance tuberculosis were sought for the national level by multiple imputation method.

The survey used a new diagnostic technology, Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing whereas the diagnostic tools of smear microscopy and solid culture had been done in previous surveys.

A high participation rate of 95% was recorded. Informed consent was obtained from 100% of the participants. The use of trained members of the central research team to conduct the screening interviews and on-site check and electronic data entry with an auto-checking system minimized data management errors.

The analysis of the survey results was conducted with technical assistance of WHO. The process was discussed and reviewed at two workshops. The first one virtual meeting was held virtually on 6th October, 2022 to members of 4th Drug Resistance Survey steering committee. The crucial decision was made on consensus of Multiple Imputation Model Analysis. The second meeting for sharing of Survey finding to Tuberculosis Technical Strategic Group members on 10th November, 2022 at Nay Pyi Taw.

17.2 Data Collection sites (Clusters)

The enrollment of tuberculosis patients by townships showed that 36 clusters/townships had more than 40 samples. Only two townships had very low tuberculosis patients' enrolment. Those townships were Ngaputaw and Paukkaung Townships. Ngaputaw township is the furthest from Pathein, capital of Ayeyarwady and Paukkaung Township is also the furthest from Pyay township, big city of Bago Region. The possible reason would be the low case detection during this period. Apart from that, most of the clusters reached the target (i.e. 40) but 7 clusters could not have the required sample size. Those clusters were as follows: Sintgaing, Lemyetnyar, Loilem, Kamaryut, Sanchaung, Oktwin, Palaw. The same reason would be happened due to low case detection.

17.3 Socio Demographic and clinical characteristics of survey participants

Overall participation showed that more males (1399, 66%) participated in the study than females (730, 34%). Twenty-three per cent of the participants in this survey were of the age between 35-44 years. Public sector constituted 89% whereas private sector constituted 11%. As a result, some townships did not complete their samples up to 40.

According to the survey, 70% of survey participants were coming from Lower Myanmar and 30% were from Upper Myanmar. It also reflected the difference of Tuberculosis disease burden between Lower Myanmar and Upper Myanmar. The difference was similar to the Annual Tuberculosis Report of 2020.

Although the proportions of survey participants from Yangon region, other regions than Yangon and States were 32%, 44% and 24% respectively, those proportions were not consistent with the TB 07 data of those areas in 2020. The contribution of State's participants and other Regions' participants were very low (24% versus 29%) (44% versus 46%) as a result, higher participation of survey patients was from Yangon Region. It was owing to the cluster selection.

The remaining characteristics were smoking and diabetes mellitus.

Survey patients with tuberculosis and Diabetes mellitus comorbidity proportion was 14% so that it was greater than proportion of diabetes mellitus among general population (10.5%) according to Myanmar STEP survey in 2014. On that account, the Diabetes mellitus of tuberculosis patients would be the one of the social determinants for contracting tuberculosis.

Furthermore, 21% of survey tuberculosis patients were smokers. Tobacco use among young adolescents in Myanmar in 2017: Findings from global youth tobacco survey pointed out that overall percentage was 13.6% of students currently used tobacco. Hence, the smoking status among tuberculosis patients was higher proportion compared to that of the article.

17.4 Tuberculosis history

The proportion of participants with previous anti-tuberculosis treatment history was (12%). It was similar to National Tuberculosis Program annual data (11.4% in 2020). The only one risk factors associated with Rifampicin Resistant Tuberculosis was the previous tuberculosis treatment history which was statistically significant with p value= 0.000165.

17.5 Laboratory results

After DNA extraction from both laboratories, DNAs were gathered at National Tuberculosis Reference Laboratory and from National Tuberculosis Reference Laboratory, they were sent to Otago University, New Zealand. The reducing number of samples tested for every step was happened. For example, while total number available for Line Probe Assay was 1899, number available for Whole Genome Sequencing was 1543.

Prevalence of Rifampicin Resistant tuberculosis among new and previously treated tuberculosis patients

By Multiple Imputation Model analysis method, the final agreed prevalence of Rifampicin Resistant tuberculosis was 3.3% (95% Confidence Interval 2.4% - 4.5%) and 13.1% (95% Confidence Interval 7.8% - 21%) among new and previously treated tuberculosis patients. According to annual tuberculosis report, 2020, while the proportion of Rifampicin Resistant tuberculosis detected among patients with MTB diagnosed by Xpert MTB/RIF was 5%, this survey indicated that the proportion of Rifampicin Resistant tuberculosis was lower than that of annual report. The possible reason was the number of patients tested was not only new but also other types of tuberculosis patient in Annual report.

By Multiple Imputation Model analysis method, final agreed prevalence of **Multi-Drug-Resistant** Tuberculosis was 3.0% (95% Confidence Interval 2.1% - 4.1%) and 11.8% (95% Confidence Interval 6.8% -19.6%) among new and previously treated patients.

By Multiple Imputation Model analysis, final agreed prevalence of **Isoniazid mono-resistance tuberculosis** was 10.2% (95% Confidence Interval 8.7% -12%) and 20.8%. (95% Confidence Interval 14.8% -28.5%) among new and previously treated tuberculosis patients. Thus, Isoniazid mono-resistance prevalence was higher than expected.

In addition, By Line Probe Assay test, the proportions of Kat G mutation were detected greatly in both Rifampicin sensitive tuberculosis and Rifampicin Resistant tuberculosis cases.

Because of presence of Kat G mutation, Isoniazid would be unlikely to be effective even at high doses in treatment for those cases.

17.6. Overall agreement and positive agreement of Rifampicin results between two tests

Line Probe Assay versus Whole Genome Sequencing

A variety of tests were performed in the survey. Microscopy, Xpert MTB/RIF, Line Probe Assay and Whole Genome Sequencing test were used to compare the agreement between two tests were calculated. Regarding the overall agreement and positive agreement of Rifampicin results between two tests, the highest agreement was observed in Line Probe Assay versus Whole Genome Sequencing. National Tuberculosis program is now using Line Probe Assay test (First Line anti-tuberculosis Drug) for the purpose of confirmation of Rifampicin Resistant tuberculosis cases when the discrepancy between two results (first and second samples) of Xpert MTB/RIF for new tuberculosis patients (having low Drug Resistant tuberculosis risk). The Drug Resistance Survey finding revealed that Line Probe Assay results provided strong proof for National Tuberculosis Program to use in current National Tuberculosis Program guidelines. In addition, same proportions of fluoroquinolone and injection Amikacin Resistance among Rifampicin Resistant Tuberculosis

cases which were discovered by Whole Genome Sequencing as well as Line Probe Assay, both tests were found to be comparable.

17.7. Isoniazid and Ethambutol Resistance among Rifampicin Resistant tuberculosis patients

Apart from that, the proportions of Anti-tuberculosis Drug Resistance among Rifampicin Resistant tuberculosis patients were crucial for National Tuberculosis Program. It was found among Rifampicin Resistant tuberculosis cases (Whole Genome Sequencing), Isoniazid and Ethambutol Resistance constituted 75% of Rifampicin Resistant tuberculosis cases. Thus, those resistance were crucial factor because first diagnosis of Rifampicin Resistant tuberculosis was currently identified by using initial Xpert MTB/RIF test, prediction could be made 3 drugs (Rifampicin, Isoniazid and Ethambutol) were already resistant in three fourth (3/4) of Rifampicin Resistant tuberculosis cases.

Additionally, the considerable proportion of resistance to levofloxacin/moxifloxacin among Rifampicin Resistant tuberculosis was 20.3%. That result gave rise to use the Line Probe Assay (Second Line anti-tuberculosis Drug) or Xpert MTB-XDR must be necessary before giving fluoroquinolone containing regimen.

In contrast to this, the proportion of resistance to injection Amikacin among Rifampicin Resistant tuberculosis was 1.1%. That very small proportion of injection Amikacin would be fortunately spare drugs for individualized regimen. If those patients could not fit for oral longer/shorter regimen, injection could be included to construct the effective regimen for them.

17.8. Prevalence of Rifampicin Resistant tuberculosis by Whole Genome Sequencing and Xpert MTB/RIF

The very interesting finding was that Xpert MTB/RIF test showed that the prevalence of Rifampicin Resistant Tuberculosis was higher than that of Whole Genome Sequencing (approximately 1%). It might lead to false negative result by Xpert MTB/RIF test. National Tuberculosis Program's physicians become aware about the Xpert MTB/RIF results. Owing to those false negative results, close monitoring of clinical symptoms, radiological finding and response to treatment would be the foremost for comprehensive management for them to achieve the good quality care.

17.9. Risk factor analysis

The only one risk factors associated with Rifampicin Resistant tuberculosis which was found out in this study was the previous tuberculosis treatment history which was statistically significant. The other factors were not associated.

17.10. Genotypic mutations conferring putative resistance to Rifampicin and other anti-tuberculosis drugs

Concerning the Rifampicin resistance, the distribution of mutations was detected in 11 genotypes by Whole Genome Sequencing. Among those different mutations, the fascinating point was noted in "I491 F" which was missed because it was outside the X pert MTB/RIF and caught only by Whole Genome Sequencing. In consequence, National Tuberculosis Program, Myanmar should understand to solve the false negative result obtained in routine Xpert MTB/RIF testing other than research purpose.

Furthermore, the commonest mutations in Streptomycin, Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, Amikacin, Fluoroquinolones and Ethionamide resistance were found in K43R, S315T, S450L, M306V, G132D/P45L, C1402Trrs/g-10a eis, A90V and C15T.

Genotype distribution

The predominate lineage was "Indo-Oceanic and EAI "in this study. According to literature review, the study on Indo-Oceanic Mycobacterium tuberculosis strains from Thailand associated with higher mortality conducted in September, 2019 pointed out that the Indo-Oceanic lineage of Mycobacterium tuberculosis increased the mortality risk compared with modern lineages or the East-Asian lineage, the latter being considered highly virulent in previous studies. Hence, the high mortality would be expected to Myanmar tuberculosis patients because of predominate Indo-Oceanic and EAI lineage.

Moreover, this study also revealed that Beijing genotype was more common than EAI in Kachin and 3 Shan States. The literature depicted that Beijing genotype was commonly associated with **Multi-Drug-Resistant** Tuberculosis. In North India study, detection of Beijing genotype of **Multi-Drug-Resistant** Tuberculosis M. tuberculosis and their association with drug resistance mutations in katG, rpoB, and embB genes showed that the **Multi-Drug-Resistant** was significantly associated with Beijing genotype. Myanmar National Tuberculosis Program therefore needed to take care on this Beijing genotype for **Multi-Drug-Resistant** Tuberculosis association. Consequently, closer collaboration in cross border tuberculosis control should be essential due to the high proportions of Beijing genotype in 4 States expressed in this study.

The study on "Distribution of Mycobacterium tuberculosis Genotypes and Drug Resistance in Upper Myanmar" depicted that the most common drug resistance mutation was streptomycin and Beijing Genotype which showed a higher number of **Multi-Drug-Resistant** Tuberculosis compared to other genotypes. Based on the finding of genotypic mutation of anti-tuberculosis drugs from this study, further studies would be necessary to be conducted for association between Mycobacterium tuberculosis genotypes and Drug Resistance tuberculosis which would support the better understanding of tuberculosis transmission and control in Myanmar and other neighboring countries.

The similar finding was observed in other countries' studies that depicted the highest proportion of **Multi-Drug-Resistant** Tuberculosis cases were associated with this Beijing Genotype.

17.11. Comparison with three previous national anti-tuberculosis Drug Resistance Survey in Myanmar

Comparing the previous three national anti-tuberculosis drug resistance Surveys, the decline of **Multi-Drug-Resistant** Tuberculosis among new and previously treated tuberculosis patients were prominently found in the fourth National anti-tuberculosis Drug Resistance Survey. The fall of **Multi-Drug-Resistant** Tuberculosis prevalence among new tuberculosis patients was due to their great effort of National Tuberculosis Program with all implementing partners for tuberculosis Control.

17.12. Comparison with recent national tuberculosis prevalence surveys in Asia

Comparison with recent national tuberculosis prevalence surveys in Asia revealed that the decline of **Multi-Drug-Resistant** Tuberculosis among new and previously treated tuberculosis patients were prominently found in the fourth National anti-tuberculosis Drug Resistance Survey.

Compared to India, Bangladesh and Timor Leste[2], the **Multi-Drug-Resistant** Tuberculosis prevalence among new tuberculosis patients in Myanmar was the highest among those Asian countries.

Hence, Myanmar must try to do more effort on emphasize the drug susceptible tuberculosis and drug resistant tuberculosis management intensively together with tuberculosis partners up to field operation.

18. Challenges

During COVID-19 lock down, transport difficulties limited official working days, inability of patient come to health center, data collection period lengthen more than planned period. The collection of the sample enrollment and sputum transportation were affected by COVID-19 pandemic. It was minimal. Only public transportation was shut down during first wave of COVID-19 in April and after the long holiday period of Thingyan (and got back to normal from 16th May. Due to difficulty in sputum transportation, some cases

were missed for enrollment. Fortunately, sample enrollment of Drug Resistance Survey could be finished successfully at the end of August. Therefore, routine laboratory work and survey work activities were affected inevitably. During this hardship period, National Tuberculosis Reference Laboratory tried to do shipment of extracted DNA sample to New Zealand as planned and laboratory staff from National Tuberculosis Reference Laboratory and upper Myanmar Tuberculosis Laboratory had to work harder than before in order to get the timely culture result for this Drug Resistance Survey.

Apart from that, not all bacteriologically confirmed tuberculosis cases from the clusters were consecutively enrolled in survey (during public transportation was shut down). Targeted patients from Population Services International, Myanmar and Myanmar Medical Association couldn't be enrolled in time (due to clinic closure and lower caseload) and substituted with National Tuberculosis Program patients. Not all rifampicin resistant tuberculosis patients in Yangon were enrolled in Lower Myanmar clusters as they were directly enrolled at main Drug resistant tuberculosis treatment initiating center, Yangon Regional Tuberculosis Center, during the sample collection period. Laboratory work burden was faced due to government staff's weekly work shift although some sputum samples couldn't be reached to culture centers within 5 days from collection.

19. Limitations of the survey and analysis

The study was targeted to bacteriologically confirmed pulmonary Tuberculosis patients over 8 years of age. Accordingly, the current findings are not fully representative to smear-negative, extra-pulmonary Tuberculosis patients and childhood Tuberculosis patients.

20. Implications

The National anti-tuberculosis Drug Resistance Survey 2020–2021 was completed successfully, with a high participation rate and fewer missing values. The survey showed that the **Multi-Drug-Resistant** Tuberculosis burden in Myanmar has declined significantly, due to the huge efforts of the National Tuberculosis Program and its partners. It could provide the sound estimation of resistant profile of all tuberculosis cases in a population. By knowing the resistant profile of anti-Tuberculosis drugs, National Tuberculosis Program could update and revise the drug resistant tuberculosis management guideline to improve treatment outcomes of the drug resistant tuberculosis patients & avert the transmission of resistance strains. The drug resistant tuberculosis guidelines would also be necessary to be revised because high proportions of mono resistance of isoniazid and fluoroquinolone were identified by Line Probe Assay as well as Whole Genome Sequencing. Fortunately, the resistance to newer anti-tuberculosis drugs (Bedaquline, Linezolid and Delamanid) were not detected so those drugs could be used in Shorter Treatment Regimen. On the other hand, it was recommended that the rapid diagnostic tests (genotypic Drug Susceptibility Testing) with higher sensitivity should be widely used in Myanmar for early detection of drug resistant tuberculosis patients. Though the Tuberculosis burden has been declining significantly, the absolute number of Tuberculosis patients who could be identified with new technologies could be higher. There is a need to lay greater emphasis on multisectoral approaches, decentralization and the integration of basic tuberculosis services into primary care facilities.

Finally, this survey could give rise to strong evidence-based documentation to coming Myanmar Tuberculosis National Strategic Plan (2026-2030).

References

1. Anamika Gupta, Savita Kulkarni, Nalin Rastogi, Shampa Anupurba.(2014). A study of Mycobacterium tuberculosis genotypic diversity & drug resistance mutations in Varanasi, north India. Indian J Med Res, 2014 Jun;139(6):892-902

2. Constantino Lopes, Debashish Kundu, Ismael Da Costa Barreto, Bernardino da Cruz, S. Siva Kumar, Sureshbabu Ramalingam, Anna S. Dean, et al. Experience on the first national anti-TB drug resistance survey (DRS) in Timor-Leste, Global Health Research and Policy,2018
3. Dodge R. Lim, Anna S. Dean, Mary Rosary Taguinod-Santiago, et al. Low prevalence of fluoroquinolone resistance among patients with tuberculosis in the Philippines: results of a national survey. Ama Eur Respir J. 2018;51:1702571. Available at: https://erj.ersjournals.com/content/ 51/3/1702571. Last accessed on 08 Feb 2022. 17. Agrawal M, Bajaj A, Bhatia V, et al.
4. Gyanshankar P. Mishra, Jasmin Mulani, First National Anti-Tuberculosis Drug Resistance Survey in India
5. Iem V, Dean A, Zignol M, 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.
6. N Smittipat and et al. (2019). Indo-Oceanic Mycobacterium tuberculosis strains from Thailand associated with higher mortality. The International Journal of Tuberculosis and Lung Disease.23(9):972-979
7. National Tuberculosis Program, Protocol for Fourth Nationwide Anti-Tuberculosis Drugs Resistance Survey in Myanmar (2020-2021)
8. National Tuberculosis Program, Myanmar, Third Nationwide Tuberculosis Drug Resistance Survey (2012-2013)
9. National Tuberculosis Program, Myanmar, Annual Report, 2018
10. National Tuberculosis Program, Guidelines for management of MDR-Tuberculosis in Myanmar (2013)
11. National Tuberculosis Program, BHS Manual for National Tuberculosis Programme, Myanmar (2019)
12. Soumya Chatterjee, Husain Poonawala, Yogesh Jain, Chatterjee S, et al.Drug-resistant tuberculosis: is India ready for the challenge? BMJ Glob Health 2018;3:e000971. doi:10.1136/bmjgh-2018-000971
13. World Health Organization, Progress reports on Fourth Nationwide Anti-Tuberculosis Drugs Resistance Survey
14. World Health Organization, Global Tuberculosis Report 2018
15. World Health Organization, Global Tuberculosis report 2019
16. World Health Organization, Global Tuberculosis Report 2020. Available at: https://www.who.int/teams/ global-tuberculosis-programme/tb-reports
17. World Health Organization, Guidelines for Surveillance of drug resistance in tuberculosis 5th Edition. Geneva (2015)
18. World Health Organization SEARO. Annual Report 2017. Bending the curve – Ending TB. Available at: http://apps.who.int/iris/bitstream/10665/254762/1/97892 9022584-eng.pdf. Last accessed on 08 Feb 2022.
19. World Health Organization, operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection. Geneva: World Health Organization. 2020. Licence: CC BY-NC-SA 3.0 IGO.
20. World Health Organization consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Geneva: