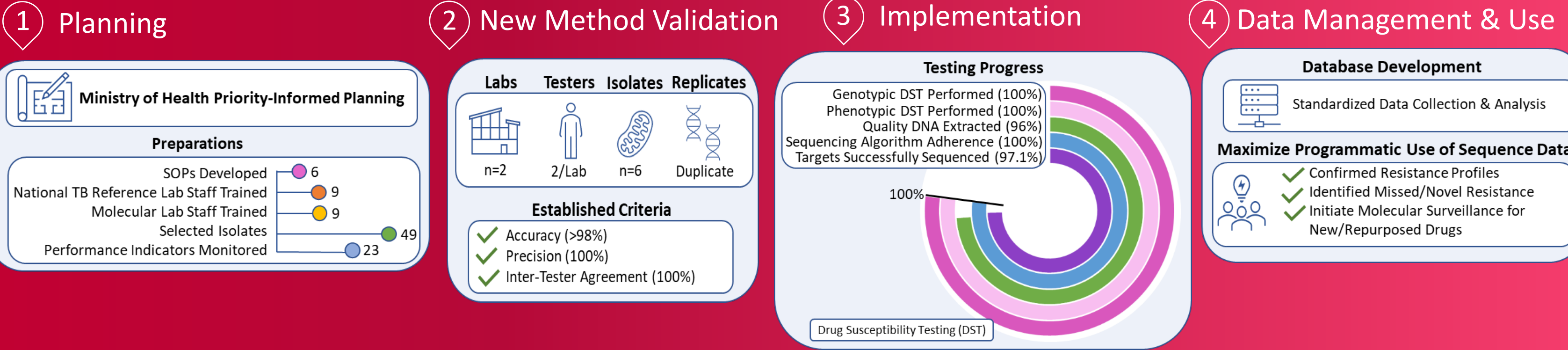


# Implementation of the First Sequencing-Based Surveillance Model for Drug-Resistant Tuberculosis in Nigeria

Heather McLaughlin<sup>1\*</sup>, Israel Audu<sup>2</sup>, Jamie Dawson<sup>1</sup>, Tola Monday<sup>3</sup>, Julius Ogwu<sup>3</sup>, Chika Onwuamah<sup>4</sup>, Patricia Hall-Eidson<sup>1</sup>, Nnaemeka Iriemenam<sup>2</sup>, McPaul Okoye<sup>2</sup>, Anyaike Chukwuma<sup>5</sup>

<sup>1</sup>International Laboratory Branch, Division of Global HIV & TB (DGHT), Global Health Center (GHC), US Centers for Disease Control and Prevention (CDC), Atlanta, USA, <sup>2</sup>Laboratory Systems Branch, DGHT, GHC, US CDC, Abuja, Nigeria, <sup>3</sup>Institute of Human Virology Nigeria (IHVN), Abuja, Nigeria, <sup>4</sup>Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria, <sup>5</sup>National Tuberculosis and Leprosy Control Programme (NTBLCP), Federal Ministry of Health, Abuja, Nigeria.

## Implementation Roadmap



## BACKGROUND

Response to drug-resistant tuberculosis (DR-TB) has been prioritized globally, including in Nigeria; a high HIV/TB- and TB-burden country. Molecular diagnostics have accelerated detection of DR-TB but is limited to interrogating narrow sections of a limited number of *Mycobacterium tuberculosis* (*Mtb*) resistance-associated genes. Sequencing-based surveillance for DR-TB offers a more comprehensive approach to resistance detection, can aid in monitoring resistance trends as TB treatment regimens are updated, and is recommended by the World Health Organization (WHO) for both surveillance and clinical reporting purposes. Implementation of multi-disease sequencing services can be resource efficient and increase speed of introduction by leveraging existing investments.

## METHODS

The first sequencing hub-and-spoke network for DR-TB surveillance, REST Hub, was established in Nigeria. Ministry of Health priorities informed strategies for isolate selection, sample referral, and sequencing algorithm design. For an efficient and cost-effective introduction, REST Hub integrated TB and HIV sequencing services by establishing referral of *Mtb* genomic DNA (gDNA) from two National TB Reference Laboratories (NTRLs) within zonal TB testing networks to two HIV sequencing laboratories (Figure 1). New method validation was conducted for Sanger sequencing of eight *Mtb* genes associated with resistance to six anti-TB drugs, including new and repurposed drugs bedaquiline and clofazimine recommended by WHO for treatment of DR-TB (Figure 2). A standardized analytics plan was developed to investigate genomic mutations against upstream drug susceptibility testing (DST) results and to maximize programmatic use of sequencing data for DR-TB surveillance.

## RESULTS

- Sample referral network establishment leveraging multi-disease sequencing services was demonstrated (Figure 1).
- Staff training, development of technical SOPs, and new method validation for *Mtb* Sanger sequencing and data analyses were successfully completed by two HIV DR laboratories.
- 47 *Mtb* isolates originating from all six geopolitical zones were sequenced.
- Sequencing analysis was used to confirm resistance patterns reported by upstream genotypic and phenotypic DST (pDST) assays and detect additional resistance (rifampicin (RIF) (n=5) and isoniazid (INH) (n=1)) missed by line probe assays (LPAs) or pDST (Table).
- No resistance-associated mutations were detected in *Rv0678* or *atpE* genes which are currently known to be affiliated with resistance to bedaquiline and clofazimine, drugs newly recommended by WHO for treatment of DR-TB.

<i>Mtb</i> isolate	LPA (gDST)	MGIT (pDST)	Sanger Sequencing	Gene (Mutation Detected)		Missed Drug Resistance
				Manual Analysis	Automated Software	
1	RIF-S	RIF-R	RIF-R	<i>rpoB</i> , L452P (Borderline)	<i>rpoB</i> , L452P (Borderline)	INH-R ► MDR (LPA Missed RIF-R)
2	RIF-R	RIF-S	RIF-R	<i>rpoB</i> , L452P (Borderline)	<i>rpoB</i> , L452P (Borderline)	S ► RIF-R (MGIT Missed RIF-R)
3	RIF-R	RIF-S	RIF-R	<i>rpoB</i> , L430P (Borderline)	<i>rpoB</i> , L430P (Borderline)	S ► RIF-R (MGIT Missed RIF-R)
4	RIF-R	RIF-S	RIF-R	<i>rpoB</i> , S450L	<i>rpoB</i> , S450L	S ► RIF-R (MGIT Missed RIF-R)
5	RIF-S	RIF-S	RIF-R	<i>rpoB</i> , H445L	<i>rpoB</i> , H445L	INH-R ► MDR (LPA & MGIT Missed RIF-R)
6	RIF-R	RIF-R	RIF-R	<i>rpoB</i> , <i>ins_ttc</i> , 1300-1303nt (Novel)	Not detected	N/A
7	RIF-R	RIF-R	RIF-R	<i>rpoB</i> , S450L (Heteroresistance)	Not detected	N/A
8	RIF-R	RIF-R	RIF-R	<i>rpoB</i> , <i>del_aac</i> , 1308-1310nt	Not detected	N/A
9	INH-S	INH-R	INH-R	<i>inhA</i> promoter, c-15t	<i>inhA</i> promoter c-15t	RIF-R ► MDR (LPA Missed INH-R)
<b>Total No. Missed Resistance</b>	<b>RIF 2 INH 1</b>	<b>RIF 4 INH 0</b>	--	--	<b>RIF 3 INH 0</b>	--

Table. Sequencing-based prediction of rifampicin (RIF) and isoniazid (INH) resistance among *Mtb* isolates (n=47), missed by genotypic DST (MTBDRplus Ver 2.0, Line Probe Assay (LPA)), phenotypic DST (BACTEC MGIT 960), or automated bioinformatics software (Bacterio-Check). Susceptible (S), Resistant (R), Multidrug Resistant (MDR).

## CONCLUSIONS

Successful REST Hub implementation demonstrates how DR-TB surveillance efforts may benefit from incorporating sequencing services and leveraging existing resources. Establishment of sequencing-based detection for new and repurposed drugs will aid in surveillance as updated DR-TB treatment regimens are scaled across Nigeria. Expansion of services may strengthen geographically targeted outbreak control, lead to enhanced selection of anti-TB drugs among TB populations, and help guide programmatic testing and treatment policies.

## FIGURES

Figure 1. Sample Referral Network

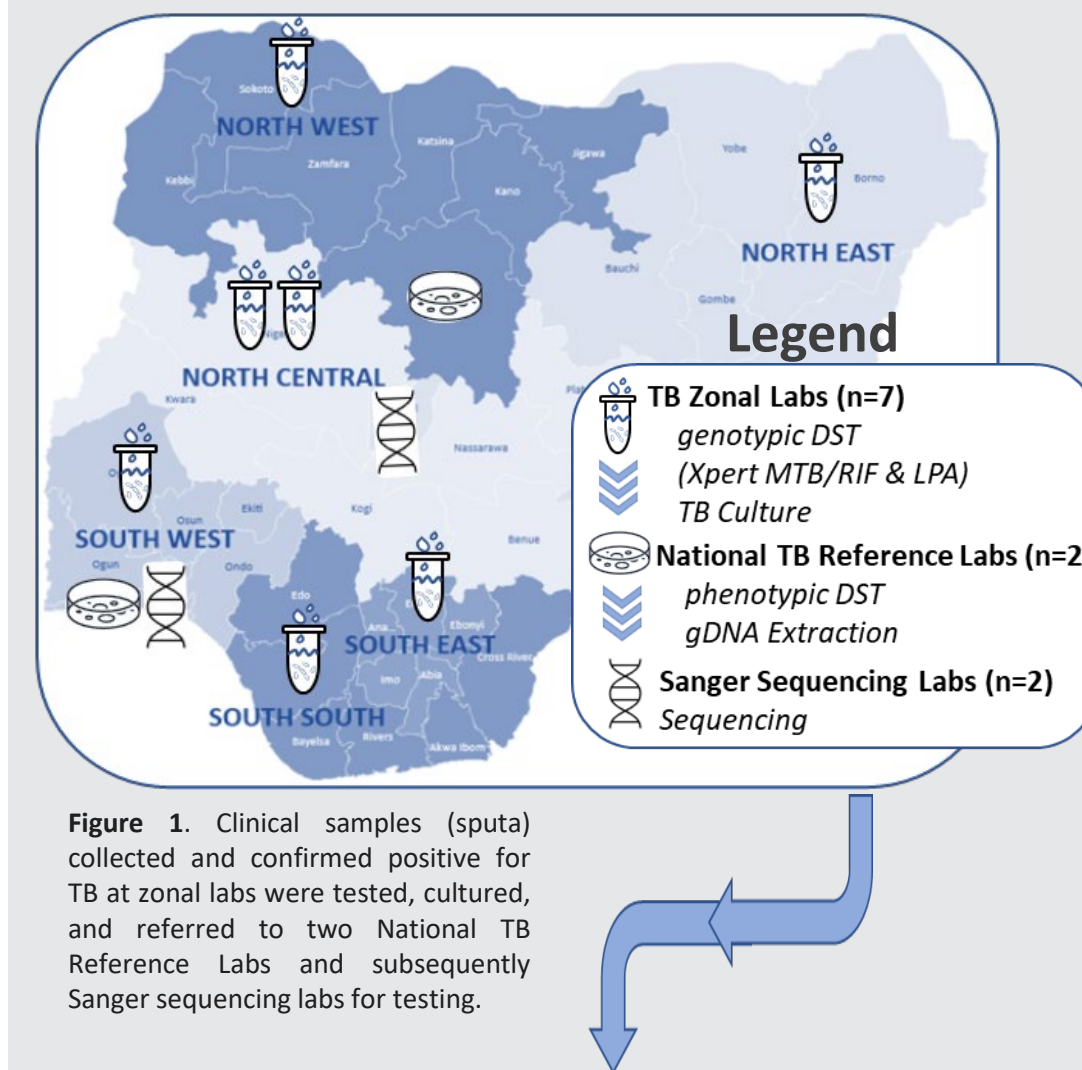


Figure 1. Clinical samples (sputa) collected and confirmed positive for TB at zonal labs were tested, cultured, and referred to two National TB Reference Labs and subsequently Sanger sequencing labs for testing.

Figure 2. Sequencing Algorithm

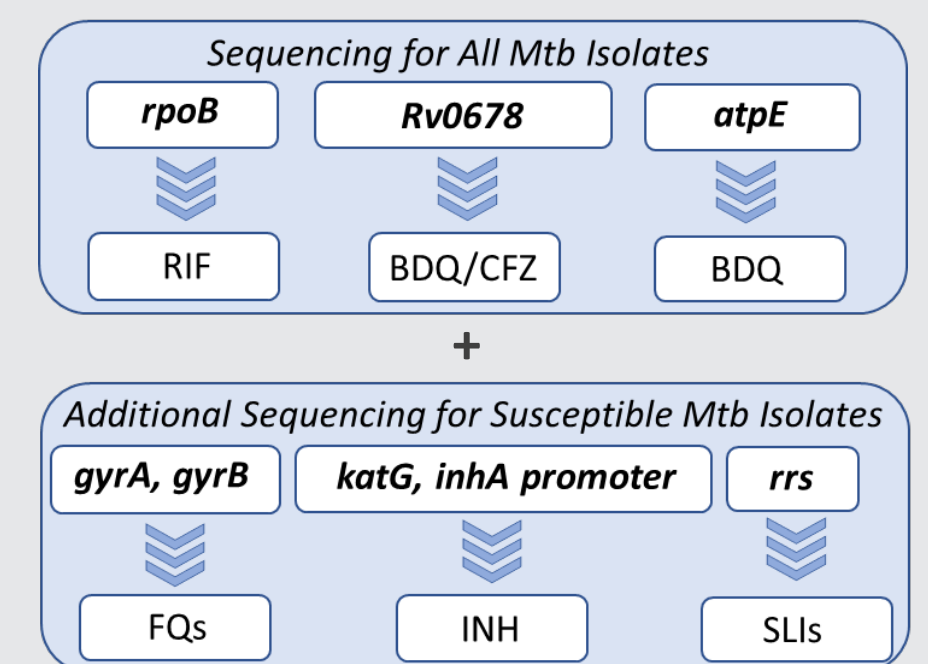


Figure 2. All *Mtb* isolates (top panel) were sequenced to confirm or identify missed resistance to rifampicin (RIF), bedaquiline (BDQ) and clofazimine (CFZ). *Mtb* isolates susceptible by LPA (bottom panel) reflexed for sequencing to additionally identify mutations associated with resistance to fluoroquinolones (FQs), isoniazid (INH), and second-line injectables (SLIs) that may have been missed given lack of routine DST for these drugs in the absence of RIF resistance.

## BRIEF SUMMARY OF CDC-SUPPORTED DR-TB SEQUENCING EFFORTS IN OTHER AFRICAN COUNTRIES:

<p><b>South Africa</b></p> <ul style="list-style-type: none"> <li>Multi-year initiative at Centre for Tuberculosis at the National Institute for Communicable Diseases</li> <li>Successful implementation of whole genome sequencing (WGS) <i>Mtb</i> isolates from 2 high DR TB-burden districts supported longitudinal DR surveillance and timely identification of high-risk transmission clusters.</li> <li>These efforts informed national TB surveillance and diagnostic strategies.</li> </ul>	<p><b>Zambia</b></p> <ul style="list-style-type: none"> <li>The National TB Program along with National and Regional TB Reference Laboratories are establishing ≥1 DR TB genomic sequencing hub to provide test results for existing and new DR TB drugs using targeted Next Generation Sequencing (tNGS) within days.</li> <li>Culture-based resistance testing will be done in parallel to ensure holistic sensitivity profiles to manage patients and track DR TB outbreaks and trends in resistance over time.</li> </ul>	<p><b>Global</b></p> <ul style="list-style-type: none"> <li>Developing a practical toolkit for technology and test implementation of tNGS for DR TB, including a User Guide for programs and laboratories and a range of customizable standardized procedures, forms, tools, and job aids that incorporate the latest international technical and quality requirements for test introduction and strengthening.</li> <li>PEPFAR-supported external quality assurance (proficiency testing) program for Xpert MTB/RIF and Xpert Ultra testing.</li> </ul>	<p>Contact info for more details, including on projects in Ethiopia and Mozambique:</p> <p>Brigette Gleason yer7@cdc.gov</p>
-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------