

MAKING GENOMICS WORK IN TB

Practical approaches and Cost-effective use of tNGS in TB Programs: Sharing Eswatini Experience

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Outline

Country context

- TB/HIV burden
- RR/MDR-TB burden

Key Steps in introducing TNGS

Cost Considerations

Clinical Use of TNGS

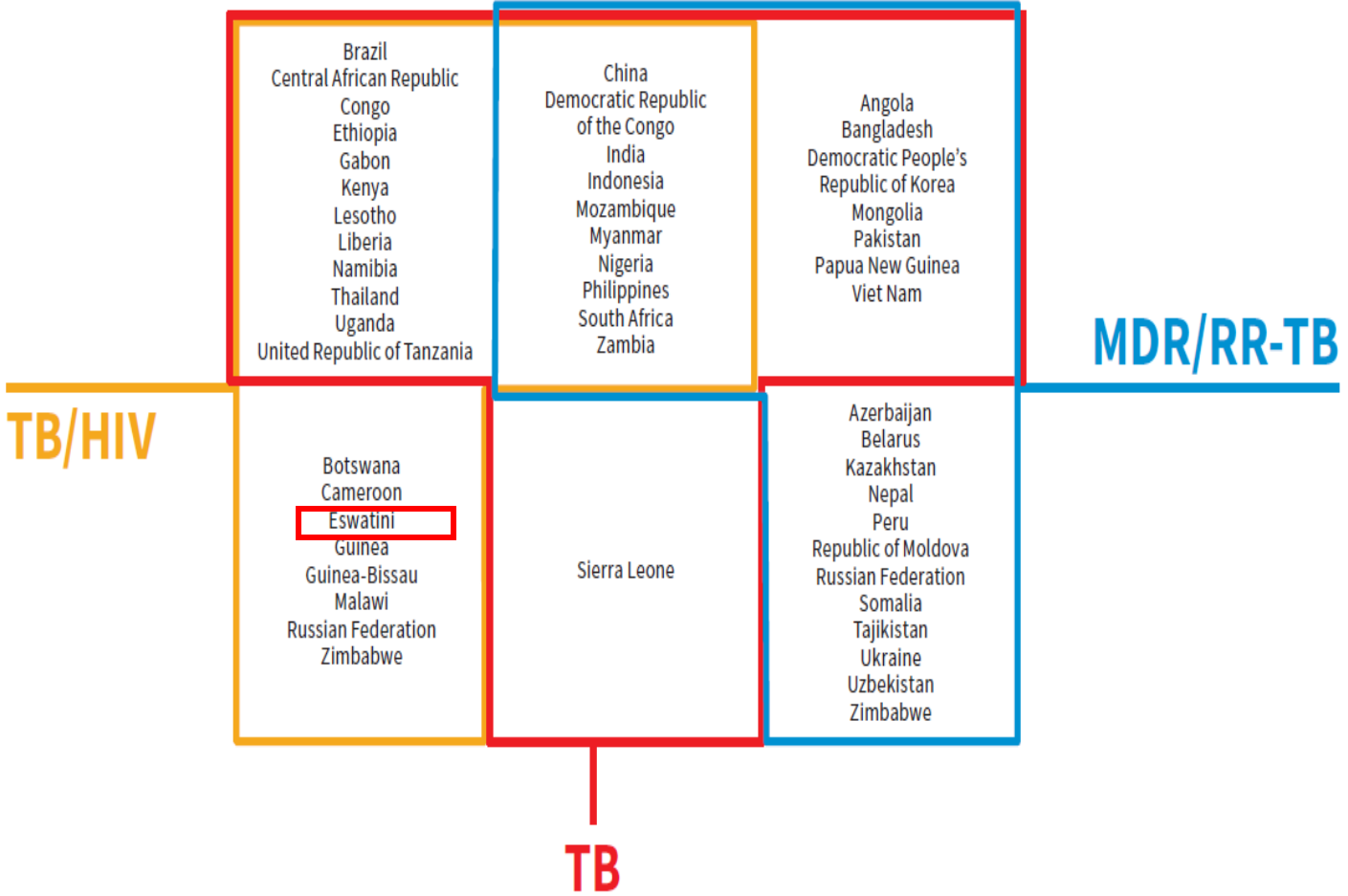
- Progress so far

Conclusion

COUNTRY CONTEXT: ESWATINI

1. Global Rankings:

- Among the 30 countries with the highest TB/HIV burden globally.
- TB incidence: 350/100,000 population (2024 Global TB Report).
- HIV prevalence: 24.8% (SHIMS 3).
 - Highest in the world
- MDR-TB prevalence: 5% in new cases; 44% in retreatment cases.



2. Co-Infection Rates:

- TB/HIV co-infection: 65%.
- DR-TB/HIV co-infection: 71%.

TB DIAGNOSTIC AND SERVICE ORGANIZATION

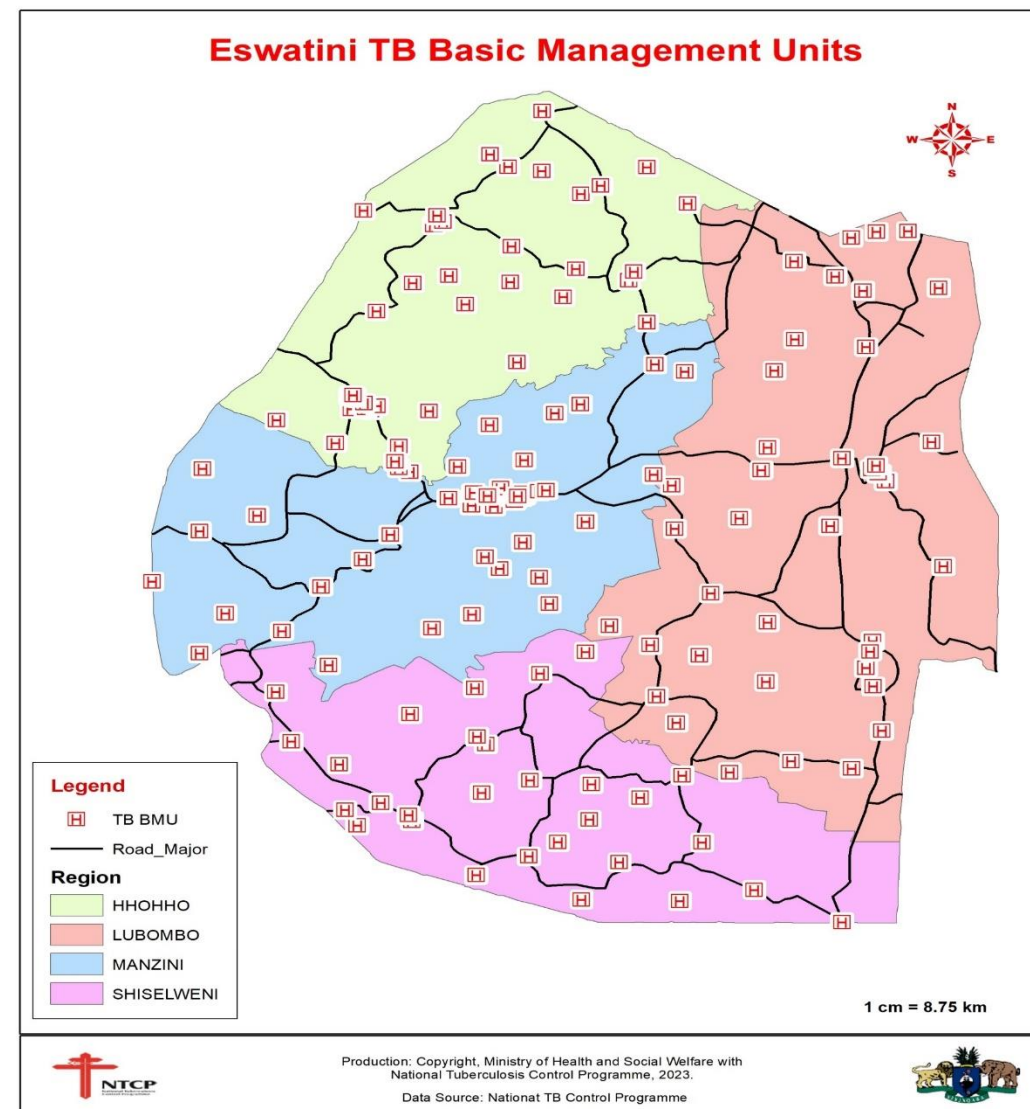
☰ TB diagnostic network:

- 35 GeneXpert sites and 1 mobile
 - 52 6-colour,
 - 14 10-colour devices(XDR Assay)
- 2 TB culture labs
 - MGIT,LPA
 - Introduced sequencing

TB service organization:

Decentralized:

- 151 Accredited TB Treatment Units
- 14 DR-TB sites
- Introduced new drugs(BDQ/DLM)- 2015/2016
- Introduced 9-12-month shorter regimen-2021
- BPaLM regimen in 2023



TB ESTIMATED INCIDENCE RATE PER 100,000

Incidence rate

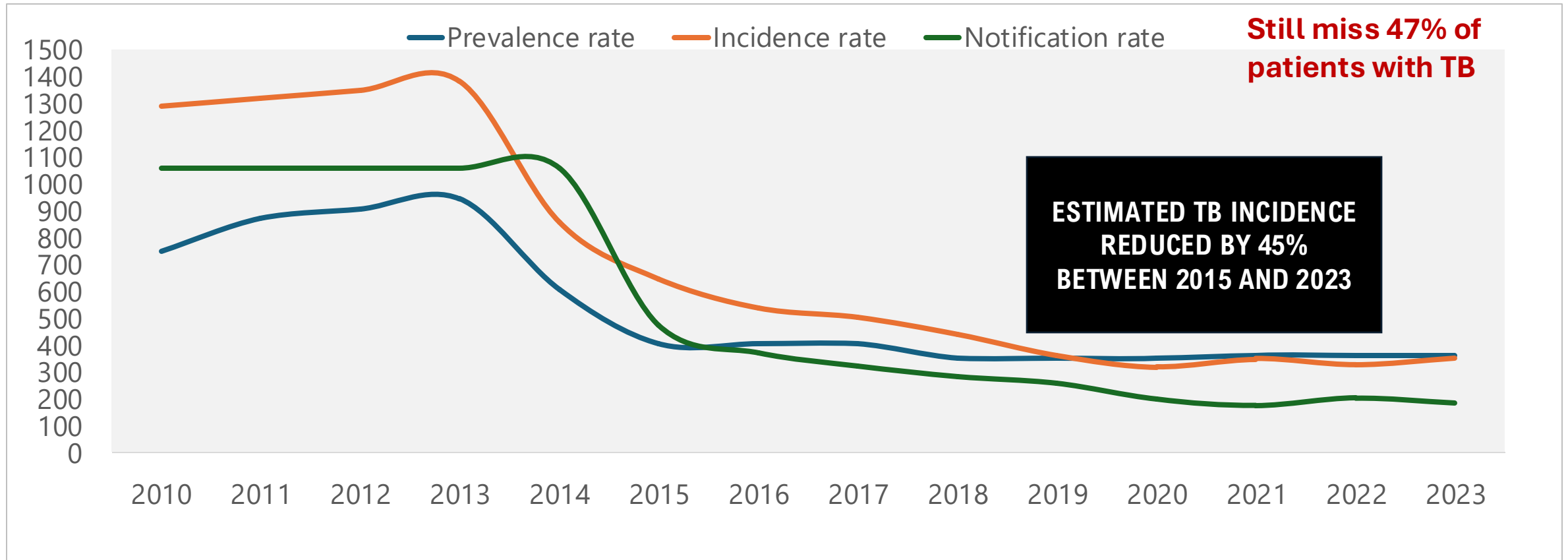
350 per 100.000 (2023)

Notification rate

186 per 100.000 (2023)

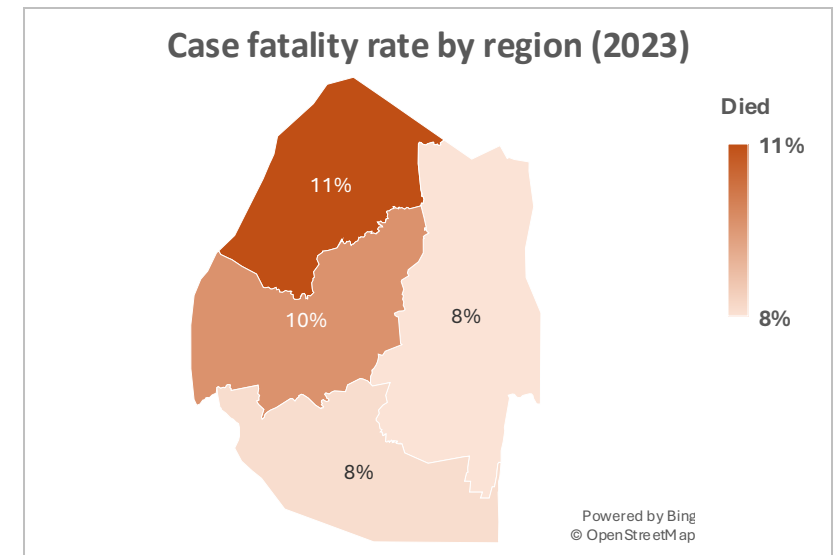
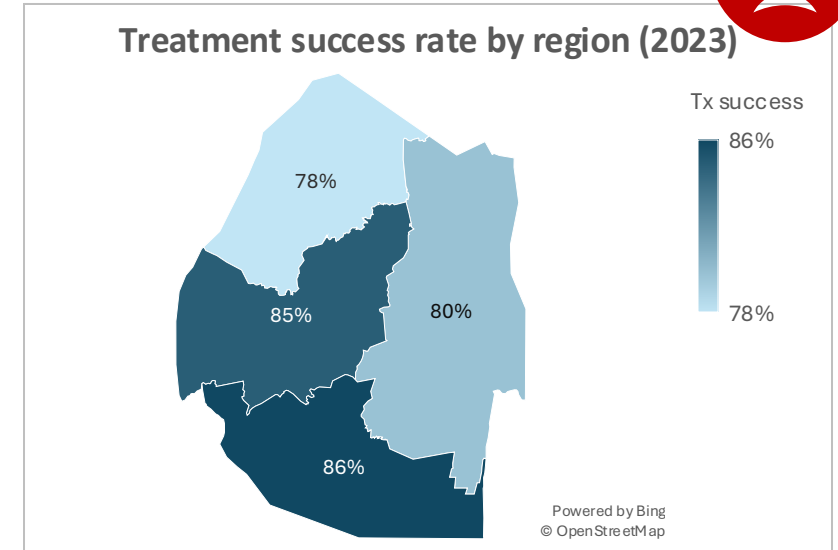
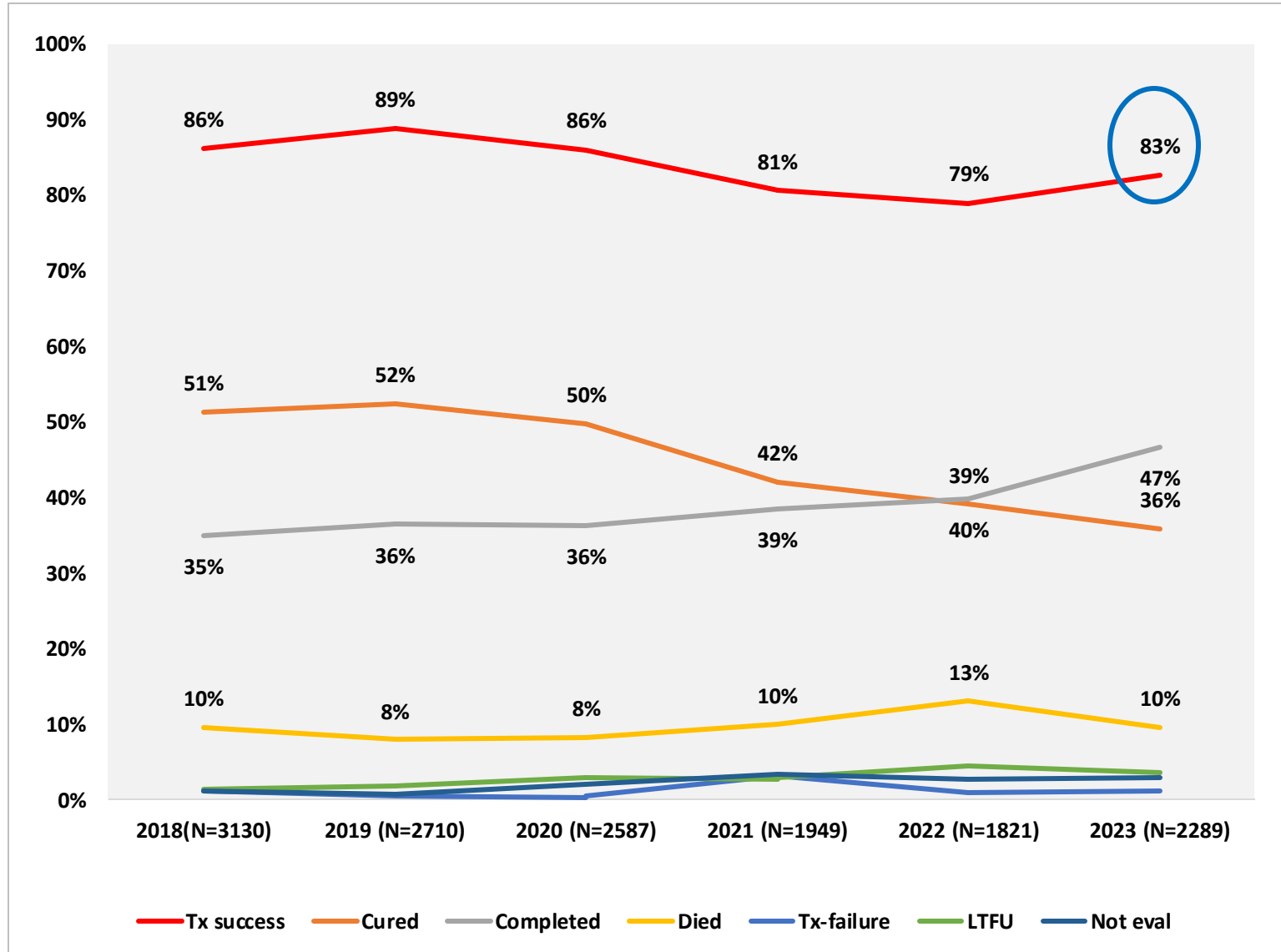
Treatment coverage

53% (37-130)



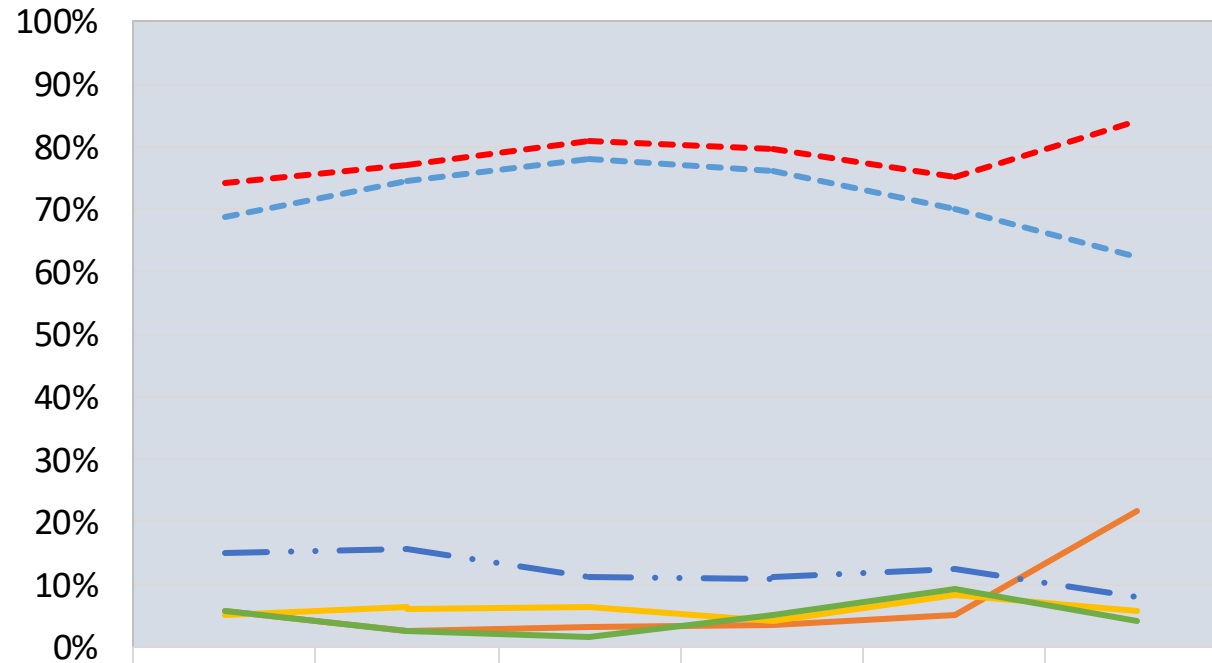
TREATMENT OUTCOMES -DSTB

Global average is 88%



TREATMENT OUTCOMES - DRTB

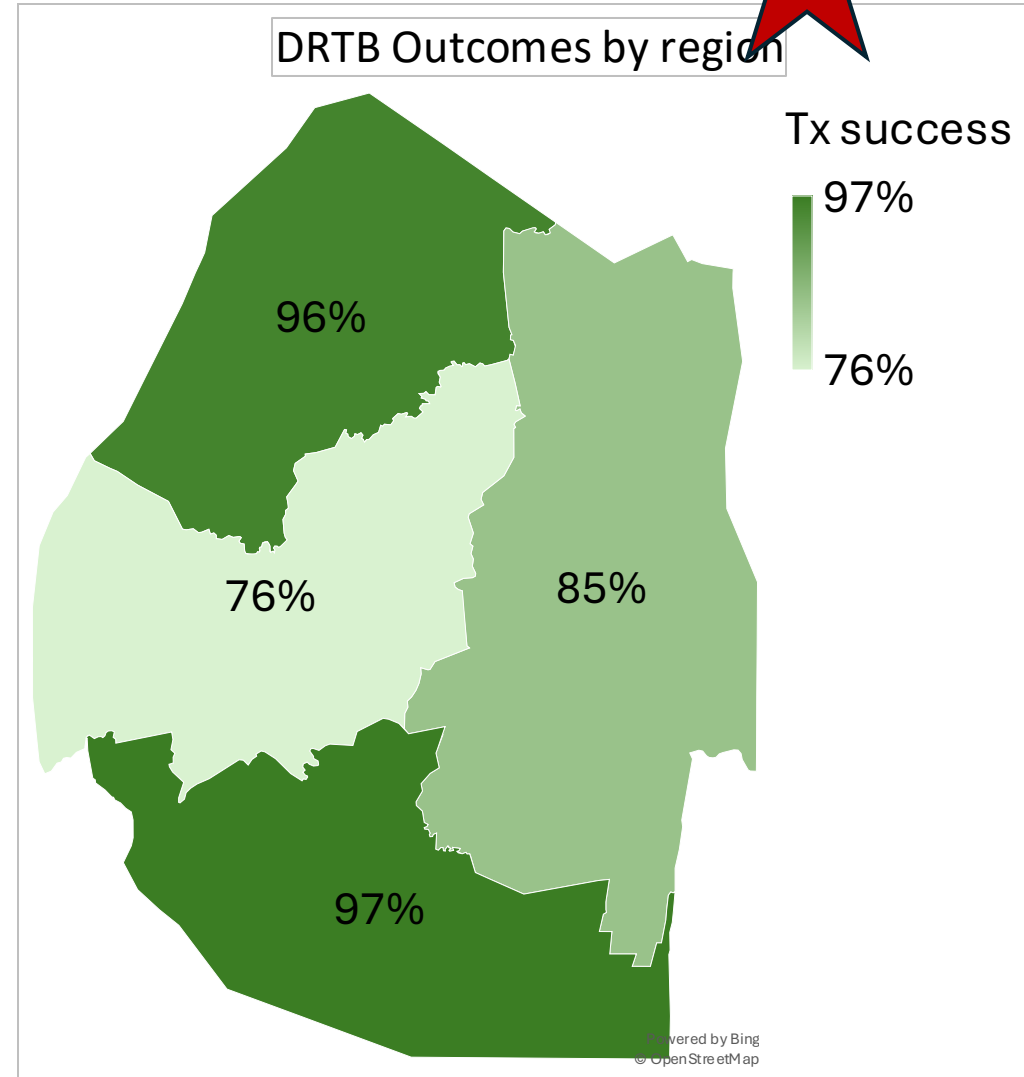
Global average is 63%



	2018 (N=463)	2019 (N=363)	2020 (N=296)	2021 (N=253)	2022 (N=157)	2023 (N=165)
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--- Cured	69%	75%	78%	76%	70%	62%
— Completed	6%	2%	3%	4%	5%	22%
- - - Tx Success	74%	77%	81%	80%	75%	84%
— Failed	5%	6%	6%	4%	8%	6%
- · - Died	15%	16%	11%	11%	12%	8%
— LTFU+Not Eval	6%	2%	2%	5%	9%	4%

DRTB Outcomes by region



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Despite all the diagnostic initiatives and Good DR-TB treatment outcomes Eswatini **still misses 63%** of individuals diagnosed of MDR/RR-TB

Incidence rate

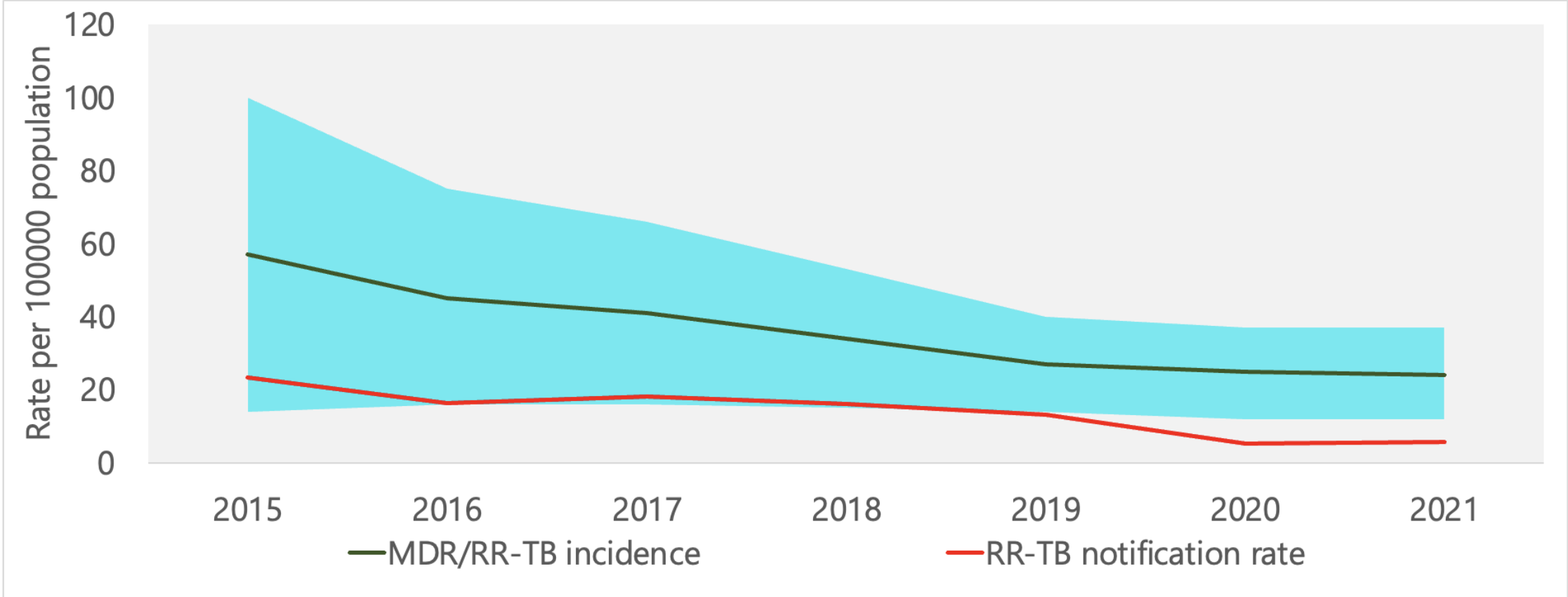
19 per 100.000

Notification rate

4 per 100.000

Treatment coverage

37%



BACKGROUND OF RIFAMPICIN-RESISTANCE IN ESWATINI

- It was not until the country conducted the Drug resistance survey-realized we had a prevalent strain with an uncommon mutation(rpoB I491F) that escapes the diagnostic techniques we have in the country, that is **GeneXpert, LPA, and even MGIT**
- **30%** of RR-TB individuals were missed by our widely decentralized GeneXpert in **2009/2010** TB Drug Resistance Survey (TBDRS)
- In response, the NTCP introduced Universal phenotypic DST testing using MGIT 960 for all samples with Xpert MTB positive test,
 - to identify resistance due to this mutation-unfortunately it was still missed.
- The prevalence increased to **~58% in 2018 TB DRS**
 - ✚ **58% of true RR-TB individuals are incorrectly diagnosed as RS-TB by GeneXpert**
 - ✚ *An important indicator for strains harboring this mutation is that most of them are resistant to isoniazid when tested using MGIT culture, Xpert MTB XDR Assay or LPA*

KEY STEPS IN IMPLEMENTATION OF TNGS(1)

- A multidisciplinary team was established to review Drug Resistance Survey (DRS) results and explore enhanced diagnostic options.

Members included:

- National TB Control Program (NTCP), TB Technical Working Group (TWG), Eswatini Health Laboratory Services (EHLS), DRS team and DR-TB experts, Implementing partners, WHO AFRO representatives, WHO Eswatini TB/HIV focal person, Research Center Borstel (Germany)

- **Diagnostic Tools Assessed**

- **Löwenstein-Jensen (LJ)** culture was considered as it better detects certain mutations than MGIT, but still has its limitations.
- **Sequencing Technologies** were discussed, including next-generation sequencing, though not yet WHO-recommended at that time.

- **Strategic Decision**

Despite WHO not yet endorsing sequencing for routine use, the team agreed that **targeted next-generation sequencing (tNGS)** offered the most promise.

- Decision was to **Implement tNGS as a pilot project (2019)**

- **Protocol Development & Implementation Support**

- A national protocol was developed to guide the pilot roll-out.
- **Funding support** through a collaborative project funded by the Global Health Protection Program (GHPP) from the German Ministry of Health.
- **Implementation by Baylor Foundation Eswatini**, in collaboration with **Baylor College of Medicine**

Germany Federal Ministry of Health Global Health Protection Programme



About the GHPP Projects Institutes News Q EN

About the Federal Ministry of Health Global Health Protection Programme

As part of its international commitments, Germany is providing partner countries with increasing support with outbreaks and the development of reliable healthcare systems. To this end, the Federal Ministry of Health has launched a Global Health Protection Programme to improve international health.

Eswatini, Mozambique, Namibia

Global Health Protection Programme

The GHPP leverages the core competencies and knowledge of specialised German institutions in the field of public health to support the prevention and management of health crises worldwide.



[Read more →](#)



KEY STEPS IN IMPLEMENTATION OF TNGS(2)

- **Strategic Partnerships & Agreements**

- Memorandum of Understanding (MoU) signed between the Eswatini Ministry of Health and the Germany Ministry of Health represented by Research Center Borstel (Germany).

- **Laboratory Infrastructure Enhancement**

- Structural adjustments made to the National TB Reference Laboratory, modifying the existing Line Probe Assay (LPA) lab to integrate tNGS into the workflow.
- Additional space created to accommodate critical equipment such as the **iSeq100 sequencer**, **PCR hood**, and other required tools.

- **Capacity Building & Sensitization**

- Laboratory team trained on **tNGS workflow and operations**.
- Sensitization sessions conducted for both laboratory and **DR-TB clinical teams** to enhance awareness and alignment on tNGS use.
- Training included the **interim diagnostic algorithm** and reflex testing of second specimen.

- **Implementation Challenges:** Project experienced delays due to:

- COVID-19 disruptions
- Procurement challenges

- **Launch & Momentum Building**

- Implementation only happened in **2021**.
- Official launch included **high-level stakeholder engagement**, boosting momentum and catalyzing mobilization of in-country resources.

COST CONSIDERATIONS IN ESWATINI

Setting Up cost

- Infrastructure, refurbishment of LPA room
- Equipment (Iseq 100, thermal cycler, waterbath, minicentrifuge, heating block etc)
- Reagents (DNA isolation reagents, Deeplex, Library Preparation reagents)

Data management

- Data base development (epi-info to RedCap)
- Data collection tools (14 Tablets)
 - Data collection related trainings
- Data storage :Network Attached Storage, Laptops, external hard drives-for back up

Technical Support

- Bioinformatics-remote by Borstel team, Automated-Deeplex website(for analysis/interpretation)
- Laboratory techniques(tNGS)-onsite and remote. More support in first 2 years due to failed runs, very limited from 2024 onwards
 - RCB, Illumina, Milan, WHO Supranational labs

Implementation

- HR costs: Three personnel were hired in addition to the staff complement at the NTRL (1 Senior Laboratory Scientist, 1 Lab tech, and 1 research assistant)
- Lab based Trainings-International, regional and In-country(France, Uganda, Mozambique,UNAM(Namibia)
- Meetings and conferences
 - Setting up of Clinical advisory Committee
 - 1 day meeting
 - Trainings (2 annual trainings in all 14 DR-TB facilities)

- Started with TB only
- Have now integrated COVID 19

Cost-effectiveness of targeted next-generation sequencing (tNGS) for detection of tuberculosis drug resistance in India, South Africa and Georgia: a modeling analysis

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Summary

Background Targeted next-generation sequencing (tNGS) is promising alternative to phenotypic drug susceptibility testing (pDST) for detecting drug-resistant tuberculosis (DR-TB). This study explored the potential cost-effectiveness of tNGS for the diagnosis of DR-TB across 3 settings: India, South Africa and Georgia.

Methods To inform WHO guideline development group (GDG) on tNGS we developed a stochastic decision analysis model and assessed cost-effectiveness of tNGS for DST among rifampicin resistance individuals. We also assessed tNGS as initial test for TB drug resistance in bacteriologically confirmed TB. Diagnostic accuracy and cost data were sourced from a systematic review conducted for GDG, covering studies published until September 2022. The primary outcome was incremental cost (2021 US\$) per disability-adjusted life year (DALY) averted.

Findings tNGS when compared with in-country DST, tNGS proved cost-effective in South Africa (ICER: \$15,619/DALY averted, WTP: \$21,165) but not in Georgia (ICER: \$18,375/DALY averted, WTP: \$15,069). In India, tNGS dominated in-country DST practice, providing greater health impact at lower cost. When comparing tNGS with universal pDST, tNGS was dominated by pDST in all three countries. In Georgia, using tNGS as initial test for TB drug-resistance compared to Xpert MTB/Rif followed by pDST appeared cost-effective. Scenario with 50% reduction in tNGS test kit costs made tNGS cost-effective across all three countries, while a high Bedaquiline resistance prevalence (30%) led to a worsening cost-effectiveness.

Interpretation tNGS may be cost-effective in India, South Africa and Georgia when comprehensive DST is not routinely performed. Thus, existing DST practice and healthcare infrastructure should be considered before implementation and scale-up of tNGS.



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<https://doi.org/10.1016/j.edinm.2024.103003>

- Explored potential Cost effectiveness of TNGS for diagnosis of DR-TB across 3 countries
- To inform WHO GDG process
- tNGS proved cost effective in South Africa but not in Georgia
- but when using tNGS as initial test vs Xpert followed by pDST- appeared cost effective.
- In India tNGS dominated in-country DST practice
- Universal pDST dominated tNGS.
- BUT how many countries have universal pDST??

Systematic Review – Cost Drivers

Four major cost drivers were identified that impact costs associated with tNGS and WGS

1) Different sequencers

- Considerations include sequencers themselves, as well as specific kits

2) Depth and breadth of coverage

- Greater depth and breadth of coverage are associated with greater costs

3) Inefficiencies in initial sample runs

- Inefficiencies unique to initial sample runs increased costs

4) Economies of scale via batching and/or multiplexing

- Lower sample volumes and less batching or cross-batching per run can be associated with a greater cost per sample

Additional cost drivers identified

1. Operational efficiency of lab
2. Availability of trained personnel
3. Sequencers being used at full capacity
4. Discounts associated with purchasing high volume from the same supplier
5. Complexity of infectious pathogen

Illumina Sequencing Systems



Focused Power



iSeq 100



MiniSeq



Flexible Power



MiSeq



NextSeq 500/550

Production Power



HiSeq 2500



HiSeq 3000/4000

Population Power



HiSeq X Five/Ten



NovaSeq

An interim algorithm was developed in 2021 to guide eligible samples for sequencing at National TB Reference Laboratory

Eligibility for sequencing test: *Informed by the DRS results*

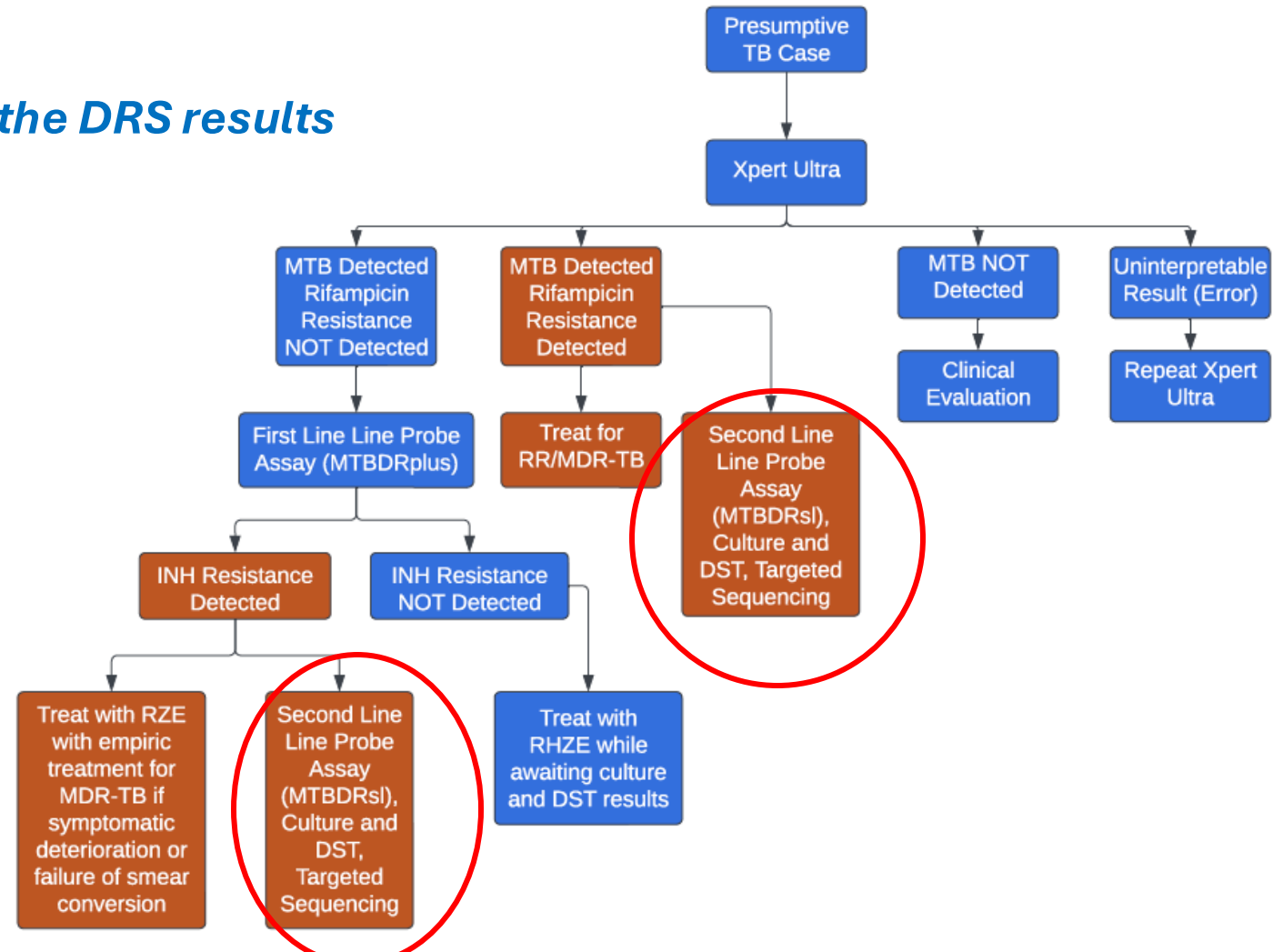
Baseline

- Hr-TB, PDR-TB by LPA or MGIT
- RR/MDR-TB by GeneXpert or MGIT

During Treatment

- At 2/3 months-non conversion.
- Culture reversion, TB relapse during the post-treatment follow-up period.

Diagnostic Testing and Treatment Algorithm for People with Presumptive Tuberculosis Symptoms

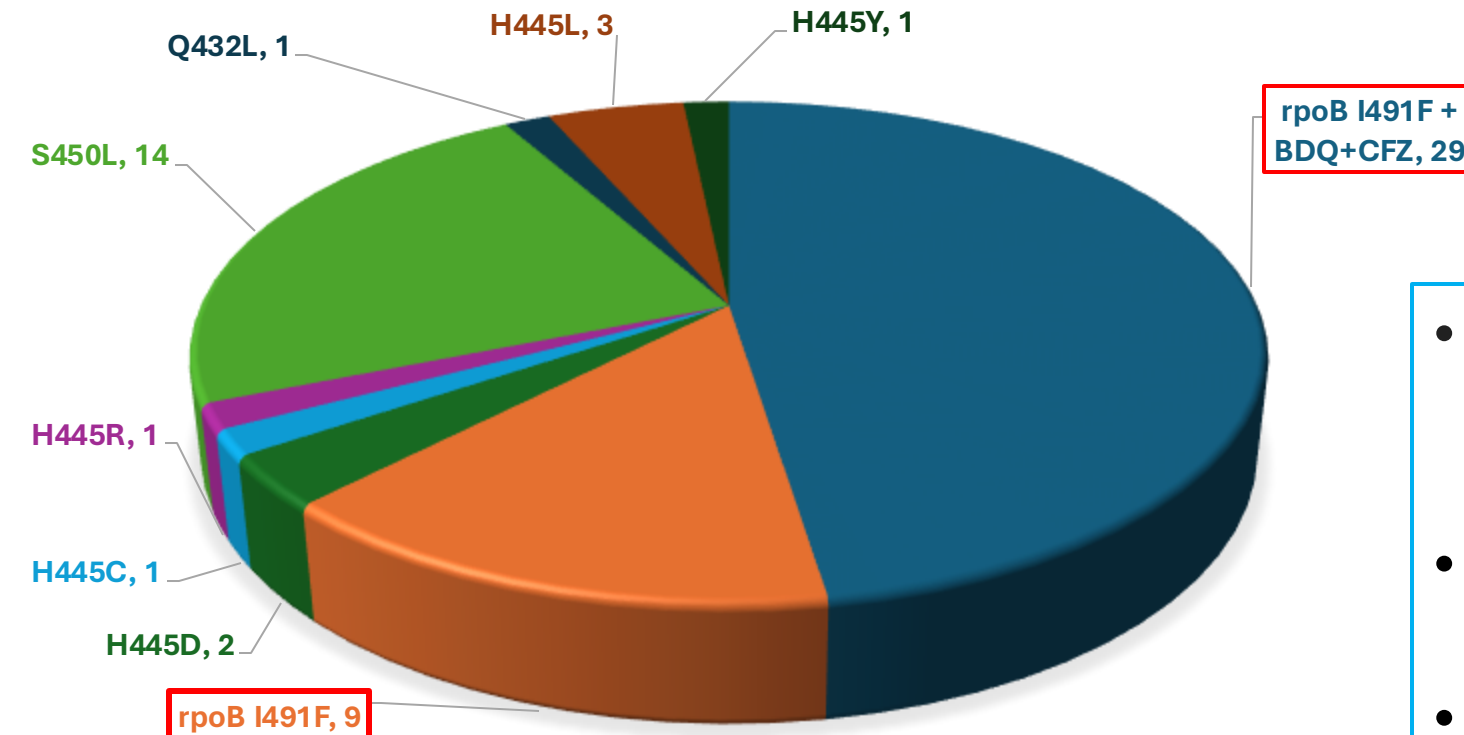


Clinical Use of TNGS results

RESULTS OF THE FIRST 85 TNGS SAMPLES FURTHER GUIDED IMPLEMENTATION

- From a total of 85 samples that were sequenced from Sept 2021
- 61 mutations were identified, 38(62%) had *rpoB* I491F mutation
- 29(76%) of the 38 *rpoB* I491F mutation had additional resistance to Bedaquiline and Clofazimine.

MUTATIONS IDENTIFIED BY TARGETTED SEQUENCING N= 61

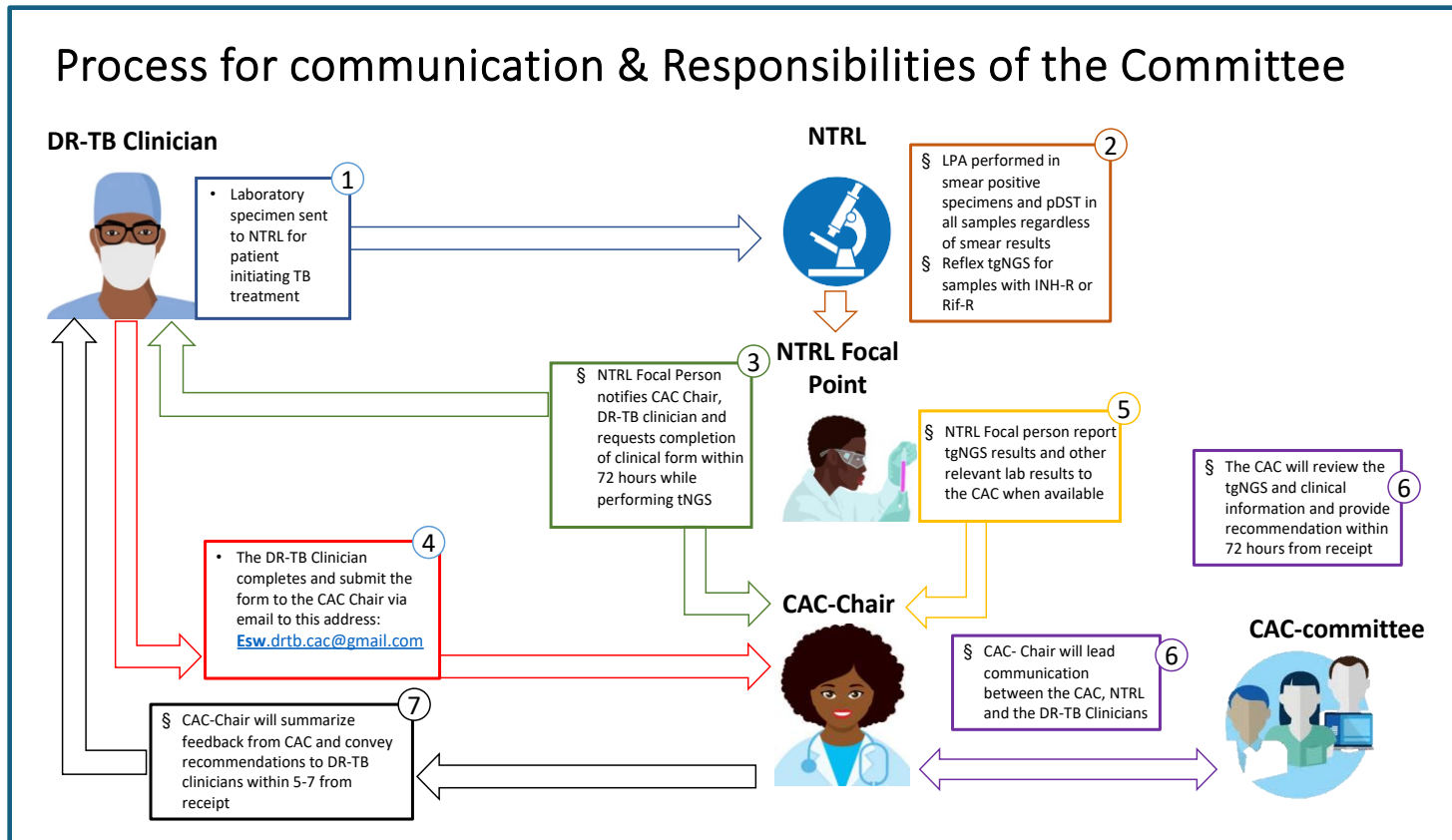


- Realised there was even a bigger problem
- Decision by NTP and TWG to use these pilot results for clinical management

- A clinical Advisory committee (CAC) was set up to guide optimization of treatment.
- This team has clinical, laboratory and public health expertise
- Includes national & international DR-TB experts

CLINICAL ADVISORY COMMITTEE

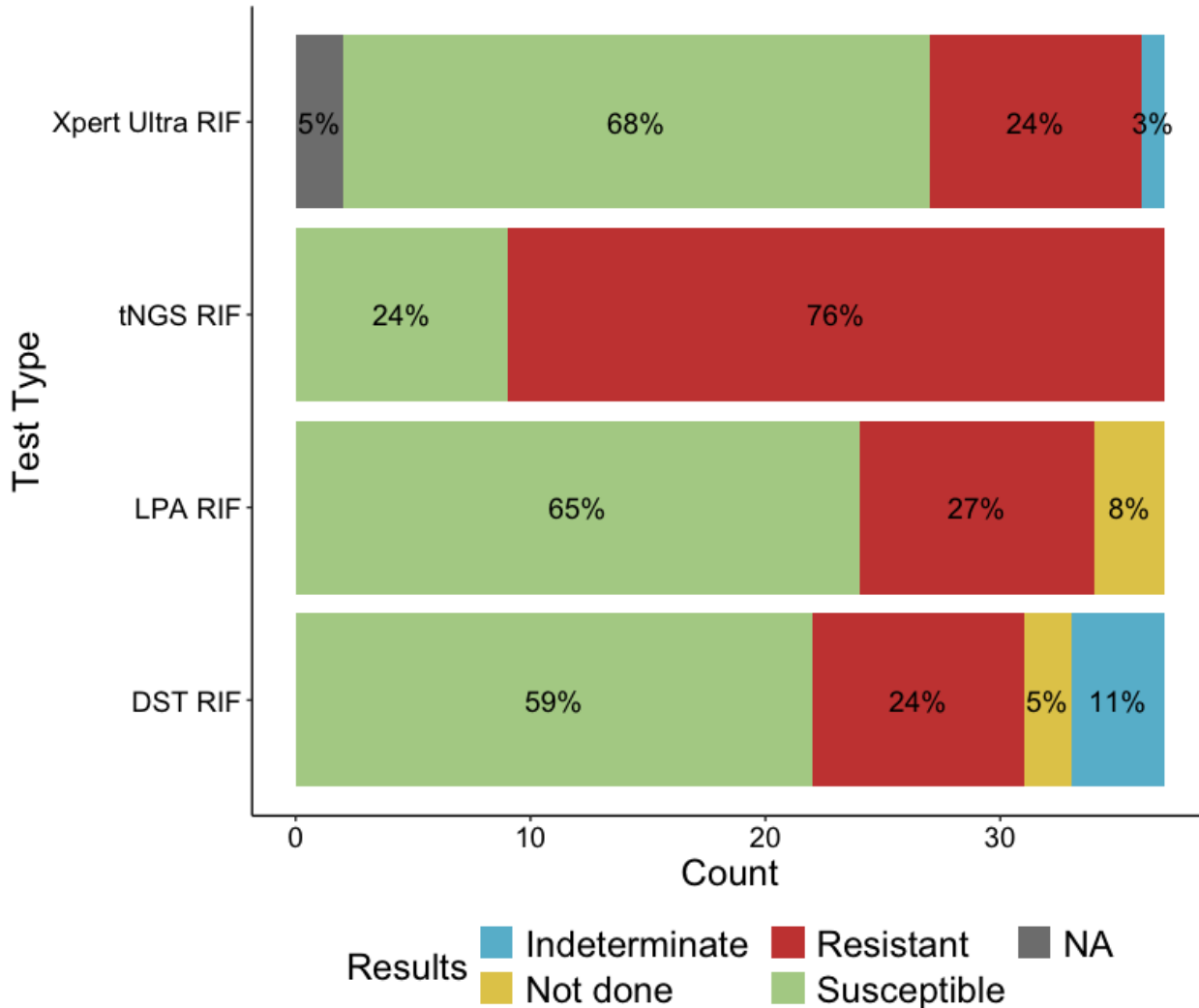
- Managed to follow up 37 patients
- Had both clinical information and tNGS results
- Submitted to the Clinical Advisory Committee for treatment guidance



The CAC review both the tgNGS and Clinical Information and provide recommendation within 72 hours from receipt

Early Clinical Sequencing Population	
	Overall (N=37)
Age	
Mean (SD)	43.3 (14.1)
Median [Min, Max]	42.0 [14.0, 76.0]
Sex	
F	11 (29.7%)
M	26 (70.3%)
Region	
Hhohho	8 (21.6%)
Lubombo	10 (27.0%)
Manzini	17 (45.9%)
Shiselweni	2 (5.4%)
HIV Status	
NR	11 (29.7%)
R	26 (70.3%)
Prior TB Hx	
1 st line	12 (32.4%)
2nd line	2 (5.4%)
New	23 (62.2%)

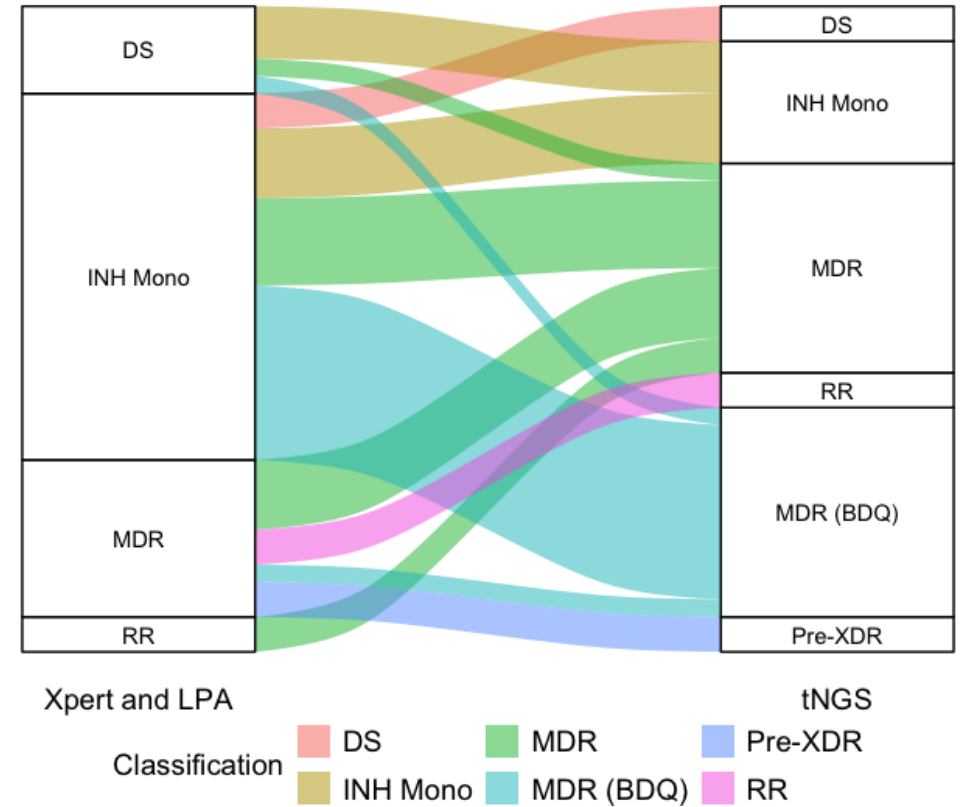
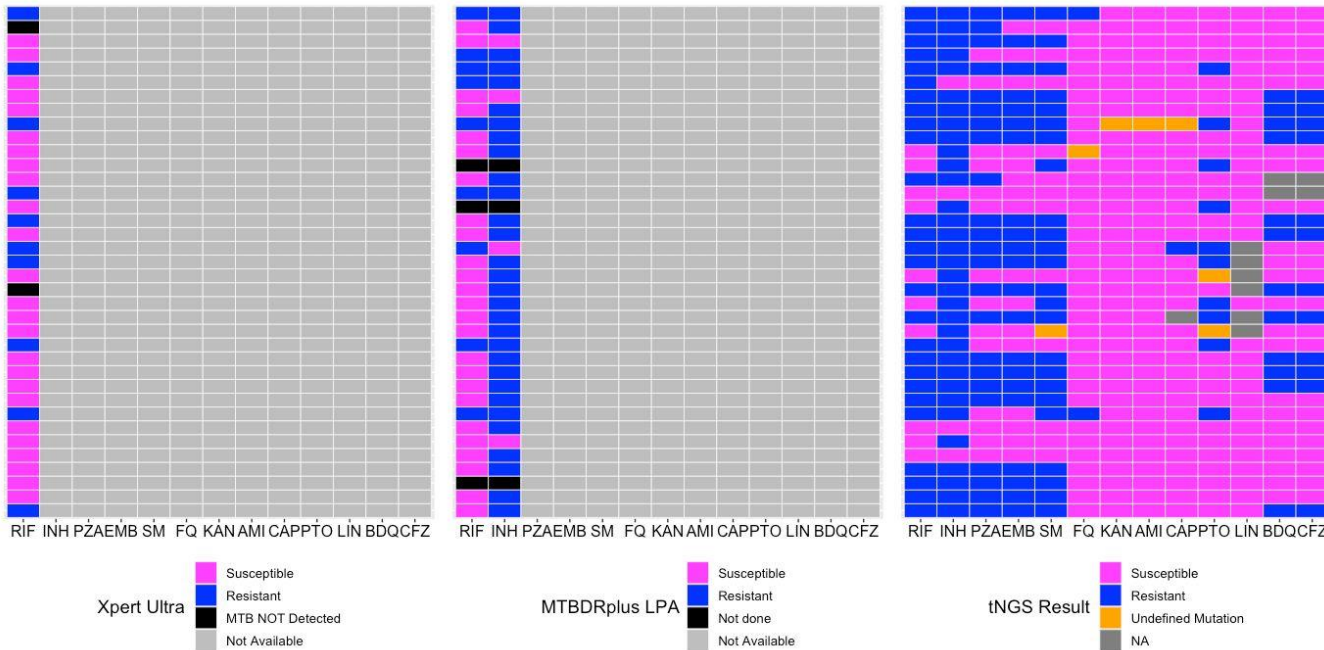
RIFAMPICIN RESISTANCE DETECTION ACROSS MODALITIES



- Demonstrates the varying performance on **rifampicin resistance** detection in Eswatini across Xpert ultra/LPA/pDST(MGIT) as compared to tNGS

Additional 46% of patients with RR/MDR-TB were detected by TNGS

1. tNGS detects the I491F mutation + additional resistance to BDQ&CFZ



- **MDR+BDQ(without FQ) = Difficult to classify as PreXDR.**
- **Very few had FQ resistance**

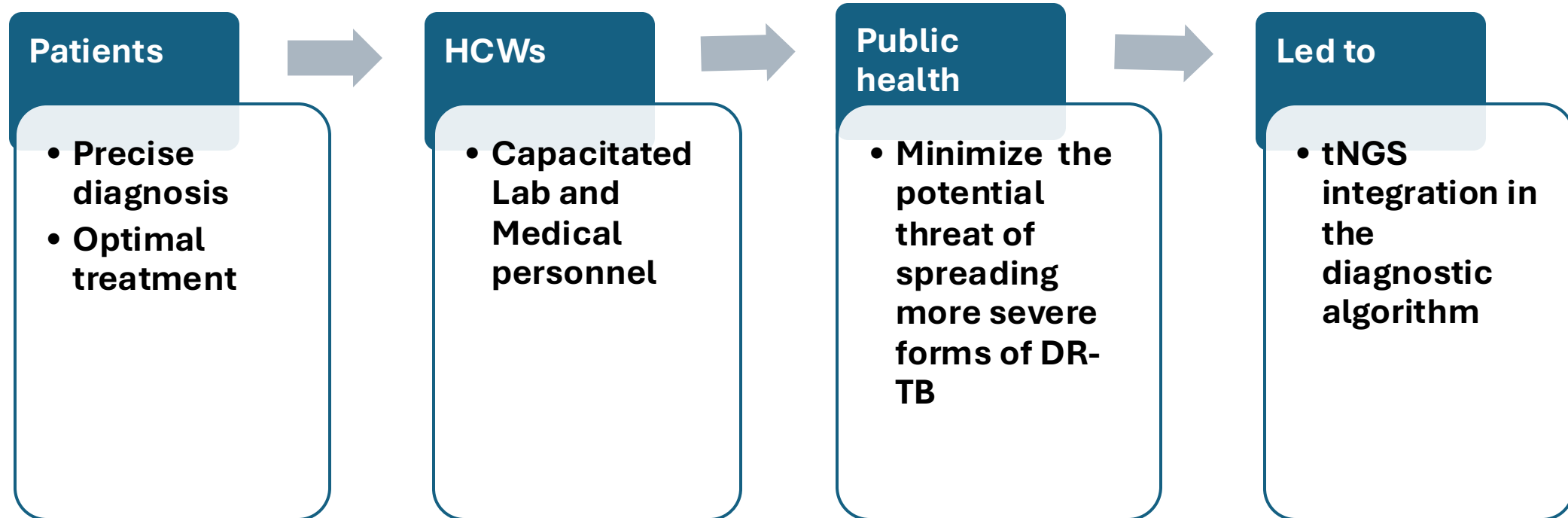
2. Comprehensive tNGS testing results in major shifts in tuberculosis resistance classifications

PATIENT OUTCOMES OF THE 37 PATIENTS

Sequencing Cohort		(N = 37)
Initial Regimen		
	BDQ/CFZ/TRD/DLM	1 (2.7%)
	BDQ/LFX/LZD/CFZ/DLM	9 (24.3%)
	BDQ/LFX/LZD/CFZ/TRD	2 (5.4%)
	RHZE	25 (67.6%)
Final Regimen		
	BDQ/LFX/LZD/CFZ/DLM	18 (48.6%)
	LFX/LZD/TRD/DLM/PTO	5 (13.5%)
	BDQ/LFX/LZD/CFZ/TRD	4 (10.8%)
	LFX/LZD/TRD/DLM/PAS/IMP-CLV	2 (5.4%)
	LFX/LZD/TRD/DLM/PTO/IMP-CLV	2 (5.4%)
	TRD/DLM/PTO/IMP-CLV	1 (2.7%)
	BDQ/LFX/CFZ/TRD/DLM	1 (2.7%)
	RHZE	3 (8.1%)
	RHZE/LFX	1 (2.7%)
Treatment Outcomes		
Completed		18 (48.6%)
Cured		13 (35.1%)
Treatment Success		31 (83.7%)
Died		5 (13.5%)
LTFU		1 (2.7%)

- ❖ Regimen change: 73% of patients.
- ❖ Main Change from RHZE to BDQ based regimen or Individualised regimen
- ❖ 84% treatment success rate

TNGS PLAYED A CRITICAL ROLE IN CLINICAL MANAGEMENT



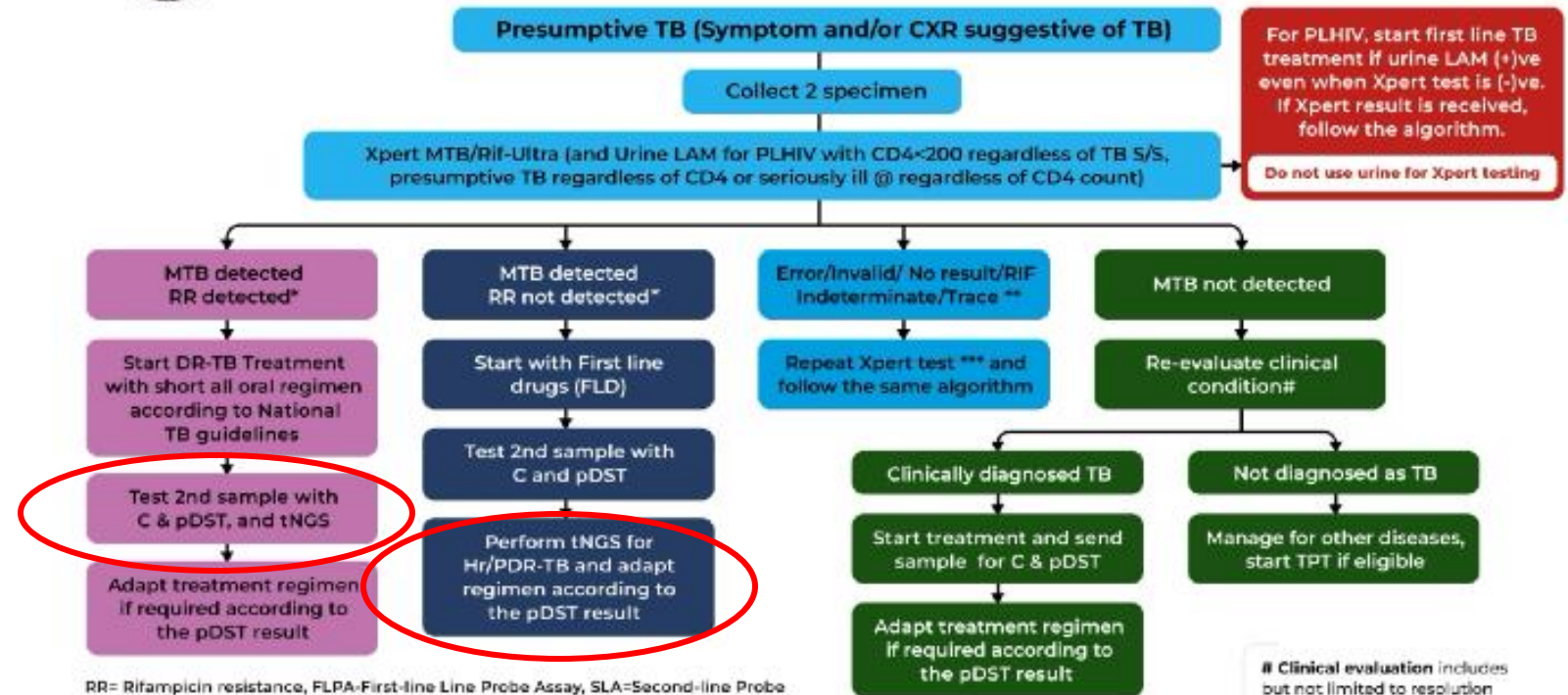
Progress so far



INTEGRATION OF TNGS IN THE NATIONAL TB DIAGNOSTIC ALGORITHM



TB Diagnostic and Treatment Decision Algorithm



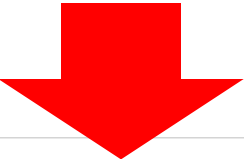
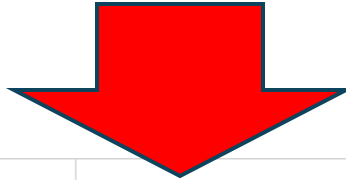
For PLHIV, start first line TB treatment if urine LAM (+)ve even when Xpert test is (-)ve. If Xpert result is received, follow the algorithm.
Do not use urine for Xpert testing

RR= Rifampicin resistance, FLPA-First-line Line Probe Assay, SLA-Second-line Probe Assay, tNGS=Next generation sequencing, C & pDST = Culture and phenotypic drug susceptibility test, Hr= Isoniazid resistance, PDR = Poly-drugs resistance, TPT = TB preventive treatment.
Note: If no culture conversion at month 4 or culture reversion, perform C&DST and tNGS.
 *If facility has the Xpert XDR Assay on site, use the sample left over from Xpert Ultra to test for Isoniazid, Fluoroquinolones and Ethionamide resistance.
 If **MTB Trace, persons evaluated for pulmonary TB & extra-pulmonary TB including PLHIV and children and no history of TB in the past 5 yrs, start FL-TB treatment and perform C,pDST & tNGS for DR-TB detection. Adapt treatment if required when pDST is received.
 If facility has Xpert XDR assay on site, perform test with residue from Xpert Rif ultra test.
 ***If RR indeterminate with melting curve showing RR, no need to repeat and start treatment as RR-TB.

@ **Seriously ill adult:** having any of danger signs: respiratory rate ≥ 30 /min; heart rate ≥ 120 /min; or unable to walk unaided, high $\geq 39^{\circ}\text{C}$,
Seriously ill child: lethargy or unconsciousness; convulsions; unable to drink or breastfeed; and repeated vomiting, high $\geq 39^{\circ}\text{C}$, age- defined tachycardia and/ or tachypnoea.

Clinical evaluation includes but not limited to resolution of clinical signs and symptoms after 1 week course of broad-spectrum antibiotic, response to nutrition therapy if malnourished especially in children, TB contact history, CXR (if accessible). After evaluation, clinical diagnosis of TB is by the discretion of attending physicians.

DATABASE



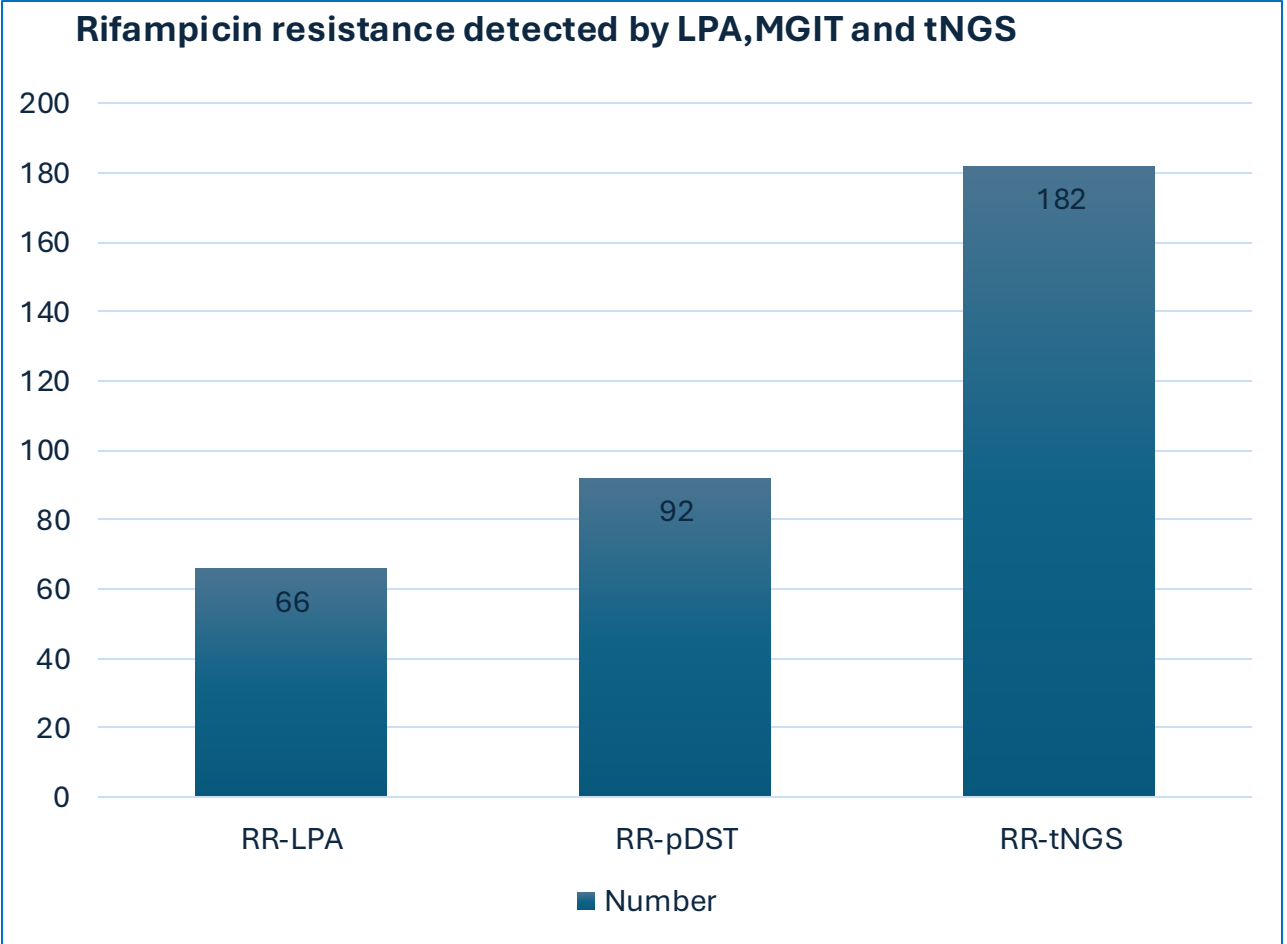
First line LPA	PHENOTYPIC DRUG SUSCEPTIBILITY TESTING First line						RIF	INI	PZA	EME	SM	FQ	KAI	AM	CAP	ETH	LIN	BDQ	CFZ	Rifampicin Mutation
Rifampicin	Isonizid	Rafamp	Isonia	nan	lt acce															
S	R by KatG	S	R	S	+	1	1	1	1	1	0	0	0	0	0	0	1	1	I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	I491F	
S	R by KatG	S	R	etermin	+	1	1	1	1	1	0	0	0	0	0	0	1	1	I491F	
S	R by KatG	S	R	R	+	1	1	1	1	1	0	0	0	0	0	0	0	0	rpoB I491F	
S	R by KatG	S	R	R	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	Not done	Not done	Not done	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB S450L rpoB I491F	
S	R by KatG	S	ndeterminate	S	+++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by INHA	S	R	S	-	1	1	1	1	1	0	0	0	low coverage	0	0	1	1	rpoB I491F	
S	R by KatG	Not done	Not done	Not done	+++	1	1	1	1	1	0	0	0	0	0	0	0	0	rpoB I491F	
S	R by KatG	S	R	S	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	S	etermin	-	1	1	1	1	1	0	0	0	low coverage	0	0	1	1	rpoB I491F	
S	R by KatG	S	Not done	R	+	1	1	1	1	1	0	0	0	0	0	0	1	1	I491F	
S	R by KatG	Indeterminate	ndeterminate	etermin	+++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	Indeterminate	ndeterminate	etermin	-	1	1	1	1	1	0	0	0	low coverage	1	Low coverage	1	1	rpoB I491F	
S	R by KatG	S	R	S	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	1	1	1	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	1	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	IND	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	-	1	1	1	1	1	0	0	0	low coverage	low coverage	low coverage	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	IND	+++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by both genes	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F rpoB S450L	
S	R by KatG	Not done	Not done	Not done	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	0	0	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	0	0	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	++	1	1	1	1	1	1	1	1	0	0	0	1	1	rpoB I491F	
S	R by KatG	Not done	Not done	Not done	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	R	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	-	1	low coverage	1	1	1	0	1	1	1	1	low coverage	1	1	rpoB I491F	
S	R by KatG	S	R	S	+++	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	
S	R by KatG	S	R	S	+	1	1	1	1	1	0	0	0	0	0	0	1	1	rpoB I491F	



RESULTS FROM 427 SAMPLES SEQUENCED-AS OF AUG 2024

Variables	Number	Percentage
Samples analysed at NTRL	427	
RR/MDR-TB by LPA	66	15%
R-Susceptible LPA	286	67%
No LPA/Indeterminate results	75	18%
INH resistance by LPA	288	67%
INH-Susceptible by LPA	62	15%
No LPA/Indeterminate results	77	18%
RR/MDR-TB by MGIT	92	22%
R-Susceptible MGIT	238	56%
Not Done/Indeterminate results	97	22%
INH resistance by MGIT	274	64%
INH-Susceptible by MGIT	59	14%
No LPA/Indeterminate results	94	22%
INH Mono resistance by LPA	246	79%
True INH Mono by tNGS	64	21%
RR/MDR-TB by tNGS	182	
I491F	132	73%
RvO678	108	82%
Fluoroquinolone resistance-tNGS	8	
Total	427	

About 50% of the rifampicin resistance samples could have been missed without tNGS



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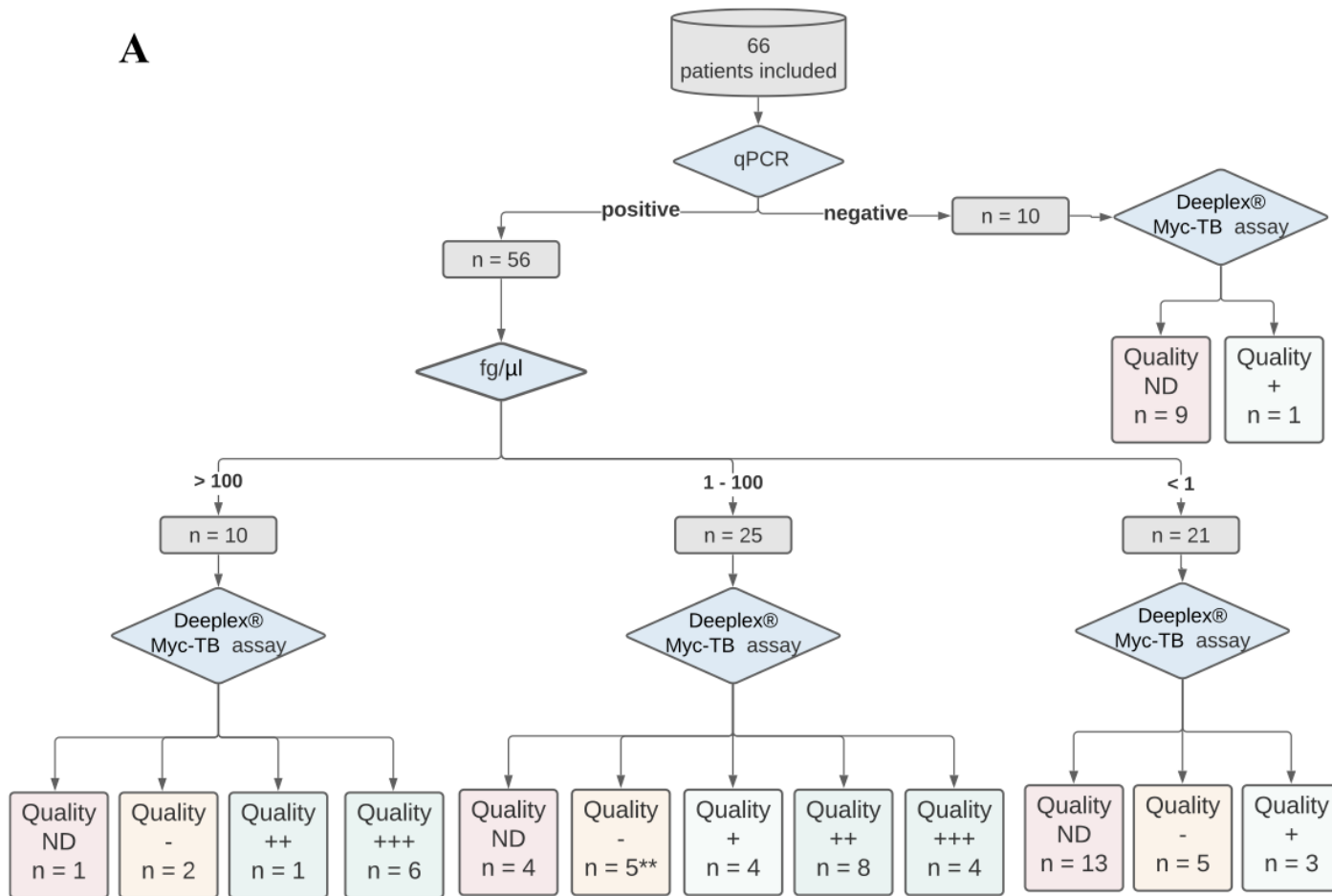
❖ 79% were initially classified as INH mono by LPA, after tNGS only 21% were true Inh mono resistance

❖ Out of the 182 samples with RR/MDR-TB, 73% (132/182) had I491F mutation

❖ 82% of the I491F strain have RV0678 mutation (BDQ and CFZ resistance)

❖ Fluoroquinolone resistance is minimal

STOOL TNGS RESULTS



- Stool is an important diagnostic specimen for tuberculosis in populations who struggle to provide sputum, such as children or PLHIV
- Studied predominantly on stored specimens in adult cohorts (inclusive of people living with HIV)
- Head to head comparisons between tNGS on sputum and stool are needed
- Only useful when a molecular stool pcr (e.g. Xpert) is positive

Rapid Diagnostic Sequencing of Stool DNA Using Targeted Nanopore Sequencing in Patients With a Pulmonary Tuberculosis Diagnosis

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Background. Approximately 1.25 million individuals died of tuberculosis in 2023, in part due to ineffective treatment. In patients with paucibacillary tuberculosis, microbiologic confirmation and drug resistance testing via respiratory specimens is challenging; hence, stool samples are increasingly used for microbiologic confirmation. Targeted next-generation sequencing (tNGS) of stool DNA may improve detection of drug-resistant (DR) tuberculosis, helping patients receive appropriate treatments.

Methods. We assessed the ability of a nanopore tNGS approach using stool to detect drug resistance in a prospective, nested cohort of consecutive participants in Eswatini with pulmonary tuberculosis confirmed via sputum culture or sputum GeneXpert Ultra from 2020 to 2023. We compared stool tNGS with (1) a composite reference standard of diagnostic tools available in the study setting, (2) sputum culture tNGS, and (3) whole-genome sequencing of sputum culture.

Results. Participants ranged in age from 2 to 80 years (median age, 28 years; interquartile range, 20–40 years), and 45.6% (26 of 57) had human immunodeficiency virus. Based on stool tNGS, 14% of our cohort (8 of 57) had drug resistance, and 8.8% (5 of 57) would have received a different treatment regimen had stool tNGS informed clinical decision making. Stool tNGS with nanopore technology was 94.4% concordant (in 17 of 18) for identifying DR mutations with whole-genome sequencing and identified 90% of resistant mutations (9 of 10) indicated by composite reference standard. Stool tNGS detected resistance not detected by standard methods, including detection of rifampicin resistance associated with *rpoB* Ile491Phe not detected with culture-based phenotypic drug susceptibility testing (pDST) and GeneXpert Ultra. Stool tNGS also detected bedaquiline, clofazimine, and ethambutol resistance not detected with culture-based pDST in Eswatini. The workflow from stool processing to nanopore tNGS report can be completed in 1 day.

Conclusions. Stool tNGS of *Mycobacterium tuberculosis* using nanopore technology provides a rapid and accurate method to inform the design of effective treatment regimens in patients with pulmonary tuberculosis in countries with high DR tuberculosis burdens and limited resources.

Keywords. *Mycobacterium tuberculosis*; nanopore; sequencing; targeted sequencing; tuberculosis.

Proof of concept: Assess ability of a nanopore tNGS approach using stool to detect drug resistance

- N=57
- Age range:2-80years,IQR:20-40years
- HIV Positive: 45.6%
- 14% had drug resistance
- Detected Rif resistance associated with *rpoB* I491F mutation
- 8.8% would have received a different treatment regimen without stool TNGS information
- Can also detect resistance to BDQ,CFZ etc
- TAT-approx. 6 hours

CONCLUSION

- TNGS is a powerful tool for rapid diagnosis of drug-resistant TB
- It offers advantages in terms of speed, cost-effectiveness, and comprehensiveness
- In Eswatini where prevalence of rpoB I491F mutation is high, without tNGS, we would miss over 50% with rifampicin resistance.
 - Leading to suboptimal treatment
- Additional resistance to Bdq and Cfz resistance makes it even more challenging especially in the context of BPaLM regimen
- Emphasizing the need for Integration of targeted sequencing into national diagnostic and treatment algorithms
- Sustainability
 - Capacity building in tNGS, wet lab and bioinformatics.
 - Transition procurement of reagents by Government-phased approach
 - Integration and Strengthening the existing sample referral system

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Eswatini Implementing Partners

Health Care workers

Recipients of Care and community



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THANK YOU

