Integration of targeted sequencing into national diagnostic and treatment algorithm in Eswatini

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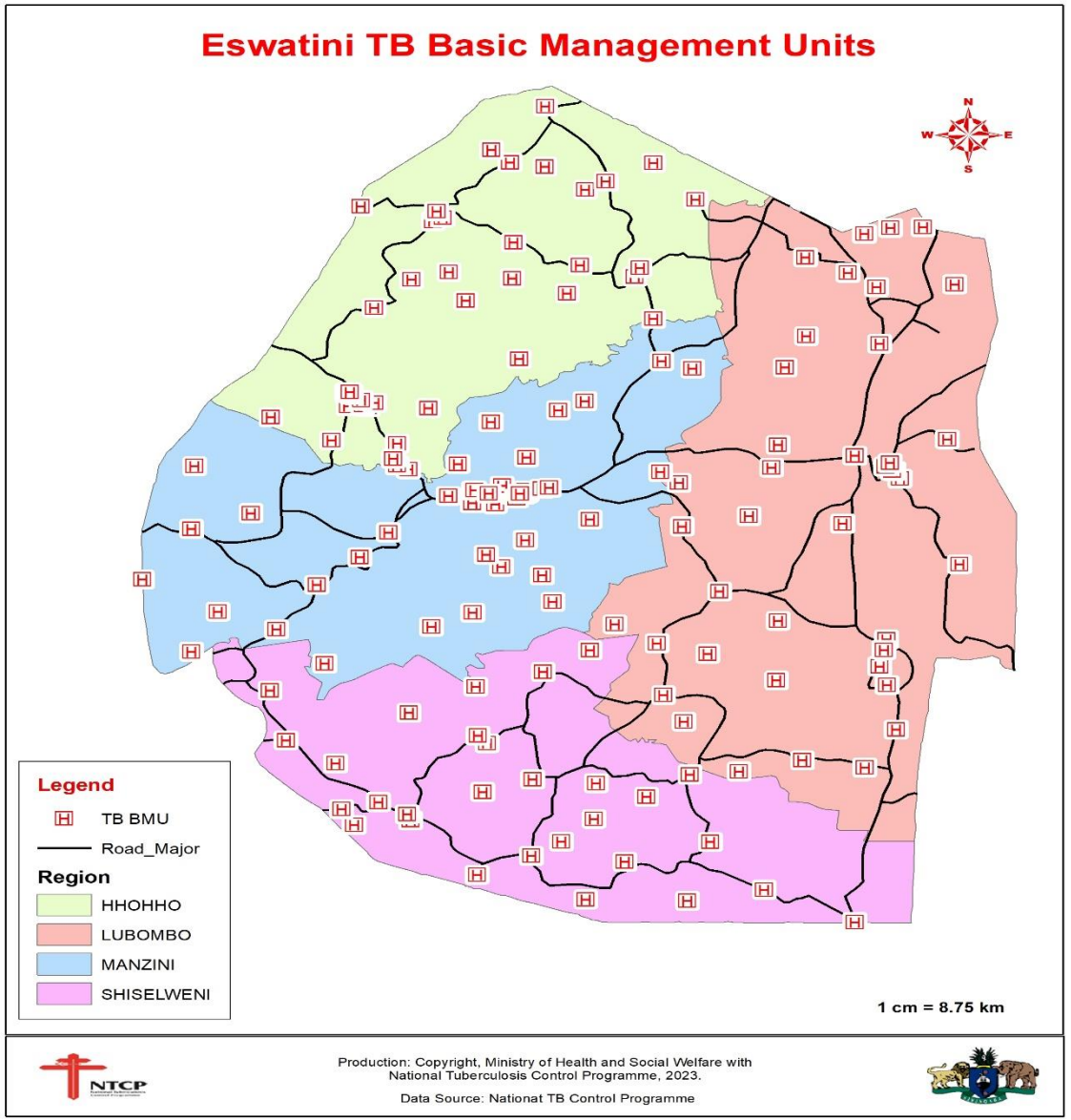
Eswatini

COUNTRY CONTEXT

- Eswatini is still among the 30 countries with the highest TB/HIV burden globally.
- Highest HIV prevalence: 24.8 %
- TB/HIV co-infection: 65%
- DR-TB/HIV co-infection: 71%
- Is divided into 4 Administrative regions
  - Manzini, Hhohho, Shiselweni, Lubombo
- Has 327 health facilities
  - 149 TB treatment sites(BMUs).
  - 14 DR-TB sites
- Diagnostic sites
  - 35 GeneXpert testing sites(6 colour)
    - 10 colour GeneXpert machines(13)
  - National reference Lab
    - FL-LPA,SL-LPA, MGIT, tNGS

Population (2022)
1 201 670



TB estimated incidence and notification rate

Incidence rate

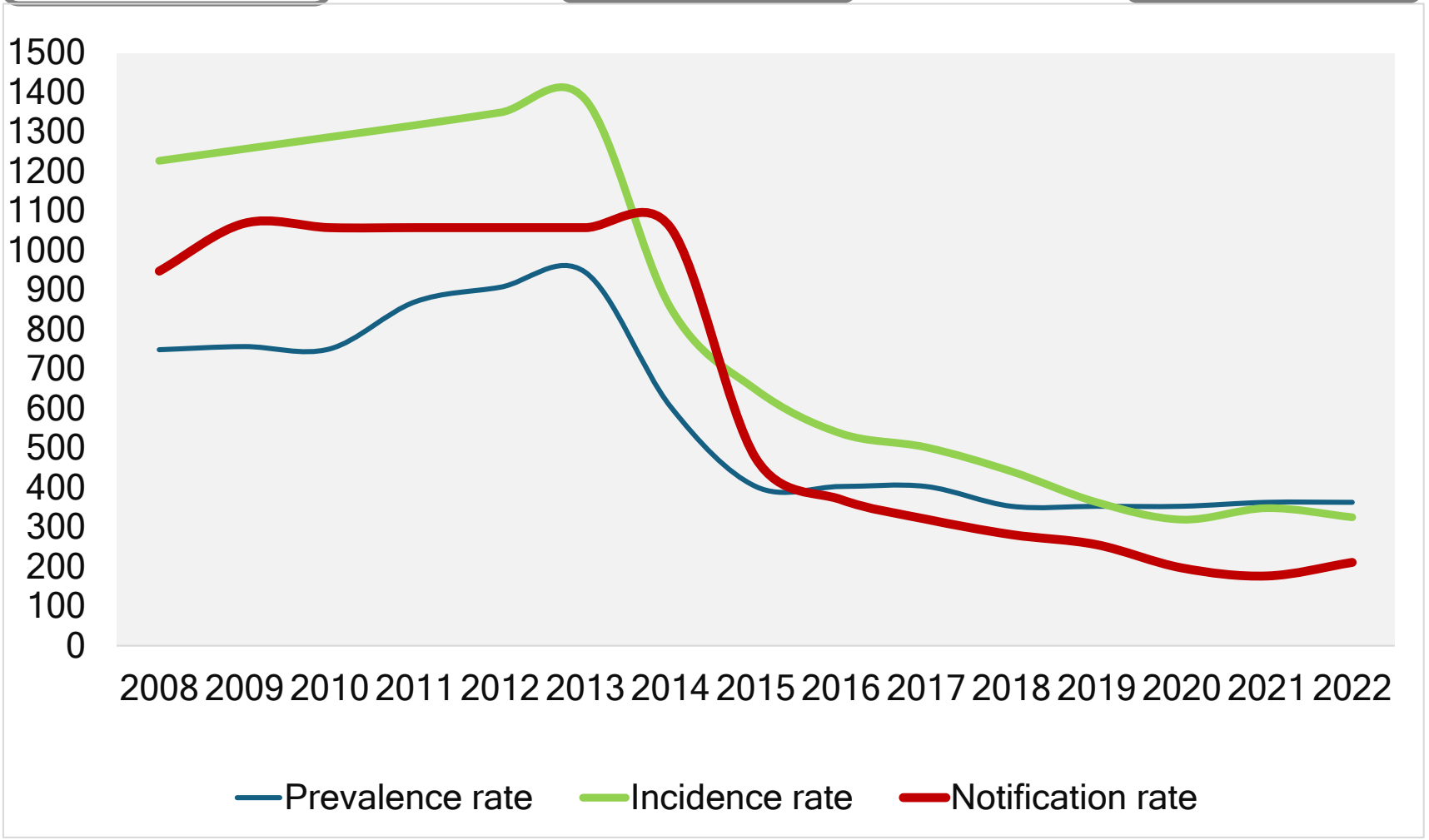
325 per 100.000

Notification rate

211 per 100.000

Treatment coverage

61% (32 - 75)



- 76% drop-TB incidence from 1382/100,000 in 2013 to 325/100,000 in 2022.
- Recovery in treatment coverage from 47% in 2021
- Community based ACF
- Facility based intensive case finding
- The system still miss 39% of individuals with TB
- TB prevalence survey revealed that these individuals
  - Do not cough
  - Not picked up by symptom-based tool

MDR/RR-TB INCIDENCE AND NOTIFICATION RATE

Incidence rate

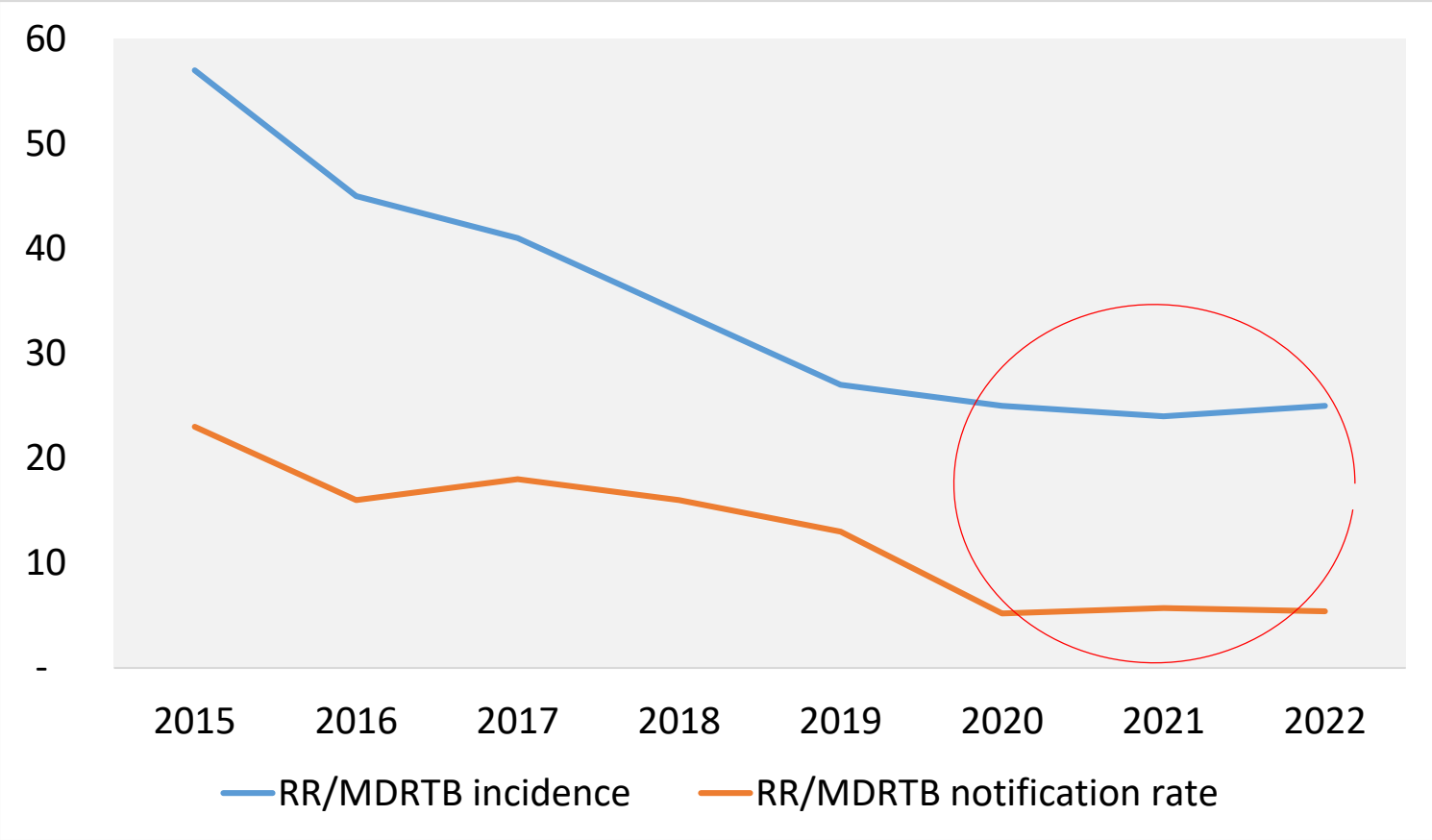
19 per 100.000

Notification rate

5.7 per 100.000

Treatment coverage

30%



4.4% Estimated RR/MDR-TB among new cases.

32% Estimated RR/MDR-TB among previously TX cases.

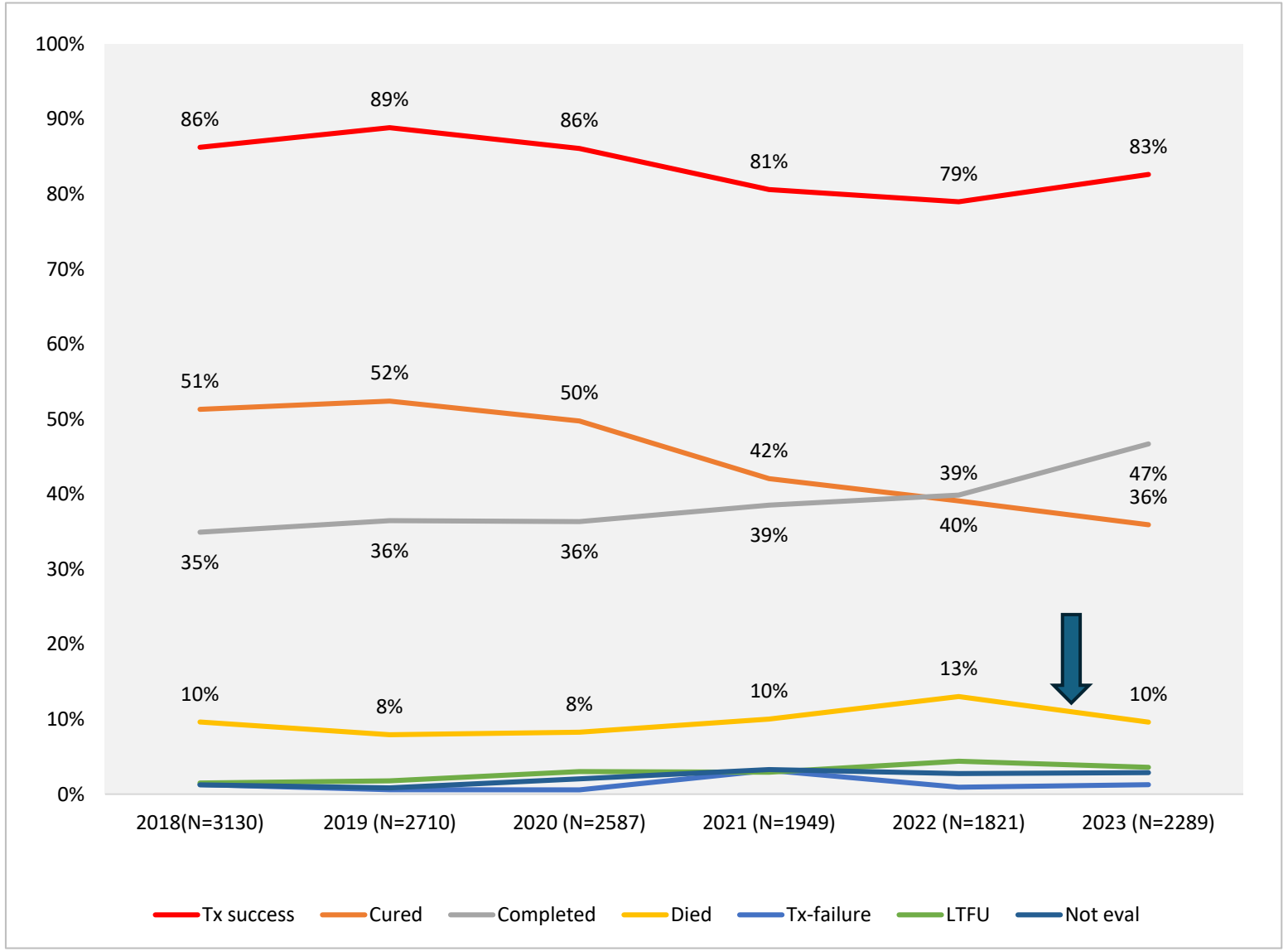
230 Estimated RR/MDR-TB among notified cases.

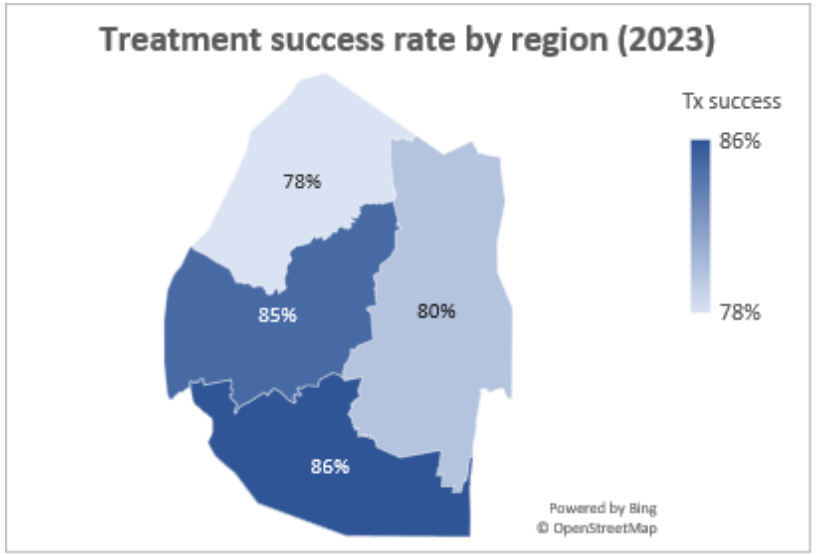
70 Lab Confirmed RR/MDR-TB cases

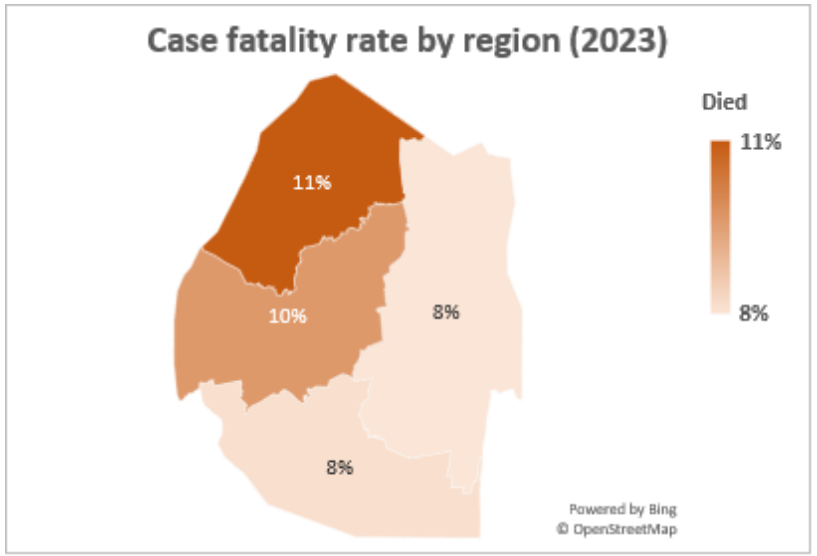
84 RR/MDR-TB patients started on second line treatment

TREATMENT OUTCOMES -DSTB

Global average is 88%

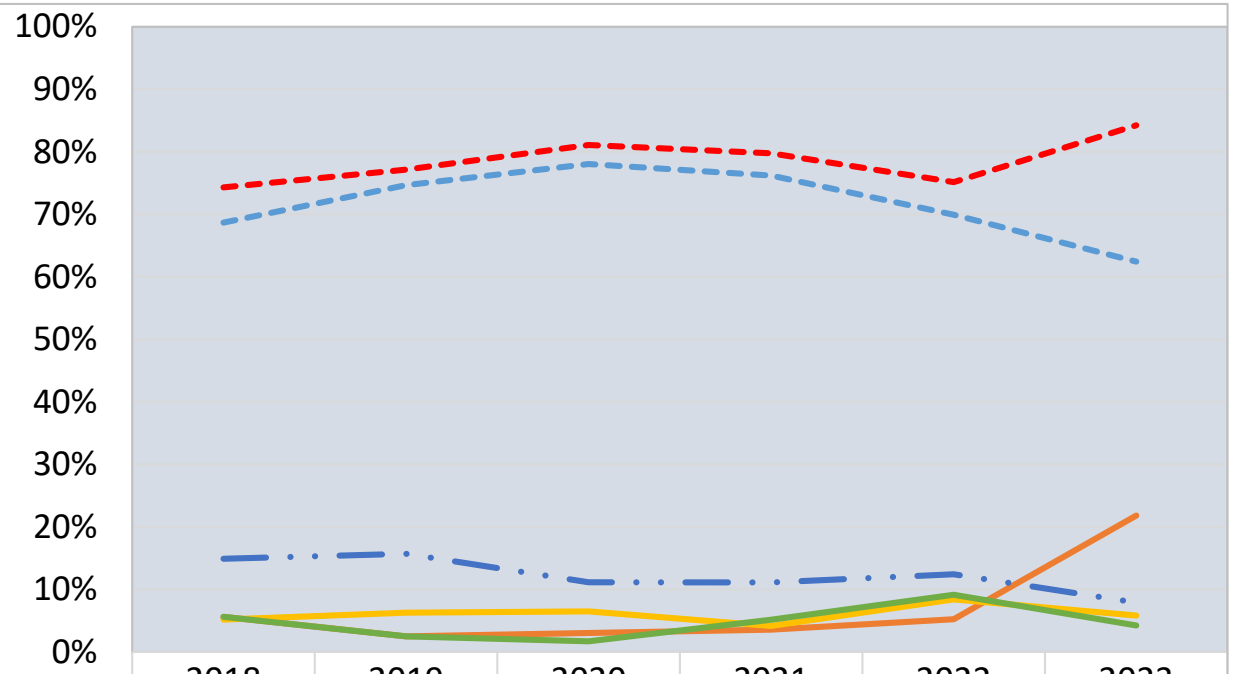




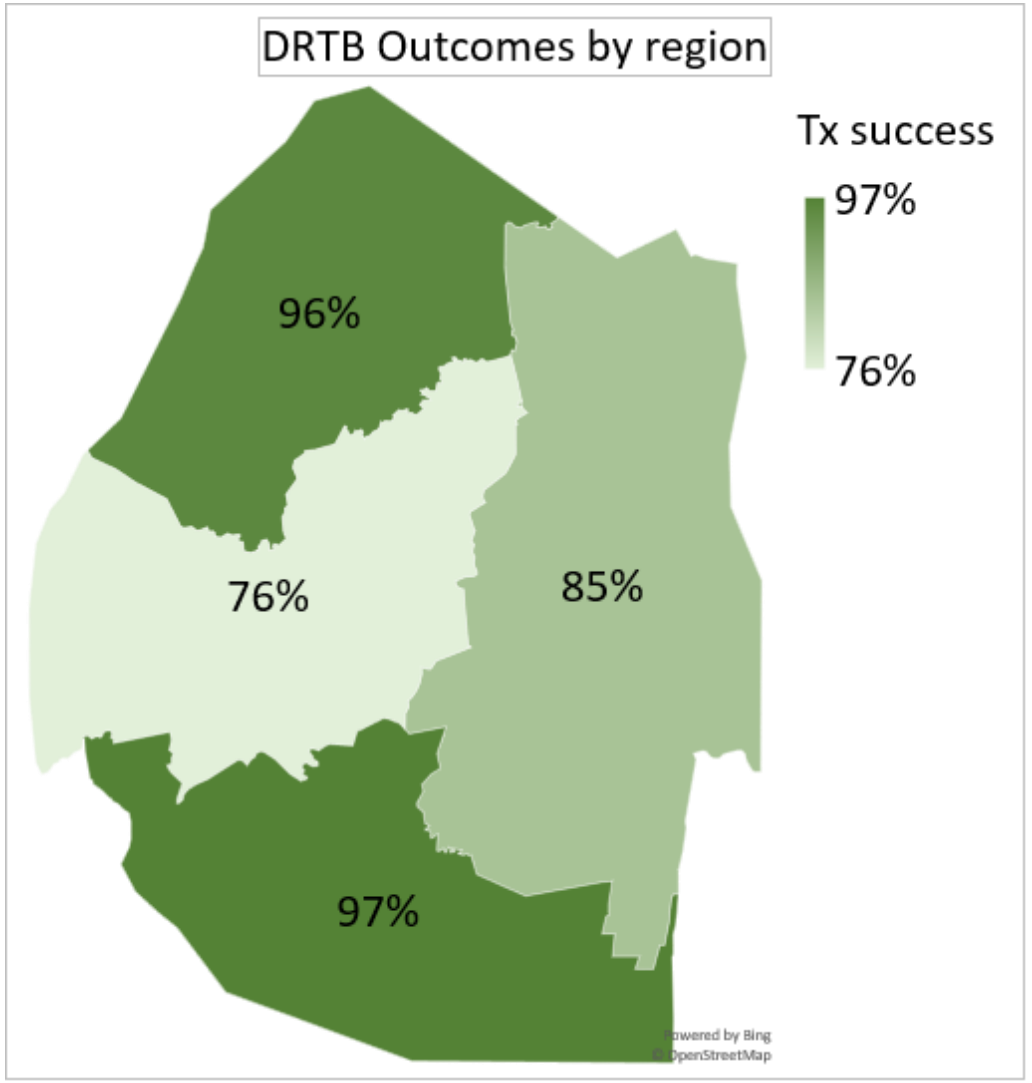


TREATMENT OUTCOMES- DRTB

Global average is 63%



	2018 (N=463)	2019 (N=363)	2020 (N=296)	2021 (N=253)	2022 (N=157)	2023 (N=165)
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%



CHALLENGES WITH RR/MDR-TB DIAGNOSIS

- Eswatini first reported the existence of a rifampicin resistant TB strain that harbors a *rpoB I491F* mutation in 2009/2010 TB Drug Resistance Survey (TBDRS)
- This mutation could not be detected by GeneXpert - 30% of RR-TB individuals were missed

- 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
    - NB:94% as INH resistance.

- All the currently available molecular rapid diagnostic techniques could not detect this mutation.
- Leading to DR-TB diagnostic gap and suboptimal treatment

WHAT WAS THE NEXT STEP?

- Decision to introduce targetted next generation sequencing(tNGS) in 2019 as a project(before WHO recommendation)
  - With support from German MOH and research center in Borstel
  - A protocol was then developed to assist with implementation
- Delays in implementation
  - COVID 19 disruptions-procurement challenges
  - Structural adjustments of the lad to accommodate sequencing
  - Training of the lab team
- Guidelines revised for early detection & empirical treatment of presumed RR/MDR-TB cases
  - Whilst waiting for implementation of the sequencing
- Implementation of tNGS in November 2021
- An interim algorithm was developed to guide eligible samples for sequencing at NTRL

ALGORITHM FOR IMPLEMENTATION OF TB SEQUENCING IN ESWATINI

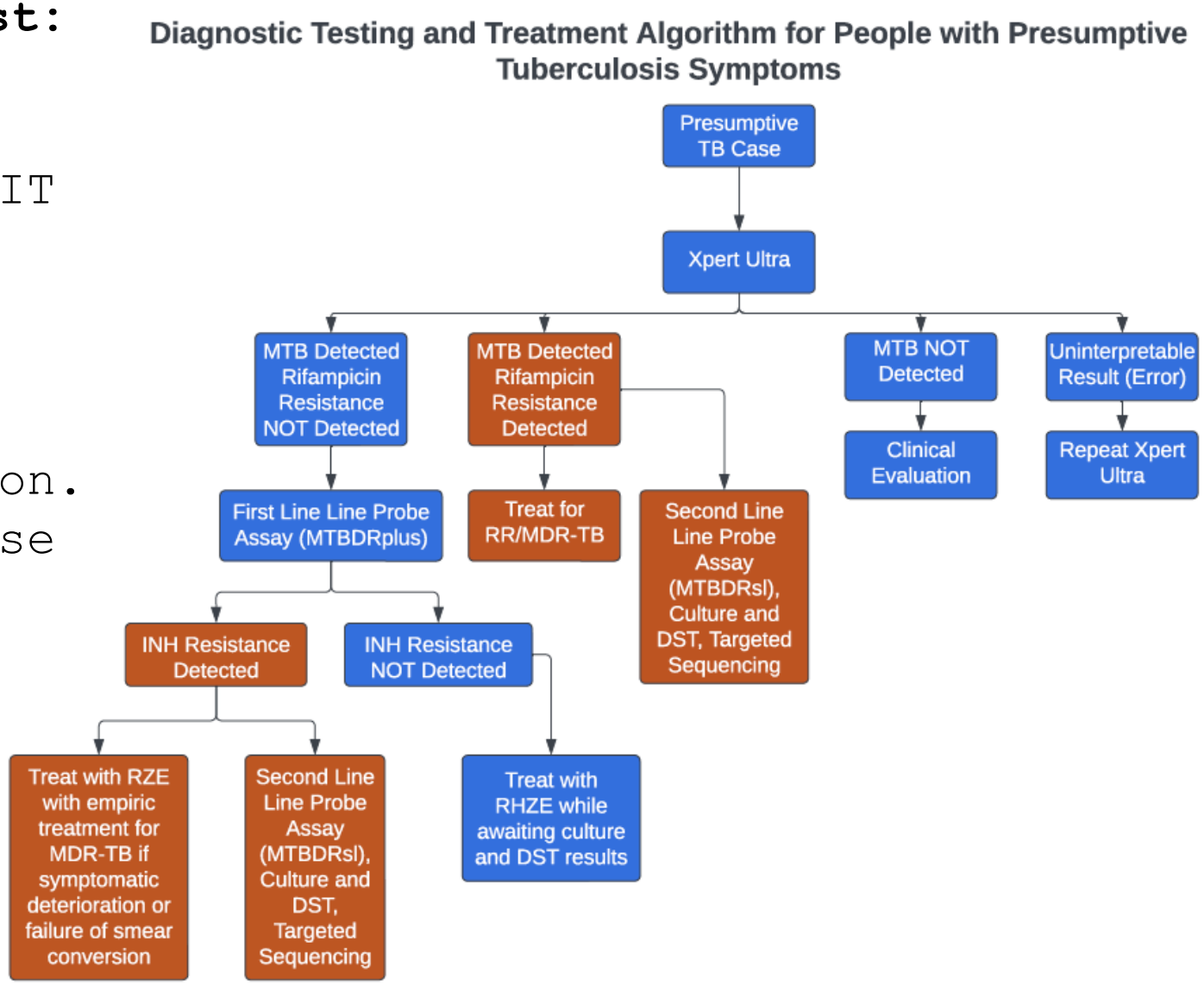
Eligibility for sequencing test:

Baseline

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

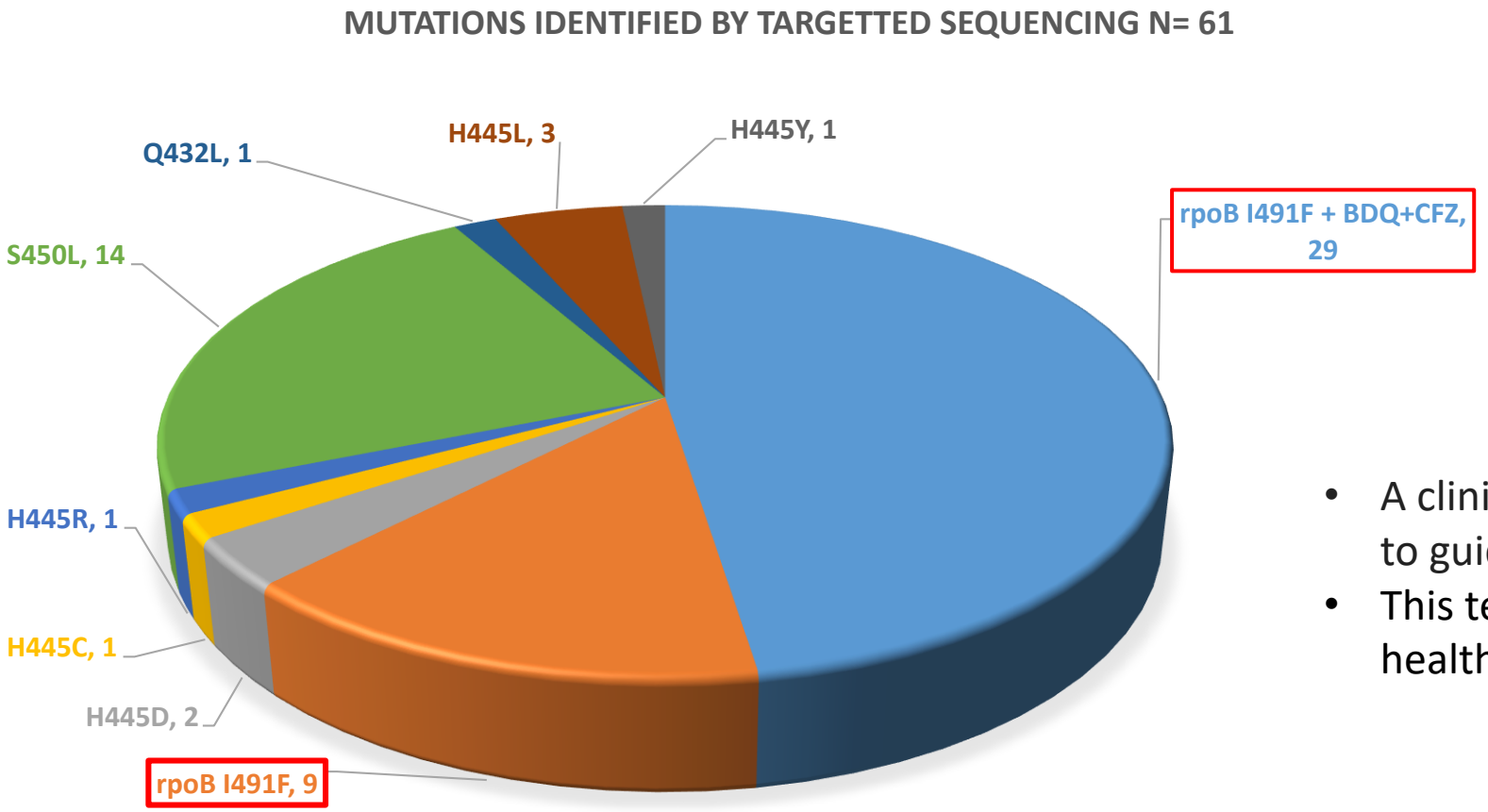
During Treatment

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



SHARING PRELIMINARY DATA FROM TARGETED SEQUENCING

- A total of 85 samples were sequenced from Nov 2021-Dec 2022
- 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.

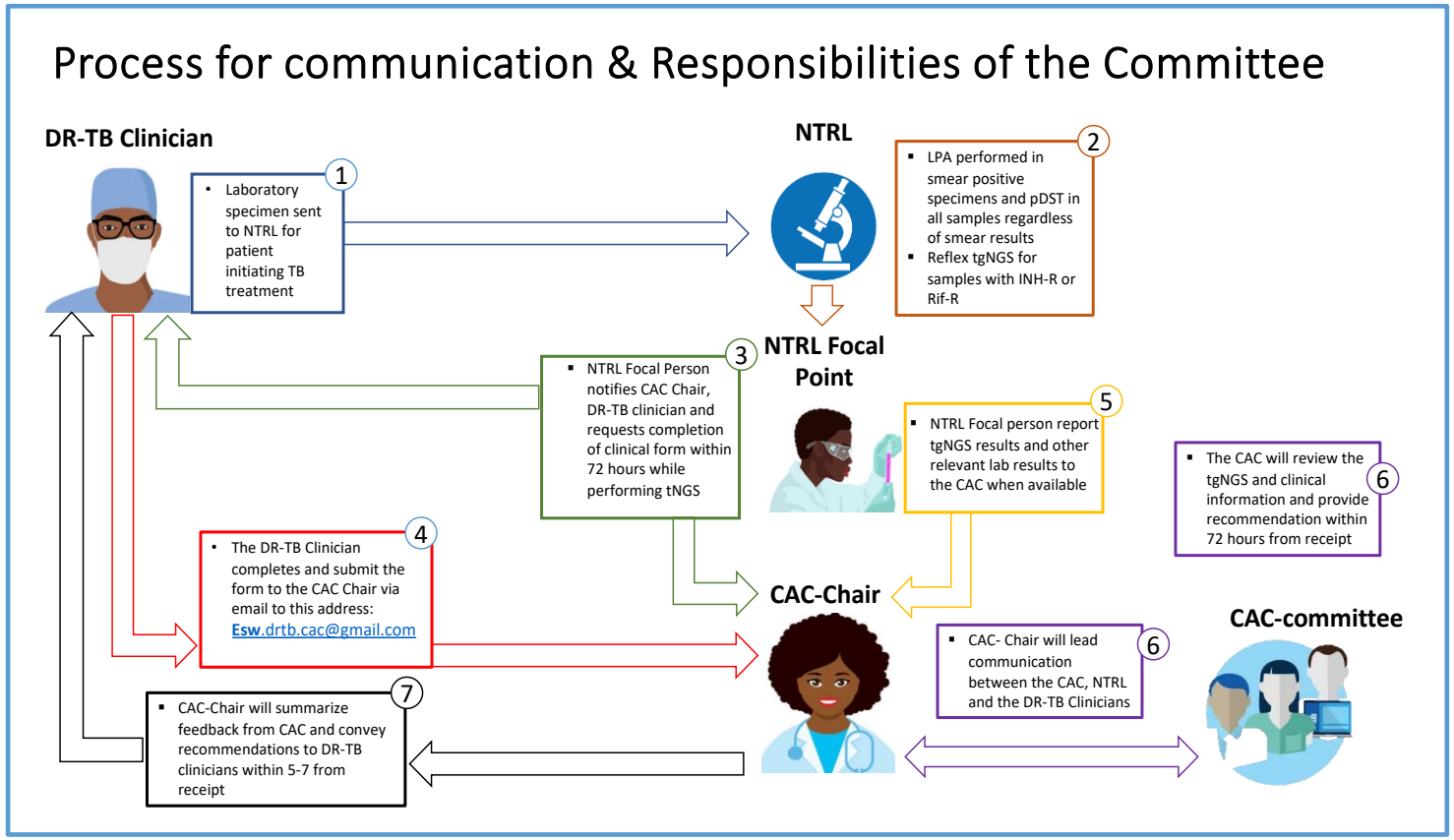


- Realised there was even a bigger problem
- Decision by NTP to use these pilot results for clinical management

- A clinical Advisory committee (CAC) was set up to guide optimization of treatment.
- This team has clinical, laboratory and public health expertise

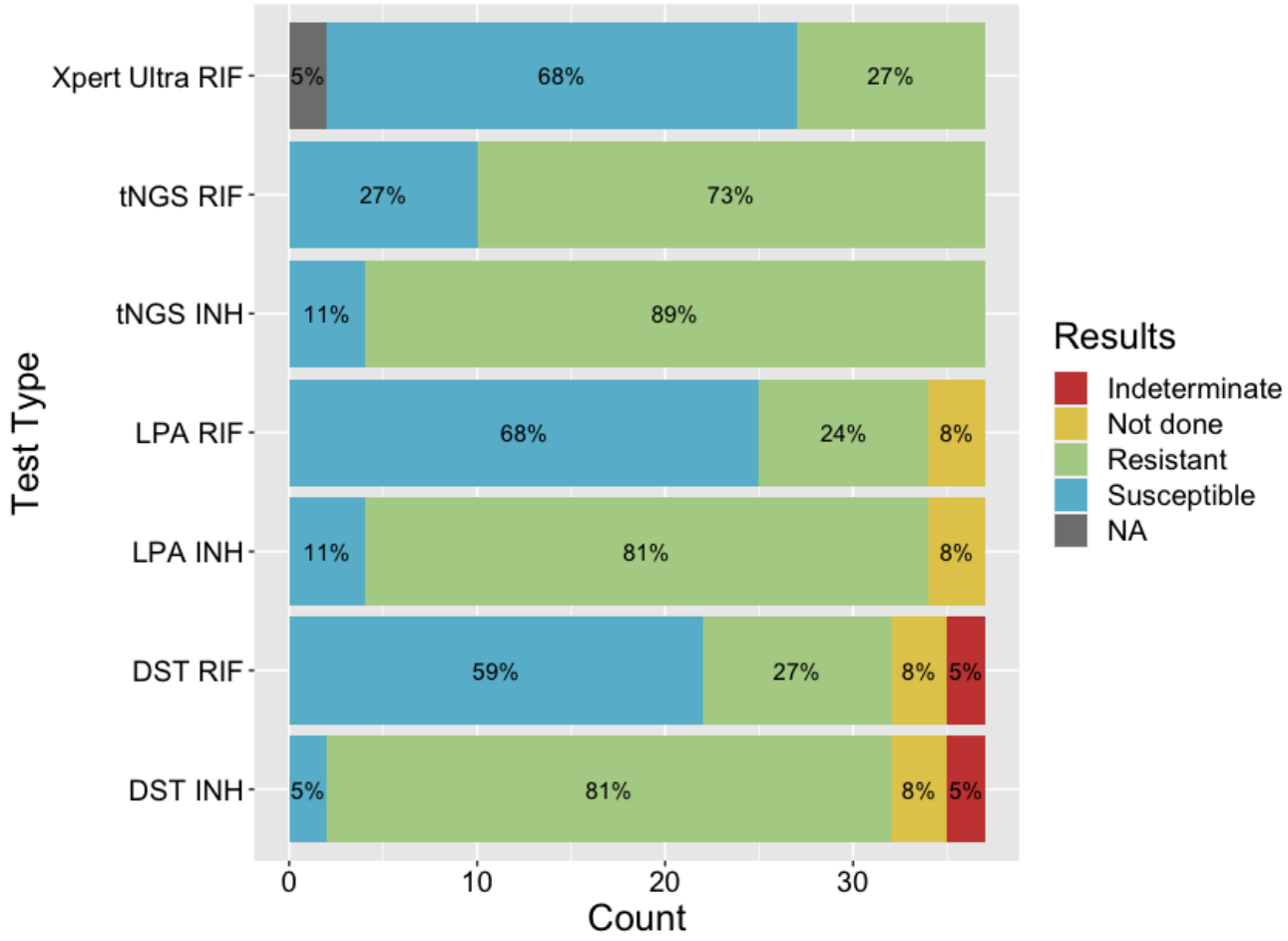
CLINICAL ADVISORY COMMITTEE

Clinical information and sequencing results for 37 patients were submitted to the Clinical Advisory Committee for treatment guidance



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%)

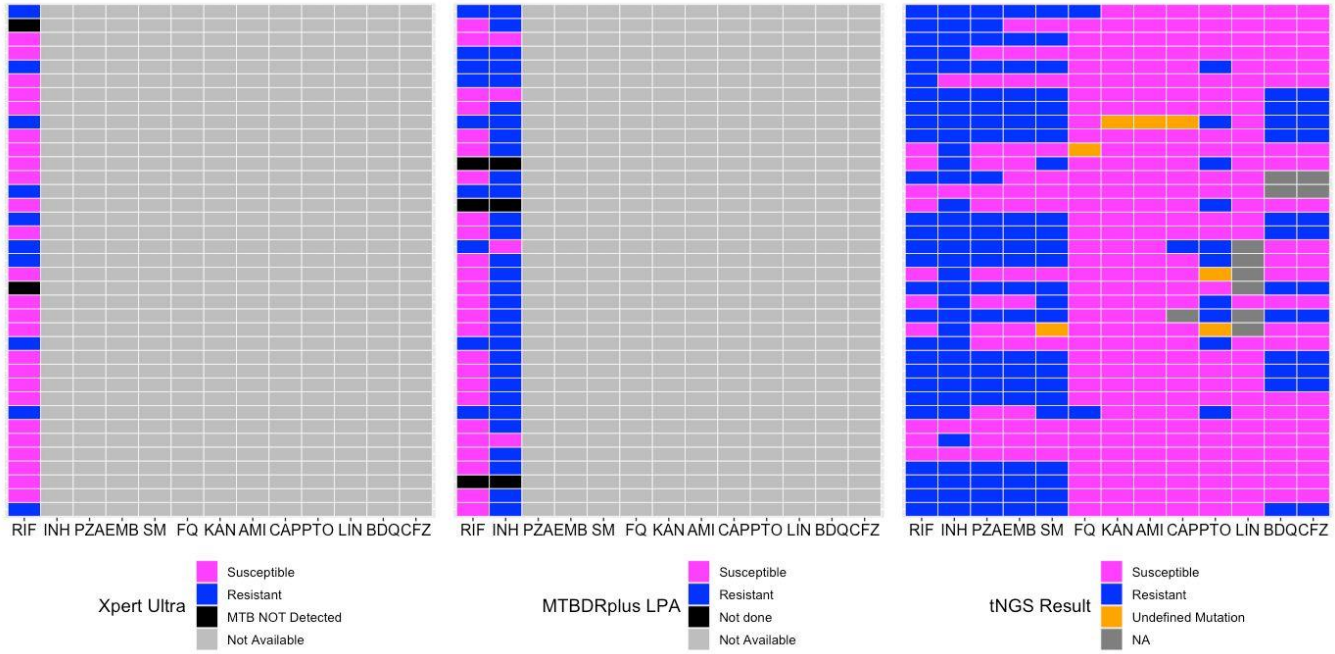
RIFAMPICIN AND ISONIAZID RESISTANCE DETECTION ACROSS MODALITIES



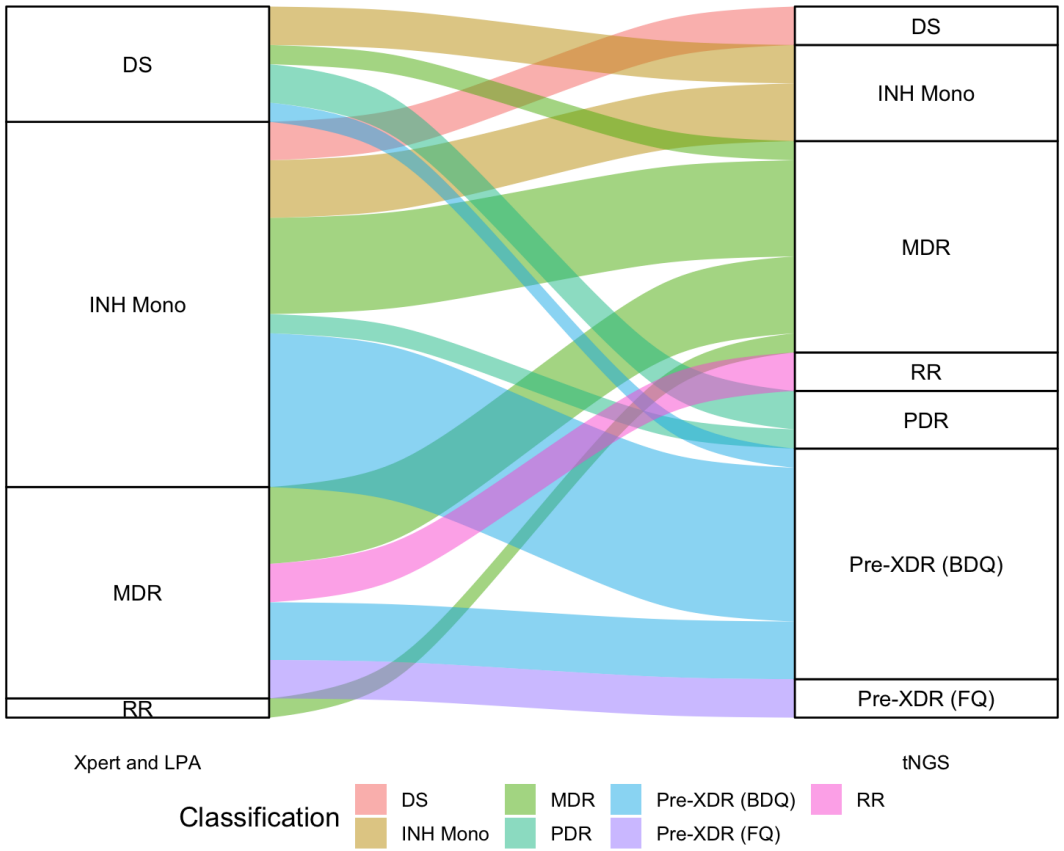
Demonstrates the varying performance on **rifampicin resistance** detection in Eswatini across LPA/Ultra/pDST as compared to tNGS

INH is fairly concordant across all modalities

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



Pre-XDR TB(**MDR+FQ** only or **MDR+BDQ** only)
???WHO Definition



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

ESWATINI DR TB TREATMENT REGIMENS

Oral MDR TB regimens	Regimen composition	Eligible group
BPaLM	6 Bdq-Pa-Lzd-Mfx	The primary treatment regimen for MDR-TB/RR-TB/PDR-TB/Inh Mono treatment failed patients ⩾ 15 years old
BPaL	6-9 Bdq-Pa-Lzd	The primary treatment regimen for Pre-XDR-TB patients ⩾15 years old
Modified shorter treatment regimen (mSTR)	**FQ-S** 9-12 Bdq/Lzd/Lfx/Cfz/Trd(Cs) **FQ-R (PreXDR)** 9-12 Bdq/Lzd/DLM/Cfz/Trd(Cs)	MDR/RR-TB, PDR and Hr-TB failed patients including children and PLHIV and pregnant women who are ineligible to BPaLM
LTR (standardized - STDTR or individualized -ITR)	**STDTR** 18-20 Bdq/Lzd/DLM/Cfz/Trd(Cs) **ITR:** composed of 4-5 drugs containing Gp A&B drugs as priority	For those who are **not eligible** for the BPaL or mSTR, XDR, Severe EP TB (meningitis, Osteo-articular, pericardial), disseminated or miliary TB
Hr-TB Regimen	6 R(H)ZE+/-Lfx	For confirmed R susceptible and H resistant TB patients

PATIENT OUTCOMES

- 31/37 (84%) completed or cured
- Regimen change-70% of patients
- Recommendations for patients with BDQ resistance were made but there was debate about the duration and construction of regimens among the CAC.
  - Clinical information of the patients guided most of the decisions

Sequencing Cohort	Overall (N=37)
Initial Regimen	
BDQ/CFZ/TRD/DLM	1 (2.7%)
BDQ/LFX/LZD/CFZ/DLM	7 (18.9%)
BDQ/LFX/LZD/CFZ/DLM-empiric MDR	2 (5.4%)
BDQ/LFX/LZD/CFZ/TRD	2 (5.4%)
RHZE	23 (62.2%)
RHZE	2 (5.4%)
Final Regimen	
BDQ/LFX/CFZ/TRD/DLM	1 (2.7%)
BDQ/LFX/LZD/CFZ/DLM	18 (48.6%)
BDQ/LFX/LZD/CFZ/TRD	4 (10.8%)
LFX/LZD/TRD/DLM/PAS/IMP-CLV	2 (5.4%)
LFX/LZD/TRD/DLM/PTO	5 (13.5%)
LFX/LZD/TRD/DLM/PTO/IMP-CLV	2 (5.4%)
RHZE	3 (8.1%)
RHZE/LFX	1 (2.7%)
TRD/DLM/IMP-CLV/PTO	1 (2.7%)
Treatment Outcome	
Completed	18 (48.6%)
Cured	13 (35.1%)
Died	5 (13.5%)
LTFU	1 (2.7%)

CONCLUSION

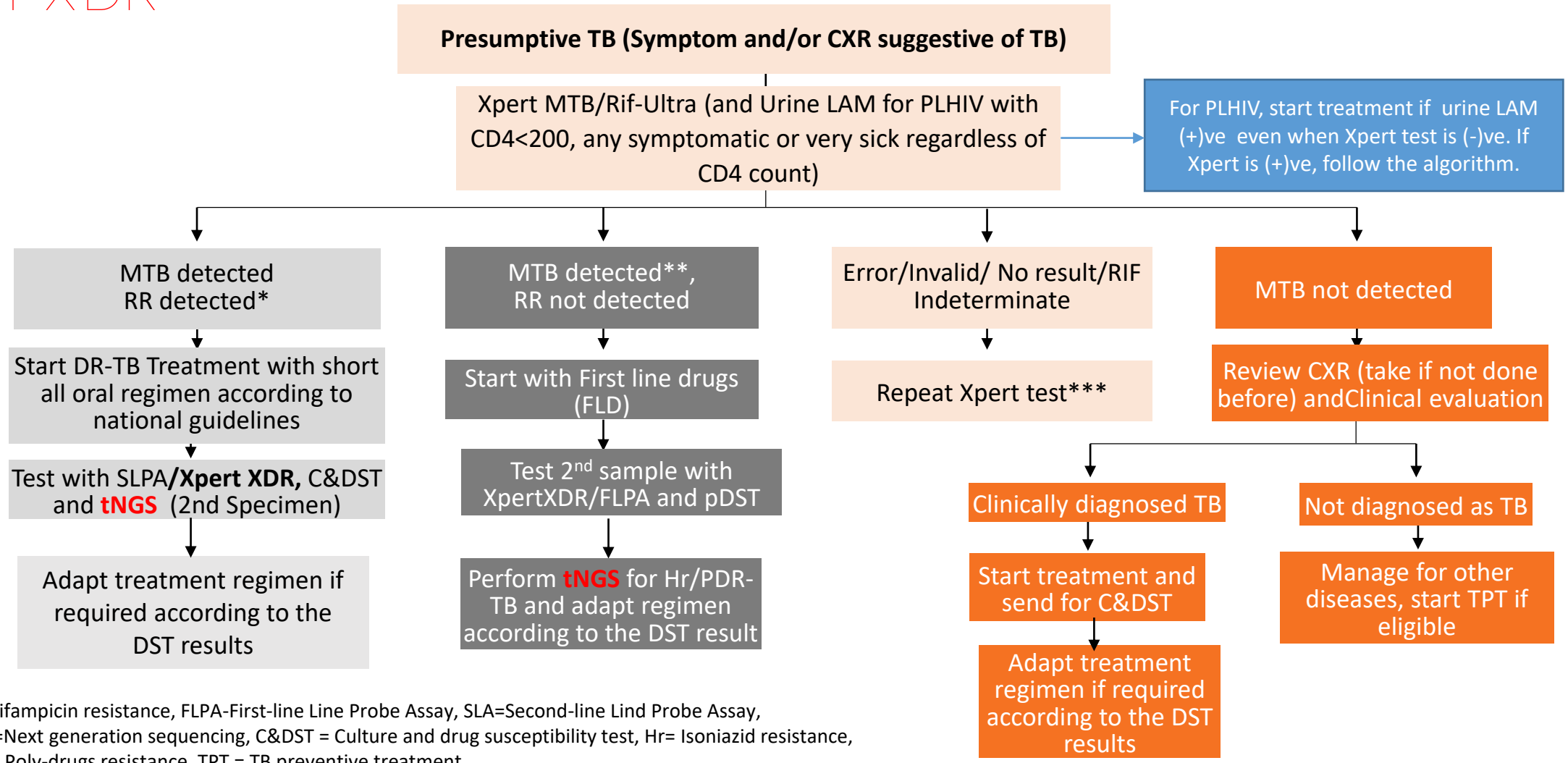
- Without use of tNGS, 38/61(62%) of MDR/RR-TB with *rpoB* I491F mutation and 29/38(76%) with additional Bdq and Cfz resistance would be missed.

- Targeted sequencing resulted in treatment adaptation in 70% of patients within this cohort and resulted in successful treatment outcomes in 84% of patients.

- This emphasizes the need for :
  - Expanding the coverage of rpoB probes to detect I491F mutation on GeneXpert
  - Integration of targeted sequencing into national diagnostic and treatment algorithms
  - New definition for MDR+BDQ-(??PreXDR or XDR)

- And the need for newer medicines cannot be over emphasized as Bedaquiline resistance is becoming more common

REVISED DIAGNOSTIC AND TREATMENT ALGORITHM: TNGS AND XPERT XDR



RR= Rifampicin resistance, FLPA-First-line Line Probe Assay, SLA=Second-line Lind Probe Assay, tNGS=Next generation sequencing, C&DST = Culture and drug susceptibility test, Hr= Isoniazid resistance, PDR = Poly-drugs resistance, TPT = TB preventive treatment

Note: If poor treatment response while on treatment , perform C&DST and tNGS.

**If MTB (trace), persons evaluated for PTB &EPTB including PLHIV and children and no history of TB in the past 5 yrs, start FL-TB treatment and perform C&pDST for DR-TB detection. Adapt treatment if required when pDST is received.

***If RR indeterminate with melting curve showing RR, no need to repeat and start treatment as RR-TB.

ACKNOWLEDGEMENTS

Ministry of Health-Eswatini

National TB Program

National reference Laboratory

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Eswatini Implementing Partners

Health Care workers

Recipients of Care and community

THANK YOU