High Genetic Diversity of Rifampicin-Resistant *Mycobacterium tuberculosis* Strains in Botswana

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BACKGROUND

- The emergence of drug-resistant *Mycobacterium tuberculosis (Mtb)* strains remains a threat to global tuberculosis (TB) prevention and care.
- There is limited data on the population structure of Rifampicin-resistant (RR) *Mtb* strains circulating in Botswana.
- The aim of this study was to utilize whole genome sequencing (WGS) technology to determine the genetic diversity of rifampicin-resistant *Mtb* strains.

METHODS

- A total of 100 *Mtb* isolates, obtained from individuals diagnosed with (RR-TB) across all Multidrug-Resistant Tuberculosis (MDR-TB) clinics in Botswana and cultured between January 2016 and December 2021, were included in this study.

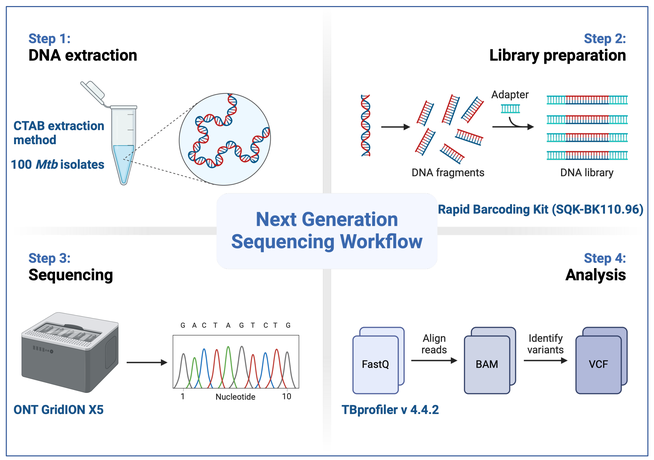


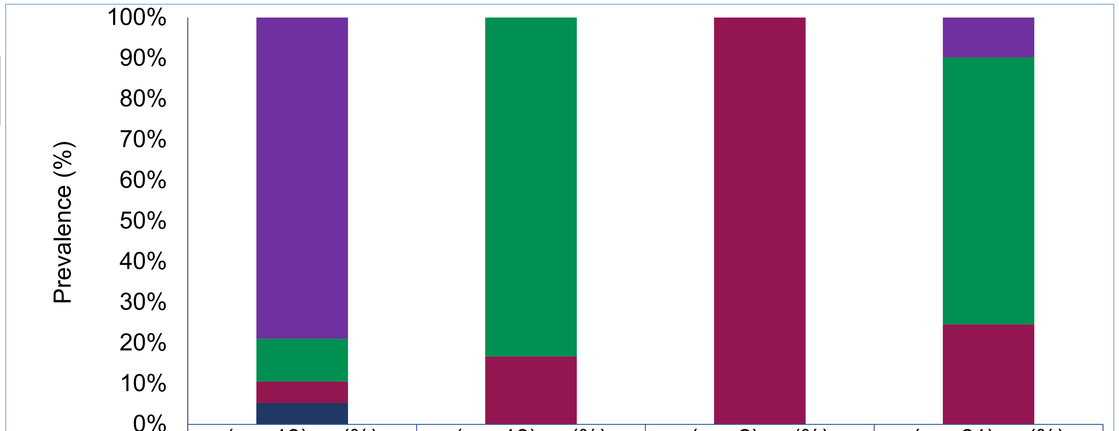
Figure 1. Identification of variants with TBProfiler after DNA extraction and whole genome sequencing

- Quality control of sequencing alignment data was examined using Qualimap software, command line; version 2.2.2
- TB-profiler was used to identify known *Mtb* lineages and drug resistance profiles.
- Logistic regression models were used to identify factors associated with unsuccessful treatment outcomes.

All four major *Mtb* lineages are present in Botswana with Lineage 4 being the most predominant.

These isolates did not harbour bedaquiline and/or delamanid resistance-associated variants.

RESULTS



	(n = 19), n (%) Lineage 1	(n = 18), n (%) Lineage 2	(n = 2), n (%) Lineage 3	(n = 61), n (%) Lineage 4
Pre-XDR-TB	78.90%	0	0	9.80%
MDR-TB	10.50%	83.30%	0	65.60%
RR-TB	5.26%	16.70%	100%	24.60%
HR-TB	5.26%	0	0	0

Pre-XDR-TB: Pre- extensively drug resistant-TB, **MDR-TB**: Multi-drug resistant TB, **RR-TB**: Rifampicin resistant-TB, **HR-TB**: Isoniazid resistant-TB

Figure 2. *Mtb* lineages stratified by drug resistance profiles.

- The mean depth of coverage, percentage reads mapped, and number of reads mapped was 103X, 98% and 218520 respectively.
- The most frequently observed mutations conferring resistance to rifampicin, isoniazid, ethambutol, pyrazinamide, and fluoroquinolones were: ***rpoB* S450L** (52%), ***katG* S315T** (60.8%), ***embA*_c.-29_-28delCT**, ***embB* Q497R** (31%), *rrs*_n.517C>T (59%), *pncA*_c.375_389delCGATGAGGTCGATGT (38%) and *gyrA* A90V (71%), respectively.
- Residing in rural areas was associated with unsuccessful treatment outcomes (adjusted odds ratios: 9.1, 95% CI: 3.0-27.9, *p*-value <0.001).

CONCLUSIONS

- Unexpectedly, we observed that most Lineage 1 strains were pre-XDR-TB while Lineage 2 and Lineage 4 strains were predominantly MDR-TB.
- Study findings provide insights into the genetic diversity and most common drug resistance-associated mutations present in RR *Mtb* strains circulating in Botswana.
- The most prevalent mutations in the *rpoB* gene were S450L (51%), in the *katG* gene were S315T (60.8%), similar to what has been previously reported in neighbouring countries such as South Africa and Namibia [1, 2]

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