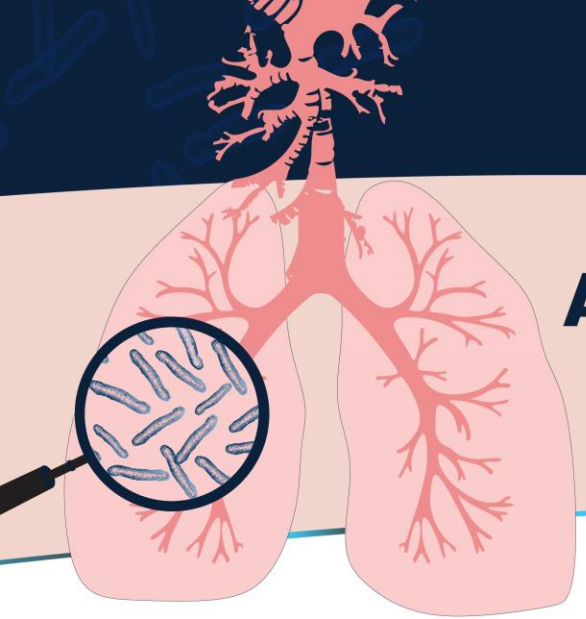

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DESCRIPTION OF MUTATIONS DETECTED IN GENETIC TARGETS ASSOCIATED WITH TUBERCULOSIS RESISTANCE TO "NEW AND REPURPOSED DRUGS" FROM SURVEILLANCE SITES IN SOUTH AFRICA

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INTRODUCTION

- Bedaquiline (BDQ), pretomanid (Pa), linezolid (LZD) and delamanid (DLM), classified as "new and repurposed drugs", have been recommended by the World Health Organization (WHO) for the management of drug-resistant tuberculosis (DR-TB)2
  - *Rv0678*, *atpE*, *pepQ mmpL5*, *mmpS5* and *Rv1979c* have emerged as commonly associated genetic markers for BDQ resistance.
  - Resistance to LZD is mediated predominantly via mutations in *rplC* and *rrl*.
  - Genetic markers associated with resistance to Pa and DLM are less well-characterised, but mutations in *ddn*, *fbiA*, *fbiB*, *fbiC* and *fgd1* have been shown to mediate resistance to either drug.

- Given the expanding use of the "new and repurposed drugs", active surveillance to monitor the emergence of resistance is critical for preserving their efficacy

- We describe all mutations detected in the aforementioned genetic targets using whole genome sequencing (WGS).

METHODOLOGY

- As part of a larger study assessing the application of WGS to enhance microbiological and epidemiological surveillance in selected districts of South Africa, DR-TB isolates were prospectively collected between 2020 and 2022.

- WGS was performed using the Illumina sequencing platform (NextSeq 550/1000) (Illumina, USA)

- Bioinformatics analysis for resequencing analysis was performed using CLC Genomics Workbench (Qiagen, Venlo, The Netherlands) which included genetic targets associated with resistance to these drugs.

- Mutations with a minimum frequency of 90% (fixed) and a coverage of at least 5x with supporting reads in both forward and reverse were reported.

- An in-house catalogue of mutations, which includes the *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance* (WHO, 2021) was used to grade mutations based on their association with drug susceptibility.

RESULTS

- A total of **2523** isolates met the eligibility criteria and were successfully sequenced
- We reported **5447** mutations across all genetic targets
- Overall, 2.0% of the mutations were graded as associated with resistance, 15.3% were graded as uncertain significance, 22.9% were graded as not associated with resistance, and no grading could be assigned to 59.8% of mutations (Figure 1)
- We observed a high level of heterogeneity and frequency of mutations observed for each genetic target, with the lowest for *mmpS5* (n=5) and the highest for *fgd1* (n=2361) (Table 1)
- Of the 2523, 1769 (68.5%) had 1924 mutations in fgd1 only (no grading). The remaining 755 isolates had 3523 mutations across the genetic targets.

RESULTS

- Resistance gradings could only be assigned to the genetic targets listed below:
  - *Rv0678:* 103/156 (66.0%)
  - *atpE:* 2/16 (12.5%)
  - *rplC:* 5/99 (5.1%)

- When analyzing the subset of 755 isolates, a WHO grading of "uncertain significance" could only be assigned to 15.3%

- Mutations graded as "not associated with resistance" were identified only in 1137/1207 (94.2%) of *mmpL5*, 74/398 (18.6%) of *rrl* and 37/585 (4.7%) of *Rv1979c* mutations observed

RESULTS

Table 1: Total number of mutations observed in genetic targets associated with the "new and repurposed drugs" (n=2523)

	Gene	Number of mutations observed
DLM/Pa	ddn	27
	fbiA	147
	fbiB	128
	fbiC	94
	fgd1	2361
BDQ	atpE	16
	mmpL5	1207
	mmpS5	5
	pepQ	22
	Rv0678	156
	Rv1979c	789
LZD	rplC	99
	rrl	398

RESULTS

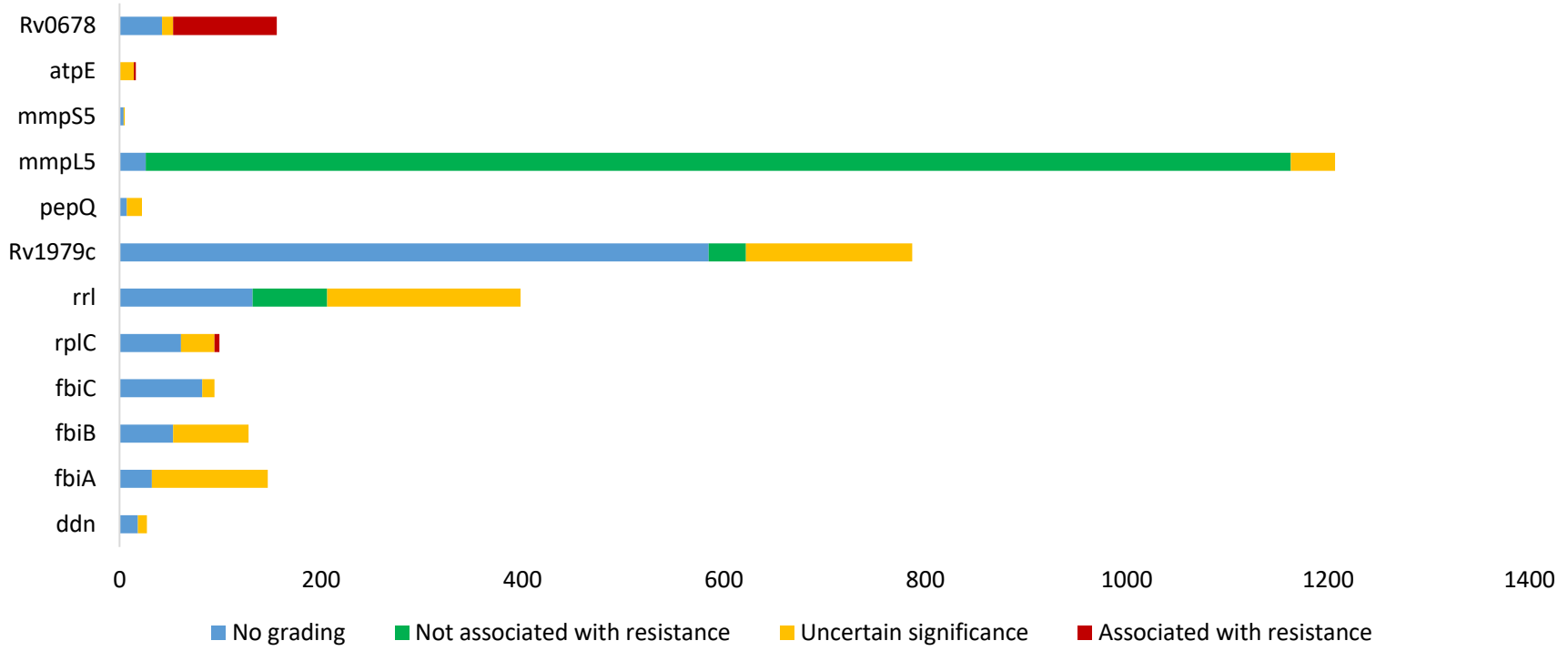

Figure 1: Mutation gradings for genetic targets associated with the "new and repurposed drugs"

CONCLUSION

- A high level of genetic diversity is observed for targets associated with resistance to the “new and repurposed drugs“
  - >50% of the mutations observed could not be assigned gradings for genotypic resistance detection due to a paucity of paired phenotypic data

- Whole genome sequencing currently for surveillance may not provide informative epidemiological data related to resistance to the "new and repurposed drugs"

- Ongoing activities such as the WHO *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance* is required to improve grading which in turn will enhance the application of WGS for surveillance.

- The study is ongoing and the role of homoplasy will be investigated to add to the global efforts in elucidating the grading of the mutations