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Genetic Basis of Resistance to BPaL Regimen Drugs: Continued Genotypic–Phenotypic Characterisation in South Africa

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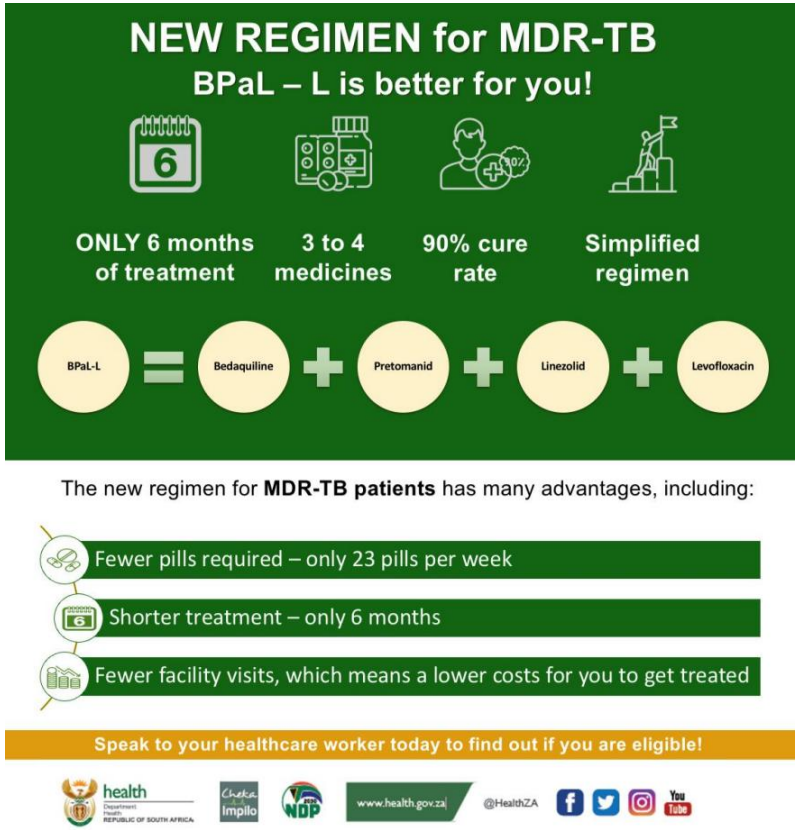
Background

- The introduction of the **BPaL (Bedaquiline, Pretomanid and Linezolid) regimen** in South Africa has transformed the management of drug resistant-TB
  - Recommended by WHO in 2022
  - Rolled out in South Africa in 2023
  - Reduced treatment duration to 6 months
  - Improved treatment outcomes

- Emergence of resistance to any of the BPaL regimen drugs threatens these gains

- Resistance detection is critical to preserve regimen efficacy and prevent resistance transmission



DOH (2023)

Background

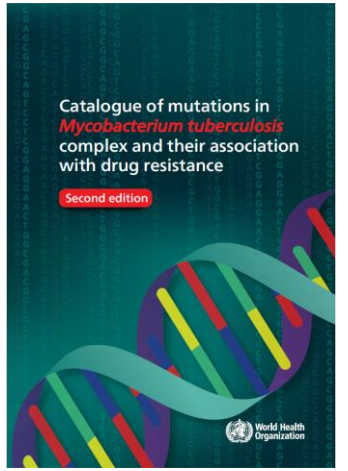
- Interpretive criteria for determining phenotypic and genotypic resistance based on WHO guidelines

	pDST: MGIT 960 Critical Concentration[1,2]	gDST: WHO Mutation Catalogue[3]
BDQ	1.0 µg/mL	*Rv0678*, *atpE*, *pepQ*, *mmpL5* and mmpS5
Pa	0.5 µg/mL* & 2.0 µg/mL	*ddn*, *fgd1*, *fbiA*, *fbiB*, *fbiC* and *Rv2983* (*fbiD*)**
LZD	1.0 µg/mL	*rplC* and *rrl*

*0.5-1.0 µg/mL: Area of concern requiring close follow-up in clinical settings
**Inferred from delamanid

- Classification of "Uncertain Significance" mutations (insufficient evidence to establish association with resistance or susceptibility) and ungraded mutations (not reported in the catalogue) represents a grey area for diagnostic interpretation

- Continuous characterisation using both phenotypic and genotypic methods is critical to comprehensively understand resistance

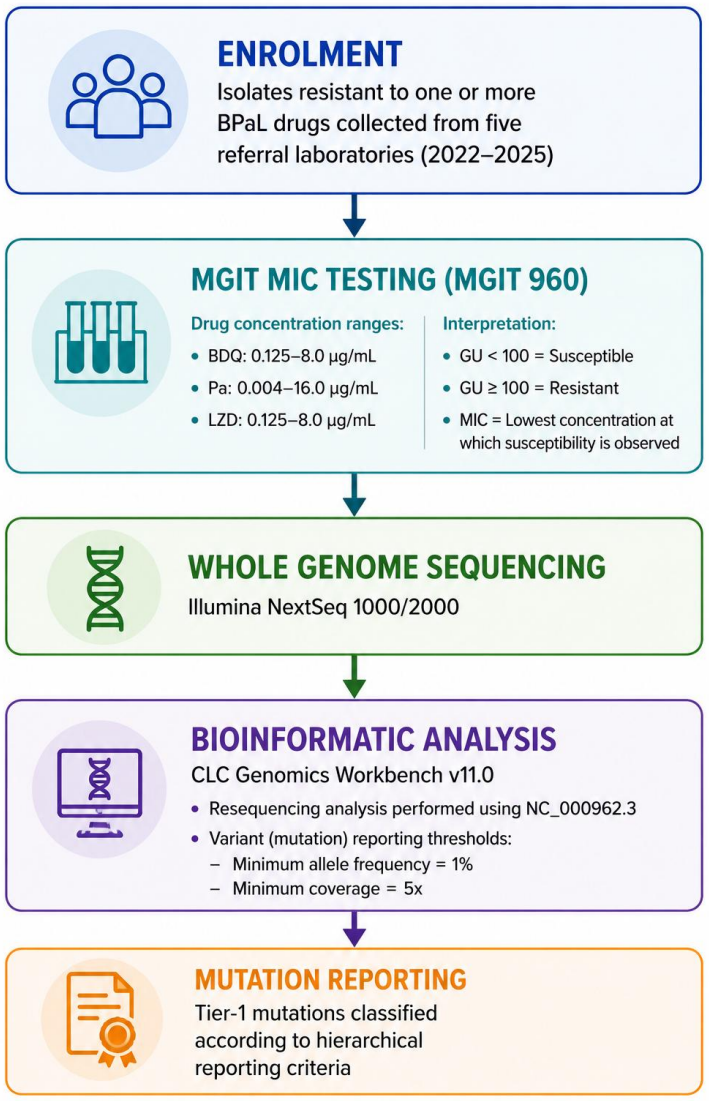


[1]WHO (2018). *Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis*
[2]WHO (2024). *Web annex B: critical concentrations for pretomanid and cycloserine: WHO policy statement*
[3]WHO (2023). *Catalogue of mutations in* Mycobacterium tuberculosis *complex and their association with drug resistance, 2nd ed*

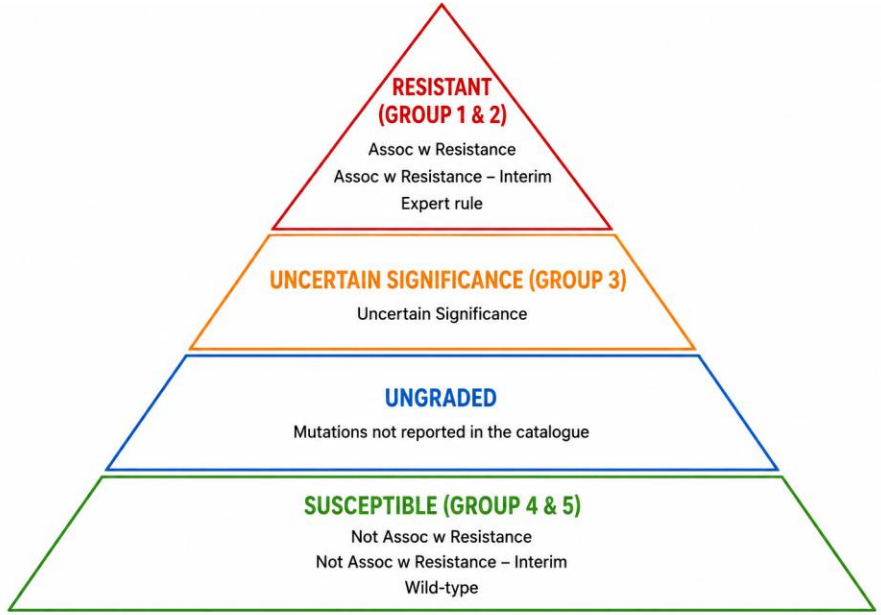
Objectives

1. To further characterise genetic determinants of resistance to the BPaL drugs
2. To assess their association with phenotype using minimum inhibitory concentration (MIC) testing

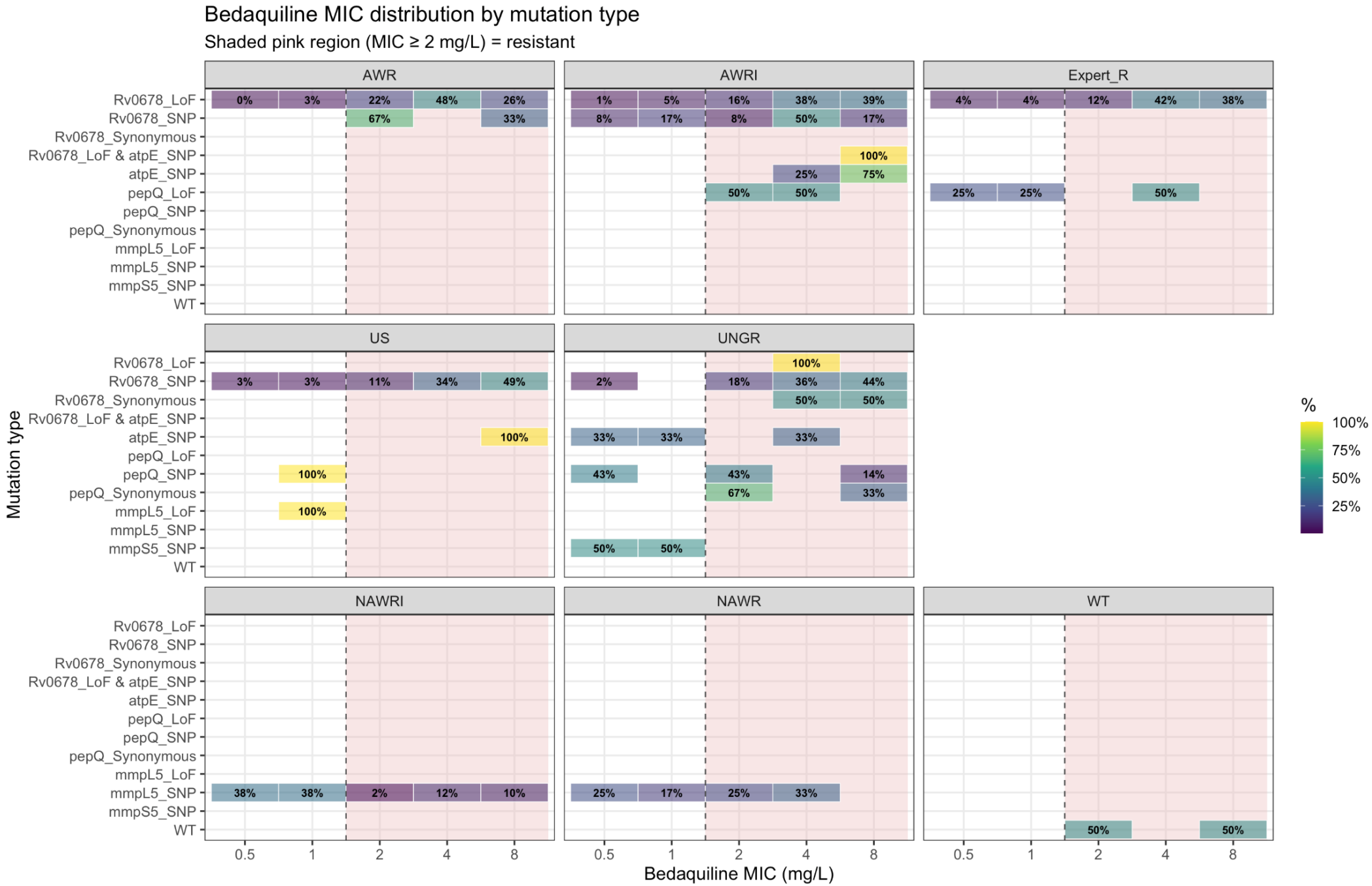
Methods

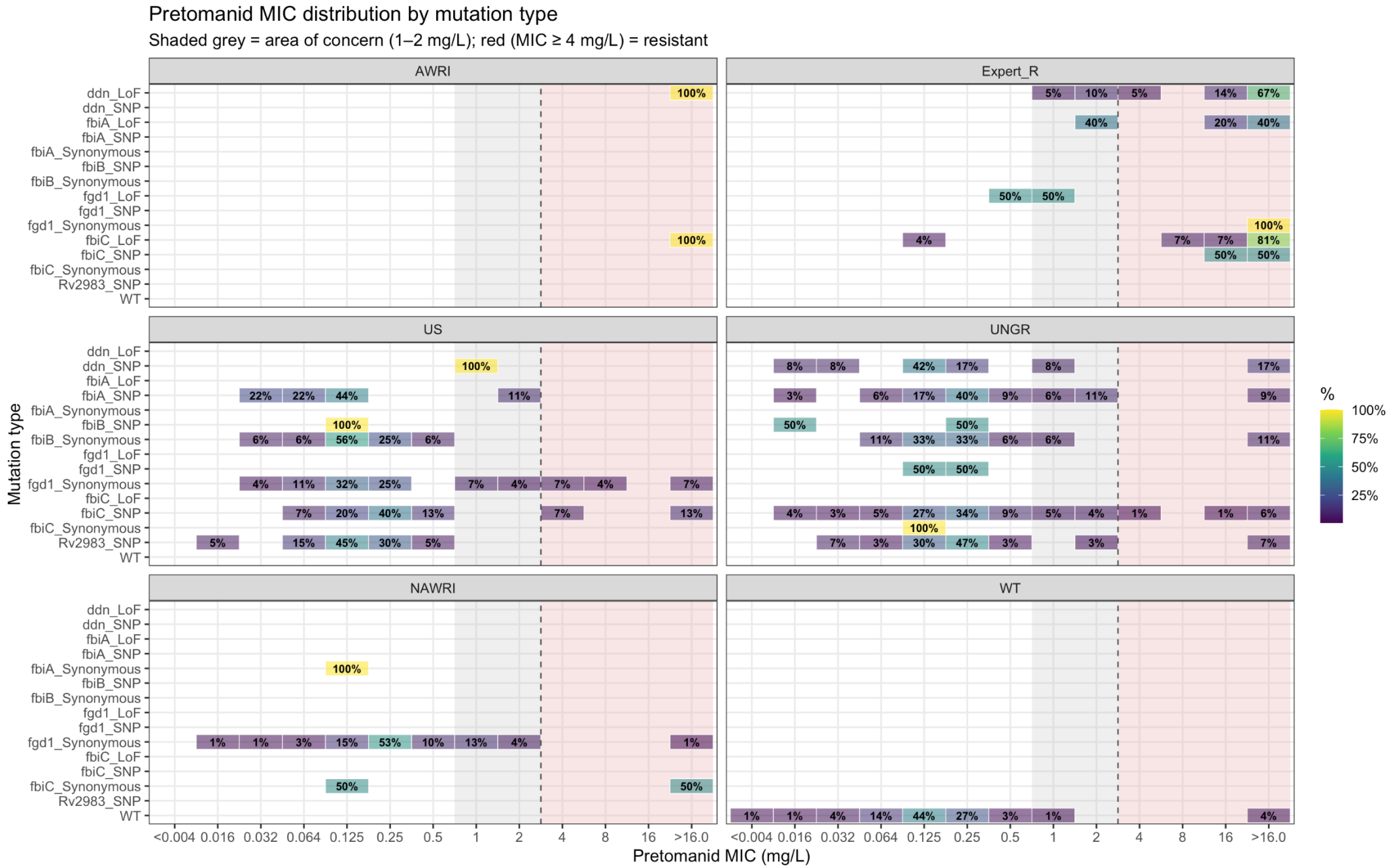


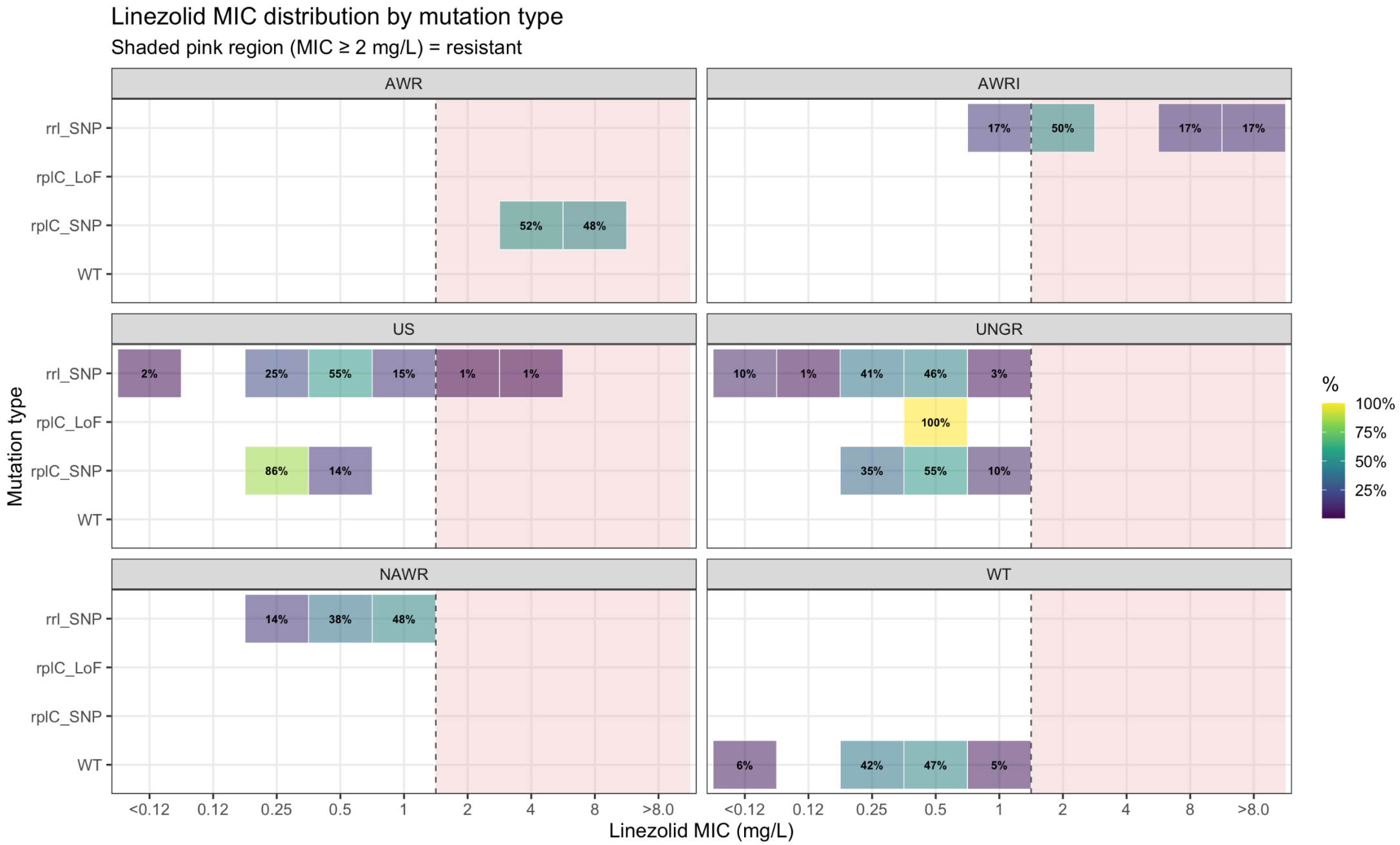
Laboratory workflow of study methods



Hierarchy for final mutation reporting


Bedaquiline MIC distribution by mutation type
Shaded pink region (MIC ≥ 2 mg/L) = resistant
AWR
AWRI
Expert_R
US
UNGR
NAWRI
NAWR
WT
Rv0678_LoF
Rv0678_SNP
Rv0678_Synonymous
Rv0678_LoF & atpE_SNP
atpE_SNP
pepQ_LoF
pepQ_SNP
pepQ_Synonymous
mmpL5_LoF
mmpL5_SNP
mmpS5_SNP
WT
Mutation type
0.5
1
2
4
8
Bedaquiline MIC (mg/L)
%
100%
75%
50%
25%


Pretomanid MIC distribution by mutation type
Shaded grey = area of concern (1–2 mg/L); red (MIC ≥ 4 mg/L) = resistant
AWRI
Expert_R
US
UNGR
NAWRI
WT
Mutation type
ddn_LoF
ddn_SNP
fbiA_LoF
fbiA_SNP
fbiA_Synonymous
fbiB_SNP
fbiB_Synonymous
fgd1_LoF
fgd1_SNP
fgd1_Synonymous
fbiC_LoF
fbiC_SNP
fbiC_Synonymous
Rv2983_SNP
WT
<0.004 0.016 0.032 0.064 0.125 0.25 0.5 1 2 4 8 16 >16.0
Pretomanid MIC (mg/L)
%
100%
75%
50%
25%


Linezolid MIC distribution by mutation type
Shaded pink region (MIC ≥ 2 mg/L) = resistant
AWR
AWRI
US
UNGR
NAWR
WT
rrl_SNP
rplC_LoF
rplC_SNP
WT
Mutation type
52%
48%
17%
50%
17%
17%
2%
25%
55%
15%
1%
1%
86%
14%
10%
1%
41%
46%
3%
100%
35%
55%
10%
14%
38%
48%
6%
42%
47%
5%
<0.12
0.12
0.25
0.5
1
2
4
8
>8.0
Linezolid MIC (mg/L)
%
100%
75%
50%
25%

Discussion

- The majority of resistance and susceptibility phenotypes observed for the BPaL drugs were explained by mutations classified by the WHO Mutation Catalogue
    - LoF expert rule critical for Pa-R determination & associated with high-level Pa resistance (MIC ≥16 µg/mL)

- Of the observed US and ungraded mutations, most were linked to elevated MICs for BDQ and susceptible phenotypes for Pa and LZD
    - Ungraded BDQ mutations associated with elevated MICs harboured mutations not currently represented in the WHO Mutation Catalogue (e.g. IS elements and synonymous mutations)
    - Highlights the need for continued refinement of the catalogue

- Several Lineage 1 isolates harboured mutations not classified as resistance and displayed Pa MICs in the area of concern
    - Supported by the fact that lineage 1 strains harbour phylogenetic polymorphisms in genes involved in the F420 pathway – essential for Pa activation

- Genomic analysis identified putative resistance markers not captured phenotypically:
    - Borderline MICs with growth observed below the thresholds for resistance reporting
    - Mutation frequencies below 20% not detected on MIC potentially due to culture bias

Conclusions

1. The current interpretive criteria captured the majority of resistance and susceptibility observed in our study for the BPaL regimen drugs
    - Additive value of expert rules for resistance detection

2. US and ungraded mutations demonstrated distinct phenotype distributions across the BPaL drugs
    - Continued global contribution of paired genotypic/phenotypic data will strengthen the evidence base for mutation classification and improve resistance calling

3. Ongoing efforts to expand and refine resistance mutation catalogues are critical to ensure accurate genotype-based prediction of susceptibility, particularly with the rollout of targeted next generation sequencing technologies reliant on genetic determination of resistance

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