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Determining Minimum Inhibitory Concentration of Drugs Against *Mycobacterium tuberculosis* Isolates with WHO–Interim Resistance Mutations

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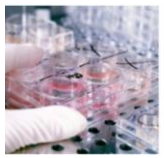

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Background

- DR-TB remains a major threat worldwide.
- **Need for Accuracy:** Rapid molecular tests require a reliable, standardized reference to link mutations to resistance.
- The WHO Catalogue of Mutations is a powerful tool used as reference standard for classifying *M. tuberculosis* drug-resistance.
- **The Current Challenge:** Some mutations remain poorly characterized, which can result in discrepancies between genotypic and phenotypic drug testing results.

Rational of the study

WHO Catalogue of Mutations v2 (2023): groups mutations by resistance evidence

Group 1 Associated with resistance

Group 2 Associated with resistance interim

Group 3 Uncertain significance

Group 4 Not associated with resistance interim

Group 5 Not associated with resistance

Group 2 Associated with resistance interim

Research Gap:

- Group 2 mutations, remain clinically unclear due to a lack of sufficient phenotypic data.
- These mutations suggest resistance but warrants further investigation, using minimum inhibitory concentration (MIC) data.

Aim of the study

To determine the MICs of **rifampicin (RIF), isoniazid (INH),** and **fluoroquinolones (FLQ)** for isolates harboring mutations associated with interim resistance (Group 2 mutations).

Study Population

Samples: 214 stored MTB isolates for CTB repository.

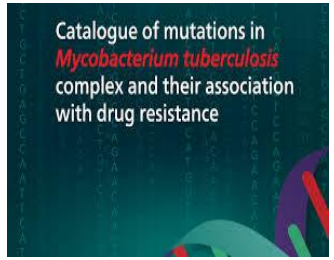
Duration: Collected between 2020 to 2024.

Study Sites:

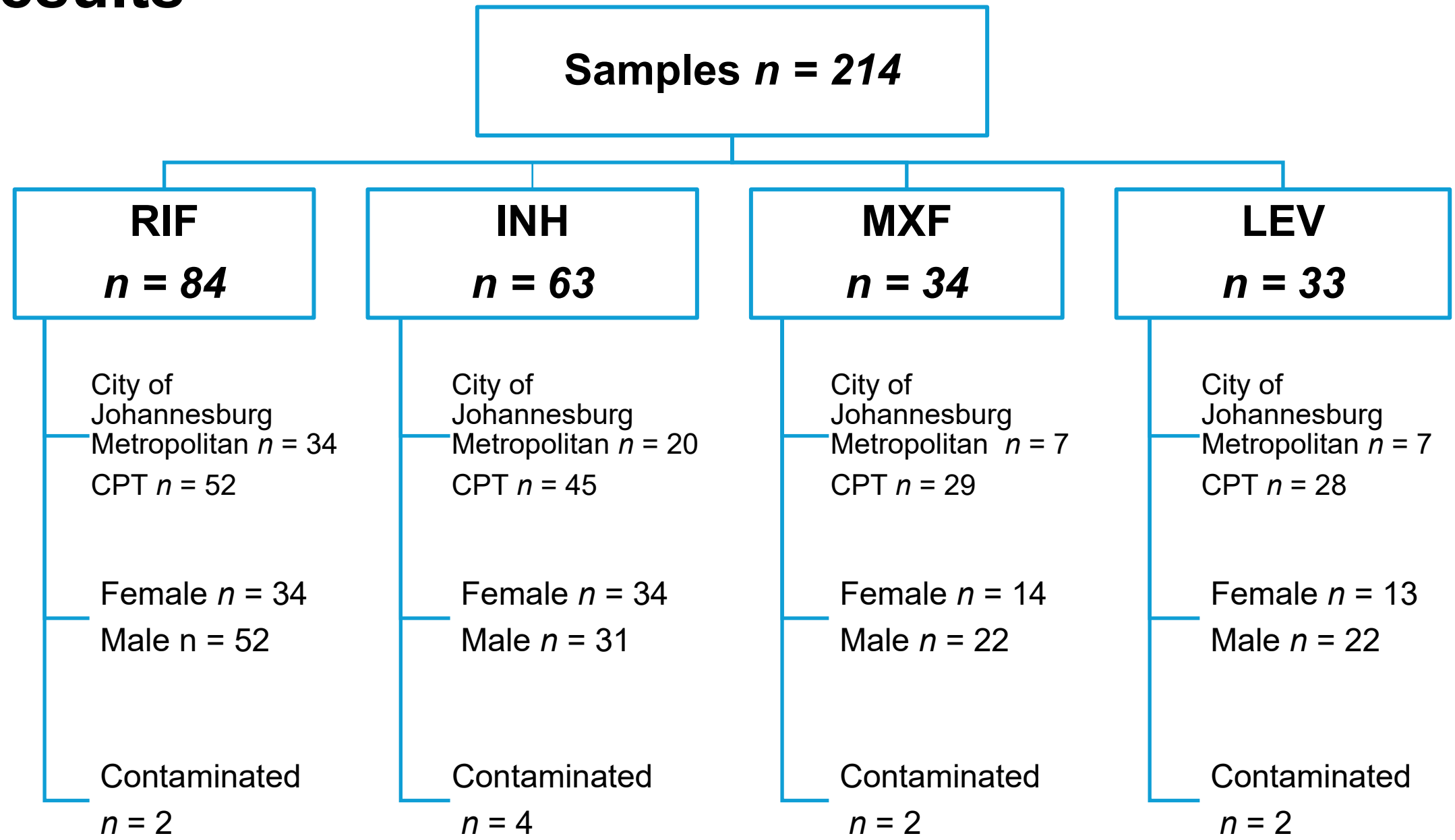
- City of Cape Town
- City of Johannesburg Metropolitan

Method- Workflow

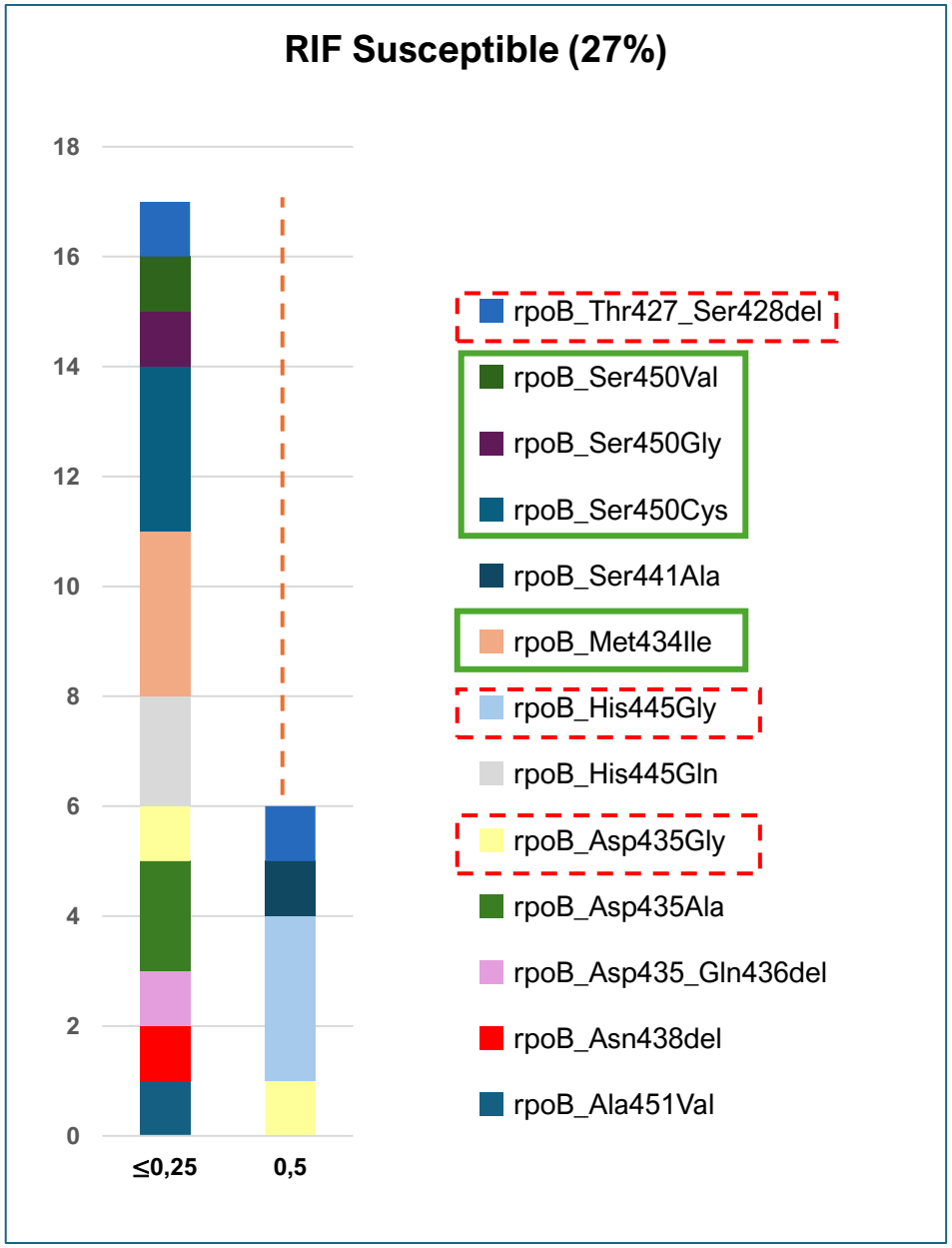
Subculture → Confirmatory Tests → MIC Testing → DNA Extraction → WGS → Analysis → Comparison

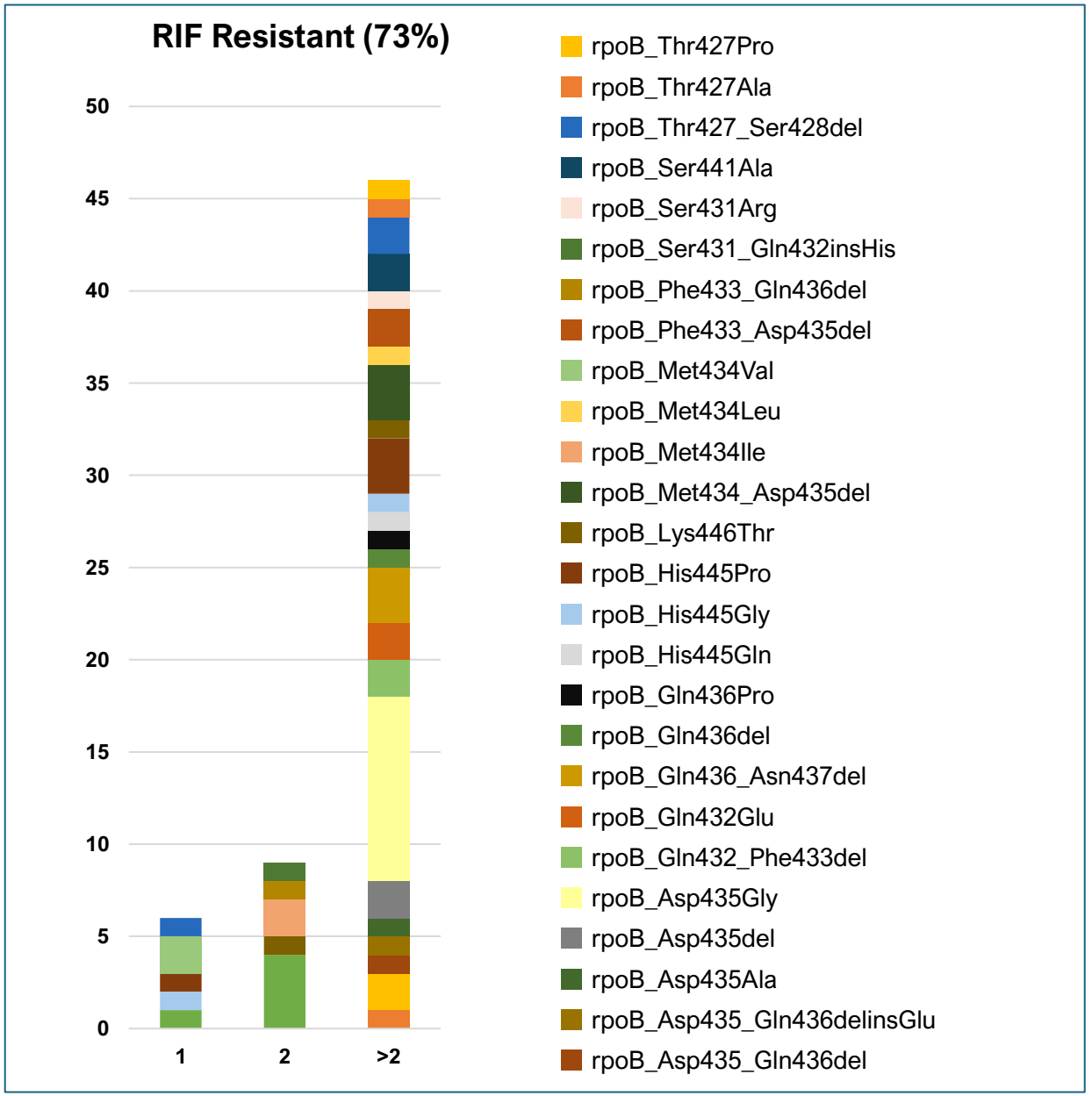


Results

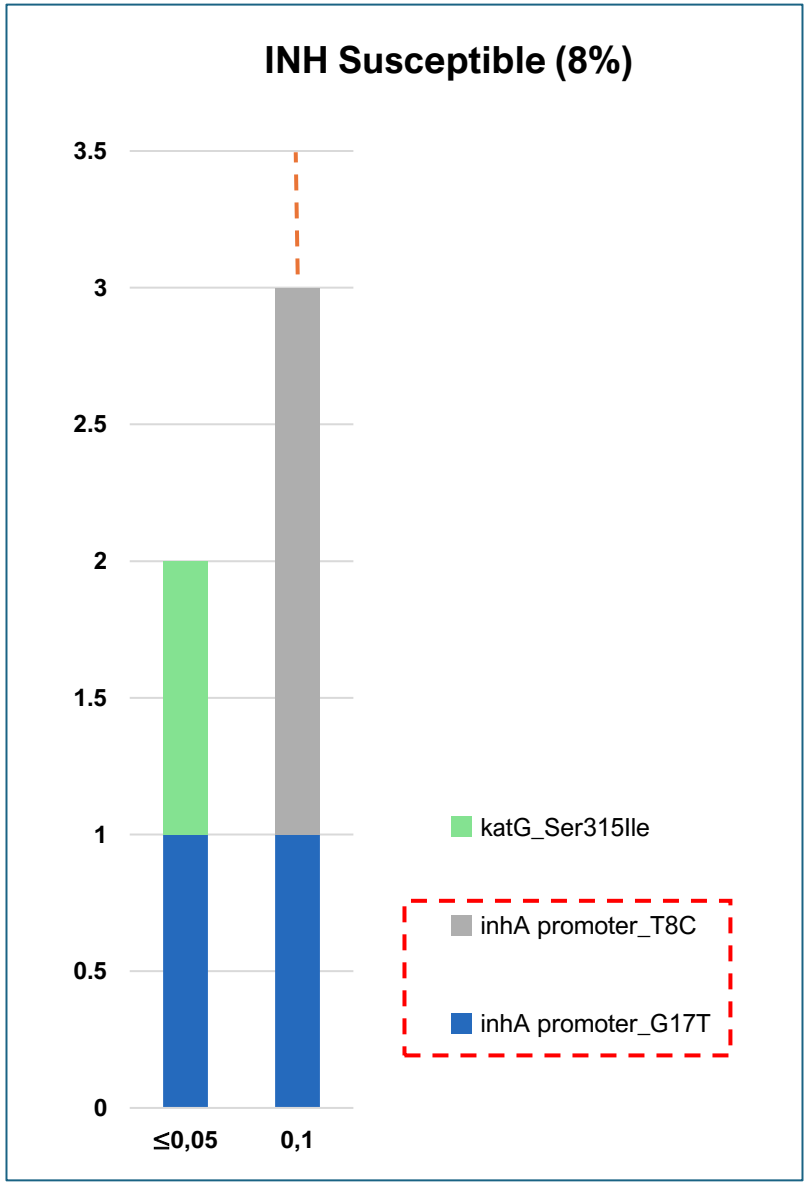

Samples n = 214
RIF
n = 84
INH
n = 63
MXF
n = 34
LEV
n = 33
City of Johannesburg Metropolitan n = 34
CPT n = 52
Female n = 34
Male n = 52
Contaminated n = 2
City of Johannesburg Metropolitan n = 20
CPT n = 45
Female n = 34
Male n = 31
Contaminated n = 4
City of Johannesburg Metropolitan n = 7
CPT n = 29
Female n = 14
Male n = 22
Contaminated n = 2
City of Johannesburg Metropolitan n = 7
CPT n = 28
Female n = 13
Male n = 22
Contaminated n = 2

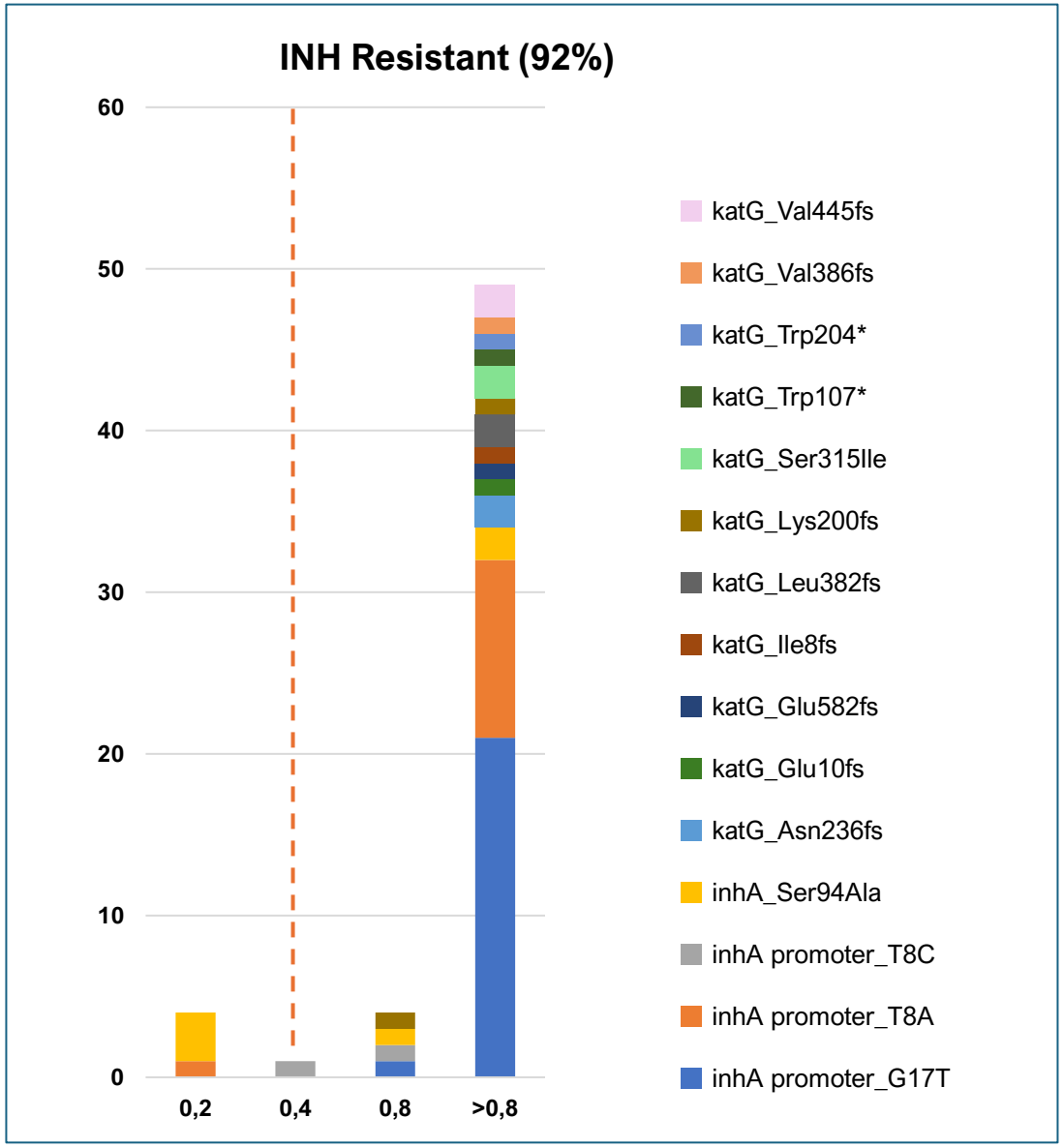
RIF MIC Distribution vs Mutation (Breakpoint 0.5 µg/mL) N=84



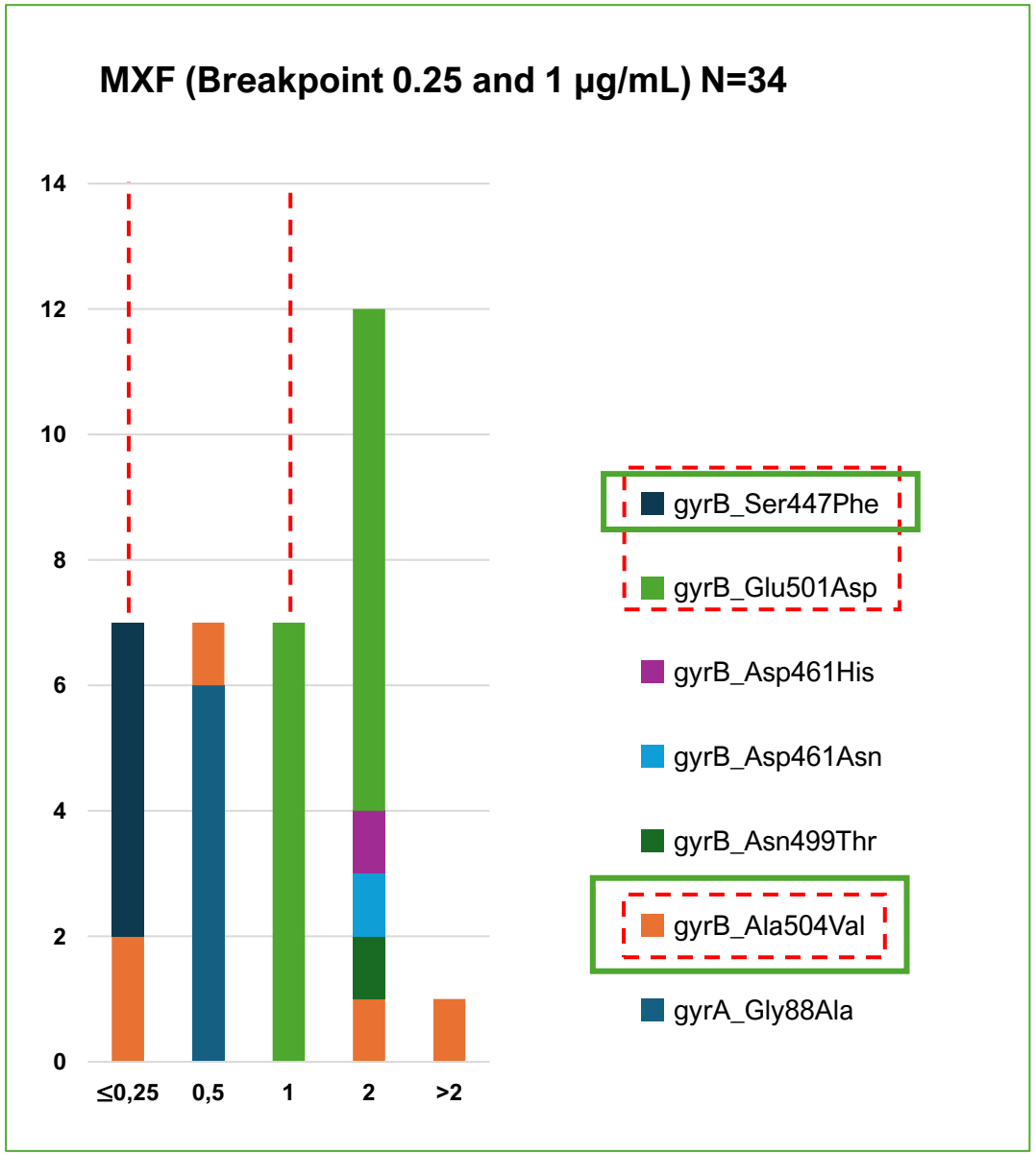


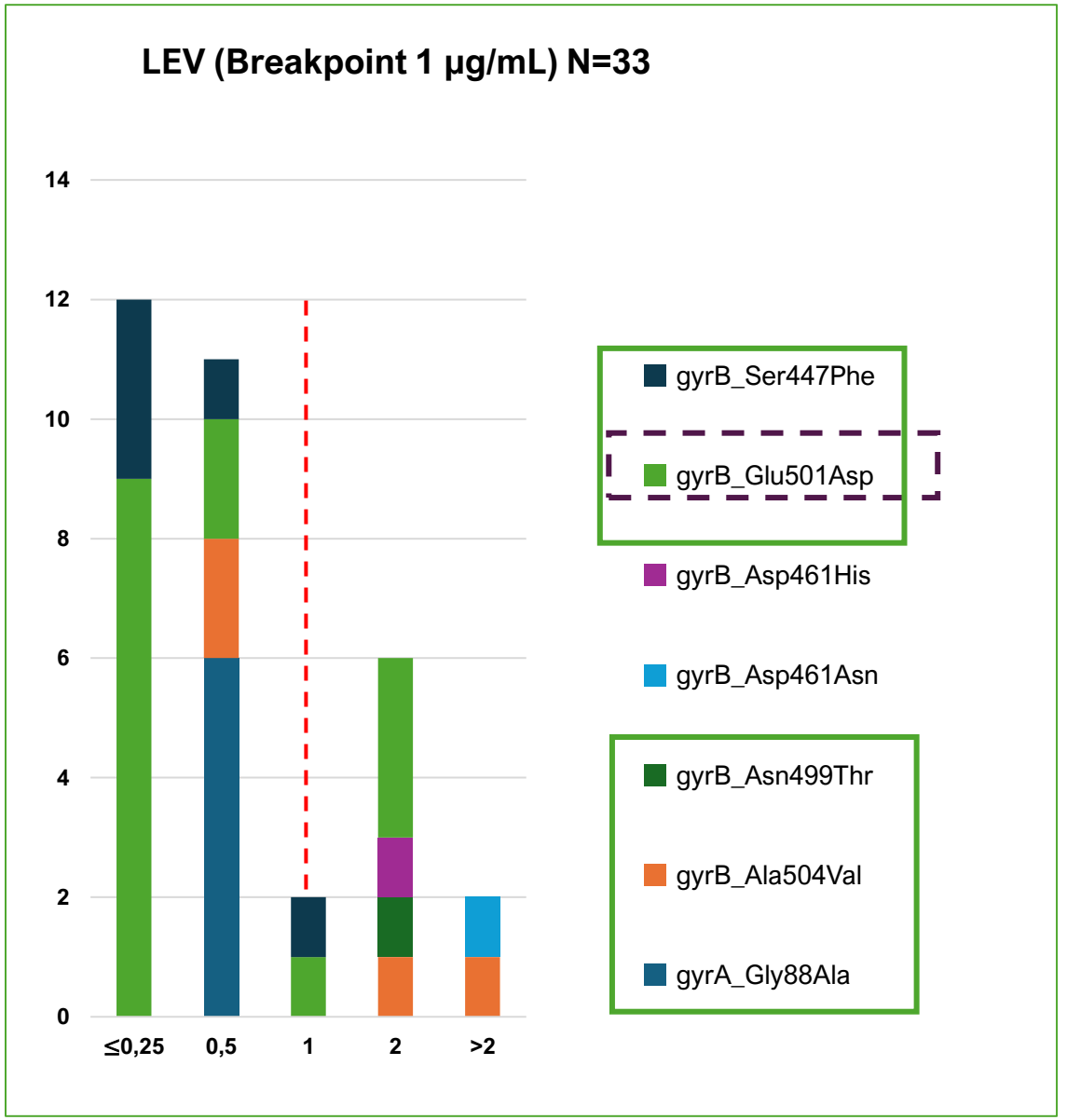
INH MIC Distribution vs Mutation (Breakpoint 0.1 and 0,4 µg/mL) N=63





FLQ MIC Distribution vs Mutation





Discussion

RIF

- 73% were resistant (Over half at high-level: ≥ 2.0 µg/mL)
- 27% susceptible (7% at the breakpoint 0.5 µg/mL).

Mutations profile (Susceptible Range)

- **Codon 450 mutations:** Ser450Cys, Ser450Gly, and Ser450Val.
- **Other mutations:** Met434Ile, His445Gln, and Asp435Ala.
- Mutations at positions **445** and **435** linked to low-level resistance/borderline MIC values.
- Isolates with **Thr427_Ser428del** showed a broad MIC range (≤0.25 to ≥2.0 µg/mL)

INH

- 92% of isolates were resistant (84% show high-level resistance ≥ 0.8 µg/mL).
- Only 8% susceptible (0.4 µg/mL MIC at the breakpoint).
- **katG** mutants: Consistent with previous studies,100% exhibit high-level resistance (≥0.8 µg/mL).
- **inhA** mutants: 67% are resistant, with 59% showing high-level resistance.
- *Historically:* **inhA** mutations linked to low-level resistance.
- *Recent Data:* Many inhA mutants confer high-level comparable to katG mutations.

FLQ (MXF & LEV)

- High MXF resistance vs LEV: 79% vs 24% of the isolates
- All the MXF susceptible isolates had MICs at the breakpoint
- **gyrB_Glu501Asp**: resistant to MXF but susceptible to LEV
- **gyrB_Ser447Phe**: susceptibility to both drugs

Conclusions

- **RIF:** 27% remained susceptible despite carrying mutation with interim resistance.
  - Further studies is required to understand the role of these mutations.
- **INH:** The majority (92%) of isolates were resistant and resistance was predominantly high-level.
  - Over half (59%) of inhA mutations caused high-level.
  - Commercial assays (e.g. Xpert XDR) detect the promoter variants of inhA mutation. This could have clinical implications as patients may be incorrectly managed with high-dose INH, which is ineffective against high-level resistance.
- **FLQ:** MXF resistance was higher than LEV resistance (79% vs 24%).
  - The gyrB_Glu501Asp mutation, confers resistance mainly to MXF while maintaining LEV susceptibility. This requires further study to understand its clinical significance for treatment.
  - gyrB_Ser447Phe maintains susceptibility to both drugs.