WGS-based Dual Strategy for the Identification of Key Targets to Enhance Beta-lactam Activity in *Mycobacterium tuberculosis*

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Meet the Challenge of Antimicrobial Resistance

Background

One-third of all antimicrobial resistance fatalities are attributed to tuberculosis (TB). Beta-lactams are the most widely used antibiotics, but their application in TB is limited by inherent characteristics of *Mycobacterium tuberculosis* (*Mtb*). These include a complex cell wall, the presence of a beta-lactamase, and a peptidoglycan cross-linked by L,D-transpeptidases. However, the development of the potent subclass of carbapenems has rekindled the interest in beta-lactams as potential rescuing antibiotics. Two carbapenems are currently included by WHO in group C of the recommended medicines against multidrug-resistant TB and the agency considers further research on the role of the class in these regimens as essential. Through a dual approach, this work aims to identify genomic markers of altered susceptibility and uncover prospective novel drug targets that may enhance the activity of beta-lactams in TB:

1. Genotype-phenotype association tests with a cohort of clinical strains → pre-existent variants
2. Selective pressure assays with *Mtb* H37Rv → induced variants

Methods

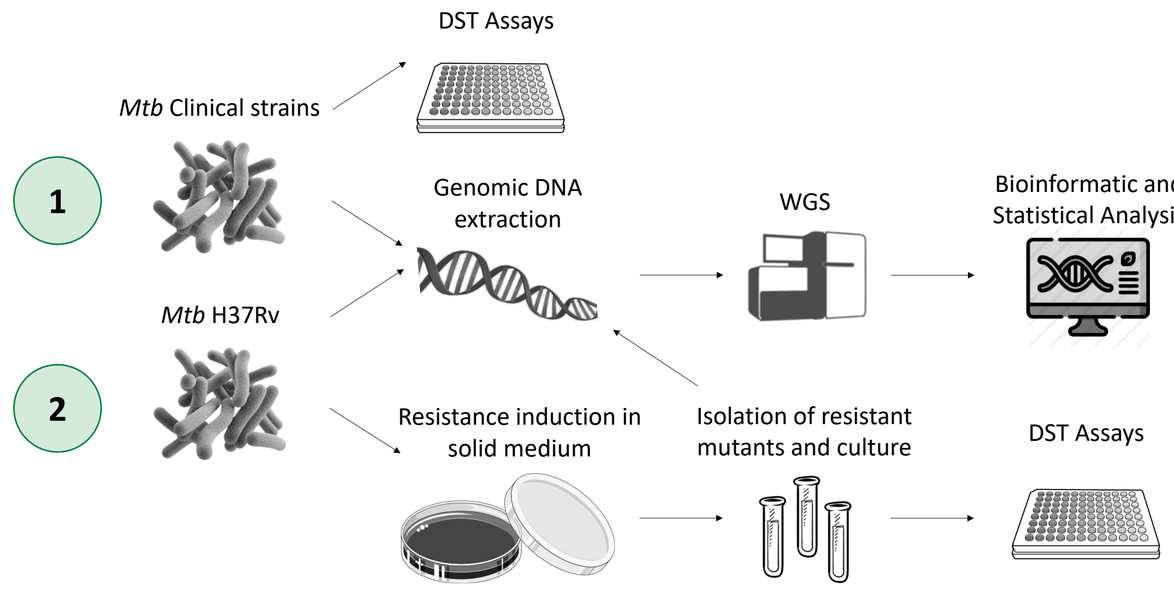

Figure 1. Schematic representation of the workflow of this study. DST, drug susceptibility testing; WGS, whole-genome sequencing.

Results

1.1. *Mtb* sublineages showed beta-lactam susceptibility variations

Sublineage 4.3.4.2 (60% MDR/pre-XDR) has significantly lower beta-lactam MICs

Beijing strains and 4.1.2.1 isolates appear to be more resistant to these antibiotics

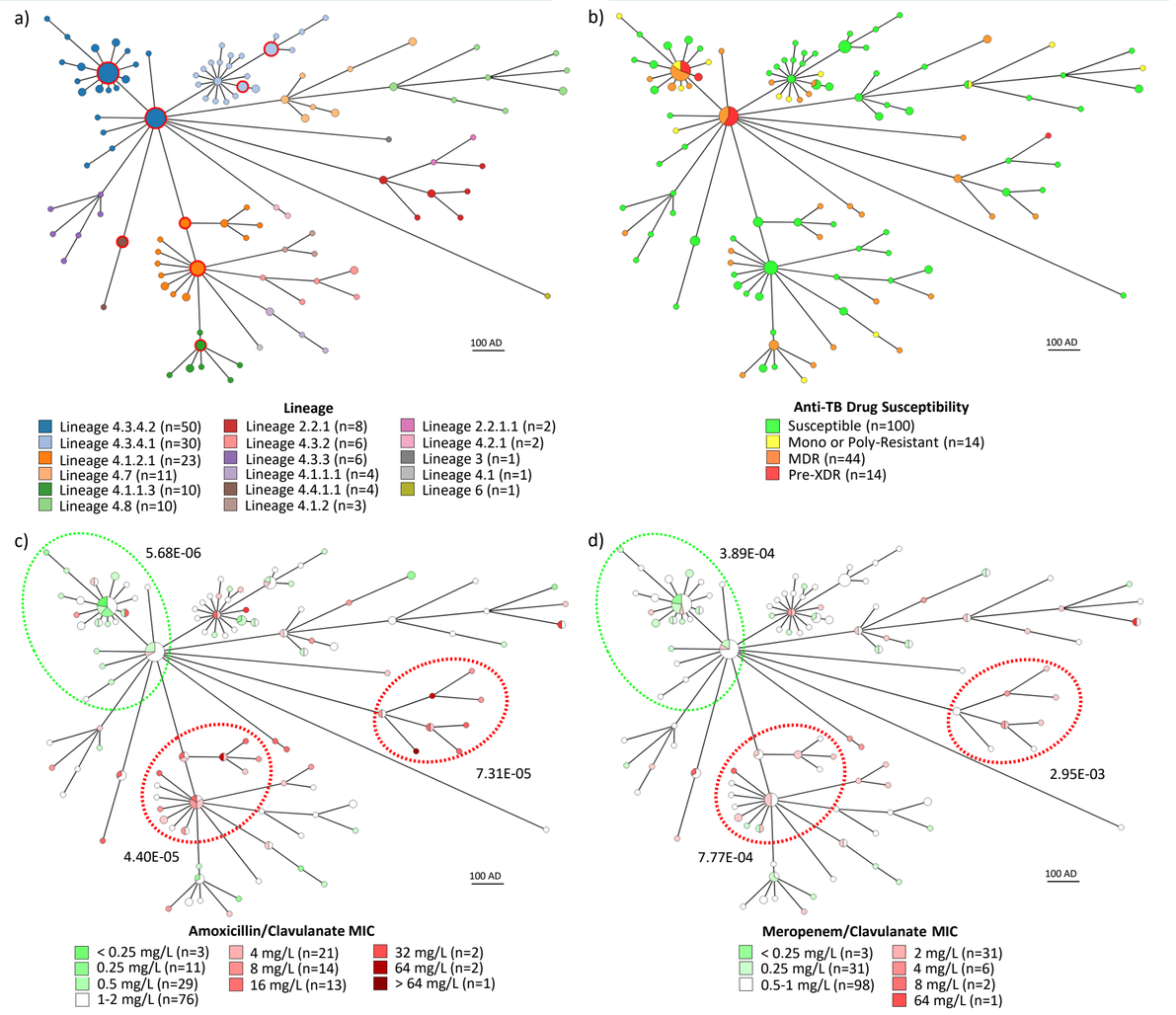

Figure 2. Minimum spanning trees generated (GrapeTree software MSTree V2) for the 172 *Mtb* strains. Branch length represents allelic differences (AD) between nodes, with strains sharing 12 or fewer variants collapsed in the same node. The dashed green or red circles define sublineages with significantly lower or higher MICs (values next to the circles correspond to *P* value obtained by the Mann-Whitney U test).

1.2. Variants in canonical target genes were not identified as putative phenotypic markers

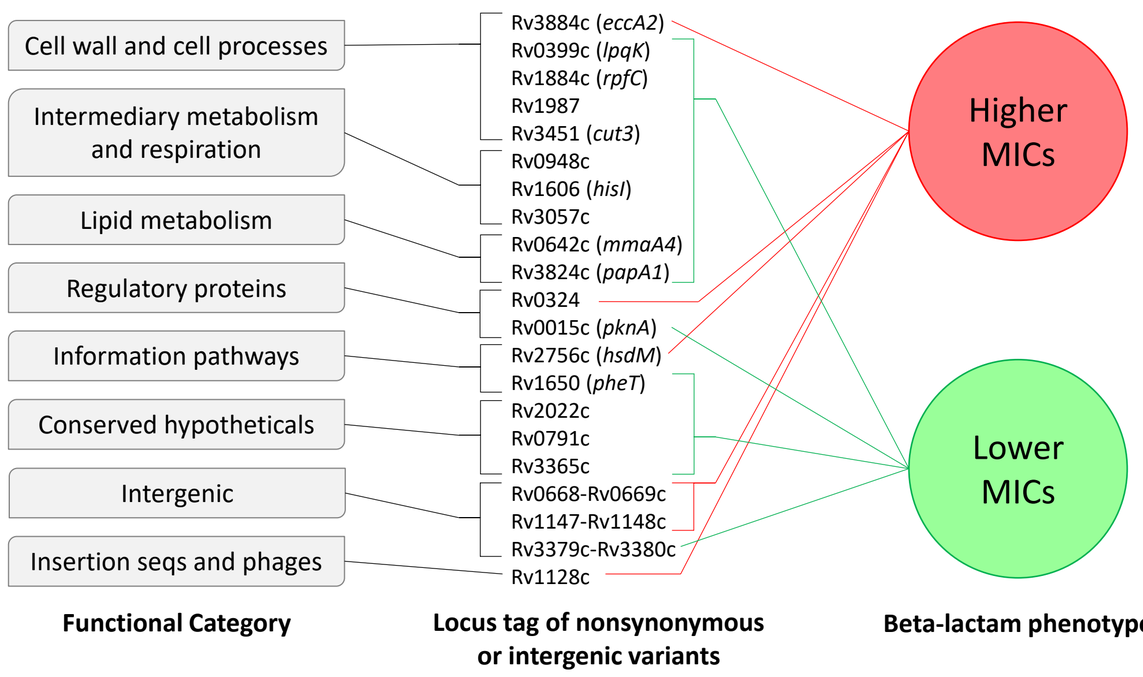

Figure 3. Locus tag of nonsynonymous or intergenic mutations identified by phenotype-genotype association tests, with respective functional category (as annotated in Mycobrowser) and associated beta-lactam phenotype for strains carrying those variants.

2.1. Selective pressure yielded isolates with beta-lactam resistance

Most notorious MIC increases were attained for carbapenems, particularly when not combined with clavulanate

MICs of cefotaxime, faropenem, unconjugated amoxicillin and anti-TB drugs were typically unchanged

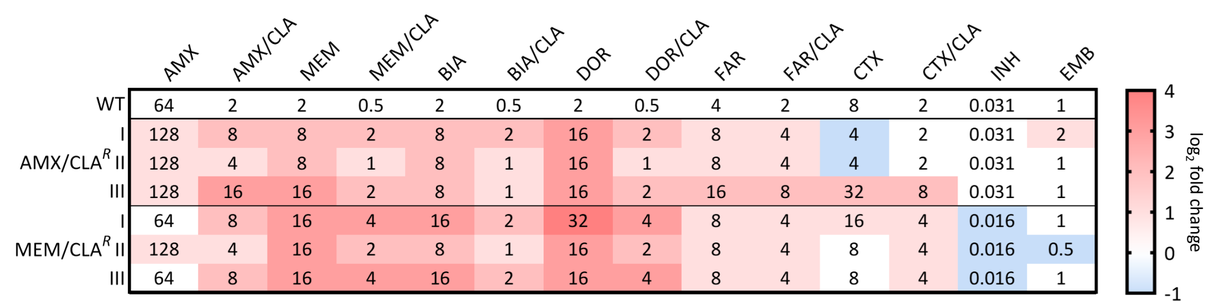

Figure 4. Heatmap of the $\log_2$ fold changes in minimum inhibitory concentrations (values in intersections) of the resistant *Mtb* H37Rv isolates. AMX, amoxicillin; BIA, biapenem; CLA, clavulanate; CTX, cefotaxime; DOR, doripenem; EMB, ethambutol; FAR, faropenem; INH, isoniazid; MEM, meropenem; WT, wild type.

2.2. Resistant mutants had different mutational patterns depending on subclass

All isolates had mutations in PhoP, part of a two-component system that regulates essential genes for complex lipid biosynthesis

Rv2864c appears to be specifically involved in decreased susceptibility to meropenem

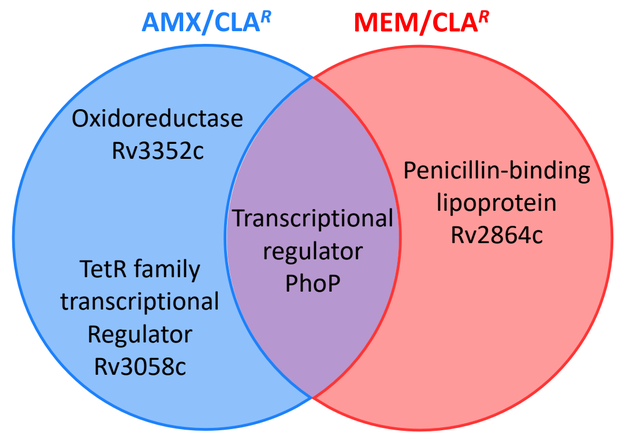

Figure 5. Genes with mutations detected by WGS of the Mtb H37Rv isolates resistant to amoxicillin/clavulanate (AMX/CLA^R) and to meropenem/clavulanate (MEM/CLA^R).

2.3. Clinical strains with mutations in Rv2864c have lower beta-lactam MICs

Variants in Rv2864c were linked to significantly lower MICs to both amoxicillin and meropenem

These mutations were more prevalent in clinical strains with previous resistance to anti-TB drugs

Mutations associated with resistance to isoniazid or ethambutol were not associated with any differences in beta-lactam MICs

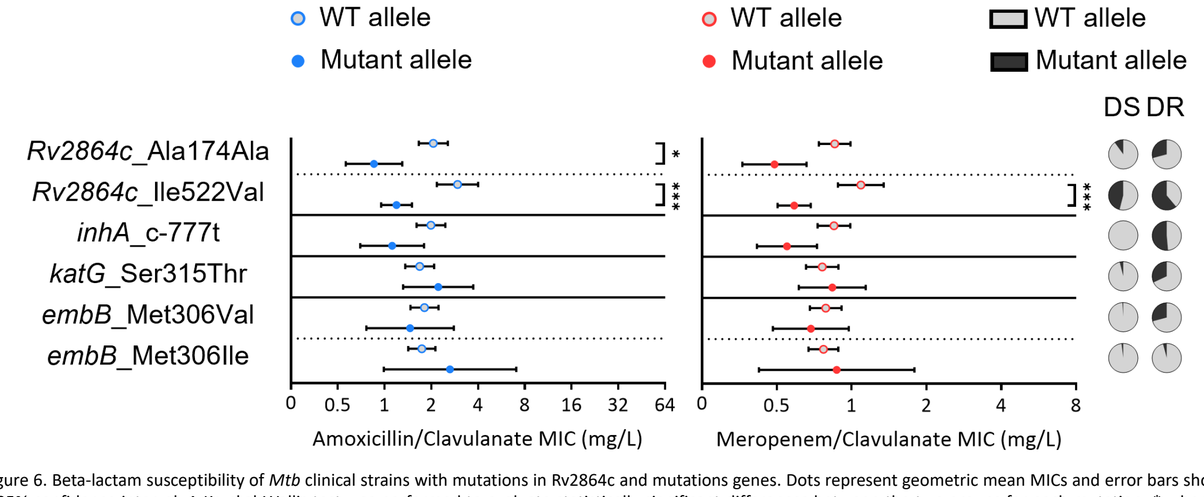

Figure 6. Beta-lactam susceptibility of *Mtb* clinical strains with mutations in Rv2864c and mutations genes. Dots represent geometric mean MICs and error bars show a 95% confidence interval. A Kruskal-Wallis test was performed to evaluate statistically significant differences between the two groups for each mutation: * when P ≤0.05; *** when P ≤ 0.001. Pie charts represent the distribution of each mutation in anti-TB drug-susceptible (DS) and drug-resistant (DR) strains.

Conclusions

- Determining if the inclusion of beta-lactams may result in better clinical outcomes concerning certain sublineages over other genotypes should be further explored.
- Lack of significant pre-existing mutations in genes encoding major penicillin-binding proteins, L,D-transpeptidases or beta-lactamases shows these targets are mostly conserved. This supports the global benefit that certain beta-lactams may add to TB therapeutics and suggests that extreme beta-lactam phenotypes may rely on more intricate mutational patterns.
- Lesser-known penicillin-binding lipoproteins LpqK and Rv2864c emerge as potentially relevant for beta-lactam susceptibility.

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