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Structural and Molecular Characterization of Drug Resistance-Associated PncA and EmbB Mutations in Mycobacterium Tuberculosis

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ABSTRACT: Drug resistance in Mycobacterium tuberculosis is driven by genetic variation that can alter protein structure and function. This study characterized sequence variants in pncA and embB from clinical M. tuberculosis isolates and evaluated their predicted structural consequences using molecular modelling. Clinically suspected pulmonary tuberculosis specimens collected at the Centre for Chest and Respiratory Diseases, Erbil, from July 2024 to March 2025 were investigated by GeneXpert MTB/RIF and Line Probe Assay, followed by PCR and bidirectional Sanger sequencing of pncA and embB. Sequences were aligned with the H37Rv reference genome and variants were assessed against the WHO catalogue of resistance-associated mutations. Missense variants were modelled with Phyre2, validated using ProSA-web, evaluated for changes in stability and flexibility with DynaMut2, and examined by molecular docking using AutoDock Vina. Three-dimensional models of wild-type and mutant proteins were generated successfully, with ProSA-web Z-scores within the range of experimentally determined protein structures. Most evaluated substitutions produced negative $\Delta\Delta G$ values, indicating predicted destabilization of the corresponding protein structures. Structural models showed local changes in atomic interactions around selected pncA and embB substitutions. These findings indicate that sequence variation in pncA and embB can produce measurable predicted changes in protein stability and local structure, supporting the use of integrated sequence and structural analyses for investigating molecular mechanisms of drug resistance in M. tuberculosis.

INTRODUCTION

Drug-resistant tuberculosis remains an important challenge because genetic variation can reduce the activity of antimicrobial agents and complicate treatment [1]. Sequence-based approaches have expanded the identification of mutations associated with resistance, but the functional interpretation of individual substitutions is often difficult when experimental structural information is unavailable [2]. *pncA* and *embB* are clinically relevant resistance-associated genes in *M. tuberculosis* [3]. Sequence variation in these genes may alter amino-acid composition, protein stability, local interactions, or molecular recognition. Combining clinical isolate sequencing with computational structural analysis can therefore provide a practical framework for describing the potential molecular effects of resistance-associated variants [4].

The present study aimed to identify *pncA* and *embB* sequence variants in clinical *M. tuberculosis* isolates and to characterize their predicted structural and stability consequences using protein modelling, stability analysis, and molecular docking.

MATERIALS AND METHODS

Study design, setting, and ethical approval

This laboratory-based cross-sectional study was conducted at the Centre for Chest and Respiratory Diseases, Erbil, Kurdistan Region, Iraq, in collaboration with the College of Medicine, Hawler Medical University and Exogen Center for Genetic Diagnosis. Eligible clinical specimens were collected from July 2024 to March 2025. The study received ethical approval from the Ethical Review Committee of the College of Medicine, Hawler Medical University (Approval No. 7; 30 June 2024). Written informed consent was obtained, and patient information was anonymized.

Molecular detection, amplification, and sequencing

Clinically suspected pulmonary tuberculosis specimens were examined using GeneXpert MTB/RIF according to the manufacturer's instructions [5]. Isoniazid resistance was additionally assessed by GenoType MTBDRplus as part of routine laboratory investigation. Genomic DNA was extracted using the SORB-B Nucleic Acid Extraction Kit. Target regions of *pncA* and *embB* were amplified using gene-specific primers and sequenced bidirectionally with the SuperDye v3.1 Cycle Sequencing Kit on a SeqStudio Genetic Analyzer.

Raw sequences were quality-checked, trimmed, and aligned against the *M. tuberculosis* H37Rv reference genome (NC_000962.3) using MEGA X and BioEdit. Nucleotide and corresponding amino-acid changes were recorded. Nonsynonymous variants were compared with the WHO Catalogue of Mutations in *Mycobacterium tuberculosis* Complex and Their Association with Drug Resistance [6]; variants without an established resistance association were treated as unclassified rather than confirmed resistance determinants.

Structural modelling and computational analysis

Missense variants were introduced into protein models generated with Phyre2 and evaluated using ProSA-web. DynaMut2 was used to estimate changes in Gibbs free energy ($\Delta\Delta G$) and predicted flexibility [7]. Molecular docking was performed with AutoDock Vina, and protein–ligand interactions were visualized using Discovery Studio Visualizer. Docking experiments were repeated 15 times, and mean $\pm$ standard deviation was used to summarize repeated runs. Data were managed in Microsoft Excel and analysed descriptively using GraphPad Prism 8.0.1.

RESULTS AND DISCUSSION

Three-dimensional models of wild-type and mutant *pncA* and *embB* proteins were generated successfully. ProSA-web analysis placed the predicted structures within the range expected for experimentally resolved proteins, supporting their use for downstream comparative analyses (Figure 1). The Z-score profiles showed variation among individual mutation models, indicating that different substitutions may influence the predicted structural environment to different extents. DynaMut2 predicted predominantly negative $\Delta\Delta G$ values among the evaluated substitutions, consistent with a destabilizing effect on protein structure (Figure 2). Similar computational approaches have demonstrated that amino-acid substitutions in *pncA* can alter protein stability, conformational dynamics, and interactions within functional regions [8,9]. Such predictions should be interpreted as computational estimates rather than direct measurements of protein stability. Nevertheless, the consistent direction of many predicted changes suggests that amino-acid substitutions identified in clinical isolates may modify the energetic landscape of the affected proteins.

Structural inspection of selected variants demonstrated changes in the local atomic environment around the substituted residues (Figure 3). Altered hydrogen-bonding, hydrophobic, and other non-covalent interactions may influence local flexibility or the configuration of functional regions. Recent investigations of *embB* variants have demonstrated that individual substitutions can be associated with different levels of ethambutol resistance and that genotype–phenotype relationships may be more complex than the presence of a mutation alone suggests [10–11]. Furthermore, large-scale investigations of genetic determinants of tuberculosis drug resistance have identified multiple variants associated with changes in antimicrobial susceptibility, emphasizing the importance of evaluating individual substitutions in their genetic and phenotypic contexts [12]. These observations provide a structural explanation for why sequence variation may have consequences beyond the nucleotide level, although the computational findings require experimental validation before individual substitutions can be considered mechanistic determinants of resistance.

Taken together, the sequence, stability, and structural analyses support an integrated approach for prioritizing resistance-associated variants for further investigation. The study also highlights the value of examining clinical sequence variation together with protein-level predictions rather than interpreting mutations solely from nucleotide changes. Because the dataset represents the eligible isolates identified during a defined study period at a single centre, the findings should be interpreted within this setting and confirmed in larger collections of *M. tuberculosis* isolates.

CONCLUSION

Clinical *pncA* and *embB* variants were successfully characterized by Sanger sequencing and evaluated using complementary structural bioinformatics approaches. Most evaluated substitutions showed predicted destabilizing effects, and selected variants produced changes in their local structural environments. These findings support the use of combined sequence and structural analyses to investigate the molecular basis of drug resistance in *M. tuberculosis* and to prioritize variants for future experimental validation.

Ethics statement

Ethical approval was granted by the Ethical Review Committee of the College of Medicine, Hawler Medical University, Erbil, Iraq (Approval No. 7; 30 June 2024). Written informed consent was obtained, and confidentiality was maintained.

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Conflict of interest

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Author contributions

The journal requires a contribution statement. The specific contributions of each author should be inserted after confirmation by all authors.

Data availability

The journal requests that datasets associated with the manuscript be accessible. The appropriate data-availability statement and repository link, if applicable, should be added before submission.

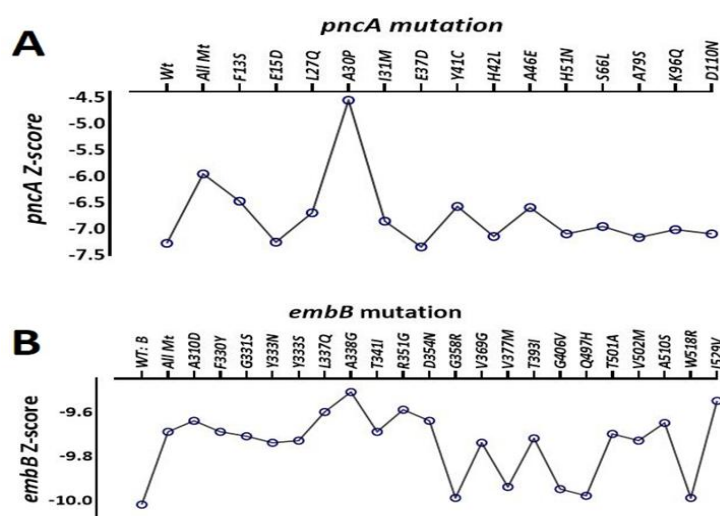


Figure 1. ProSA-web Z-score profiles of *pncA* (A) and *embB* (B) wild-type and mutant protein models. The plots show the predicted Z-score for the wild-type model and individual mutation variants; the values are used to assess the structural plausibility of the predicted models.

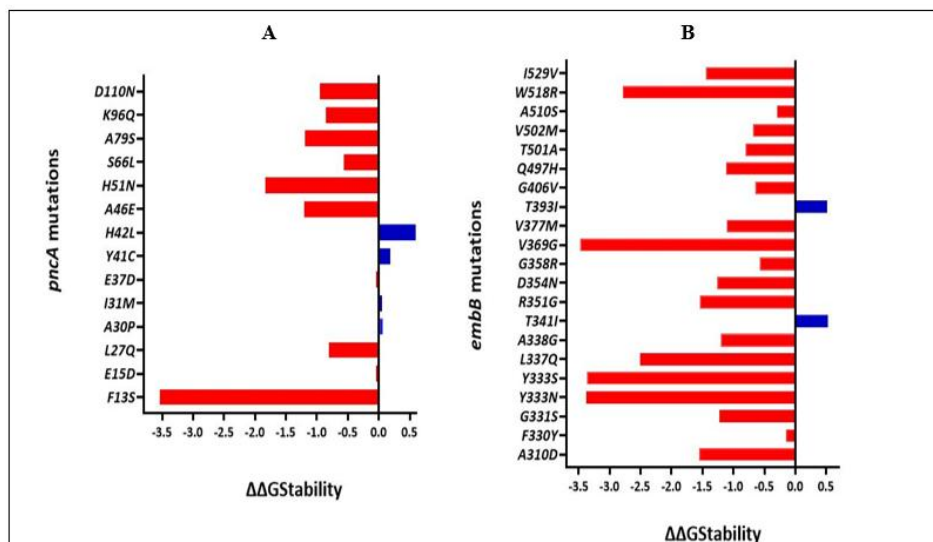


Figure 2. Predicted changes in protein stability ($\Delta\Delta G$) for *pncA* (A) and *embB* (B) variants calculated with DynaMut2. Negative $\Delta\Delta G$ values indicate predicted destabilization, whereas positive values indicate predicted stabilization.

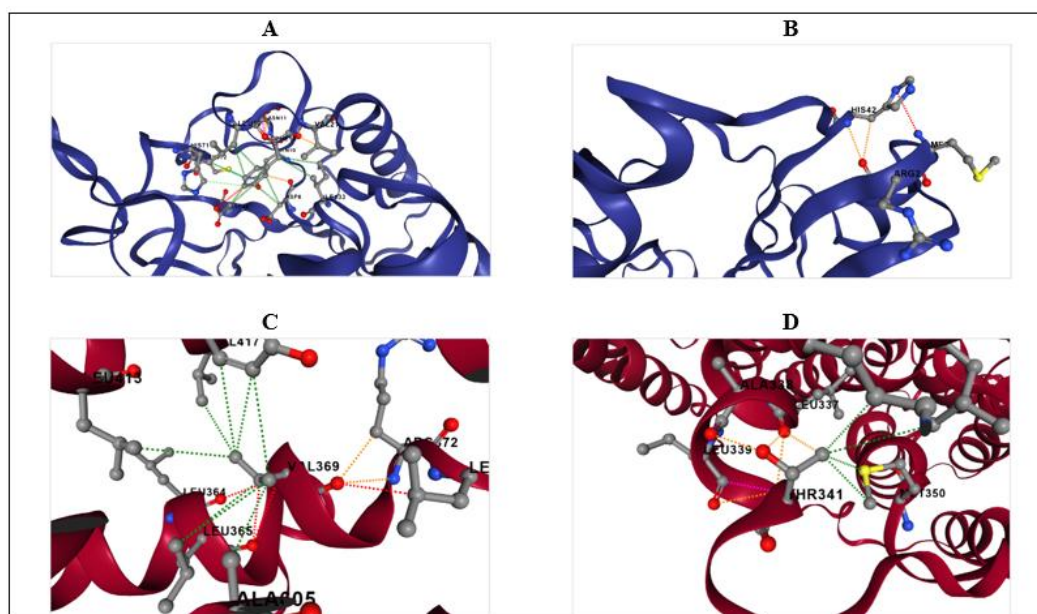


Figure 3. Structural visualization of selected *pncA* and *embB* mutation models generated with DynaMut2. Panels A–B show selected *pncA* models and panels C–D show selected *embB* models destabilizing and stabilizing respectively. Dashed lines represent predicted local interatomic interactions, including hydrogen bonds and other stabilizing contacts, around the substituted residues.

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