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NOVEL MUTATIONS IN PNCA, ASSOCIATED WITH CATALYTIC CENTRE, MIGHT BE RELATED WITH PZA HIGH RESISTANCE PROFILE

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Tuberculosis (TB) remains a serious health problem worldwide, and the greatest threat to TB control is the emergence of multidrug (MDR) Mycobacterium tuberculosis (Mtb) strains. Pyrazinamide (PZA) is an important first-line anti-TB drug. The emergence of isolates resistant to PZA, associated with MDR resistance, difficult TB treatment options. In order to evaluate mutations that might be responsible for PZA resistance, 30 clinical isolates collected in Lisbon Health Region, were screened for mutations in pncA, pyrazinamidase (PZase) coding gene, which convert the pro-drug PZA into its active form. We found 12 mutations in 23 resistant isolates. Among these mutations, six occurred in residues from catalytic centre or play an important role in PZase–PZA binding. We highlighted mutations that have never been described before: 3bp deletion (Del) at position 412, and 13 base par (bp) deletion between residues Ala146 and Gly150. The Del 412 3bp observed corresponds to entire deletion of Cys138 residue. Cys138 is a conserved residue in the active site, responsible for maintaining a functional conformation and is directly involved in drug donor binding. We also found a 13bp deletion between residues Ala146 and Gly150, resulting in a frameshift mutation plus four residues absence (Val147, Arg148, Asn149 and Gly150). There are no references for mutations in these residues, however it has been reported that residue Ala146 interacts with Cys138 to establish a functional folding. Evidences from mutational directed studies showed that amino acid substitution in catalytic centre residues produced some degree of conformational changes, but substitutions in Cys138 was associated with large folding alterations. Altogether, the results let us to hypothesize that the novel mutations presented might confer significant differences in protein folding. The loss of four amino acid residues plus frameshift near Ala146 residue could alter stability of PZase, and interfere with Cys138 normal interaction, resulting in a diminished PZase activity. Moreover, absence of the entire Cys138 residue would drastically alter the catalytic centre conformation, resulting not only in a different folding and reduction of protein stability but could also depleted PZase activity, and culminate with mycobacteria survival in the presence of PZA. Nevertheless, more studies are needed to evaluate the influence of such mutations in resistance to PZA.