

Retrospective Analysis of Bactec MGIT 960-based Pyrazinamide Monoresistance Results from a Referral Hospital in Hunan, China

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Abstract

Background: Pyrazinamide (PZA) monoresistance, traditionally linked with *Mycobacterium bovis*, is uncommon in clinical practice and warrants further investigation. **Methods:** Eighteen clinical *Mycobacterium tuberculosis* complex (MTBC) isolates, reported as PZA monoresistant by repeating Bactec MGIT 960 PZA assay using the inoculum prepared from the original seed MGIT tubes, were repeated again using the inoculum prepared from growth on solid medium (Middlebrook 7H10). Sequencing of PZA resistance-associated genes (*pncA*, *rpsA*, and *panD*) was performed to identify mutations linked to resistance. Spoligotyping was performed to identify the isolates. **Results:** When using the inoculum from solid medium, only nine isolates exhibited PZA monoresistance. Sequencing revealed that all true PZA-monoresistant isolates carried nonsynonymous mutations in *pncA* or *panD*, but not in *rpsA*, and those harboring nonsynonymous mutations in *pncA* did not show pyrazinimidease (PZase) activity. Six out of seven nonsynonymous mutations in *pncA* (T142M, S59P, I90S, D136G, T76P, and G132S) were associated with resistance according to the WHO mutation catalogue. All PZA-monoresistant isolates were identified as *Mycobacterium tuberculosis* by spoligotyping. **Conclusions:** The concentration of inoculum must be strictly controlled when confirming PZA-monoresistance using the Bactec MGIT 960 PZA assay. Additionally, laboratory differentiation of *Mycobacterium bovis* from PZA-monoresistant *Mycobacterium tuberculosis* complex isolates is unnecessary in routine diagnostic work.

Keywords: Bactec MGIT 960 PZA assay; *pncA* mutation; *Mycobacterium tuberculosis*; *Mycobacterium bovis*