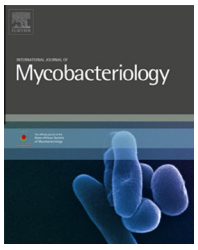
HOSTED BY

ELSEVIER

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/IJMYCO



Detection of pyrazinamide heteroresistance in *Mycobacterium tuberculosis*

Mikaela Glader [a], *Sven Hoffner* [b,c], *Jim Werngren* [b,*]

[a] *Dept. of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden*
[b] *Dept. of Microbiology, Public Health Agency of Sweden, Solna, Sweden*
[c] *MTC, Karolinska Institute, Stockholm, Sweden*

ARTICLE INFO

Article history:
Received 19 September 2014
Accepted 23 September 2014
Available online 31 October 2014

Keywords:
M. tuberculosis
Pyrazinamide
Drug susceptibility testing
Heteroresistance

ABSTRACT

Aim: Pyrazinamide (PZA) is a first-line key agent in the effective treatment of tuberculosis (TB), including PZA susceptible multidrug-resistant tuberculosis (MDR-TB). Occasionally, TB patients might have mixed infections with both drug-sensitive and -resistant strains. This is termed heteroresistance. If 10% of the bacterial population is resistant to PZA, there is an increased risk for poor treatment outcome. The aim of this study is to evaluate the ability of the three established drug susceptibility testing (DST) techniques – BACTEC MGIT 960, Wayne's pyrazinamidase test and Sanger sequencing of the *pncA* gene – to detect 10% PZA heteroresistance.

Methods: Mixed cultures of the fully drug susceptible *Mycobacterium tuberculosis* H37Rv reference strain and two laboratory-generated isogenic H37Rv mutants (with C475G and T254C *pncA* mutations, respectively) were made in proportions of 100%, 10%, 5% and 1% of the PZA-resistant (PZA-R) strain. Corresponding mixed cultures were also made using one drug-susceptible and one PZA-resistant MDR clinical isolate with the T62G *pncA* mutation, both belonging to one specific MIRU cluster. Additional mixes of 50%, 75%, 90% and 99% of the PZA-R strains were prepared for the Wayne's test. Tests were for all methods performed in duplicates at two separate occasions.

Results: Using the MGIT system, the *in vitro*-generated PZA-R strains were generally detected at a 5% proportion while the clinical PZA-R isolate only was detected at the critical 10% proportion, except for one test occasion. Sanger sequencing was unable to detect 10% PZA heteroresistance. Wayne's test also failed to detect the critical level of 10% PZA resistance; instead it displayed misguiding results determining highly resistant samples as susceptible.

Conclusion: Heteroresistance is caused by present drug-resistance development and/or dual infections with one resistant and one susceptible strain. Mixed infections with resistant strains may occur in up to 20% of all TB cases in high burden areas, according to limited data. This study showed that only the phenotypic BACTEC MGIT system was capable in determining the critical proportion of 10% PZA resistance, whereas neither the Sanger nor the Wayne's test were successful in this respect. This indicates a need for diagnostic tools with increased sensitivity to determine heteroresistance in *M. tuberculosis*.

* Corresponding author.

http://dx.doi.org/10.1016/j.ijmyco.2014.09.013