Published in final edited form as:

Tuberculosis (Edinb). 2016 July ; 99: 128–130. doi:10.1016/j.tube.2016.05.003.

Prevalence of pyrazinamide resistance across the spectrum of drug resistant phenotypes of *Mycobacterium tuberculosis*

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Abstract

Pyrazinamide resistance is largely unknown in the spectrum of drug resistant phenotypes. We summarize data on PZA resistance in clinical isolates from South Africa. PZA DST should be performed when considering its inclusion in treatment of patients with rifampicin-resistant TB or MDR-TB.

Keywords

Mycobacterium tuberculosis; pyrazinamide; *pncA* gene; DNA sequencing

Pyrazinamide (PZA) is a critical component of the first-line tuberculosis (TB) treatment regimen [1]. The unique ability of PZA to target subpopulations of persister bacteria has significantly reduced the treatment duration from 9–12 to 6 months [2]. The importance of PZA is further highlighted by its inclusion in the latest WHO recommended multi-drug resistant (MDR)-TB treatment regimen [3] as well as most novel MDR-TB regimens presently under investigation [4].

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Conflict of Interest: None

Little is known regarding the prevalence of PZA drug resistance, largely because drug susceptibility testing (DST) of PZA is not yet standardized and thus not routinely implemented in high burden countries. The BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system PZA (BD Diagnostic Systems, NJ, USA) recently became the reference technique, but concerns remain since false resistance may occur with this system [5]. False resistance may occur due to inoculum size or the presence of bovine serum albumin in the media of the MGIT 960 DST.[6]

Mutations in *pncA* are the primary mechanism of resistance to PZA [7]. It is hypothesized that these mutations decrease pyrazinamidase (PZase) activity, thereby limiting the conversion of PZA to the active form of pyrazinoic acid (POA) [7]. A high sensitivity (92%) and specificity (93%) for PCR-DNA sequencing when compared to the phenotypic DST supports the use of genotypic DST for the identification of PZA resistance [8]. Extensive DNA sequencing studies of *pncA* have revealed that mutations occur across the entire length of the gene, making it a complex target for a molecular diagnostic to identify all causal mutations [9, 10]. Furthermore, not all non-synonymous mutations cause phenotypic resistance [6], and mutations in the *pncA* gene are absent in a small percentage of phenotypically PZA resistant isolates [11] suggesting that resistance could be conferred via an alternative mechanism(s), such as the gene *rpsA* or *panD* [12, 13].

Several studies have investigated the prevalence of PZA resistance in drug susceptible as well as MDR-TB isolates. In a recent meta-analysis, the prevalence of PZA resistance was 0 – 9% among drug susceptible and 31 – 89% among MDR-TB isolates [8]. Data is scarce on the prevalence of PZA resistance in RIF mono-resistant, INH mono-resistant, extensively drug resistant (XDR) and pre-XDR TB isolates.

This study aimed to determine the association between mutations in the *pncA* gene and phenotypic resistance to PZA and to investigate the prevalence of PZA resistance across the spectrum of drug resistant isolates, from pan-susceptible TB to XDR-TB.

Materials and Methods

Study samples

A convenience sample of 775 drug resistant clinical isolates classified as INH mono-resistant, RIF mono-resistant or MDR-TB by the National Health Laboratory Services (NHLS) was selected. Routine DST for INH and RIF at the NHLS was done using either MGIT culture or the GenoType MTBR*plus* (Hain LifeScience) line probe assay (LPA).

Phenotypic DST of INH, RIF and PZA

At the MRC Centre for Tuberculosis Research laboratory, all selected isolates were subjected to phenotypic INH, RIF and PZA susceptibility testing using the BACTEC MGIT 960 method (BD Diagnostic Systems, NJ, USA). Growth at a critical concentration of 0.1μg/ml INH, 1.0μg/ml RIF, and 100μg/ml PZA was used to define resistance [14].

Genotypic classification of isolates

DNA sequencing of the *gyrA* and *rrs* gene was done to identify pre-XDR-TB and XDR-TB, as previously described [15]. DNA sequencing of the *pncA, panD* and *rpsA* genes were done to identify PZA resistance, as previously described [12, 13, 16]. No restriction fragment length polymorphism (RFLP) genotyping was performed.

Statistics

We used the Cochran-Armitage test in R to assess the presence of a trend in prevalence of PZA resistance by levels of resistance to other drugs.

Results

Phenotypic and genotypic characterisation of isolates

Based on phenotypic and genotypic DST performed at the research laboratory, 80 (10.3%) isolates were classified as pan-susceptible, 98 (12.7%) as INH mono-resistant; 279 (36.0%) as RIF mono-resistant, 224 (28.9%) as MDR-TB and 94 (12.1%) as pre-XDR and XDR-TB. Of the 80 pan-susceptible and 98 INH mono-resistant samples, 96 were confirmed rifampicin susceptible by molecular methods (MTBDRplus, Xpert MTB/RIF, *rpoB* sequencing). Low level rifampicin resistance may have been missed among the 82 samples evaluated only by MGIT960 DST, possibly resulting in some misclassification of samples as rifampicin susceptible.

Correlation of genotypic and phenotypic PZA resistance by level of resistance to other TB drugs

Overall, 246 (31.7%) isolates had a polymorphism in the *pncA* gene: 29 (11.8%) synonymous single nucleotide polymorphisms (SNP), 126 (51.2%) non-synonymous SNPs, 80 (32.5%) insertions and 11 (4.5%) deletions. Among all 775 isolates, 15 had discordant results between genotypic and phenotypic DST after considering SNPs known to not confer resistance to PZA at 100μg/ml [6]. Most discrepancies (10/15) were phenotypically resistant despite the presence of wild type *pncA* gene. The remaining 5 discrepancies were phenotypically susceptible with a non-synonymous change in the *pncA* gene (TTC13TGC, TGC72GGC, GCG102GTG, GTG139GGG). Using the phenotypic DST results as the reference standard, genotypic DST had an overall sensitivity of 95.0 (95% CI 92.1 – 98.0) and specificity of 99.1 (95% CI 98.4 – 99.9). The strong correlation between phenotypic PZA-resistance and changes in the *pncA* gene was observed independent of the resistance profile to other TB drugs. DNA sequencing of the *panD* gene failed to identify any mutations, sequencing of the *rpsA* gene identified one synonymous mutation (CGA212CGC) not associated with resistance [12].

Phenotypic PZA resistance by level of resistance to other TB drugs

Using the BACTEC MGIT 960 system, the number of isolates resistant to PZA at critical concentration of 100μg/ml was: 0 of 80 (0.0%) pan-susceptible isolates, 2 of 98 (2.0%; 95% CI 0, 4.8) INH mono-resistant isolates, 21 of 279 (7.5%; 95% CI 4.4, 10.6) RIF mono-resistant isolates, 88 of 224 (39.3%; 95% CI 32.9, 45.7) MDR isolates, and 91 of 94 (96.8%;

95% CI 93.2, 100) pre-XDR-TB and XDR-TB isolates (Figure 1). The proportion of isolates with PZA resistance increased significantly with increasing resistance to other drugs (Cochran-Armitage test, p =< 0.0005).

Discussion

We found a significant increase in the prevalence of PZA resistance with increasing level of resistance (pan-susceptible (0%) to pre-XDR and XDR-TB (96.8%)). These findings extend prior observations of low prevalence of PZA resistance in pan-susceptible but high prevalence of PZA resistance in MDR-TB isolates [8, 10]. Our findings strongly suggest that patients with pan-susceptible and INH mono-resistance benefit from inclusion of PZA in their treatment regimen and that PZA DST should be routinely performed in patients with RIF mono-resistance and MDR-TB. Lastly that the inclusion of PZA in treatment regimens for pre-XDR and XDR regimen is likely to cause more harm due to side effects than benefit since 97% (95% CI 93, 100) of all pre-XDR and XDR-TB isolates were resistant to PZA.

Similar to other studies, we found an excellent correlation between phenotypic and genotypic DST with an overall sensitivity of 95.0% (95% CI 92.1 – 98.0) and a specificity of 99.1% (95% CI 98.4 – 99.9). We further observed that this strong correlation was independent of the resistance profile to other TB drugs. Taken together, these observations provide mounting evidence supporting the use of genotypic instead of the slow and cumbersome phenotypic DST for PZA [10]. Use of genotypic DST could however result in some misclassification due to the existence of silent mutations and resistance not conferred by mutations in the *pncA* gene [6]. In our study, the occurrence of discordances was rare, similar to other studies findings [11]. Finally, the role of some *pncA* mutations is unclear as two mutations (GCG102GTG, GTG139GGG) were found to be susceptible in our study but resistant in another study [10].

Conclusion

The prevalence of PZA resistance is strongly correlated with resistance to rifampicin and increases with additional resistance in MDR and XDR-TB strains. The presence of rifampicin-resistance could then be considered as an indicator to perform DST to PZA.. Consideration should also be given when defining the role of PZA in future TB treatment regimens.

Acknowledgments

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under the Award Number #R01AI099026. SLS is funded by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation (NRF) of South Africa, award number UID 86539. The findings and conclusions in this report are those of the authors and do not necessarily represent the official views of the National Institutes of Health, the NRF.

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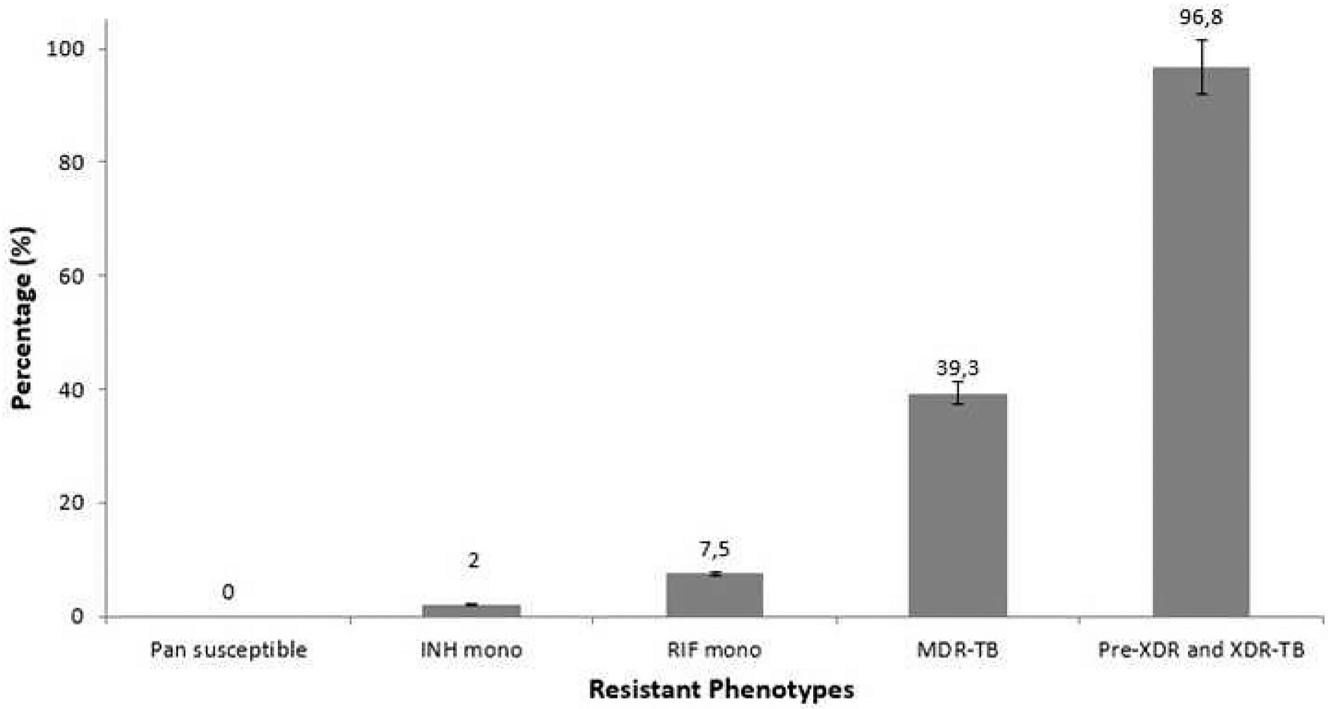

Figure 1.
PZA resistance prevalence in different resistant phenotypes.