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REVIEW

'Z^S-MDR-TB' versus 'Z^R-MDR-TB': improving treatment of MDR-TB by identifying pyrazinamide susceptibility

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Indispensable for shortening treatment of drug-susceptible tuberculosis (TB), pyrazinamide (PZA, Z) is also essential in the treatment of multidrug-resistant (MDR)-TB. While resistance to PZA in MDR-TB is associated with poor treatment outcome, bacillary susceptibility to PZA along with the use of fluoroquinolone (FQ) and second-line injectable drugs (SLIDs) may predict improved treatment success in MDR-TB. Despite a high prevalence of PZA resistance among MDR-TB patients (10%–85%), PZA susceptibility testing is seldom performed because of technical challenges. To improve treatment of MDR-TB, we propose to: (i) classify MDR-TB into PZA-susceptible MDR-TB (Z^S-MDR-TB) and PZA-resistant MDR-TB (Z^R-MDR-TB); (ii) use molecular tests such as DNA sequencing (*pncA, gyrA, rrs*, etc.) to rapidly identify Z^S-MDR-TB versus Z^R-MDR-TB and susceptibility profile for FQ and SLID; (iii) refrain from using PZA in Z^R-MDR-TB; and (iv) explore the feasibility of shortening the treatment duration of Z^S-MDR-TB with a regimen comprising PZA plus at least two bactericidal agents especially new agents like TMC207 or PA-824 or delamanid which the bacilli are susceptible to, with one or two other agents. These measures may potentially shorten therapy, save costs, and reduce side effects of MDR-TB treatment.
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Drug-resistant tuberculosis (TB), especially multidrug-resistant TB (MDR-TB), defined by bacillary resistance to at least isoniazid (INH) and rifampin (RIF), and extensively drug-resistant TB (XDR-TB), poses an increasing challenge for TB control.[1] XDR-TB refers to MDR-TB with additional bacillary resistance to fluoroquinolones (FQs) and one or more of the three second-line injectable drugs (SLIDs)—kanamycin, amikacin and capreomycin. WHO estimates that 500 000 MDR-TB cases occur every year.[1] Treatment of MDR-TB is difficult with an average cure rate of only around 62% in the best clinics.[2] In addition, the recommended treatment duration of MDR-TB, which is at least 18–24 months, is expensive and toxic in a substantial proportion of patients.

UNIQUE ROLE OF PZA IN THE TREATMENT OF TB AND MDR-TB

Pyrazinamide (PZA) plays a unique role in modern TB chemotherapy.[3] Inclusion of PZA enables considerable shortening of the treatment period from the previously 9–12 months to 6 months, thus the drug plays a pivotal role in the current short-course chemotherapy for drug susceptible TB.[4] The powerful sterilizing activity of PZA is due to its ability to kill a population of persister tubercle bacilli that are not killed by other TB drugs.[4] Studies in the mouse model of TB showed that substitution of PZA, but not INH and RIF, invariably led to poorer treatment outcomes.[5–7] Furthermore, the synergistic activity of PZA with newly developed agents such as the diarylquinoline bedaquiline suggests that the use of PZA in regimens including novel agents could improve efficacy substantially, if the organism retains susceptibility to PZA.[8,9]

FEASIBILITY OF ESTABLISHING A SIMPLE AND SHORTENED TREATMENT REGIMEN FOR PZA-SUSCEPTIBLE MDR-TB

There is fairly good evidence from animal and human studies that the treatment duration of Z^S-MDR-TB can be shortened to a minimum of 9 months with a regimen comprising PZA accompanied by two bactericidal drugs. McCune *et al.*[10,11] demonstrated in the mouse model that murine TB could be better sterilized with PZA plus a companion drug, especially a bactericidal one. In the treatment of drug-susceptible TB, the 2-year relapse rates of 9-month regimens comprising streptomycin, INH and PZA given daily or intermittently were only 5%–6%.[12,13] A small retrospective study suggested that inclusion of PZA in the treatment regimen was associated with a favorable outcome.[14] A recent observational study among second-line treatment-naive

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MDR-TB patients suggested that the treatment duration of MDR-TB could be shortened to a minimum of 9 months with a gatifloxacin-based regimen that contained PZA and clofazimine throughout with kanamycin, high-dose INH and prothionamide given for at least 4 months in the initial phase.[15] Although the impressive treatment outcome was partially attributed to clofazimine by conjecture,[15] interpretation of findings could have been confounded by PZA susceptibility, which was not checked and might be present in at least 31% of the study sample according to a systematic review.[16] The feasibility of shortening TB treatment for selected MDR-TB patients was further corroborated by a recent report.[17] Subsequent retrospective analysis with the same updated dataset suggests that PZA use with *in vitro* activity alongside later-generation FQs and SLID may considerably increase the proportion with three-month sputum culture conversion, and marginally increase that with two-year treatment success. (Chang KC *et al*., unpublished). However, the above results are preliminary and future prospective studies are required to assess the possibility that PZA, alongside two or three bactericidal agents might improve the treatment of Z^S-MDR-TB.

PROBLEM WITH PZA SUSCEPTIBILITY TESTING: PHENOTYPIC TESTS VERSUS MOLECULAR TESTS

Despite the potential importance of PZA resistance in MDR-TB treatment outcome,[14,18] standard phenotypic PZA susceptibility testing is seldom performed owing to technical challenges.[3] This also explains why PZA resistance data are generally unavailable in TB drug resistance surveys. However, a number of studies have demonstrated a high prevalence of PZA resistance among MDR-TB patients in different localities, ranging from 10% in Papua New Guinea[19] and 25% in Turkey,[20] to 49% in Thailand,[21] 50% in Central Africa,[22] 52% in South Africa,[23] 53% in Japan,[24] 55% in Taiwan,[25] 77% in Pakistan[26] and 85% in South Korea[27] and in India.[28] Bacillary resistance to PZA is generally higher in XDR-TB than MDR-TB cases, ranging from 72% in Chongqing, China (Zhang WH, unpublished), 86% in South Korea,[27] to 93% in FQ-resistant pre-XDR-TB in Cambodia.[29] The reason for the high PZA resistance rates in many high-burden areas may be partly related to widespread use of PZA in retreatment regimens without drug susceptibility guidance or maybe false resistance. It is also possible that some of the above studies that reported very high PZA resistance among MDR-TB overestimated the PZA resistance frequency due to false resistance. However, the XDR-TB studies that reported very high PZA resistance were all based on molecular test of *pncA* sequencing rather than conventional PZA susceptibility testing.

There are different methods for PZA susceptibility testing such as Lowenstein–Jensen medium and 7H10/11 agar at pH 5.5, BACTEC 460 and MGIT 960 or BacT/ALERT systems at pH 6.0.[30] However, PZA susceptibility testing is prone to errors,[3,31,32] which arise from: (i) acidity of the medium required for PZA activity inhibits the growth of *Mycobacterium tuberculosis*—about 20%–25% of clinical isolates do not grow on acidic 7H10 plates (pH 5.5), and even with pH 6.0 in BACTEC 460 liquid medium, 3.5% of the strains did not grow;[33] and (ii) use of too large an inoculum (over 10^7 bacilli/ml) leads to increase in medium pH, which then inactivates PZA.[34] In a recent study, the MGIT 960 PZA susceptibility testing method was found to be even less reliable than the radioactive BACTEC 460 method giving rise to more false resistant results, presumably due to the larger inoculum used in the MGIT method. The authors suggested retesting of PZA-resistant strains by the 'gold standard' BACTEC 460 method and *pncA* sequencing of PZA-resistant strains identified by the MGIT method.[32] Automated PZA susceptibility testing methods, including the BACTEC 460 method, are not exempt from false resistance owing to the use of either a lower resistance breakpoint (100 μg/ml) or an inadvertently large inoculum. According to the Henderson–Hasselbalch equation, the minimum inhibitory concentration (MIC) cutoff for PZA resistance should be at least 156 μg/ml,[34] rather than 100 μg/ml, which is the currently used breakpoint for PZA resistance in MGIT 960 or BACTEC 460.

To circumvent the above problems, use of nicotinamide at high concentrations (0.5–2 mg/ml) at neutral pH has been proposed as a surrogate method for PZA susceptibility testing in acidic Lowenstein–Jensen medium,[3,35,36] with promising results.[36–38] The nicotinamide test can be used potentially as an inexpensive alternative for PZA susceptibility testing in clinical microbiology laboratories, but it has a long (several weeks) turnaround time. The PZase enzyme test (the Wayne test), using PZase as a surrogate of PZA susceptibility[39] may also give rise to false resistance, due to the need for a sufficiently large inoculum that inevitably increases its turnaround time.

Mutation in the *pncA* gene encoding PZase[40] is the major mechanism for PZA resistance in *M. tuberculosis.*[40–42] Although a lower percentage of *pncA* mutations in PZA-resistant strains, i.e. 64%[43] and 72%,[44] has been reported, these studies did not retest PZA-resistant strains without *pncA* mutations to rule out false resistance. Because of the problem of PZA susceptibility testing discussed above, the lower percentage of PZA-resistant strains, i.e., 64%[43] and 72%,[44] with *pncA* mutations is most likely due to false resistance, lack of vigorous retesting to rule out false resistance, the low resistance breakpoint (100 μg/ml PZA in BACTEC or MGIT) used, or the small number of strains analyzed in the study.[43] A recent systematic review with meta-analysis showed no significant difference between *pncA* sequencing and the Wayne PZase test by sensitivity and specificity in detecting PZA resistance,[16] indicating good correlation between *pncA* mutations and lack of PZase activity and PZA resistance. In analysis of PZA-resistant strains, *pncA* mutations were found in an average of 87% of PZA-resistant strains[16] and sometimes in as high as 99% of PZA-resistant strains.[45] However, some studies suggest that a few PZA susceptible strains have *pncA* mutations that do not appear to alter the PZase enzyme activity,[46–48] indicating that false resistance can potentially occur by the sequencing approach. In addition, a few PZ-resistant strains with no PZase activity did not have *pncA* mutations,[42] indicating a potential regulatory gene of *pncA* that may have acquired a mutation. A few genuine low level PZA-resistant strains do not have *pncA* or *rpsA* mutations.[3,41,49] However, the above three situations are rare[24,41,50] and do not pose a significant problem for use of *pncA* sequencing for rapid detection of PZA susceptibility or resistance. Nevertheless, it would be of interest to develop a database of rare mutations that are not associated with PZA resistance to guide clinical treatment. In view of the good correlation of *pncA* mutations and PZA resistance.[41,42,45] the extremely diverse *pncA* mutations that are impossible to be included in current molecular tests such as MTBDR*plus* (Hain Lifescience) and GeneXpert (Cepheid) and new advances in sequencing technology and increasing affordability of DNA sequencing, we propose *pncA* sequencing as the best available molecular test for rapid PZA susceptibility testing. Although various molecular tests such as PCR single stranded conformation polymorphism (PCR-SSCP),[41] microarray,[51] expression of PncA protein followed by PZase activity testing,[52] and line-probe assay[53] have been used to detect *pncA* mutations in PZA-resistant strains, these tests are generally more onerous and expensive than *pncA* sequencing. As phenotypic PZA susceptibility testing is prone to false resistance,

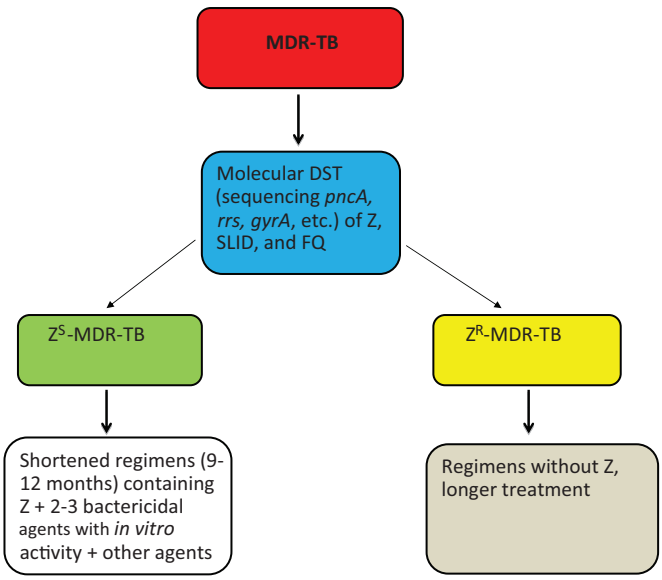

Figure 1 Classification of MDR-TB into PZA-susceptible and PZA-resistant MDR-TB and the potential to shorten the treatment of PZA-susceptible MDR-TB. DST, drug susceptibility test; Z, PZA.

pncA sequencing can be more sensitive and specific than the BACTEC 460 or MGIT 960 method. Indeed, a recent study showed a disturbingly low sensitivity of MGIT 960 PZA susceptibility testing in comparison with the molecular test owing to a high false resistance rate of 68%.[54] Clinical studies comparing these two tests and the molecular test with treatment outcome are needed.

PROPOSITION

In the area of drug-resistant TB, emphasis has previously been focused on INH and RIF resistance as in MDR-TB. In addition, in the management of MDR-TB, attention has been focused on the use of FQs and SLID. However, in view of the potentially important role of PZA in treatment outcome of MDR-TB, its unique sterilizing activity, and a considerable proportion of MDR-TB strains that are susceptible to PZA (about 50% resistance on average), we propose to classify MDR-TB based on PZA susceptibility into Z^S-MDR-TB and PZA-resistant MDR-TB (Z^R-MDR-TB) (Figure 1). This classification may allow Z^S-MDR-TB treatment to be shortened without compromising cure rates and also will improve evaluation of treatment outcomes of novel regimens in observational studies. Because of the good correlation between *pncA* mutations and PZA resistance, we further propose to use molecular tests such as sequencing of *pncA* (and FQ and SLID mutations, e.g., *gyrA, rrs*) to rapidly identify Z^S-MDR-TB and Z^R-MDR-TB with backup phenotypic tests to guide therapy. Moreover, we propose to sequence the *pncA* gene for all drug-resistant TB, including MDR/XDR-TB, even INH- or RIF-resistant TB. As PZA may cause hepatotoxicity,[55] it may be prudent to omit PZA in the treatment of Z^R-MDR-TB. Finally, with the implication of PZA susceptibility on treatment outcome of MDR-TB in human studies and its superior sterilizing activity, we suggest actively exploring a simple and shortened treatment regimen for Z^S-MDR-TB (possibly 9–15 months) comprising PZA plus at least two bactericidal agents including new agents like TMC207[9] or PA-824[56] or delamanid[57] as companion drugs, with one or two other agents. The above measures may potentially help to shorten therapy, protect against development of resistance to PZA, reduce costs and ameliorate side effects in MDR-TB treatment. Future clinical studies are needed to validate these propositions for better MDR-TB treatment.

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