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Pyrazinamide Resistance among Multidrug-Resistant *Mycobacterium tuberculosis* Clinical Isolates in Myanmar

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The emergence and spread of multidrug-resistant tuberculosis (MDR-TB) have been a serious threat in the control of TB. This situation has been augmented by the emergence of more-severe extensively drug-resistant tuberculosis (XDR-TB). MDR-TB strains have become resistant to all fluoroquinolones (FQ; specifically, ofloxacin, levofloxacin, moxifloxacin, and gatifloxacin) and all of the second-line injectable drugs (amikacin, capreomycin, and kanamycin). In Myanmar, TB incidence was estimated at 365/100,000 population, and there were 2,793 laboratory-confirmed cases of MDR-TB and rifampin-resistant TB and 11 laboratory-confirmed cases of XDR-TB in 2015 (1).

Pyrazinamide (PZA) is a standard component of short-course anti-TB treatment regimens and also of second-line regimens for MDR-TB and XDR-TB (2, 3). PZA is also one component of new regimens: novel rifampin-sparing anti-TB regimens and a shorter MDR-TB treatment regimen (4). There are limited data on PZA resistance because routine drug susceptibility testing (DST) has rarely been performed due to technical difficulties (5, 6). We identified PZA resistance in 66 clinical MDR-TB isolates which were collected at the Yangon and Mandalay TB Centers during 2015 and 2016. Those isolates were used for phenotypic PZA DST by the BACTEC 960 mycobacterial growth indicator tube (MGIT 960) (Becton, Dickinson, Sparks, MD) system at the concentration of 100 μg/ml, and the *Mycobacterium tuberculosis* H37Rv strain was used as the reference strain (7). Mutations in the *pncA* gene and its promoter region (*pncA* region) were identified using DNA sequencing to determine genotypic resistance to PZA. The 756-bp *pncA* gene was sequenced with forward (CGGATTTGTCGCTCACTACA) and reverse (TCCGCCGCCGAACAGTTCATCCCGGT) primers using an ABI 3500XL genetic analyzer (Applied Biosystems, USA). The wild-type *pncA* gene from *M. tuberculosis* H37Rv (Gene ID 888260) was used as the reference sequence, and sequences were aligned using Bioedit version 7.2.6.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Analysis of the sequences was performed on the basis of sequences in the available literature and online databases (8, 9, 10).

Of 66 MDR-TB isolates from Myanmar, 40 (60.6%) were PZA resistant and all of them showed mutations in the *pncA* region. There was good concordance between phenotypic PZA DST and sequencing results (0.968 kappa coefficient). This finding coincides with those of other studies (6, 11). Forty different types of mutations were distributed in the *pncA* region, and 10 types were first found in this study. We found 10 FQ-resistant pre-XDR and 7 XDR strains among 40 PZA-resistant isolates (Table 1).

A recent multicountry survey reported 3.0 to 42.1% PZA resistance among patients with rifampin resistance (12). Our study showed relatively higher PZA resistance (60.6%) among MDR-TB isolates, and some of them were XDR- and pre-XDR-TB strains. This

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TABLE 1 Correlation between phenotypic pyrazinamide susceptibility and *pncA* gene-sequencing results

Wild type or MDR-TB isolates with *pncA* gene mutations[a]	No. of strains that were phenotypically PZA[b]:		Total (% agreement)
	Susceptible	Resistant	
Wild type	25	0	25 (100)
Mutants			41 (97.6)
Single mutation	0	32	
Double mutations	1	7	
Triple mutations	0	1	
Total	26	40	66 (98.5)

[a]MDR-TB, multidrug-resistant tuberculosis.
[b]PZA resistance was determined by the BACTEC MGIT 960 system. Kappa coefficient = 0.968.

finding supported the recommendation that routine PZA DST be incorporated into the current MDR-TB treatment monitoring scheme. The presence of combined resistance to FQ and PZA suggests the need to evaluate effective treatment regimens for MDR/XDR-TB. The possible synergistic action of PZA with other anti-TB drugs should be assessed in larger studies linking PZA resistance to patient outcomes. MGIT 960 PZA DST is a useful and reliable method, but DNA sequencing can be considered an alternative method to replace phenotypic DST with a long turnaround time.

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