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Spectrum of *pncA* Mutations in Multidrug-Resistant *Mycobacterium tuberculosis* Isolates Obtained in Latvia

Pyrazinamide (PZA) is an effective antituberculous agent (1) that becomes active when bacterial pyrazinamidase converts it to pyrazinoic acid, which is toxic to mycobacteria (4). In *Mycobacerium tuberculosis*, PZA resistance is associated with the loss of pyrazinamidase activity, mainly due to mutations in the *pncA* gene coding region or in the putative regulatory area upstream of it (7, 9).

The molecular basis of resistance to PZA in *M. tuberculosis* has been extensively studied in recent years. Nevertheless, more studies should be done in order to evaluate molecular methods for PZA susceptibility testing, which is complicated (10). For that purpose, we examined the *pncA* gene in 28 randomly selected PZA-resistant and 10 randomly selected PZA-susceptible single-culture isolates of multidrug-resistant (MDR) *M. tuberculosis* from 38 patients in Latvia, where a high MDR level persists (12). Cultures were collected in the State Centre of Tuberculosis and Lung Diseases between 2001 and 2002; they represented about 10% of all MDR cultures isolated annually. All 38 cultures were resistant to rifampin, isoniazid, and streptomycin. Drug susceptibility was determined by the BACTEC method (Becton Dickinson, Sparks, Md.) (3). Native DNA was isolated as previously described (13). To amplify the 720-bp fragment containing the 561-bp *pncA* gene and the surrounding regions, the P1–P6 primer set and *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania) were used (10). PCR products were analyzed by automatic nucleotide sequencing of both DNA chains by using the ABI PRISM 3100 DNA analyzer (Applied Biosystems, Inc., Foster City, Calif.).

Results from sequencing of the 28 PZA-resistant MDR *M. tuberculosis* isolates are shown in Table 1. Altogether, point mutations were found in 23 (82%) of the 28 PZA-resistant isolates and were located in 10 different codons of the *pncA* gene, leading to amino acid changes. One mutation resulted in premature termination of synthesis. Codons T76 and Y103 were most frequently affected (43%). One isolate showed a mixture of the wild-type and mutant sequences, with mutations at codons C14 and Y103, which arose independently, as this isolate had a unique pattern (data not shown). Five isolates showed the wild-type *pncA* gene sequence, indicating an alternative mechanism for PZA resistance. No mutations were found in the PZA-susceptible MDR isolates.

TABLE 1. Mutations detected in the *pncA* gene of PZA-resistant *M. tuberculosis* isolates

No. (%) of isolates	Change(s) in:	
	Nucleotide sequence[a]	Amino acid[b]
5 (18)	A→C at position 226	T76→P
5 (18)	T→C at position 307	Y103→H
2	A→G at position 188	D63→G
2	C→D at position 216	C72→W
2	T→G at position 254	L85→R
1	C→T at position 28	Q10→Ter
1	G→A at position 41	C14→Y
1	C→A at position 184	P62→T
1	A→T at position 308	Y103→S
1	G→A at position 413	C138→Y
1	G→T at position 537	V180→F
1	WT/C→G at position 42 + T→C at position 307	WT/C14→W + Y103→H
5 (18)	WT	WT

[a] WT, wild type.
[b] Ter, chain synthesis-terminating codon.

We have detected mutations at three novel codons, C14Y, D63G, and V180F, by sequencing of both DNA strands from two independent PCRs; the remaining seven mutated codons have been described previously (2, 5–8, 10, 11). Here, mutations occurred most frequently in codons T76 and Y103 but were not found in codons R140, L85, and T47, reported previously (2, 5–8, 10, 11). Seven of 10 mutated codons (Q10, C14, P62, D63, C72, T76, and C138) were located in the three "hot" regions of the *pncA* gene as suggested by Scorpio et al. (10), partially confirming their hypothesis.

The relatively high percentage of mutations found in the *pncA* gene by sequencing should contribute to its further use for PZA susceptibility testing. However, it should be kept in mind that 18% of PZA-resistant isolates had no *pncA* mutations.

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