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 Mycobacterium 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 more studies should be done in order to evaluate molecular methods for PZA susceptibility testing, which is complicated.

For that purpose, we examined the methods for PZA susceptibility testing, which is complicated. 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, 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.

We have detected mutations at three novel codons, C14Y, D63G, and V180F, by sequencing of both DNA strands from two independent PCR products: the remaining seven mutated codons have been described previously (2, 5–8, 10, 11). Here, mutations occurred most frequently in codons. 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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### TABLE 1. Mutations detected in the pncA gene of PZA-resistant M. tuberculosis isolates

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

* WT, wild type.

** Ter, chain synthesis-terminating codon.

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