Ethyambutol (EMB) [(S,S')-2,2'-(ethylenediimino)di-1-butanol] is a first-line drug used for antituberculosis therapy. It is often used in combination with isoniazid, rifampin, pyrazinamide, and streptomycin. Membrane-associated arabinosyl transferases have been implicated as the targets for EMB (2, 3, 4). Arabinosyltransferases often used in combination with isoniazid, rifampin, pyrazinamide, and streptomycin. Membrane-associated arabinosyl transferases have been implicated as the targets for EMB (2, 3, 4).

Novel Mutations within the embB Gene in Ethambutol-Susceptible Clinical Isolates of Mycobacterium tuberculosis

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Genetic analysis of the embB gene revealed mutations in 17 (68%) of 25 ethambutol (EMB) resistant isolates (M306I, M306V, M306L, Q497R) but also in 4 (20%) of 20 EMB-susceptible isolates of Mycobacterium tuberculosis, namely, an ATG→ATM substitution resulting in M306I, G406N, and the novel alterations M423I and A659T.

DNA extracted from the isolates was analyzed by amplifying four fragments, using the PCR primers shown in Table 1. The PCR products were purified (QiAquick PCR purification kit or QiAquick gel extraction kit; QiAGEN) and directly sequenced using the BigDye Terminator sequencing kit and the ABI PRISM 377 automated sequencer (PE Biosystems, Branchburg, N.J.). Confirmation of mutations was done by reamplification and resequencing.

IS6110 profiling was done according to standard procedures to determine if the isolates were epidemiologically independent (28). All isolates with the same nucleotide substitutions in this study were deemed to be epidemiologically unassociated as they had distinct IS6110 fingerprints.

Overall, mutations in the embB gene were detected in 17 (68%) of the 25 EMB-resistant isolates (Table 2). Mutations at embB306 were detected in 12 of these 25 (48%) EMB-resistant isolates. Notably, all of the 12 EMB-resistant isolates with embB306 mutations were also resistant to isoniazid. All five EMB-resistant isolates with mutations at codon 497 were resistant to at least three antituberculosis drugs. Three isolates mono- resistant to EMB had no detectable mutations in embB. This isolate was resistant to both isoniazid and rifampin.

In addition, three other alterations were also detected in EMB-susceptible isolates, G406D and two novel mutations, M423I and A659T. The isolate with the G406D alteration was also resistant to isoniazid and rifampin, while the isolate with the M423I alteration was mono-resistant to isoniazid and the isolate with the A659T alteration was mono-resistant to streptomycin. In total, alterations in the embB gene were detected in 4 (20%) of the 20 EMB-susceptible isolates (Table 2).

There is a possibility that these mutations may have occurred in susceptible isolates due to cross-contamination of the PCR product, heteroresistance involving mixed cultures, or errors in the susceptibility testing, though every effort was undertaken to avoid this.

Alterations in embB in EMB-susceptible isolates at codons other than codon 306 have been documented in only two isolates with the G406D alteration (20) and one isolate with the
S347T alteration (8). This paucity of information is due in part to some studies targeting only embB306 (17, 29), several reports not noting (1, 12, 19, 23), and others not including EMB-susceptible isolates (22, 31).

Interestingly, all three EMB mono-resistant isolates in this present study did not have any detectable alterations in embB. In contrast, all 58 EMB-susceptible isolates in this and other studies with embB alterations were resistant to other antituberculosis drugs as well (8, 17, 20, 29). These observations support the hypothesis that a target other than EmbB may exist for EMB which may be activated during combination treatment with other first-line antituberculosis drugs, resulting in susceptibility to EMB (17).

Importantly, if all alterations of embB306 are considered polymorphisms, then only a minority of EMB-resistant isolates would be mutated. A similar scenario was observed in studies defining the role of the katG gene in isoniazid resistance in M. tuberculosis. Members of our group and others have shown that the predominant alteration in katG is R463L, which is detected in both isoniazid-resistant and -susceptible isolates and hence is considered a polymorphism and an unreliable indicator of isoniazid resistance (11, 13, 27). Thus, it is imperative for all studies elucidating the molecular mechanisms of drug resistance in M. tuberculosis to include drug-susceptible isolates as controls.

Another interesting finding of this study was the presence of resistance to other antituberculosis drugs when alterations of embB were present. Further investigations are necessary in order to understand the involvement of these drugs in the molecular mechanism of EMB resistance.

In conclusion, alterations at embB306 may not confer resistance to EMB but may be common polymorphisms in clinical isolates of M. tuberculosis. The clinical significance of this alteration is dubious, and further evaluation of EMB-susceptible isolates from other geographic regions is warranted.

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### REFERENCES


### TABLE 1. Oligonucleotide primer sequences for amplification of the embB gene

<table>
<thead>
<tr>
<th>Primer</th>
<th>Description</th>
<th>Sequence</th>
<th>Nucleotides</th>
<th>Annealing temp (°C)</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>embB1(F)</td>
<td>First fragment, sense</td>
<td>5’ CTG AAA CTG CTG GCG ATC AT</td>
<td>7601–7620</td>
<td>58.0</td>
<td>415</td>
</tr>
<tr>
<td>embB1(R)</td>
<td>First fragment, antisense</td>
<td>5’ GGT CTG GCA GGC GCA TCC</td>
<td>8015–7998</td>
<td>58.0</td>
<td>451</td>
</tr>
<tr>
<td>embB2(F)</td>
<td>Second fragment, sense</td>
<td>5’ TGG AGG CCA GCA AAC CGG</td>
<td>8082–8099</td>
<td>62.0</td>
<td>528</td>
</tr>
<tr>
<td>embB2(R)</td>
<td>Second fragment, antisense</td>
<td>5’ TAG TAG TAA CGG AGC TTC TC</td>
<td>8532–8513</td>
<td>62.0</td>
<td>565</td>
</tr>
<tr>
<td>embB3(F)</td>
<td>Third fragment, sense</td>
<td>5’ GCT GTT CGC CGC CGT AGG</td>
<td>8743–8760</td>
<td>62.0</td>
<td>585</td>
</tr>
<tr>
<td>embB3(R)</td>
<td>Third fragment, antisense</td>
<td>5’ GAA CCC GAA TCG CCG TCC AG</td>
<td>9270–9251</td>
<td>62.0</td>
<td>591</td>
</tr>
<tr>
<td>embB4(F)</td>
<td>Fourth fragment, sense</td>
<td>5’ TTC GCC CGA GCA AAG ATG</td>
<td>9752–9769</td>
<td>62.0</td>
<td>591</td>
</tr>
<tr>
<td>embB4(R)</td>
<td>Fourth fragment, antisense</td>
<td>5’ TCG CCG GAC AGG TAG GTG</td>
<td>10119–10102</td>
<td>62.0</td>
<td>591</td>
</tr>
</tbody>
</table>

**a** The M. tuberculosis sequence used to design the primers was obtained from GenBank, accession number U68480.


