Research Article

Mutations Found in \textit{embCAB}, \textit{embR}, and \textit{ubiA} Genes of Ethambutol-Sensitive and -Resistant \textit{Mycobacterium tuberculosis} Clinical Isolates from China

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To better understand the molecular mechanisms of Ethambutol (EMB) resistance, the mutant hot spot region of five genes (\textit{embB}, \textit{embA}, \textit{embC}, \textit{embR}, and \textit{ubiA}) was amplified and sequenced in 109 EMB-resistant and 153 EMB-susceptible clinical isolates from China. Twenty-seven EMB-susceptible isolates were found to have nonsynonym mutations, 23 of which were in \textit{embB}. The mutations occurred most frequently in \textit{embB} (85.3%, 93) and were seldom in \textit{embC} (2.8%, 3), \textit{embA} (3.7%, 4), \textit{embR} (3.7%, 4), and \textit{ubiA} (8.3%, 9) in EMB-resistant isolates. For the \textit{embB} gene, 63 isolates showed mutations at \textit{embB} 306, 20 at \textit{embB} 406, nine at \textit{embB} 497, and five at \textit{embB} 354 in EMB-resistant isolates. In addition, the particular mutants at \textit{embB} 406 and \textit{embB} 497 indicated both high levels of EMB resistance (MICs > 5 μg/mL) and broad anti-TB drug resistance spectrums. Our data supported the facts that \textit{embB} 306 could be used as a marker for EMB resistance with a sensitivity of 57.8% and a specificity of 78.8%.

1. Introduction

Ethambutol (EMB) is an antituberculosis drug that is widely used for treating drug resistance, and it is also commonly used for treating multidrug-resistant tuberculosis [1]. The collective results of the EMB drug susceptibility test (DST) of clinical \textit{M. tuberculosis} strains, which has been extensively reviewed in many countries, indicate that many of the strains are resistant to EMB [2–4]. Due to the numerous EMB-resistant strains, the mechanisms underlying EMB resistance, namely, mutations related to EMB target genes, have been both investigated and summarized [4–8].

EMB appears to inhibit arabinosyl transferases encoded by the \textit{embCAB} operon, which is involved in polymerizing arabinose into the arabinan components of arabinogalactan and lipoarabinomannan. The mutations in the \textit{embCAB} operon are responsible for its resistance, especially the “canonical” mutations in codons 306, 406, or 497 of \textit{embB} [4, 9, 10]. Belanger et al. (1996) reported that \textit{embR} modulates the level of arabinosyltransferase activity \textit{in vitro}, which might confer EMB resistance [11]. \textit{embR} may control arabinosyltransferase activity in \textit{M. tuberculosis} in a phosphorylation-dependent fashion, acting downstream of the Ser/Thr-kinase PknH [11]. Recently, Safi et al. found that mutations on the gene \textit{ubiA} were associated with high-level resistance and had multiplicative effects with \textit{embB} mutations on minimum inhibitory concentrations (MICs) [4]. The \textit{ubiA} gene encoding 5-phospho-alpha-d-ribose-1-diphosphate:decaprenyl-phosphate 5-phosphoribosyltransferase is known to be essential for the growth of \textit{M. tuberculosis}, and EMB was found to inhibit other steps in arabinan biosynthesis [12]. Although EMB does not directly inhibit \textit{ubiA}, \textit{ubiA} mutations have been shown to increase DPA synthesis, causing the MICs to increase in
both a wild-type background and an embB codon 306 mutant background [4].

The embB mutations that are related to the EMB target genes have been extensively studied, but studies regarding embA, embC, embR, and ubiA mutations are lacking. Moreover, less data has been generated on the simultaneous presence of these particular gene mutations in a large amount of clinical isolates. In this study, we sequenced the five genes to find the concomitant existence of the mutations in 109 clinical isolates. This study was an important step towards gaining a full understanding of the molecular mechanisms of EMB resistance and the mutation patterns in clinical isolates from China.

2. Materials and Methods

2.1. Bacterial Strains and Susceptibility Testing. M. tuberculosis H37Rv (ATCC 27294), which was used as the control for the antibiotic susceptibility test, was obtained from the Beijing Bio-Bank of Clinical Resources on Tuberculosis for the isolates. From January 1, 2009, to December 31, 2009, a total of 109 EMB-resistant clinical M. tuberculosis isolates were collected from 1,048 isolates. The absolute two-concentration method was conducted twice in order to determine the low or high resistance levels on Lowenstein-Jensen (L-J) slants [13]. The 109 EMB-resistant and 153 randomly selected EMB-susceptible isolates included in this study were also subjected to susceptibility testing for isoniazid (INH), rifampicin (RFP), streptomycin (SM), EMB, ofloxacin (OFX), Capreomycin (CM), para-aminosalicylic acid sodium (PAS), and amikacin (AMK) by the absolute concentration method [9].

3. Results

3.1. Antibiotic Susceptibility Testing. Among the 1,048 isolates collected between January 1, 2009, and December 31, 2009, a total of 109 clinical M. tuberculosis isolates were EMB-resistant, of which 67 were MDR-TB isolates, 11 were XDR-TB isolates, 26 were resistant to INH or RFP, and the remaining 5 were resistant to neither INH nor RFP. The results of the drug susceptibility tests are shown in Table 2. The absolute two-concentration, concentration DST results showed that 34 isolates had a high EMB concentration level (MICs ≥ 5), and the remaining 75 EMB-resistant isolates had a low EMB concentration level (2 ≤ MICs < 5).

To further investigate the drug-resistant spectrum, the 109 EMB-resistant isolates and 153 randomly selected EMB-sensitive isolates were also subjected to susceptibility testing for INH, RFP, SM, EMB, OFX, CPM, PAS, and AMK. Results showed that the EMB-resistant isolates were resistant to an average of 3.49 ± 1.59 (mean ± SD) of the eight tested anti-TB drugs, while the EMB-sensitive isolates were resistant to an average of 0.72 ± 0.41 (mean ± SD) of the eight tested anti-TB drugs. The highly EMB-resistant isolates were resistant to an average of 4.58 ± 1.96, whereas the isolates with low EMB resistance were resistant to an average of 2.94 ± 1.02. The 153 EMB-sensitive isolates were resistant to an average of 0.73 ± 0.42 of the eight tested anti-TB drugs.

3.2. Mutations in the Tested Genes. Of all the 153 EMB-susceptible isolates, only one or two were found to have nonsynonym mutations in embC, embA, embR, and ubiA. Among the 109 EMB-resistant isolates, there were one, four, four, and six isolates with nonsynonym mutations in the embC, embA, embR, and ubiA, respectively. The mutation pattern in embA included V343L, L125S, and R380P, for EMB-resistant, and V343L, R380P for EMB-susceptible isolates. In embR, nonsynonym mutations occurred at P49A, S104N, and P243S in EMB-resistant isolates and at L125S and R230W in EMB-susceptible isolates. Nonsynonym mutations occurred at S244T, I797T, E419D, and A38T in EMB-resistant isolates and none in EMB-susceptible isolates in ubiA. Only one nonsynonym mutation was found in embC at E305D, which was found in EMB-resistant isolates, and a synonym mutation occurred at E305E, which was found in both EMB-resistant and EMB-susceptible isolates.

The embB mutation rate in 109 EMB-resistant M. tuberculosis strains was 85.3% (93/109) but was only 15.0% (23/153) in EMB-susceptible strains, of which 17 were at the site of embB306 (Table 3). Other embB mutation patterns were also found at codons 328 (3), 354 (5), 406 (20), and 497 (9) in
EMB-resistant isolates and at codons 246 (1), 307 (1), 318 (1), 336 (1), 406 (1), and 439 (1) in 153 EMB-susceptible isolates. Eleven isolates had double mutations in EMB-resistant isolates. Of these 11, 10 carried the mutation at the site of embB306 combined with either embB406, embB497, embB354, or embB328.

Mutations at embB306 were most common, as they were found in both EMB-resistant (63) isolates (Table 2) and EMB-susceptible (17) isolates (Table 3). The wild type codon ATG in embB306 changed into GTG, CTG, TTG, ATA, ATT, or ATC, of which GTG was the most frequent (39), followed by ATA (11), CTG (8), TTG (2), ATT (2), and ATC (1) (Table 2).

### 3.3. Correlation between Mutations and Drug Resistance.

Mutations at embB497, embB354, and embB328 were found only in EMB-resistant clinical isolates, and they were considered to correlate to EMB resistance. Mutations at embB406 and embB306 were also found mainly in EMB-resistant isolates, and they were correlated to EMB resistance with an odd ratio (OR) of 50.7 \((P < 0.001)\) and 46.5 \((P < 0.001)\), respectively.

Of all the 109 EMB-resistant isolates, the percentage of isolates showing high levels of resistance to EMB (MICs > 5 \(\mu g/mL\)) was not significantly dependent on the presence (39.7%, 25/63) or absence (37.5%, 12/32) of the embB306 mutation \((OR = 1.09, P = 0.84)\). The difference was statistically significant in relation to the presence (60%, 12/20) or absence (33.8%, 25/77) of an embB406 mutation \((OR = 3.12, P = 0.02)\) as well as the presence (77.8%, 7/9) or absence (34.9%, 30/86) of an embB497 mutation \((OR = 6.53, P = 0.01)\). Mutations at embB328 \((P = 0.78)\) and embB354 \((P = 0.70)\) were not found to be correlated to high EMB resistance. Regression analysis could not be performed in this study, as there were so few EMB-sensitive and -resistant isolates with mutations at embA, embC, embR, and ubiA.

Of all the 109 EMB-resistant isolates, more than 18 isolates were found to have mutations (including synonym mutations) in at least two of the five tested genes. When mutations occurred in more than two of the five tested genes, high levels of EMB resistance occurred \((OR = 6.2, P = 0.001)\); isolates with mutations in two or more of the tested genes were resistant to more anti-TB drugs \((5.87 \pm 1.60)\) than those with mutations in only one of the tested genes \((3.03 \pm 1.37)\). Some strains with certain mutation patterns showed broad anti-TB drug-resistant spectrums. The average number of resistant anti-TB drugs for the mutant at embB306, embB328, embB354, embB406, and embB497 was 2.96 \(\pm 1.07\), 4.33 \(\pm 1.53\), 2.60 \(\pm 0.89\), 5.19 \(\pm 1.23\), and 5.75 \(\pm 0.88\), respectively.

### 4. Discussion

EMB is an important antimycobacterial drug and is recommended to treat tuberculosis as well as opportunistic infections by M. avium in patients with acquired immunodeficiency syndrome [14]. However, EMB resistance has been reported frequently in many countries. The traditional views of the mechanisms for EMB resistance mainly focus on the mutations of the embB gene, which creates resistance by altering drug-protein interaction. Including the embCAB operon, the transcriptional regulators embR and ubiA have also been associated with EMB-resistant M. tuberculosis [15]. In the present study, we sequenced the embCAB operon, embR, and ubiA in 109 EMB-resistant and 153 EMB-sensitive M. tuberculosis isolates to find the relationships between the mutations and drug resistance.

Our data supported that mutations in codon embB were the predominant mechanism associated with EMB resistance, since 85.3% (93/109) were found to be mutated in EMB-resistant isolates and 15% (23/153) were found to be mutated in EMB-susceptible isolates. High mutation frequencies in
Table 2: Characteristics of the mutants in *embB*, *embA*, *embC*, *embR*, and *ubiA* within the EMB-resistant isolates.

| Types | embB   | embA change | embC change | embR change | ubiA change | Number (n=109) | High resistance (n=37) | Resistant spectrum (mean ± SD = 3.49 ± 1.59) | MDR/XDR |
|-------|--------|-------------|-------------|-------------|-------------|----------------|------------------------|-----------------------------------------------|---------|
| 1     | WT     | WT          | WT          | WT          | WT          | 14             | 0                      | 1.8                                           | 6 none, 8 MDR |
| 2     | M306V  | WT          | WT          | WT          | WT          | 22             | 5                      | 1.5                                           | 9 none, 13 MDR |
| 3     | M306L  | WT          | WT          | WT          | WT          | 8              | 3                      | 1.9                                           | 2 none, 6 MDR |
| 4     | M306L  | WT          | WT          | WT          | WT          | 2              | 1                      | 2.2                                           | 1 none, 1 MDR |
| 5     | M306I  | WT          | WT          | WT          | WT          | 8              | 2                      | 3.8                                           | 3 none, 4 MDR, 1 XDR |
| 6     | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | 1 MDR |
| 7     | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 5                                             | MDR |
| 8     | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | MDR |
| 9     | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | MDR |
| 10    | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | MDR |
| 11    | M306I  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | MDR |
| 12    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 4                                             | MDR |
| 13    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 7                                             | XDR |
| 14    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 5                                             | MDR |
| 15    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 8                                             | XDR |
| 16    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 3                                             | None |
| 17    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 7                                             | XDR |
| 18    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 3                                             | MDR |
| 19    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 3                                             | MDR |
| 20    | M306V  | WT          | WT          | WT          | WT          | 1              | 1                      | 6                                             | MDR |
| Types | embB | Locus, nucleotide change, and amino acid change | embA | embC | embR | ubiA | Number (n = 109) | High resistance (n = 37) | Resistant spectrum (mean ± SD = 3.49 ± 1.59) | MDR/XDR |
|-------|------|-----------------------------------------------|------|------|------|------|-----------------|------------------------|--------------------------------------------|--------|
| 21    | M306V (ATG-GTG) | G406P (GGC-CCG) A439A (GCA-GCG) M306V (ATG-GTG) | WT   | WT   | WT   | E149D (GAA-GAC) | 1              | 1                       | 7                  | XDR     |
| 22    | G406P (GGC-CCG) A439A (GCA-GCG) M306V (ATG-GTG) | WT   | WT   | WT   | 1260I (ATC-ATT) | 1              | 1                       | 6                  | MDR     |
| 23    | G406A (GGC-GCC) M306V (ATG-GTG) Q497R (CAG-CGG) D531D (GAC-GAT) | WT   | WT   | WT   | WT   | 1              | 1                       | 3                  | MDR     |
| 24    | M306V (ATG-GTG) Q497R (CAG-CGG) D531D (GAC-GAT) | WT   | WT   | WT   | E305E(GAG-GAA) | WT   | WT                       | 1              | 1                       | 4                  | MDR     |
| 25    | M306V (ATG-GTG) D531D (GAC-GAT) | WT   | WT   | WT   | WT   | 4              | 1                       | 5.5                | 2 none, 2 MDR |
| 26    | D328H (GAT-CAT) | WT   | WT   | WT   | WT   | 1              | 0                       | 4                  | MDR     |
| 27    | R354S (AGA-AGC) | WT   | WT   | WT   | WT   | 3              | 0                       | 2                  | 1 none, 2 MDR |
| 28    | R354S (AGA-AGC) | WT   | WT   | WT   | WT   | 1              | 0                       | 3                  | None    |
| 29    | G406P (GGC-CGG) | WT   | WT   | WT   | WT   | 1              | 1                       | 7                  | XDR     |
| 30    | G406P (GGC-CGG) A439A (GCA-GCG) | WT   | WT   | WT   | WT   | 2              | 0                       | 5.5                | MDR     |
| 31    | G406D (GGC-GAC) | WT   | WT   | WT   | WT   | 4              | 2                       | 5                  | 3 MDR, 1 XDR |
| 32    | G406A (GGC-GCC) | WT   | WT   | WT   | WT   | 6              | 2                       | 5.5                | 5 MDR, 1 XDR |
| 33    | G406A (GGC-GCC) | WT   | WT   | WT   | WT   | 1              | 0                       | 5                  | MDR     |
| 34    | G406C (GGC-TGC) | WT   | WT   | WT   | WT   | 1              | 1                       | 4                  | MDR     |
| 35    | A439A (GCA-GCG) | WT   | WT   | WT   | WT   | 2              | 0                       | 3.5                | 2 none    |
| 36    | Q497R (CAG-CGG) | WT   | WT   | WT   | WT   | 5              | 3                       | 5.4                | 2 none, 2 MDR, 1 XDR |
| 37    | Q497K (CAG-AAG) | WT   | WT   | WT   | WT   | 1              | 1                       | 6                  | MDR     |
| 38    | Q497R (CAG-CGG) T496N (ACC-AAC) | WT   | WT   | WT   | E179T (ATC-ACC) | WT   | 1              | 1                       | 7                  | XDR     |
| 39    | Q497R (CAG-CGG) | WT   | WT   | WT   | P49A (CCC-GCC) | WT   | 1              | 1                       | 6                  | None    |
| 40    | WT | WT | WT | WT | P49A (CCC-GCC) P243S (CCC-TCC) | WT | 1 | 0 | 4 | MDR |
| 41    | WT | WT | WT | WT | S244T (AGC-ACC) | WT | 1 | 0 | 5 | MDR |

WT: wild type; MDR: multidrug resistance; XDR: extensively drug resistance.
embB were found at embB306 (63), embB406 (20), embB497 (9), and embB354 (5) in EMB-resistant isolates. Other embB mutation patterns, such as codons 297, 304, 313, 319, 330, 332, 334, 368, 378, 423, 424, 434, 469, and 508 were not found in this study [4, 16–20]. Previous studies have demonstrated that mutations occur at the embB codon 306 in 27% to 87% of EMB-resistant clinical isolates [7, 9, 11, 15, 16, 19, 21–23]. In this study, mutations occurred at the embB codon in 55% of the EMB-resistant clinical isolates. Our data supported the facts that embB306 could be used as a marker for EMB-resistance with a sensitivity of 57.8% and a specificity of 78.8%. A different frequency of the mutation patterns in the embB gene was reported in India. Of all the 52 different positions that were investigated, the most commonly found mutations were located at codon 378 (11), followed by mutations at codons 368 (9), 306 (8), 380 (7), and 406 (6) [21]. This discrepancy may be due to heterogeneity in the methodologies used (e.g., drug susceptibility testing methods) or to the intrinsic molecular variability between isolates from diverse geographical regions.

In this study, all the EMB-resistant isolates with embB497 or embB406 mutations were MDR-TB, which was consistent with the facts reported by Shi et al. and Srivastava et al. [17, 21]. Moure et al. also reported that the percentage of multidrug resistance among isolates with at least one embB406 substitution was significantly higher than that found in the group of isolates without mutations in this codon (100% versus 73.1%, \( P = 0.035 \)). In our report, both higher drug resistance level and broader anti-TB drug spectrum were found in EMB-resistant isolates with embB497 or embB406 mutations than in those with embB306, embB328, or embB354 mutations.

Table 3: Mutants in embB, embA, embC, embR, and ubiA within the EMB-sensitive isolates.

| Types | Locus, nucleotide change, and amino acid change | Number (n = 153) |
|-------|-------------------------------------------------|----------------|
| 1     | WT WT WT WT WT WT WT WT WT WT WT WT WT WT WT WT | 124 |
| 2     | M306V (ATG-GTG) WT WT WT WT WT WT WT | 11 |
| 3     | M306L (ATG-CTG) WT WT WT WT WT WT WT | 4 |
| 4     | M306I (ATG-ATA) WT WT WT WT WT WT WT | 1 |
| 5     | M306I (ATG-ATT) WT WT WT WT WT WT WT | 1 |
| 6     | G406P (GGC-CCG) WT WT WT WT WT WT WT | 1 |
| 7     | G246R (GGC-GGC) WT WT WT WT WT WT WT | 1 |
| 8     | A307G (GCC-GGC) WT WT WT WT WT WT WT | 1 |
| 9     | N318S (AAC-AGC) WT WT WT WT WT WT WT | 1 |
| 10    | A439A (GCA-GGC) WT WT WT WT WT WT WT | 1 |
| 11    | L336P (CTG-CCG) WT WT WT WT WT WT WT | 1 |
| 12    | WT V343L (GTG-TTG) WT WT WT WT WT WT | 1 |
| 13    | WT R380P (CGT-CCT) WT WT WT WT WT WT | 1 |
| 14    | WT WT E305E (GAG-GAA) WT WT WT WT | 1 |
| 15    | WT WT L125S (TTG-TCG) WT WT WT WT | 1 |
| 16    | WT WT R230W (CGT-TGG) WT WT WT WT | 1 |
| 17    | WT WT WT WT WT WT WT WT WT WT WT WT WT | 1 |

embA, embC, embR, and ubiA. In this study, the mutations were found mostly in embB (85.3%) and less in ubiA (8.26%), embA (3.7%), embC (4.6%), and embR (3.7%) in EMB-resistant isolates. In congruence with similar studies conducted in Taiwan, nonsynonymous mutations in embC (1), embA (4), and embR (3) were only rarely encountered in this study [24]. Ramaswamy et al. first reported two nonsynonymous nucleotide substitutions in embR resulting in C110W and Q379D replacements [23]. Later, several EMB resistance-associated polymorphisms in embR (16/44; 36.3%) were found in India [21]. In mainland China, 2 of 77 EMB-resistant MDR isolates and 4 of 56 EMB-sensitive isolates were found to have mutations in embC, and 5 of 74 EMB-resistant MDR-TB and 6 of 54 EMB-sensitive MDR-TB were found to have mutations in embA [25]. In New York, USA, embA had only 2 EMB resistance-associated nonsynonymous, N394D and R738E, in 75 EMB-resistant samples, and 8 EMB resistance-associated amino acid replacements were identified in embA [23]. No mutation was identified in the embA gene isolated in India [26], but novel mutations at A254, L235R, T270I, and 297 (11/44) were found in embC [21, 27, 28], which we did not find in this study. Some evidence also supported that T270I changed on its own and plays no role in EMB resistance in embC [28] and that T270I is not a marker for EMB resistance in the M. tuberculosis complex [29]. Mutations in ubiA were reported in 19 of 63 that were randomly selected from the World Health Organization Special Programme for Research and Training in Tropical Disease strain bank and in 17 of the 89 isolates from China [30].

To confirm the mutations in the various genes described in the aforementioned literature, allelic exchange experiments were carried out. Safi et al. confirmed that mutations of M306V, M306L, M306I (ATA), and M306I (ATC) all caused
EMB resistance (MIC = 4 mg/L) when incorporated into wild-type strains 210 and 5310 [31]. The fold increase in EMB MIC was also investigated for M306V, M306I (ATA), and M306I (ATC) that had been introduced into H37Rv by Starks et al. [26] and Plinke et al. [32]. Safi et al. also looked at the role of common mutations found in clinical strains with high-level EMB resistance at the embB 406 and 497 codons [10]. By introducing the point mutation in embC, Goude et al. verified that the mutations D294G, M300L, and M300V increased susceptibility to EMB and that mutation M300I had no resistance effect [28]. The introduction of Rv3806c mutations into either codon 18, 188, 237, 240, 249, 174, 176, or 175 caused the increase of EMB MIC [4, 30], but not into codon 149 [4]. Newly found mutations at codons 38, 254, 198, and 249 of Rv3806c must be studied further.

In this study, no mutations were found at the tested sites in 14 of the total 109 isolates and 17 EMB sensitive isolates were found with the mutations of embB306. The discrepancy in drug susceptibility between the phenotype and the genotype was multifactorial. Those factors included the overlapping of the MIC distributions between the wild-type and mutant strains [33], the heteroresistance from the bacterial population [22, 34], the limitation of the current DST [35, 36], and the bacterial itself changes in the cell wall thickness, the efflux pump activity and mutations at other genes not included in this study [25, 38].

5. Conclusion

In conclusion, we have demonstrated that mutations were frequently found in the embB gene, especially in EMB-resistant M. tuberculosis strains. The embB306, embB497, and embB406 mutation patterns were ranked as the top three in mutation frequency and were found to be associated with EMB resistance. In addition, the particular mutants at embB406 and embB497 indicated both high levels of EMB resistance (MICs > 5 μg/mL) and broad anti-TB drug resistance spectrums. The features of EMB resistance revealed in this study will increase our understanding of the distribution and frequency of mutations in M. tuberculosis isolates with EMB resistance in TB patients from China.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] American Thoracic Society, CDC, and Infectious Diseases Society of America, “Treatment of tuberculosis,” Morbidity and Mortality Weekly Report—Recommendations and Reports, vol. 52, no. RR11, pp. 1–77, 2003.
[2] A. Y. Coban, A. Deveci, A. T. Sunter, and A. Martin, “Nitrate reductase assay for rapid detection of isoniazid, rifampin, ethambutol, and streptomycin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis,” Journal of Clinical Microbiology, vol. 52, no. 1, pp. 15–19, 2014.
[3] Y. Feng, S. Liu, Q. Wang et al., “Rapid diagnosis of drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol using genotype MTBDRsl Assay: a meta-analysis,” PLoS ONE, vol. 8, no. 2, Article ID e55292, 2013.
[4] H. Safi, S. Lingaraju, A. Amin et al., “Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenylphosphoryl-β-D-arabinose biosynthetic and utilization pathway genes,” Nature Genetics, vol. 45, no. 10, pp. I190–I197, 2013.
[5] E. Radmacher, K. C. Stansen, G. S. Besra et al., “Ethambutol, a cell wall inhibitor of Mycobacterium tuberculosis, elicits L-glutamate efflux of Corynebacterium glutamicum,” Microbiology, vol. 151, no. 5, pp. 1359–1368, 2005.
[6] Y. F. Yen, M. S. Chung, H. Y. Huet al., “Association of pulmonary tuberculosis and ethambutol with incident depressive disorder: a nationwide, population-based cohort study,” Journal of Clinical Psychiatry, vol. 76, no. 4, pp. 505–511, 2015.
[7] S. Sriewatsan, K. E. Stockbauer, X. Pan et al., “Ethambutol resistance in Mycobacterium tuberculosis: critical role of embB mutations,” Antimicrobial Agents and Chemotherapy, vol. 41, no. 8, pp. 1677–1681, 1997.
[8] Y. Xu, Z. Zhang, and Z. Sun, “Drug resistance to Mycobacterium tuberculosis: from the traditional Chinese view to modern systems biology,” Critical Reviews in Microbiology, vol. 41, no. 3, pp. 399–410, 2015.
[9] C. Plinke, H. S. Cox, N. Zarkua et al., “embCAB sequence variation among ethambutol-resistant Mycobacterium tuberculosis isolates without embB306 mutation,” Journal of Antimicrobial Chemotherapy, vol. 65, no. 7, Article ID dkk120, pp. 1359–1367, 2010.
[10] H. Safi, R. D. Fleischmann, S. N. Peterson, M. B. Jones, B. Jarrahi, and D. Alland, “Allelic exchange and mutant selection demonstrate that common clinical embCAB gene mutations only modestly increase resistance to ethambutol in Mycobacterium tuberculosis,” Antimicrobial Agents and Chemotherapy, vol. 54, no. 1, pp. 103–108, 2010.
[11] A. E. Belanger, G. S. Besra, M. E. Ford et al., “The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antitymbacidal drug ethambutol,” Proceedings of the National Academy of Sciences of the United States of America, vol. 93, no. 21, pp. 11919–11924, 1996.
[12] H. Huang, M. S. Scherman, W. D’Haeze et al., “Identification and active expression of the Mycobacterium tuberculosis gene encoding 5-phospho-D-ribosyl-1-diphosphate: decaprenyl-phosphate 5-phosphoribosyltransferase, the first enzyme committed to decaprenylphosphoryl-D-arabinos e synthesis,” The Journal of Biological Chemistry, vol. 280, no. 26, pp. 24539–24543, 2005.
[13] Z. Sun, J. Zhang, H. Song et al., “Concomitant increases in spectrum and level of drug resistance in Mycobacterium
tuberculosis isolates,” The International Journal of Tuberculosis and Lung Disease, vol. 14, no. 11, pp. 1436–1441, 2010.

[14] H. Masur, “Recommendations on prophylaxis and therapy for disseminated Mycobacterium avium complex disease in patients infected with the human immunodeficiency virus,” The New England Journal of Medicine, vol. 329, no. 12, pp. 898–904, 1993.

[15] R. Moure, M. Españañol, G. Tiduño et al., “Characterization of the embB gene in Mycobacterium tuberculosis isolates from Barcelona and rapid detection of main mutations related to ethambutol resistance using a low-density DNA array–authors’ response,” Journal of Antimicrobial Chemotherapy, vol. 69, no. 8, pp. 2299–2300, 2014.

[16] Z. Bakula, A. Napórkowska, J. Bielecki, E. Augustynowicz-Kopeć, Z. Zwolska, and T. Jagiełski, “Mutations in the embB gene and their association with ethambutol resistance in multidrug-resistant mycobacterium tuberculosis clinical isolates from Poland,” BioMed Research International, vol. 2013, Article ID 167954, 5 pages, 2013.

[17] D. Shi, L. Li, Y. Zhao et al., “Characteristics of embB mutations in multidrug-resistant Mycobacterium tuberculosis isolates in Henan, China,” Journal of Antimicrobial Chemotherapy, vol. 66, no. 10, Article ID dkr284, pp. 2240–2247, 2011.

[18] J.-H. Yoon, J.-S. Nam, K.-J. Kim et al., “Molecular characterization of drug-resistant and -susceptible Mycobacterium tuberculosis isolated from patients with tuberculosis in Korea,” Diagnostic Microbiology and Infectious Disease, vol. 72, no. 1, pp. 52–61, 2012.

[19] J. Perdigão, R. Macedo, A. Ribeiro, L. Brum, and I. Portugal, “Genetic characterisation of the ethambutol resistance-determining region in Mycobacterium tuberculosis: prevalence and significance of embB306 mutations,” International Journal of Antimicrobial Agents, vol. 33, no. 4, pp. 334–338, 2009.

[20] F. Brossier, N. Veziris, A. Aubry, V. Jarlier, and W. Sougakoff, “Detection by GenoType MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates,” Journal of Clinical Microbiology, vol. 48, no. 5, pp. 1683–1689, 2010.

[21] S. Srivastava, A. Ayyagari, T. N. Dhole, K. K. Nyati, and S. D. Shi, L. Li, Y. Zhao et al., “Characterization of drug-resistant and -susceptible Mycobacterium tuberculosis clinical isolates from India,” International Journal of Antimicrobial Agents, vol. 33, no. 5, pp. 483–486, 2009.

[22] R. Goude, A. G. Amin, D. Chatterjee, and T. Parish, “The arabinosyltransferase EmbC is inhibited by ethambutol in Mycobacterium tuberculosis,” Antimicrobial Agents and Chemotherapy, vol. 53, no. 10, pp. 4138–4146, 2009.

[23] C. U. Köser, D. K. Summers, and J. A. C. Archer, “Thr270Ile in embC (Rv3793) is not a marker for ethambutol resistance in the Mycobacterium tuberculosis complex,” Antimicrobial Agents and Chemotherapy, vol. 55, no. 4, article 1825, 2011.

[24] L. He, X. Wang, P. Cui et al., “ubiA (Rv3806c) encoding DPPR synthase involved in cell wall synthesis is associated with ethambutol resistance in Mycobacterium tuberculosis,” Tuberculosis, vol. 95, no. 2, pp. 149–154, 2015.

[25] H. Safi, B. Sayers, M. H. Hazbon, and D. Alland, “Transfer of embB codon 306 mutations into clinical Mycobacterium tuberculosis strains alters susceptibility to ethambutol, isoniazid, and rifampin,” Antimicrobial Agents and Chemotherapy, vol. 52, no. 6, pp. 2027–2034, 2008.

[26] C. Plinke, K. Walter, S. Aly, S. Ehlers, and S. Niemann, “Mycobacterium tuberculosis embB codon 306 mutations confer moderately increased resistance to ethambutol in vitro and in vivo,” Antimicrobial Agents and Chemotherapy, vol. 55, no. 6, pp. 2891–2896, 2011.

[27] K. Ångeby, P. Juréen, G. Kahlmeter, S. E. Hoffner, and T. Schön, “Challenging a dogma: antimicrobial susceptibility testing breakpoints for Mycobacterium tuberculosis,” Bulletin of the World Health Organization, vol. 90, no. 9, pp. 693–698, 2012.

[28] X. Zhang, L. Liu, Y. Zhang, G. Dai, H. Huang, and Q. Jina, “Genetic determinants involved in p-aminosaliclyic acid resistance in clinical isolates from tuberculosis patients in northern China from 2006 to 2012,” Antimicrobial Agents and Chemotherapy, vol. 59, no. 2, pp. 1320–1324, 2015.

[29] Z. Zhang, Y. Wang, Y. Pang, and K. M. Kam, “Ethambutol resistance as determined by broth dilution method correlates better than sequencing results with embB mutations in multidrug-resistant Mycobacterium tuberculosis isolates,” Journal of Clinical Microbiology, vol. 52, no. 2, pp. 638–641, 2014.

[30] Z. Mei, Z. Sun, D. Bai et al., “Discrepancies in drug susceptibility test for tuberculosis patients resulted from the mixed infection and the testing system,” BioMed Research International, vol. 2015, Article ID 659180, 7 pages, 2015.

[31] M. Sareen and G. K. Khuller, “Cell wall and membrane changes associated with ethambutol resistance in Mycobacterium tuberculosis H37Ra,” Antimicrobial Agents and Chemotherapy, vol. 34, no. 9, pp. 1773–1776, 1990.

[32] S. Ahmad, E. Mokaddas, and A.-A. Jaber, “Rapid detection of ethambutol-resistant Mycobacterium tuberculosis strains by PCR-RFLP targeting embB codons 306 and 497 and mINa codon 501 mutations,” Molecular and Cellular Probes, vol. 18, no. 5, pp. 299–306, 2004.