Frequency of Codon 306 Mutations in embB Gene of Mycobacterium tuberculosis Resistant to Ethambutol: A Systematic Review and Meta-Analysis

Abstract

Background: Ethambutol (EMB) resistance is a major concern in patients with tuberculosis (TB). The aim of this study was to determine the frequency rate of mutations in the embB306 gene of Mycobacterium tuberculosis (M. tuberculosis) resistant to EMB, based on a systematic review and meta-analysis. Methods: Thirty-seven original articles (1997–2015) that have been published in valid databases were considered for this research. The articles were systematically reviewed for the prevalence and rate of mutations in embB306 in EMB-resistant M. tuberculosis. Data were analyzed using meta-analysis and random effects models (CI 95%, P < 0.10). Results: With a 6,931 sample size in 37 original articles, the lowest rate was related to EMB resistance that was observed in 2014 with 0.05 (95% CI: 0.04–0.07) and the highest prevalence rate was 0.84 (95% CI: 0.68–1.01), observed in 1997. Lowest and highest prevalence rates of embB306 gene mutation in M. tuberculosis were 0.03 (95% CI: 0.01–0.07) in 2014 and 0.78 (95% CI: 0.71–1.84) in 2005, in the USA, respectively. Conclusions: The present study revealed the prevalence and association of mutations in the embB306 gene of M. tuberculosis with resistance to EMB. Detecting EMB-resistant M. tuberculosis can help in controlling and correcting the administration of drugs for patients with TB.

Keywords: Codon 306, embB gene, ethambutol, mutations, Mycobacterium tuberculosis

Introduction

Tuberculosis (TB) disease occurs during infection by Mycobacterium tuberculosis (M. tuberculosis). This disease damages the lungs, central nervous system, and lymphatic circulatory systems. Other parts of the body such as the brain, intestines, kidneys, or the spine can be damaged by TB. Chronic cough, pain in the chest, hemoptysis, weakness, fatigue, weight loss, fever, and sleep hyperhidrosis can be observed in this disease. It is one of the most serious diseases that threaten human health worldwide. Eight million new TB cases are reported each year and over 2 million deaths are caused by M. tuberculosis.[1,2] The human populations with TB are a major reservoir for the transmission of this disease. Drugs that are used to treat TB include first-line drugs (isoniazid [INH], rifampicin [RIF], pyrazinamide [PZA], ethambutol [EMB], and streptomycin [STR]) and alternative or second-line agents (aminoglycosides such as kanamycin [KAN] and amikacin [AMK], fluoroquinolones such as ciprofloxacin [CIP], D-cycloserine [DCS], and ethionamide [ETA], polypeptides such as capreomycin [CAP]).[3,4] On the other hand, emergence and spread of antibiotic-resistant strains, and especially multidrug-resistant (MDR) M. tuberculosis, are among the biggest challenges in TB treatment.[5,6] According to a global report, about 0.5 million cases of patients are affected by MDR M. tuberculosis worldwide. For example, in Bangladesh, the MDR rate among new cases of TB patients was observed to be 3.5%, and it was 20% among patients that had been previously treated.[7] EMB is used as the first-line drug in the treatment of TB around the world. This drug was first found effective in 1961.[7,8] EMB’s target is the cell wall and this drug interferes with arabinosyl transferase. This enzyme is coded by embCAB operon and it is associated with the biosynthesis of arabinogalactan and lipoarabinomannan.[9,10] The emb operon contains three genes, namely embA, embB, and embC that have 65% similarity.
Mutation in the genes embA and embC happens very rarely, but mutation can be involved in resistance to EMB. In most studies, EMB resistance is associated with the embB gene, especially codon 306.\textsuperscript{[11‑13]} Approximately 50–70\% of M. tuberculosis strains that are resistant to EMB have mutations in embB306.\textsuperscript{[10]} Resistance to EMB has been reported in 4\% of isolates of M. tuberculosis that were isolated from patients.\textsuperscript{[9]} Moreover, it was observed in a report that among 131 isolates of M. tuberculosis, 54.19\% were EMB-resistant.\textsuperscript{[11]} Several meta-analyses have been conducted on mutations in embB306 of M. tuberculosis around the world: In a study in China by the Lowenstein-Jensen proportion method, sequencing, it was reported that out of 56\% of the strains of M. tuberculosis that were resistant to EMB, 79.10\% of MDR-TB isolates contained mutations in embB306.\textsuperscript{[11]} Using the Lowenstein-Jensen proportion method and polymerase chain reaction (PCR)-sequencing, it was observed that 14\% of M. tuberculosis strains were resistant to EMB in Iran and 85.71\% of the resistant isolates had embB306 mutations.\textsuperscript{[7]} In another study in Iran using Lowenstein-Jensen proportion method, PCR- single-strand conformation analysis (SSCP; is a post-PCR technique that can be used to screen for mutations that are not limited to a single hot spot but are randomly distributed throughout the exons) and direct sequencing, it was observed that 2/32 of M. tuberculosis strains were resistant to EMB. The sequencing of the embB306 gene in this study showed that both isolates of EMB-resistant M. tuberculosis were mutated.\textsuperscript{[14]} In a review study in Saudi Arabia, it was revealed that mutation in embB at codon 306 was related to EMB resistance. In addition, mutations in cell wall synthesis-associated genes aftA and ubiA cause overexpression of embC and embCAB and resistance to EMB.\textsuperscript{[15]} According to different reports by researchers, MDR-TB has been observed in 3.7\% of the new cases and it is a major problem in TB treatment and control.\textsuperscript{[2,14]} On the other hand, in MDR-TB, 50–70\% are EMB-resistant and harbor mutations in codon 306 in embB genes.\textsuperscript{[14]} Moreover, drug resistance is rising in EMB-resistant M. tuberculosis, especially with mutations in codon 306 in embB genes. Transmission of EMB-resistant M. tuberculosis between individuals by TB can easily occur.\textsuperscript{[16]} EMB resistance and mutation in the genes related to it are among the most important problems in patients because they increase morbidity and mortality. Therefore, identifying the factors associated with this issue and determining the prevalence of EMB resistance in these patients around the world is of great importance. Given the role of M. tuberculosis infection in patients with respiratory problems worldwide, comprehensive studies are needed to determine the prevalence of this type of infection, and the degree of antibiotic resistance in this bacterium. Considering the possibility of interaction between a mutation in embB306 of M. tuberculosis and resistance to EMB, determining and detecting such mutations in different communities is important and can help to control and plan suitable therapies. The purpose of this study was to evaluate the frequency of embB gene mutations in codon 306 of M. tuberculosis isolates that were resistant to EMB worldwide in a period between 1997 and 2014, using a systematic review and meta-analysis study.

**Methods**

**Search strategy**

The current systematic review and meta-analysis of the study were conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.\textsuperscript{[16]} Literature databases with original articles that were published in the time span of 1997–2014 in the English language with regard to determining the prevalence and occurrence rates of mutation in M. tuberculosis embB306 in populations from around the globe were obtained from valid and credible websites as follows: PubMed (http://www.ncbi.nlm.nih.gov), Science Direct (http://www.sciencedirect.com), Google Scholar (http://www.scholar.google.co.uk), and ISI web of knowledge (http://www.ics.webofknowledge.com), plus some Iranian database: Scientific Information Database (SID) (https://www.sid.ir/En/Journal/), Islamic World Science Citation Center (ISC) (https://isc.gov.ir/en), Mag Iran (https://www.magiran.com/), and Iran Research Information System (http://idml.research.ac.ir/). This research was conducted in the microbiology department of Kurdistan University of Medical Sciences located in Sanandaj city, Kurdistan province, Iran. For searching on different websites, the following keywords were used: TB, molecular method, MDR, embB gene, and codon 306. In cases where access to the full text of the papers required a specific username and password, only their abstracts were used that were available free of charge. All the articles and papers used in this study were surveyed by the authors. After searching by the EndNote software, all the articles were read and reviewed by each author. Two authors were committed to searching independently and they defined and arranged each time period on their own. Moreover, the authors surveyed the results of these articles meticulously and accurately. In the screening process, each of the two researchers evaluated the articles of their search to see if they met the qualification in order to be included in the study. In the selection process, a third researcher reviewed and selected the articles. At the evaluation stage, the quality of the articles was reviewed by a relevant expert in the field. After any disagreement between the authors regarding a survey, for selecting specific articles, data were entered in Excel data sheets (CEB603, Chino- Excel Technology). Then, a statistics consultant surveyed all the data in Excel datasheets and analyzed the same. The following data were extracted from original publications: a number of cases, websites, author, study location, year of the research, sample size, prevalence of embB306, and occurrence of mutations (yes or no).\textsuperscript{[21]}
Inclusion and exclusion criteria

Inclusion criteria were as follows: 1) research articles with full-text; 2) articles with abstract in English; 3) original articles. Excluded studies were as follows: 1) review and meta-analysis articles; 2) congress abstracts; 3) studies that were in languages other than English (abstract in English was acceptable); 4) studies that were not available for the authors in abstract or full-text; 5) studies that their sampling location and time of the study was unclear; 6) studies that were performed at the location of sampling and immediately after it; 7) studies that their data were not clear; and 8) letters to editors.²

Qualitative assessment of studies

PRISMA checklist consists of 27 different sections that are used for an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses.²

Data extraction

The variables of this study were the rate of mutation of the embB gene codon 306 and EMB resistance in patients with TB in different countries (1997–2015). According to different studies that were performed in various countries, it was concluded that high rates of prevalence of the mutation in embB306 can exist in different countries. Hence, the prevalence of embB306 in 37 studies was the main goal. The diversity of this prevalence was computed using the binomial distribution (confidence interval [CI]: 95%). Meta-analysis with the random effect model was applied to combine the prevalence among studies. There was sensitivity (how the uncertainty in the output of a mathematical model or system can be apportioned to different sources of uncertainty in its inputs) and heterogeneity among studies.

Statistical analysis

I² and Q (P < 0.10) statistical tests were used to assess this heterogeneity (I² static is the percentage of observed total variation across studies that are due to heterogeneity rather than chance. It is calculated as $I^2 = 100\% \times (Q − df)/Q$, where $Q$ is Cochran’s heterogeneity statistic and df degrees of freedom. Negative values of $I^2$ are put equal to zero so that $I^2$ lies between 0% and 100%. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. $Q$ is weighted of squares on a standardized scale. It is reported with a $P$ value with low $P$ values indicating the presence of heterogeneity. This test, however, is known to have potency in detecting heterogeneity, and it is suggested to use a value of 0.01 as a cutoff for significance. Conversely, $Q$ has too much power as a test of heterogeneity if the number of studies is large. Subgroup analyses were performed using Chi-square tests, and they were done for continents. Stratified analyses were subsequently performed with embB gene codon 306 isolates. Meta-analysis was carried out using the software package Meta R (Version 2.13.2, copyright 2011, and The R foundation for statistical computing).³ Publication bias in the subsample of studies was proved in this research. Some of these studies did not find a positive association between embB306 mutation and EMB resistance. This should be mentioned that excluded studies were considered as publication bias.

Results

Study information

According to the search database, out of a total of 100 articles, 37 were included and 63 were excluded for this meta-analysis, all of which are presented in Flowchart 1.

The authors searched for articles from 13 countries located in different continents. Thirty-seven original articles (published from 1997 to 2015) were reviewed from 100 articles. The total population obtained from the included articles in this meta-analysis was 6,931. Table 1 shows the major risk factors in this study that were considered for mutations in the embB306 gene of M. tuberculosis that was resistant to EMB. The risk factors were as follows: year of study, country, continent, methods, sample size, a mutation in embB306 (%), and a number of EMB-resistant cases.

Frequency of mutations in embB306 gene of M. tuberculosis resistant to EMB in the worldwide study population

According to Table 1, the highest rate of mutations in the embB306 gene of M. tuberculosis resistant to EMB was in...


Table 1: Data extracted from published documents about the frequency of mutations in *embB* of *Mycobacterium tuberculosis* resistant to ethambutol (EMB)

| References | Year of study | Country | Continent | Methods | Sample Size | Mutation *embB*306 (%) | Ethambutol resistant (no) |
|------------|--------------|---------|-----------|---------|-------------|------------------------|--------------------------|
| [17]       | 2011         | China   | Asia      | MIRU-VNTR, PCR, and DNA sequencing | 138 | 54.7 | 86 |
| [18]       | 2004         | Singapore | Asia     | PCR and DNA sequencing | 45 | 48 | 25 |
| [19]       | 2004         | Kuwait   | Asia      | PCR-RFLP and DNA sequencing | 25 | 85 | 50 |
| [20]       | 2007         | China    | Asia      | PCR and DNA sequencing | 74 | 45.2 | 17 |
| [21]       | 2001         | Germany  | Europe    | PCR-RFLP and DNA sequencing | 24 | 25 | 12 |
| [22]       | 2005         | USA      | North America | PCR-RFLP and DNA sequencing | 1,020 | 10 | 108 |
| [23]       | 2013         | Cuba     | South America | PCR | 30 | 53 | 10 |
| [24]       | 2006         | Germany  | Europe    | PCR and DNA sequencing | 159 | 68 | 101 |
| [25]       | 2013         | Poland   | Europe    | PCR and DNA sequencing | 50 | 47.05 | 17 |
| [26]       | 2014         | China    | Asia      | PCR | 158 | 61.7 | 81 |
| [27]       | 2009         | USA      | North America | PCR and DNA sequencing | 88 | 55 | 58 |
| [28]       | 2005         | China    | Asia      | PCR | 197 | 62.2 | 90 |
| [29]       | 2004         | Lativa   | Europe    | PCR and SSCP sequencing | 66 | 52 | 33 |
| [30]       | 1997         | USA      | North America | PCR and SSCP analysis | 19 | 42.1 | 16 |
| [31]       | 2012         | South Korea | Korea | PCR and DNA sequencing | 217 | 52.6 | 93 |
| [32]       | 2014         | Spain    | Europe    | PCR and DNA sequencing and LD-EMB array | 755 | 53.7 | 52 |
| [33]       | 2010         | China    | Asia      | PCR-SSCP and PCR-RFLP | 104 | 56.5 | 69 |
| [34]       | 2002         | South Korea | Asia | PCR and DNA sequencing | 26 | 26 | 21 |
| [35]       | 2009         | Russia   | Asia      | PCR and DNA sequencing | 254 | 41.5 | 183 |
| [36]       | 2000         | USA      | North America | PCR and sequencing | 75 | 68 | 28 |
| [37]       | 2005         | USA      | North America | PCR and sequencing and spoligotyping | 157 | 77.6 | 67 |
| [38]       | 2009         | Germany  | Europe    | PCR and sequencing | 63 | 73.3 | 36 |
| [39]       | 2001         | South Africa | Africa | PCR-RFLP and DNA sequencing | 70 | 31 | 11 |
| [40]       | 2010         | China    | Asia      | PCR and sequencing | 223 | 44.4 | 42 |
| [41]       | 2010         | France   | Europe    | PCR and sequencing | 52 | 50 | 28 |
| [42]       | 2014         | Spain    | Europe    | PCR and sequencing and LD-EMB array | 755 | 53.7 | 41 |
| [43]       | 2006         | South Africa | Africa | ARMS-PCR | 352 | 47 | 235 |
| [44]       | 2012         | Sierra Leone | Africa | PCR and sequencing | 97 | 46.7 | 15 |
| [45]       | 2010         | China    | Asia      | Multiplex PCR | 242 | 38 | 92 |
| [46]       | 2015         | China    | Asia      | PCR and sequencing | 262 | 57.8 | 109 |
| [47]       | 2015         | China    | Asia      | PCR and sequencing | 139 | 53.2 | 79 |
| [48]       | 2015         | USA      | North America | PCR | 175 | 20 | 61 |
| [49]       | 2009         | Germany  | Europe    | PCR | 109 | 4 | 60 |
| [50]       | 2002         | Russia   | Asia      | PCR-RFLP | 183 | 14 | 29 |
| [51]       | 2007         | USA      | North America | PCR | 201 | 30 | 14 |
| [52]       | 2009         | China    | Asia      | PCR and DNA sequencing | 101 | 34 | 51 |
| [53]       | 2004         | China    | Asia      | PCR and TDI-FP | 82 | 3 | 5 |

MIRU: Mycobacterial interspersed repetitive units, VNTR: Variable number of tandem repeats, PCR: Polymerase chain reaction, DNA: Deoxyribonucleic acid, RFLP: Restriction fragment length polymorphism, SSCP: Single-stranded conformation polymorphism, LD: Low-density, ARMS: Amplification-refractory mutation system, TDI: Templat ed-directed dye-terminator incorporation, FP: Fluorescence polarization

Kuwait in the Asia continent in 2004 (85%). PCR-RFLP in this study showed that 50 isolates were resistant to EMB. The lowest rate of mutations was in China in the Asia continent in 2004 (3%). PCR and fluorescence polarization-template-directed dye-terminator incorporation assay (FP-TDI) revealed that five isolates were resistant to EMB.

**Results of EMB resistant based on a year**

Graph 1 shows all 37 studies that were entered in this research based on the year of study (1997–2015). Final event rate was 0.41 (95% CI: 0.34–0.49). Moreover, the lowest event rate of EMB resistance was 0.05 (95% CI: 0.04–0.07) in Spain in 2014, and the highest event rate was 0.84 (95% CI: 0.68–1.01) in the USA in 1997. According to Graph 2, there was no significant relationship between the years of study and EMB resistance (*P* > 0.05). EMB resistance in *M. tuberculosis* has been decreased in recent years (*P* > 0.00). Publication bias occurs when articles that have a small sample size, very low rate of prevalence or incidence of outcomes, or those that do not present meaningful relationships between variables, have a smaller chance of being published. Therefore, publication bias can
Mohammadi, et al.: Mutations in embB306 Gene of Mycobacterium tuberculosis

Results of codon 306 mutations in \textit{embB} gene based on a year

The final occurrence rate for \textit{emb}306B gene mutations based on year was 0.43 (95% CI: 0.36–0.51). The lowest
occurrence rate of mutations was 0.03 (95% CI: 0.01–0.07) in China in 2014. The highest occurrence rate was 0.78 (95% CI: 0.71–1.84) and was observed in the USA in 2005. These results are demonstrated in Graph 5. There is a significant relationship between the years and the embB306 gene mutation rate at codon 306. Graph 6 shows that the mutation in this gene has increased in recent years ($P < 0.00$).

**Discussion**

Nowadays, EMB-resistant TB is a major challenge and problem related to the treatment of patients with TB. Diagnosis of EMB resistance may enhance patient management, as standardized treatment of MDR-TB, because EMB-resistant *M. tuberculosis* has a prominent role in EMB resistance. According to different research studies, prominent risk factors of mutations in the *embB* gene and resistance to EMB can include the following: resistance to three or more drugs, gender, diabetes, malnutrition, anemia, alcohol addiction, tobacco addiction, and retreatment.$^{[48,54]}$ Mutations in the *embB* gene have been reported in EMB-resistant *M. tuberculosis* isolates using different molecular methods.$^{[55]}$ The aim of this study was to determine the rate of frequency of mutations in the *embB306* gene of *M. tuberculosis* that was resistant to EMB, according to a systematic review and meta-analysis. Results in this study showed that the highest and lowest rates of mutations in the *embB306* gene of *M. tuberculosis* resistant to EMB had occurred.
in 2005 (77.6%) and 2004 (3%), respectively. Point mutations in \textit{embB} codon 306, which occur in 30–69% of EMB-resistant clinical strains, are associated with resistance to EMB. In addition, in retreated TB patients, the rate of resistance to EMB was observed to be 50% in some regions.\cite{56} According to the 2014 national report on Mycobacteriosis, out of 16,000 cases of pulmonary TB that are diagnosed in Mexico each year, approximately 200 (1.3%) cases are drug-resistant TB.\cite{57} In general, the final occurrence rate of codon 306 mutations in the \textit{embB} gene of EMB-resistant \textit{M. tuberculosis} isolates in this study was estimated to be 0.43 with 95% CI: 0.36–0.51. Researchers have shown that 18–78% of \textit{M. tuberculosis} isolates with \textit{embB} mutations have an \textit{embB} codon 306 substitutions. On the other hand, 30–50% of EMB resistance is without mutation in \textit{embB} codon 306, so they are not detectable by molecular methods.\cite{58} Moreover, according to the current work’s results, the lowest prevalence rate of codon 306 mutations in \textit{embB} gene of EMB-resistant \textit{M. tuberculosis} isolates was 0.03 (95% CI: 0.01–0.07) that was observed in China in 2014; and the highest prevalence rate was 0.78 (95% CI: 0.71–1.84) in the USA in 2005. The phenotype and genotype of EMB resistance are correlated with the mutations in the \textit{embCAB} operon. These point mutations change the nucleotide positions and amino acid residue positions that can include A → G (918) (Met → Val (306)), G → A (918) Met → Ile (306), G → C (918) Met → Ile (306). However, the majority of \textit{embB} mutations are observed in small sections of codons 306–497.\cite{59} Genotypic analysis in Armenia showed that 173 drug-resistant TB isolates were resistant to INH and RIF or both. In a study conducted by Margaryan \textit{et al.}, mutations at codon 306 of the \textit{embB} gene were proven in 20.8% and the missing band of the wild type was proven in 12.71% of the isolates.\cite{60} In another study that was conducted in Iran, it was reported that out of 50 strains of \textit{M. tuberculosis} that were isolated from patients with TB, a mutation in the \textit{embB} gene was detected in all of the seven EMB-resistant isolates and 42.71% of the cases were detected as MDR.\cite{61} A study in the USA proved that MDR strains of \textit{M. tuberculosis} were related to a mutation in the \textit{embB} gene.\cite{62} In the results of the present work, a symmetric distribution was detected in the rate of \textit{embB} gene mutation at codon 306 among EMB-resistant isolates and mutation in this gene has been increased in recent years. Mutant \textit{embB}306 could serve as a marker for TB cases that are at increased risk for developing MDR.\cite{63} This mutant gene can be transferred between different communities, and risk factors such as poor living conditions, unhealthy work environments, lack of access to proper medical care, HIV, and air pollution can have effects on it.\cite{64} The lowest occurrence rate of codon 306 mutation in the \textit{embB} gene that was resistant to EMB in this study was in the European continent with a 0.458 occurrence rate and the highest occurrence rate was 0.516 in the Asia continent. The final occurrence rate of EMB resistance was 0.41% with 95% CI: 0.34–0.49. On the other hand, the lowest and highest occurrence rates of EMB resistance were 0.05 (95% CI: 0.04–0.07) in Spain in 2014 and 0.84 (95% CI: 0.68–1.01) in the USA in 1997, respectively. There was no significant relationship between the year of occurrence of mutation and EMB resistance. Differences between data in different studies are probably due to regional variations in the mutation frequencies in the EMB-resistant isolates.\cite{65} In a study conducted in Iran in 2019, phenotypic susceptibility testing showed that 3.25% of 307 clinical isolates of TB were resistant to EMB. PCR showed that the mutation rate in 10 EMB-resistant TB strains was 20% (10% mutation in \textit{embB} codon 306 Met–Val and 10% in \textit{embC} codon 270 Thr–Ile).\cite{66} According to other research studies in Iran that were performed in 2015, the prevalence of TB was 66.4%, among which 7% had extrapulmonary TB.\cite{67} Efficiency of the Bacillus Calmette-Guerin (BCG) vaccine has been proved, but the type, dose, and strength of the vaccine strain, age, and inoculation technique are different and cause Mendelian susceptibility to mycobacterial disease (MSMD) primary immunodeficiency (PID).\cite{68} According to a study in Pakistan in 2019, the majority of \textit{M. tuberculosis} isolates were CAS/Delhi strain-type and MDR (76.5%), and mutations with resistance were observed in katG, \textit{rpoB}, \textit{pncA}, \textit{embB}, \textit{gyrA}, \textit{rrs}, \textit{rpsL}, and \textit{rrs} genes.\cite{69} Drug-resistant TB isolates and their genes circulate among the population and they are transmitted between different communities worldwide. Therefore, performing more epidemiological studies and designing appropriate disease control plans are of great importance.\cite{70} In this study, \textit{embB} codon 306 was investigated. Since other codons of this gene contribute to the development of EMB resistance as well, thus, it is advisable to research the rate of association of other codons with EMB resistance, in terms of systematic review and meta-analysis, to obtain comprehensive results. There are more articles on this topic that need...
to be reviewed. However, they are only available after purchase, the same as the articles used in this research. In fact, access to all the articles was restricted. In addition, extra funds were needed to carry out the study and pay the researchers. In this study, the risk factors such as year, different cities of the world, and different countries were investigated for a prevalence rate of mutation of embB codon 306 of M. tuberculosis resistant to EMB and the study included the world population over a span of several years. Therefore, comprehensive and complete results of the trend of this gene’s prevalence in the world were obtained in this study.

Conclusions
According to the current study’s results, the highest rate of mutation in the embB306 gene of M. tuberculosis that was resistant to EMB was observed in the Asia continent in 2004. EMB resistance in M. tuberculosis has been decreased over the past few years, but a mutation in this gene has been increased. The association between a mutation in the embB306 gene of M. tuberculosis and resistance to EMB was proved in this study. Therefore, the results of this study can have a positive impact on the control and prevention of MDR M. tuberculosis among different communities.

Ethical considerations
Ethical issues (including plagiarism, data fabrication, double publication) were carefully monitored by the authors and they were eliminated to the best of their capabilities.

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Conflicts of interest
There are no conflicts of interest.

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References
1. Zaman K. Tuberculosis: A global health problem. J Health Popul Nutr 2010;28:111-3.
2. Ramazanzadeh R, Roshani D, Shakib P, Rouhi S. Prevalence and occurrence rate of Mycobacterium tuberculosis Haarlem family multi-drug resistant in the worldwide population: A systematic review and meta-analysis. J Res Med Sci 2015;20:76-88.
3. Leibert E, Rom WN. New drugs and regimens for treatment of TB. Expert Rev Anti Infect Ther 2010;8:801-13.
4. Chihabra N, Aseri ML, Dixit R, Gaur S. Pharmacotherapy for multidrug resistant tuberculosis. J Pharmacol Pharmacothe 2012;3:98-104.
5. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 2014;6:25-64.
6. Smith T, Wolff KA, Nguyen L. Molecular biology of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol 2013;374:53-80.
7. Mohammadi B, Mohajeri P, Rouhi S, Ramazanzadeh R. The relationship between embB306 and embB406 mutations and ethambutol resistant in Mycobacterium tuberculosis isolated from patients in west of Iran. Med J Islam Repub Iran 2018;32:117.
8. Adebayo OA, Adesanoye OA, Abolaji OA, Kehinde AO, Adaramoye OA. First-line antituberculosis drugs disrupt endothelial balance and induce ovarian and uterine oxidative stress in rats. J Basic Clin Physiol Pharmacol 2018;29:131-40.
9. Angala SK, Belardinelli JM, Huc-Claustre E, Wheat WH, Jackson M. The cell envelope glycoconjugates of Mycobacterium tuberculosis. Crit Rev Biochem Mol Biol 2014;49:361-99.
10. Alderwick LJ, Harrison J, Lloyd GS, Birch HL. The Mycobacterial cell wall—peptidoglycan and arabinogalactan. Cold Spring Harb Perspect Med 2015;5:a021113.
11. Brossier F, Sougakoff W, Bernard C, Petrou M, Adeyema K, Pham A, et al. Molecular analysis of the embCAB locus and embR gene involved in ethambutol resistance in clinical isolates of Mycobacterium tuberculosis in France. Antimicrob Agents Chemother 2015;59:4800-8.
12. Dookie N, Rambaran S, Padayatchi N, Mahomed S, Naidoo K. Evolution of drug resistance in Mycobacterium tuberculosis: A review on the molecular determinants of resistance and implications for personalized care. J Antimicrob Chemother 2018;73:1138-51.
13. Zhao L, Sun Q, Liu H, Wu X, Xiao T, Zhao X, et al. Analysis of embCAB mutations associated with ethambutol resistance in multidrug-resistant Mycobacterium tuberculosis isolates from China. Antimicrob Agents Chemother 2015;59:2045-50.
14. Naser Esfahani B, Zarkesh FS, Rezaei Yazdi H, Radaee T. Detection of embB gene mutations in EM-resistant Mycobacterium tuberculosis isolates from Isfahan province by PCR-SSCP and direct sequencing. Jundishapur J Microbiol 2016;9:e39594.
15. Al-Saeedi M, Al-Hajoj S. Diversity and evolution of drug resistance mechanisms in Mycobacterium tuberculosis. Infect Drug Resist 2017;10:333-42.
16. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med 2009;6:339.
17. Shi D, Li L, Zhao Y, Jia Q, Li H, Coulter C, et al. Characteristics of embB mutations in multidrug-resistant Mycobacterium tuberculosis isolates in Henan, China. J Antimicrob Chemother 2011;66:2240-7.
18. Lee AS, Othman SNK, Ho YM, Wong SY. Novel mutations within the embB gene in ethambutol-susceptible clinical isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2004;48:4447-9.
19. Ahmad S, Mokaddas E, Jaber AA. Rapid detection of ethambutol-resistant Mycobacterium tuberculosis strains by PCR-RFLP targeting embB codons 306 and 497 and iniA codon 501 mutations. Mol Cell Probes 2004;18:299-306.
20. Shi R, Zhang J, Otomo K, Zhang G, Sugawara I. Lack of correlation between embB mutation and ethambutol MIC in...
Mycobacterium tuberculosis clinical isolates from China. Antimicrob Agents Chemother 2007;51:4515-7.

21. Rinder H, Mieskes KT, Tortoli E, Richter E, Casal M, Vaquero M, et al. Detection of embB codon 306 mutations in ethambutol resistant Mycobacterium tuberculosis directly from sputum samples: A low-cost, rapid approach. Mol Cell Probes 2001;15:37-42.

22. Hazbon MH, del Valle MB, Guerrero MI, Vara-Masíl M, Fillioli I, Cavatore M, et al. Role of embB codon 306 mutations in Mycobacterium tuberculosis revisited: A novel association with broad drug resistance and IS6110 clustering rather than ethambutol resistance. Antimicrob Agents Chemother 2005;49:3794-802.

23. Guerrero E, Lemus D, Yzquierdo S, Vílchez G, Munoz M, Montoro E, et al. Association between embB mutations and ethambutol resistance in Mycobacterium tuberculosis isolates from Cuba and the Dominican Republic: Reproducible patterns and problems. Rev Argent Microbiol 2013;45:21-6.

24. Plinke C, Rüscher-Gerdes S, Niemann S. Significance of mutations in embB codon 306 for prediction of ethambutol resistance in clinical Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother 2006;50:1900-2.

25. Bakula Z, Napierkowska A, Bielecki J, Augustynowicz-Kopiec E, Zwolńska Z, Jagiełski T. Mutations in the embB gene and their association with ethambutol resistance in multidrug-resistant Mycobacterium tuberculosis clinical isolates from Poland. Biomed Res Int 2013;2013:167954.

26. Zhang Z, Wang Y, Pang Y, Kam KM. Ethambutol resistance as determined by broth dilution method correlates better than sequencing results with embB mutations in multidrug-resistant Mycobacterium tuberculosis isolates. J Clin Microbiol 2014;52:638-41.

27. Starks AM, Gumusboga A, Plikaytis BB, Shinnick TM, Posey JE. Mutations at embB Codon 306 are an important molecular indicator of ethambutol resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2009;53:1061-6.

28. Wu XQ, Liang JQ, Zhang JX, Lu Y, Li HM, Zhang GY, et al. Detection and evaluation of the mutations of embB gene in ethambutol-susceptible and resistant Mycobacterium tuberculosis isolates from China. Chinese Med J 2005;118:1739-41.

29. Tracevská T, Jansone I, Nodieva A, Marga O, Skenders G, Baumanis V. Characterisation of rpsL, rrs and embB mutations associated with streptomycin and ethambutol resistance in Mycobacterium tuberculosis. Res Microbiol 2004;155:830-34.

30. Sreevatsan S, Stockbauer KE, Pan X, Kreiswirth BN, Mohgazeh SL, Jacobs W, et al. Ethambutol resistance in Mycobacterium tuberculosis: Critical role of embB mutations. Antimicrob Agents Chemother 1997;41:1677-81.

31. Kim SH, Park M, Woo H, Tharmalingam N, Lee G, Rhee KJ, et al. Inhibitory effects of anthocyanins on secretion of Helicobacter pylori CagA and VacA toxins. Int J Med Sci 2012;9:838-42.

32. Moure R, Espanol M, Tudo G, Vicente E, Coll P, Gonzalez-Martin J, 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. J Antimicrob Chemother 2014;69:2299-300.

33. Li W, Xue X, Chu Y, Qiu Y, Song J, Zheng J. Correlation between ethambutol-resistance and embB gene mutation in Mycobacterium tuberculosis. Wei Sheng Yan Jiu 2010;39:506-8.

34. Lee H, Myoung HJ, Bang HE, Bai GH, Kim SJ, Kim JD, et al. Mutations in the embB locus among Korean clinical isolates of Mycobacterium tuberculosis resistant to ethambutol. Yonsei Med J 2002;43:59-64.

35. Afanas'ev MV, Borovskaia AD, Il'ina EN, Smirnova TG, Lariosova EE, Kuz'min AV, et al. Detection of mutations in codon 306 of the embB gene for molecular genetic characterization of clinical Mycobacterium tuberculosis strains. Probl Tuberk Bolezn Legk 2009;2009:48-53.

36. Ramaswamy SV, Amin AG, GökSEL S, Stager CE, Dou SJ, El Sahly H, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2000;44:326-36.

37. Parsons LM, Salfinger M, Clobridge A, Dormandy J, Mirabello L, Polletta VL, et al. Phenotypic and molecular characterization of Mycobacterium tuberculosis isolates resistant to both isoniazid and ethambutol. Antimicrob Agents Chemother 2005;49:2218-25.

38. Hillemann D, Rüsch-Gerdes S, Richter E. Feasibility of the GenoType MBBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 2009;47:1767-72.

39. Van Rie A, Warren R, Mashanga I, Jordaan AM, Van Der Spuy G, Richardson M, et al. Analysis of a limited number of gene codons can predict the majority of drug resistance of Mycobacterium tuberculosis in a high incidence area. J Clin Microbiol 2001;39:636-41.

40. Hu Y, Hoffner S, Jiang W, Wang W, Xu B. Genetic characterisation of drug-resistant Mycobacterium tuberculosis in rural China: A population-based study. Int J Tuberc Lung Dis 2010;14:210-16.

41. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by MBBDRsl test of complex resistance mechanisms to second-line drugs and ethambutol in multidrug-resistant strains of Mycobacterium tuberculosis. J Clin Microbiol 2010;48:1689-93.

42. Moure R, Español M, Tudó G, Vicente E, Coll P, Gonzalez-Martin J, 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. J Antimicrob Chemother 2014;69:947-54.

43. Johnson R, Jordaan A, Pretorious L, Engelcke E, Van Der Spuy G, Kewley C, et al. Ethambutol resistance testing by mutation detection. Int J Tuberc Lung Dis 2006;10;68-73.

44. Feuerriegel S, Oberhauser B, George AG, Dafae F, Richter E, Rüscher-Gerdes S, et al. Sequence analysis for detection of first-line drug resistance in Mycobacterium tuberculosis strains from a high-incidence setting. BMC Microbiol 2012;12:90.

45. Luo T, Zhao M, Li X, Xu P, Gui X, Pickering S, et al. Selection of mutations to detect multidrug-resistant Mycobacterium tuberculosis strains in Shanghai, China. Antimicrob Agents Chemother 2010;54:1075-81.

46. Xu Y, Jia H, Huang H, Sun Z, Zhang Z. Mutations found in embCAB, embR, and ubiA genes of ethambutol-sensitive and-resistant Mycobacterium tuberculosis clinical isolates from China. Biomed Res Int 2015;2015:951706.

47. Zhao LL, Sun Q, Liu HC, Wu XC, Xiao TY, Zhao XQ, et al. Analysis of embCAB mutations associated with ethambutol resistance in multidrug-resistant Mycobacterium tuberculosis isolates from China. Antimicrob Agents Chemother 2015;59:2045-50.

48. Cuevas-Córdoba B, Juárez-Eusebio DM, Almaraz-Velasco R, Muñiz-Salazar R, Laniado-Laborin R, Zenteno-Cuevas R. Mutation at embB codon 306, a potential marker for the identification of multidrug resistance associated with ethambutol in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2015;59:5455-62.
49. Perdigao J, Macedo R, Ribeiro A, Brum L, Portugal I. Genetic characterisation of the ethambutol resistance-determining region in Mycobacterium tuberculosis: Prevalence and significance of embB306 mutations. Int J Antimicrobial Agents 2009;33:334-8.

50. Mokrousov I, Narvskaya O, Limeschenko E, Otten T, Vyshnevskiy B. Detection of ethambutol-resistant Mycobacterium tuberculosis strains by multiplex allele-specific PCR assay targeting embB306 mutations. J Clin Microbiol 2002;40:1617-20.

51. Shen X, Shen GM, Wu J, Gui XH, Li X, Mei J, et al. Association between embB codon 306 mutations and drug resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2007;51:2618-20.

52. Lin H, Liu J, Chen L, Jing KH, Shen J, Zhan J, et al. Molecular characterization of embB306 gene in Mycobacterium tuberculosis isolates from tuberculosis patients in Chongqing municipality. Zhonghua Yu Fang Yi Xue Za Zhi 2009;43:223-6.

53. Wang Y, Zhang WH, Zhao JR, Luo M, Zhang ZX, Bai YJ, et al. Detect embB306 gene mutation associated with EMB resistance of M. tuberculosis by TDI-FP technology. Chinese J Lab Diagn 2004;27:179-82.

54. Margaryan H, Rüsch-Gerdes S, Hayrapetyan A, Mirzoyan A. Ethambutol-resistance testing by mutation detection using MTBDRsl. Int J Mycobacteriol 2016;5:S50.

55. Ramazanzadeh R, Mohammadi B, Mohajeri P. Mutations in embB gene associated with resistance to ethambutol in Mycobacterium tuberculosis strains isolated from TB patients in the west of Iran (2014–15). Int J Mycobacteriol 2016;5:S140.

56. Munir S, Mahmood N, Shahid S, Khan MI. Molecular detection of isoniazid, rifampin and ethambutol resistance to M. tuberculosis and M. bovis in multidrug resistant tuberculosis (MDR-TB) patients in Pakistan. Microb Pathog 2017;110:262-74.

57. Goude R, Amin AG, Chatterjee D, Parish T. The arabinosyltransferase EmbC is inhibited by ethambutol in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2009;53:4138-46.

58. Operario DJ, Koeppel AF, Turner SD, Bao Y, Pholwat S, Banu S, et al. Prevalence and extent of heteroresistance by next generation sequencing of multidrug-resistant tuberculosis. PLoS One 2017;12:e0181284.

59. Khosravi A, Sirous M, Abdi M, Ahmadihosravi N. Characterization of the most common embCAB gene mutations associated with ethambutol resistance in Mycobacterium tuberculosis isolates from Iran. Infect Drug Resist 2019;12:579-84.

60. Babamahmoodi F, Alikhani A, Yazdani Charati J, Ghovvati A, Ahangarkani F, Delavarian L, Babamahmoodi A. Clinical epidemiology and paraclinical findings in tuberculosis patients in north of Iran. Biomed Res Int 2015;2015:1-5.

61. Rezai MS, Ahangarkani F, Sadeghi R, Mahdavi MR. Evaluation of children with complication of BCG vaccination in north of Iran. Int J Pediatr 2017;5:4479-88.

62. Jabbar A, Phelan JE, de Sessions PF, Khan TA, Rahman H, Khan SN, et al. Whole genome sequencing of drug resistant Mycobacterium tuberculosis isolates from a high burden tuberculosis region of North West Pakistan. Sci Rep 2019;9:14996.