Review Article

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

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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 embB*306 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 *embB*306 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 *embB*306 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 *embB*306 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.[1] 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.

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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.[11-13] Approximately 50–70% of *M. tuberculosis* strains that are resistant to EMB have mutations in *embB*306.[10] Resistance to EMB has been reported in 4% of isolates of *M. tuberculosis* that were isolated from patients.[5] Moreover, it was observed in a report that among 131 isolates of *M. tuberculosis*, 54.19% were EMB-resistant.[11] Several meta-analyses have been conducted on mutations in *embB*306 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 *embB*306.[13] 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 *embB*306 mutations.[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 *embB*306 gene in this study showed that both isolates of EMB-resistant *M. tuberculosis* were mutated.[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.[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.[2,14] On the other hand, in MDR-TB, 50–70% are EMB-resistant and harbor mutations in codon 306 in *embB* genes.[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.[6] 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 *embB*306 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.[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 embB*306 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.pcs.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 codon306. 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 *embB*306, and occurrence of mutations (yes or no).[2]

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 *embB*306 can exist in different countries. Hence, the prevalence of *embB*306 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 I2 = 100% × (Q − df)/Q, where Q is Cochran's heterogeneity statistic and df degrees of freedom. Negative values of I2 are put equal to zero so that I2 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).[2] Publication bias in the subsample of studies was proved in this research. Some of these studies did not find a positive association between *embB*306 mutation and EMB resistance. This should be mentioned that excluded studies were considered as publication bias.

Results

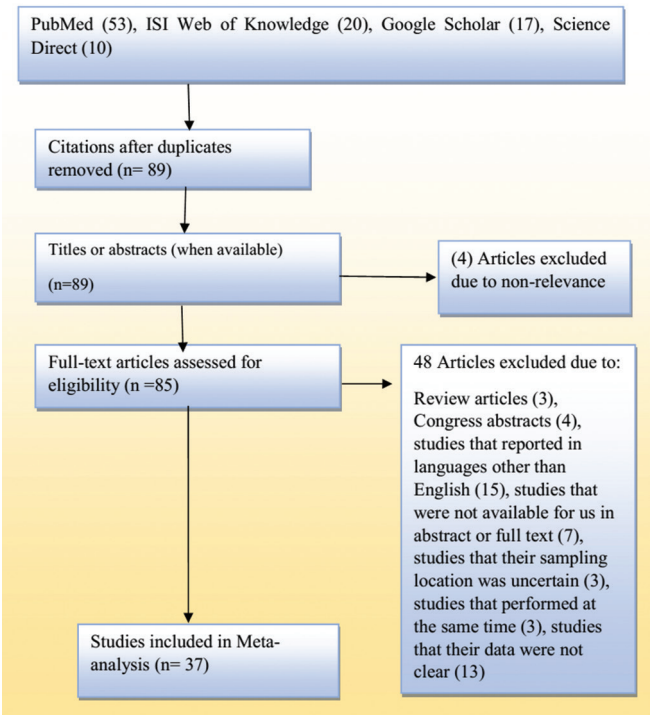
Study information

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 *embB*306 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 *embB*306 (%), and a number of EMB-resistant cases.

Frequency of mutations in *embB*306 gene of *M. tuberculosis* resistant to EMB in the worldwide study population

According to Table 1, the highest rate of mutations in *the embB*306 gene of *M. tuberculosis* resistant to EMB was in



Flowchart 1: Flow diagram for study selection

Table 1: Data extracted from published documents about the frequency of mutations in *embB*306 of *Mycobacterium tuberculosis* resistant to ethambutol (EMB)

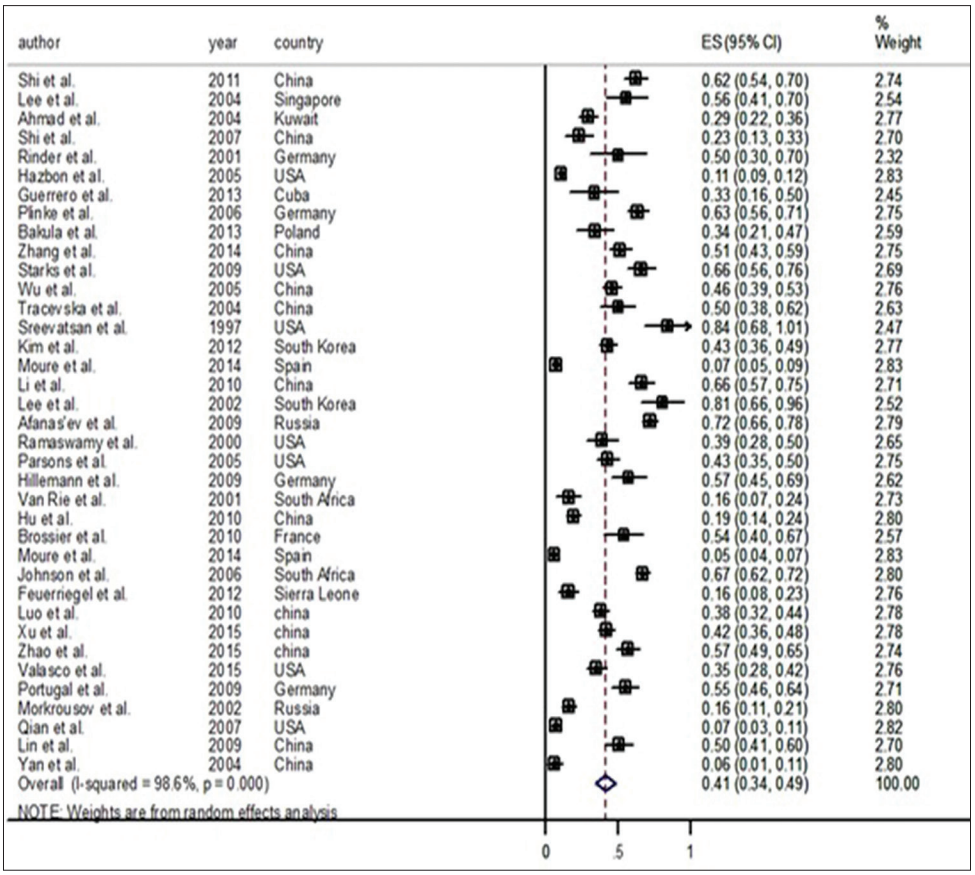
References	Year of study	Country	Continent	Methods	Sample Size	Mutation *embB*306 (%)	Ethambutol resistant (no)
[17]	2011	China	Asia	MIRU-VNTR, PCR, and DNA sequencing	138	54.7	86
[18]	2004	Singapore	Asia	PCR and DNA sequencing	45	48	25
[19]	2004	Kuwait	Asia	PCR-RFLP and DNA sequencing	25	85	50
[20]	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	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	Asia	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	56.52	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: Templatedirected dye-terminator incorporation, FP: Fluorescence polarization

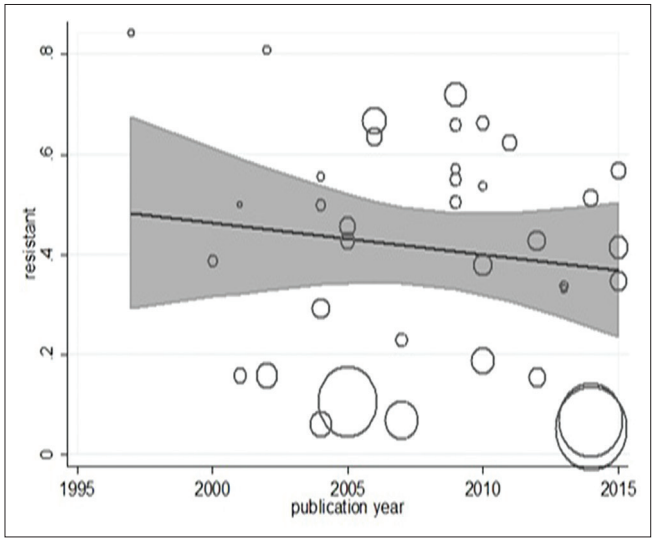
Kuwait in the Asia continent in 2004 (85%). PCR-RFLP in this study showed that 50 isolates were resistant to EMB. The lowest rate of mutations was in China in the Asia continent in 2004 (3%). PCR and fluorescence polarization-template-directed dye-terminator incorporation assay (FP-TDI) revealed that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

Results of EMB resistant based on a year

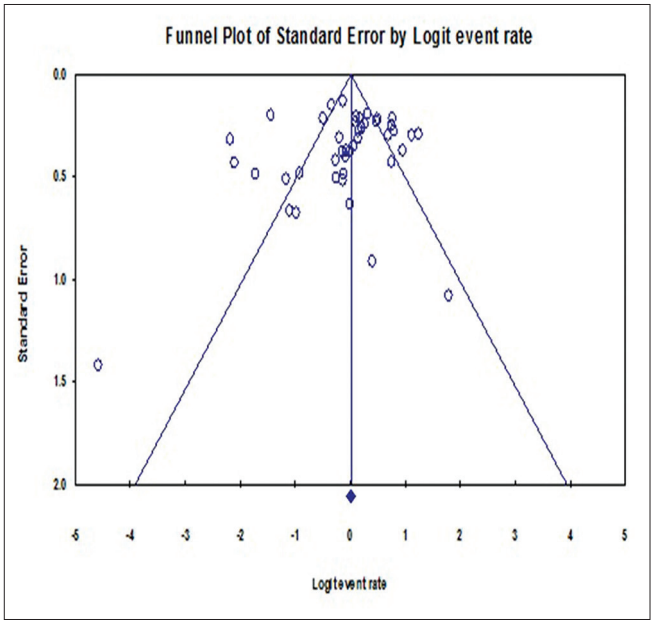
Graph 1 shows all 37 studies that were entered in this research based on the year of study (1997–2015). Final event rate was 0.41 (95% CI: 0.34–0.49). Moreover, the lowest event rate of EMB resistance was 0.05 (95% CI: 0.04–0.07) in Spain in 2014, and the highest event rate was 0.84 (95% CI: 0.68–1.01) 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



Graph 1: The random effect model for prevalence of ethambutol (EMB) resistance in various studies during the period from 1997 to 2015



Graph 2: Linear relationship between EMB resistance and year of study



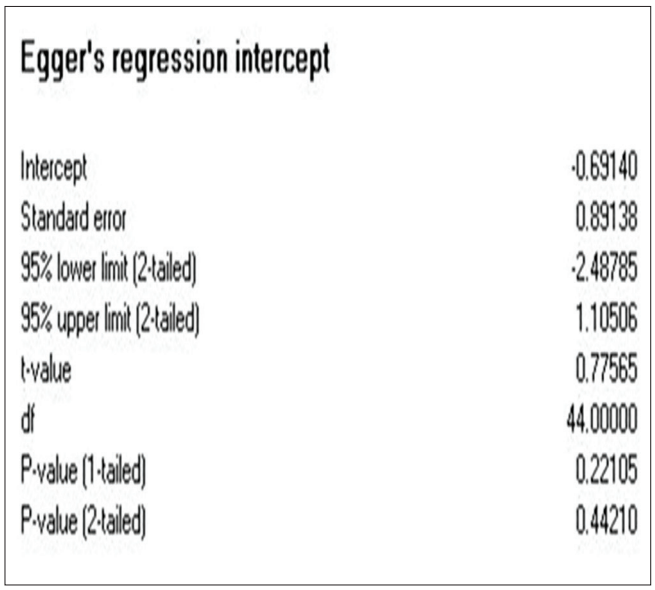
Graph 3: Funnel plot representing the proportional and symmetric distributions in this study

skew the results of a meta-analysis study on the wrong side. In Graph 3, the funnel plot drawn for the final papers selected in this study shows a perfectly symmetrical form that means there is no publication bias in the selected articles. In support of this claim, the Egger's test results in Graph 4 show that the assumption of publication bias in the study is not signifiant (bias = −0.69, *P* value = 0.442).

Results of codon 306 mutations in *embB* gene based on a year

The final occurrence rate for *emb*306B gene mutations based on year was 0.43 (95% CI: 0.36–0.51). The lowest

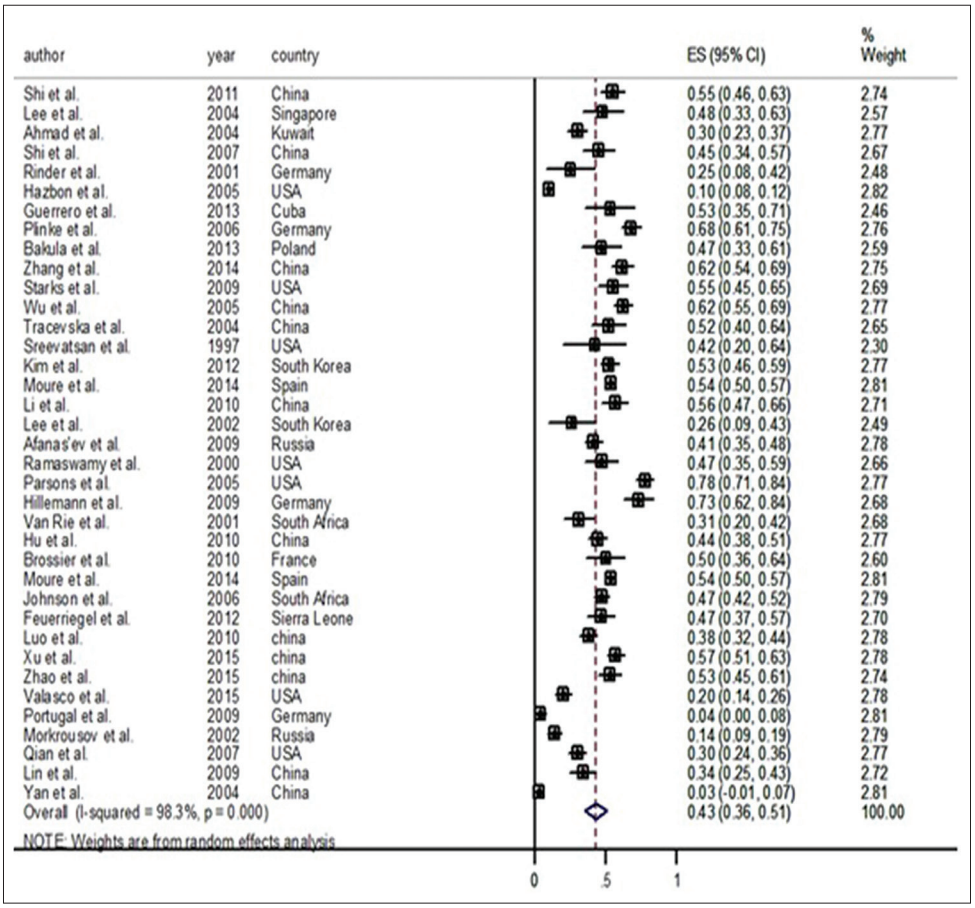
occurrence rate of mutations was 0.03 (95% CI: 0.01–0.07) in China in 2014. The highest occurrence rate was 0.78 (95% CI: 0.71–1.84) and was observed in the USA in 2005. These results are demonstrated in Graph 5. There is a significant relationship between the years and the *embB*306 gene mutation rate at codon 306. Graph 6 shows that the mutation in this gene has increased in recent years ($P < 0.00$).



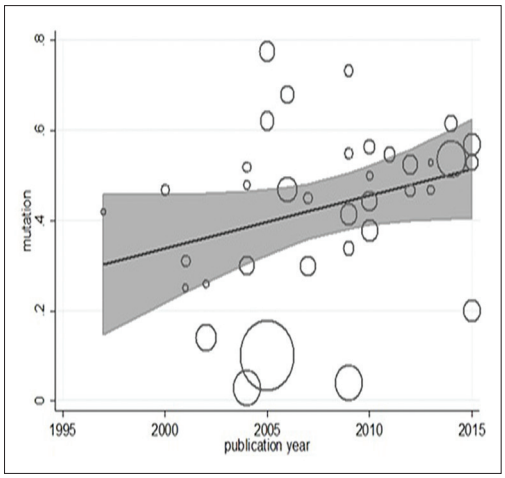
Graph 4: Bias determination results

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 *embB*306 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 *embB*306 gene of *M. tuberculosis* resistant to EMB had occurred



Graph 5: The random effect model for prevalence of *embB*306 gene mutation in various studies in a period between 1997 and 2015



Graph 6: Linear relationship between *embB*306 gene mutations with a year of study

in 2005 (77.6%) and 2004 (3%), respectively. Point mutations in *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.[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.[57] In general, the final occurrence rate of codon 306 mutations in the *embB* gene of EMB-resistant *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 *M. tuberculosis* isolates with *embB* mutations have an *embB* codon 306 substitutions. On the other hand, 30–50% of EMB resistance is without mutation in *embB* codon 306, so they are not detectable by molecular methods.[27] Moreover, according to the current work's results, the lowest prevalence rate of codon 306 mutations in *embB* gene of EMB-resistant *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 *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 *embB* mutations are observed in small sections of codons 306–497.[25] 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 *et al*., mutations at codon 306 of the *embB* gene were proven in 20.8% and the missing band of the wild type was proven in 12.71% of the isolates.[54] In another study that was conducted in Iran, it was reported that out of 50 strains of *M. tuberculosis* that were isolated from patients with TB, a mutation in the *embB* gene was detected in all of the seven EMB-resistant isolates and 42.71% of the cases were detected as MDR.[55] A study in the USA proved that MDR strains of *M. tuberculosis* were related to a mutation in the *embB* gene.[58] In the results of the present work, a symmetric distribution was detected in the rate of *embB* gene mutation at codon 306 among EMB-resistant isolates and mutation in this gene has been increased in recent years. Mutant *embB*306 could serve as a marker for TB cases that are at increased risk for developing MDR.[56] 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.[2] The lowest occurrence rate of codon 306 mutation in the *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.[13] 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 *embB* codon 306 Met–Val and 10% in *embC* codon 270 Thr–Ile).[59] 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.[60] 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).[61] According to a study in Pakistan in 2019, the majority of *M. tuberculosis* isolates were CAS/Delhi strain-type and MDR (76.5%), and mutations with resistance were observed in *kat*G, *rpo*B, *pnc*A, *embB*, *gyr*A, *rrs*, *rps*L, and *rrs* genes.[62] 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.[2,62] In this study, *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 embB*306 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 *embB*306 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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