Diagnostic accuracy of a molecular drug susceptibility testing method for the anti-tuberculosis drug, ethambutol: a systematic review and meta-analysis
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Abstract
Ethambutol (EMB) is a first-line anti-tuberculosis drug; however drug resistance to EMB has been increasing. Molecular drug susceptibility testing (DST), based on the embB gene, has recently been used for rapid identification of EMB resistance. The aim of this meta-analysis was to establish the accuracy of molecular assay for detecting drug resistance to EMB. PubMed, Embase and Web of Science were searched according to a written protocol and explicit study selection criteria. Measures of diagnostic accuracy were pooled using a random effects model. A total of 34 studies were included in the meta-analysis. The respective pooled sensitivity and specificity were 0.57 and 0.93 for PCR-DNA sequencing that targeted the embB 306 codon; 0.76 and 0.89 for PCR-DNA sequencing that targeted the embB 306, 406, and 497 codons; 0.64 and 0.70 for detecting M. tuberculosis isolates, 0.55 and 0.78 for detecting M. tuberculosis sputum specimens using the GenoType MTBDRsl test; 0.57 and 0.87 for Pyrosequencing, and 0.35 and 0.98 for PCR-RFLP. The respective pooled sensitivity and specificity were 0.55 and 0.92 when using the lower EMB concentration as the reference standard, 0.67 and 0.73 when using the higher EMB concentration as the reference standard, and 0.60 and 1.0 when using multiple reference standards. PCR-DNA sequencing using multiple sites of the embB gene as detection targets, including embB 306, 406 and 497, can be a rapid method to preliminarily screen for EMB resistance, but does not fully replace phenotypic DST. Of the reference DST methods examined, the agreement rates were the best using MGIT 960 for molecular DST and using PM on Middlebrook 7H10 media.

Introduction
Tuberculosis (TB) is one of the most serious infectious diseases in the world. According to the 2013 Global Tuberculosis report by the World Health Organization (WHO), in 2012 an estimated 450 000 people developed multidrug-resistant TB (MDR-TB) and there were approximately 170 000 deaths due to MDR-TB worldwide [1]. MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) are among the greatest threats to the success of TB control in the world [2, 3]. Ethambutol (EMB) is one of the first-line drugs included in the directly observed, treatment short-course antitubercular regimen recommended by the WHO [3]. EMB is commonly used in combination with isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA) to treat TB, particularly when treating MDR-TB and XDR-TB [3]. EMB has also been found to protect companion drugs against resistance, particularly INH [4]. Initially, EMB was effective for preventing treatment failures caused by M. tuberculosis isolates resistant to other anti-tuberculosis drugs; however, the resistant rate of EMB has gradually increased in some regions and is close to 50% in TB patients that are re-treated [5-7]. In China, the
resistance rate for EMB increased from 6.52% in 2007 to 17.18% in 2010 [8]. Therefore, rapid and effective methods of drug susceptibility testing (DST) for \textit{M. tuberculosis} resistance to EMB are vital so that clinicians can make appropriate, rational decisions regarding drugs that will be most effective for treatment. Conventional, phenotypic DST of EMB is the most commonly used approach in many countries. The WHO describes phenotypic DST as the gold standard testing method; however phenotypic DST is not efficient when used clinically, due to the long turnaround time. Recently, the development of molecular technology has allowed molecular assay testing methods based on the detection of the \textit{embB} gene, to become more widely used for diagnosing TB drug resistance. These methods are attractive, since they can shorten the turnaround time for testing to less than 1 day [9]. Many previous studies have examined the performance of molecular assays, when testing for EMB resistance based on the \textit{embB} gene; however, the sensitivity and specificity results have been inconsistent. In the current study, a systematic review and meta-analysis was performed, evaluating the overall accuracy of using molecular assays to test for EMB resistance in \textit{M. tuberculosis} isolates and sputum samples. Factors associated with the heterogeneity of findings between studies were also identified and the effects of study and test characteristics on diagnostic accuracy were assessed.

\section*{Methods}

\subsection*{Systematic Review}

This systematic review was performed according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) set by the PRISMA Group [10].

\subsection*{Search strategy and selection criteria}

A search for biomedical articles in English, which had been published between January 1990 and September 2013, was conducted using the electronic databases PubMed, Embase and Web of Science. The search terms used were as follows: ethambutol, \textit{embB}, and tuberculosis. Selected studies met the following inclusion criteria: The studies (1) used molecular assays for testing the susceptibility of \textit{M. tuberculosis} to EMB; (2) used the \textit{embB} gene as the detection target of molecular detection assays in clinical TB specimens or \textit{M. tuberculosis} isolates; (3) evaluated the accuracy (sensitivity and specificity) of the molecular assays; and (4) had one or more reference standards that were recommended by the WHO. The reference standards included the proportion method (PM) on Lowenstein-Jensen (LJ) media (EMB critical concentration: 2 \(\mu\)g/ml), Middlebrook 7H10 media (EMB critical concentration: 5 \(\mu\)g/ml), Middlebrook 7H11 media (EMB critical concentration: 7.5 \(\mu\)g/ml), radiometric BACTEC 460 media (EMB critical concentration: 2.5 \(\mu\)g/ml) and MGIT 960 media (EMB critical concentration: 5 \(\mu\)g/ml).

Studies were excluded if they met the following predetermined criteria: (1) the study was a review or the sensitivity and specificity data were grouped for meta-analysis by assay category and/or (2) the full-text of the study was not available in English. Studies with less than 20 samples were also excluded in order to reduce selection bias.

\subsection*{Data extraction and quality assessment}

Two reviewers independently assessed the final set of articles and extracted the data using a pilot data extraction form. Initially, both reviewers read the titles and abstracts of all studies. The two reviewers then evaluated the studies that were considered as possibly eligible. The full-text of each
paper was carefully read, according to the inclusion criteria, to assess whether the paper should be included. Disagreements were resolved by consensus and the authors of any papers in question were contacted to obtain more detailed information. The data extracted from the articles included first author, year of publication, sample size, specimen type, and values of true positives (TP), false positives (FP), false negatives (FN) and true negatives (TN). Additionally, because embB 306 was the main mutation codon, data targeting the embB 306 codon was primarily extracted; however data were also extracted from some studies that also targeted embB 406 and embB 497 by PCR-DNA sequencing. Two blinded reviewers assessed the quality of the studies using QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies), the revised tool for QUADAS. It is used in systematic reviews to evaluate the risk of bias in, and the applicability of, diagnostic accuracy studies. It is comprised of 4 domains: patient selection, index test, reference standard, and flow and timing. Each domain is assessed for risk of bias and the first 3 domains are also assessed for applicability. Signaling questions are included to help judge the risk of bias [11]. Risk of bias is judged as ‘low’, ‘high’, or ‘unclear’. If the answers to all signaling questions for a domain are ‘yes’, then the risk of bias is judged to be low. If any signaling question is answered ‘no’, the potential for bias exists. The ‘unclear’ category should only be used when insufficient data are reported to make a judgment [11]. Applicability was judged as ‘low’, ‘high’ or ‘unclear’ using similar criteria.

**Statistical analysis**

Analyses were done using two software programs: the Meta-Disc, version 1.4 (XI Cochrane Colloquium, Barcelona, Spain) and Cochrane RevMan 5.2. For each study, measures of test accuracy were computed using standard methods as follows: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV) and diagnostic odds ratio (DOR); these measures were pooled using the random effects model [12-13]. The area under the summary receiver operating characteristic (SROC) curve is a global measure of overall performance; therefore, the SROC curve was used to evaluate the effect of the assay, with an area under the curve of 1 indicating perfect discriminatory ability [14]. Heterogeneity was analyzed using chi-square and I-square tests [12].

**RESULTS**

Figure 1 outlines the study selection process. A total of 339 potentially relevant citations were identified from all searches and a final total of 28 [9, 15-41] eligible articles were included in the meta-analysis. Since some articles used more than one detection technique or more than one sample type, 34 independent studies were defined in the meta-analysis. Both PCR-DNA sequencing and GenoType MTBDRs/ were used in two articles [24, 27] and both PCR-DNA sequencing and GenoType MTBDRs/ were used to detect clinical isolated strains and sputum specimens, respectively, in another two articles [23, 29]. An additional 311 studies were excluded for the following reasons: the study was a duplicate among the PubMed, Embase and Web of Science databases, the reference testing of the study did not meet eligibility criteria, the study was a review, or the diagnostic 2 x 2 table could not be extracted.

**Study characteristics**
The overall sample size from the 34 selected studies was 5212, which included 2404 EMB-resistant isolates and 2808 EMB-susceptible isolates (table 1). The molecular assays included PCR-DNA sequencing (n = 16), GenoType MTBDRsl (n = 11), pyrosequencing (n = 3), PCR and restriction fragment length polymorphism (PCR-RFLP) (n = 2) and others methods (n = 2). The other methods included a one-step amplification refractory mutation system and variable-number tandem repeats of mycobacterial interspersed repetitive units. Among the PCR-DNA sequencing group (n = 16), 13 studies used embB 306 as the detection target and 3 studies used embB 306, 406 and 497 as the detection targets. Seven studies used the LJ PM for the reference test, 2 studies used Bactec MGIT 960, 1 study used Bactec 460, and 6 studies used other reference standards including, Bactec 460 or MGIT 960. Among the GenoType MTBDRsl group (n = 11), 8 studies involved detection of clinical isolated strains and 3 studies involved clinical sputum specimens.

Data extraction and quality assessment

All extracted data was double-checked by a second author and filled a 2 x 2 table (Table 1) as shown in the study report. Quality assessment of all included studies is shown in figure 2. Overall, the quality of the study was satisfactory. As shown in the figure 2, 9 (26%) studies were at low risk, 7 studies (20%) were of unclear risk and 18 studies (52%) were at high risk for patient selection bias due to inconsecutive or nonrandom patient selection. Most of the studies were at low risk for index test (n = 22, 64%) and reference standard (n = 28, 82%) bias. A total of 23 studies (67%) were at high risk for flow and timing bias. One reason for this was the fact that not all selected patients were included in the diagnostic analysis and the other reason was that the patients did not receive the same reference standards. As for applicability, 20 studies (59%) were at high risk of the patient selection, however, all select studies (n = 34, 100%) were at low risk of reference standard and index test.

Group analysis according to detection methods

PCR-DNA sequencing group

A total of 16 studies that used a single site, amino acid replacement at position 306 of the embB gene as the molecular marker for detection of EMB drug susceptibility met the inclusion criteria. The pooled sensitivity and specificity estimate for the 16 studies were 0.57 (95% confidence interval [CI]: 0.54-0.60) (Figure 3A) and 0.93 (95% CI: 0.91-0.94) (Figure 3B), respectively. PLR and NLR were 10.19 (95% CI: 4.69-22.10) and 0.48 (95% CI: 0.42-0.55), respectively. DOR was 21.28 (95% CI: 9.55-47.43), PPV was 0.85 (95% CI: 0.82-0.88) and NPV was 0.75 (95% CI: 0.73-0.77) (Table 2). The area under the SROC curve was 0.5643 and $Q^*$ was 0.5483 (Figure 3E).

A total of 3 studies used multiple single sites (amino acid replacement at positions 306, 406, and 497) of the embB gene as the molecular markers for the molecular detection of EMB drug susceptibility. The pooled sensitivity and specificity for the 3 studies were 0.76 (95% CI: 0.70-0.81) (Figure 3C) and 0.89 (95% CI: 0.83-0.93) (Figure 3D), respectively. PLR and NLR was 10.18 (95% CI: 1.28-80.99) and 0.27 (95% CI: 0.22-0.33), respectively. DOR was 33.69 (95% CI: 4.53-250.90). PPV was 0.92(95% CI: 0.88-0.95) and NPV was 0.69 (95% CI: 0.63-0.65) (Table 2). The area under the SROC curve was 0.8293 and $Q^*$ was 0.7620 (Figure 3F).

GenoType MTBDRsl group

Isolates subgroup
Eight studies detected EMB resistant isolates using the GenoType MTBDR<sub>sl</sub> assay. The pooled sensitivity and specificity was 0.64 (95% CI: 0.60-0.67) (Figure 4A) and 0.70 (95% CI: 0.67-0.74) (Figure 4B), respectively. PLR and NLR was 5.17 (95% CI: 1.95-13.66) and 0.46 (95% CI: 0.34-0.61), respectively. PPV and NPV was 0.69 (95% CI: 0.65-0.73) and 0.65 (95% CI: 0.61-0.69), respectively. DOR was 12.85 (95% CI: 3.52-46.96) (Table 2). The area under the SROC curve was 0.6455 and Q* was 0.6102 (Figure 4E).

### Sputum subgroup

Three studies used the Genotyping MTBDR<sub>sl</sub> assay to detect EMB resistance on sputum. The pooled sensitivity and specificity was 0.55 (95% CI: 0.47-0.63) (figure 4C) and 0.78 (95% CI: 0.69-0.85) (Figure 4D), respectively. PLR and NLR was 2.86 (95% CI: 0.98-8.29) and 0.56 (95% CI: 0.47-0.68), respectively. PPV and NPV was 0.76 (95% CI: 0.67-0.84) and 0.57 (95% CI: 0.49-0.65), respectively. DOR was 5.52 (95% CI: 2.07-14.71) (Table 2). The area under the SROC curve was 0.7078 and Q* was 0.6591 (Figure 4F).

### Pyrosequencing group

The pooled sensitivity and specificity for detection of resistance to EMB was 0.57 (95% CI: 0.49-0.65) (Figure 5A) and 0.87 (95% CI: 0.82-0.92) (Figure 5B), respectively, with Pyrosequencing. PLR and NLR was 4.16 (95% CI: 2.80-6.19) and 0.54 (95% CI: 0.40-0.72), respectively. DOR was 8.87 (95% CI: 5.14-15.30). PPV and NPV was 0.80 (95% CI: 0.71-0.84) and 0.70 (95% CI: 0.63-0.76), respectively (Table 2). The area under the SROC curve was 0.7549 and Q* was 0.6975 (Figure 5C).

### PCR-RFLP group

The pooled sensitivity and specificity for detection of resistance to EMB was 0.35 (95% CI: 0.24-0.46) (Figure 6A) and 0.98 (95% CI: 0.94-1.00) (Figure 6B), respectively with PCR-RFLP. PLR and NLR was 12.84 (95% CI: 4.29-38.46) and 0.68 (95% CI: 0.57-0.80), respectively. DOR was 18.53 (95% CI: 5.68-60.45). PPV and NPV was 0.90 (95% CI: 0.74-0.98) and 0.72 (95% CI: 0.65-0.78), respectively (Table 2).

### Other methods group

The pooled sensitivity and specificity for detection of resistance to EMB was 0.85 (95% CI: 0.75-0.92) (Figure 7A) and 0.96 (95% CI: 0.92-0.96) (Figure 7B), respectively using the other methods. PLR and NLR was 38.44 (95% CI: 1.17-8495.37) and 0.10 (95% CI: 0.00-9.95), respectively. DOR was 378.81 (95% CI: 0.27-522235.40), PPV and NPV was 0.79 (95% CI: 0.69-0.88) and 0.97 (95% CI: 0.96-0.99), respectively (Table 2).

### Group analysis according to reference method

Some studies reported a high agreement between GenoType MTBDR<sub>sl</sub> and PCR-DNA sequencing [24, 25, 28, 31]. Pyrosequencing can provide the same accuracy as the sequencing method [38], thus we considered the studies that used the three molecular assays for detection of EMB resistance as a group in order to stratify by type of reference standard. All of the studies were divided into three groups, according to the drug concentration of the reference tests. The ‘low’ concentration group, included all of the studies that used LJ PM with a drug concentration of 2 µg/ml or on Bactec 460 medium with a drug concentration of 2.5 µg/ml. The ‘high’ concentration group, included all the studies that used the MGIT 960, PM on Middlebrook 7H10 medium with a drug concentration 5 µg/ml.
The ‘multiple’ concentration group included the studies that used more than one reference standard. The pooled accuracy measures are shown in Table 3.

**Group analysis according to specimen resource regions**

All of the studies were divided into two groups, according to the region (Asia, Europe) from which the samples originated. The pooled accuracy measures are shown in Table 3.

**Heterogeneity**

The heterogeneity test results of pooled accuracy measures are shown in Table 4. When the studies were stratified by type of index test, there was homogeneity in all of the pooled sensitivity data and NLRs, with the exception of the GenoType MTBDRsl assay detecting EMB isolates and the other group. However, significant heterogeneity was observed in most of pooled specificity data and PLRs. When the studies were analyzed by drug concentration reference standards, homogeneity was present in all of the pooled measures in the low drug concentration group. Homogeneity was observed in samples from Asia when the sensitivity, PLR, NLR and DOR of selected studies were pooled.

**Discussion**

Rapid and effective drug susceptibility testing for *M. tuberculosis* has been a hot topic in research worldwide. Early detection of drug resistance in TB patients can contribute to TB control and management and reduce the prevalence and transmission of TB. WHO has called for research into a fast and accurate drug susceptibility testing method to reduce the spread of *M. tuberculosis* and drug-resistant TB in order to reduce the global TB burden [3]. The use of molecular methods has been recommended as an effective way to decrease the turnaround time for the detection of drug-resistance in *M. tuberculosis* [36, 42]. Recently, molecular methods for drug susceptibility testing of *M. tuberculosis* have begun to be more widely used. Molecular assays for drug susceptibility testing to RIF and INH, based on the *rpoB* gene and *katG* gene, have become more effective. The turnaround time for PCR-Single-Strand Conformational Polymorphism analysis (PCR-SSCP) assay and Pyrosequencing is less than 48 h, making it substantially faster than conventional drug susceptibility testing methods [43-44]. Earlier studies suggested that mutations in the *embB* gene, in particular amino acid replacements at position 306, were the major mechanism for acquisition of resistance to EMB in *Mycobacterium tuberculosis* [45-48]. Consequently, in recent years, many studies have focused on molecular drug susceptibility testing of EMB based on the *embB* gene for detection of drug-resistant *M. tuberculosis* strains. The most common methods have been PCR-DNA sequencing, GenoType MTBDRsl, PCR-RFLP, and pyrosequencing. Therefore, it is important that the overall accuracy of the methods used for the detection of EMB resistance be explored.

*EmbB* 306 is the main reason for EMB resistance in *M. tuberculosis* [45-48] thus, in most of the selected studies, in this review, the detection target was only *embB* 306. Therefore, the diagnostic value of different detection methods, based on *embB* 306 as detection target, was initially done in this study. The specificities of every molecular technique were more than 0.7 and the specificity of PCR-DNA sequencing and PCR-RFLP were more than 0.93; these are similar to the specificities reported for rifampicin and isoniazid [43, 44]. This suggests that these techniques are good for detecting *M. tuberculosis* strains susceptible to EMB. Of all of the detection methods examined, the sensitivity of GenoType MTBDRsl was the highest, while the sensitivity of PCR-RFLP was the lowest. These results
suggest that GenoType MTBDRsl is best when *embB* 306 is the only detection target. However, the pooled sensitivity for detection of EMB resistance by GenoType MTBDRsl was still lower than that of RIF and INH, which use *rpoB* and *katG* as detection targets, respectively. Therefore, resistant strains can easily be judged as susceptible, when using *embB* 306 as the detection target, by mistake. The specificity showed large variations among different studies in genotype GenoType MTBDRsl group and lower pooled specificity than other groups, due to the low specificities (0.55, 0.56) of two included studies [29, 31] with large sample size. In Said HM’s study, all the isolates are MDR, Hazbón et al’s [54] reported that *embB*306 mutations may has be associate with MDR and MDR isolates which are phenotypically susceptible to EMB carried mutations at codon 306 of the *embB* gene, this maybe explained the reason of low the specificity of Said HM’s study. Although the *embB* 306 mutation is the mutant hot site in EMB resistant strains [45-48], it was not enough to cover a single detection site for *embB* 306 [49-50]. Other studies used multiple sites of the *embB* gene as detection sites when using molecular detection techniques [16-17, 26-28]. Consequently, in the current study, the diagnostic value of using multiple sites of *embB* as detection targets was compared to using a single site of *embB* as a detection target. The sensitivity and PPV of using multiple sites as detection target were 19% and 7% higher, respectively than when using only *embB* 306 (0.57 vs. 0.76, p < 0.05; 0.85 vs. 0.92, p < 0.05), and 12% and 23% higher, respectively than when using GenoType MTBDRsl that targeted *embB* 306 (0.64 vs. 0.76, p < 0.05; 0.69 vs. 0.92, p < 0.05). As has been reported in some studies, *embB* 406 and 497 mutations often occur in strains that do not have *embB* 306 mutations [45, 50-51], thus using multiple sites as targets can enhance the sensitivity. Although the pooled sensitivity of multiple sites was still not high enough (0.76), the PPV was high (0.92). This suggests high accuracy when judging resistant strains using multiple sites as detection targets. Multiple-site detection, using rapid PCR-DNA sequencing, had better agreement with the reference methods compared with the other molecular DST methods examined and could be a quick method to preliminarily screen for EMB resistant strains.

Although the sensitivity of multi-site (*embB* 306, 406 and 497) detection was higher than the sensitivity of single site (*embB* 306) detection, the sensitivity and specificity of multi-site detection still did not satisfy the clinical requirement. This depends on EMB resistance mechanisms. EMB resistance is regulated by both gene mutations and gene expression [50]. *EmbB* 306, 406 and 497 were only shown to be associated with *embB* mutations and EMB resistance. It has been reported that EMB resistance is regulated by multiple genes such as, *embA*, *embB*, *embC* and Rv3792 [53]. When the EMB concentration of a reference method is less than 5 μg/ml, the *embB* mutation rate in EMB resistant strains is not high enough to produce a high enough sensitivity. In addition, although the association between *embB* 306 mutations and EMB resistance in clinical *M. tuberculosis* isolates is so strong that it has been proposed as a marker for EMB resistance in diagnostic tests, another study reported that in some *M. tuberculosis* strains, *embB* 306 mutations do not cause EMB resistance, but predispose *M. tuberculosis* to become resistant to any antibiotic and to become multidrug resistant [54]. This may explain the reason why *embB* 306 mutations occur in EMB susceptible strains and also suggests that *embB* 306 mutations can serve as a marker for TB cases that are at increased risk for the development of drug resistance.
The MIC range of EMB susceptible and resistant strains was narrow [55] and the concentrations of different reference methods were different. It has been reported that the results of the BACTEC 460 method and the Agar PM were discordant [55-56]. It was reported that false susceptibility to EMB is of little consequence in settings of susceptible *M. tuberculosis* isolates [56]. Thus, the effect of different drug concentrations on the evaluation of molecular detection of EMB resistance was analyzed. For example, when *emb*B 306 was the target, the result showed a lower sensitivity in the group with the low drug concentration in reference methods, compared to the other groups. This suggests that different reference methods with different drug concentrations affect the evaluation of the molecular detection of EMB resistance based on *emb*B 306. The agreement between molecular DST and MGIT 960 and between molecular DST and PM on Middlebrook 7H10 media was better compared to all other reference DST methods examined. This result also supports Parsons’ speculation [50] that the mutation rate of *emb*B 306 is higher in higher-level resistant strains.

The rates of gene mutations related to drug resistance in *M. tuberculosis* were varied depending on the region. The meta-analysis of pyrosequencing for the rapid detection of rifampicin resistance in *M. tuberculosis* showed that the sensitivity of molecular detection for isoniazid resistance, based on *katG*, was lower in Asia than in Europe [43]. The present result showed that the sensitivity and specificity of using molecular techniques to identify EMB resistance, based on *emb*B 306 as the detection target, was higher in Europe than in Asia. This suggests that using molecular detection to identify EMB resistance, based on *emb*B 306, works better in Europe.

Some of summary measures were significantly heterogeneous in the current study. Therefore group analyses and subgroup analyses were used to examine the reasons for heterogeneity. The results suggested that the variability in the reference and detection methods among studies could partly explain the heterogeneity. In the low reference drug concentration group, all of the studies used a consistent LJ PM reference, with the exception of 2 studies that used Bactec 460 and one study that used LJ PM and Bactec 460. Even so, heterogeneity persisted in some of the summary estimates. Since any factor can affect the heterogeneity in a diagnostic meta-analysis other factors such as variations in the study, population (e.g. severity of disease and co-morbidities), the design method, and/or sample collection method (consecutive or random collection of samples), likely resulted in variations in the accuracy estimates in this study [57].

There were some limitations to our review. First, since only abstracts and only in 3 databases were searched, some studies were not included in this review. Secondly, due to the linguistic abilities of our team, only studies that were published in English were included. Thirdly, some studies with missing data were excluded since the authors could not be contacted. The effects of factors such as laboratory infrastructure and expertise with molecular detection of EMB resistance could not be analyzed as this information was not available.

**Conclusion**

Molecular drug susceptibility testing methods, using *emb*B 306 as a single detection target, are not good for detection of EMB resistance due to low sensitivity. PCR-DNA sequencing, using multiple sites of the *emb*B gene, including *emb*B 306, 406 and 497 as detection targets, could be a rapid method to preliminarily screen for EMB resistant strains, however the drug susceptibility results using
PCR-sequencing were not strictly accurate; therefore, the molecular DST cannot fully replace phenotypic DST. Molecular DST with MGIT 960 and the PM on Middlebrook 7H10 media provided the best agreement rates.

**Author Contributions**

Experiment conception and design: SC, ZLC and ZYH. Data Analysis: SC, YYL and ZLC. Paper writing: SC, ZLC, ZYH.

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**Competing Interests**

The authors have declared that no competing interests exist.


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Figure legends

Figure 1. Flow chart of study selection

Figure 2. Quality assessment of included studies

Figure 3. Forest plots of the pooled sensitivity and specificity and SROC curve of PCR-DNA sequencing for detection of EMB drug susceptibility. Each solid circle indicates the point estimate of sensitivity and specificity from each study, the size of the solid circle indicates the size of each study. Both error bars and discontinuous lines indicate 95% confidence intervals. Diamond indicates the pooled sensitivity and specificity for all the studies. The curve is the regression line that summarizes the overall diagnostic accuracy.

A: Sensitivity of PCR-DNA sequencing used embB 306 codon as the target.
B: Specificity of PCR-DNA sequencing used embB 306 codon as the target.
C: Sensitivity of PCR-DNA sequencing used embB 306, 406 and 497 codon as the target.
D: Specificity of PCR-DNA sequencing used embB 306, 406 and 497 codon as the target.
E: SROC curve of PCR-DNA sequencing used embB 306 codon as the target.
F: SROC curve of PCR-DNA sequencing used mbB 306, 406 and 497 codon as the target.

Figure 4. Forest plots of the pooled sensitivity and specificity and SROC curve of GenoType MTBDRsl for detection of EMB drug susceptibility. Each solid circle indicates the point estimate of sensitivity and specificity from each study, the size of the solid circle indicates the size of each study. Both error bars and discontinuous lines indicate 95% confidence intervals. Diamond indicates the pooled sensitivity and specificity for all the studies. The curve is the regression line that summarizes the overall diagnostic accuracy.

A: Sensitivity of GenoType MTBDRsl for detection of EMB drug susceptibility in isolates.
B: Specificity of GenoType MTBDRsl for detection of EMB drug susceptibility in isolates.
C: Sensitivity of GenoType MTBDRsl for detection of EMB drug susceptibility in sputum specimens.
D: Specificity of GenoType MTBDRsl for detection of EMB drug susceptibility in sputum specimens.
E: SROC curve of GenoType MTBDRsl for detection of EMB drug susceptibility in isolates.
F: SROC curve of GenoType MTBDRsl for detection of EMB drug susceptibility in sputum specimens.

Figure 5. Forest plots of the pooled sensitivity and specificity and SROC curve of pyrosequencing for detection of EMB drug susceptibility.
Each solid circle indicates the point estimate of sensitivity and specificity from each study, the size of the solid circle indicates the size of each study. Both error bars and discontinuous lines indicate 95% confidence intervals. Diamond indicates the pooled sensitivity and specificity for all the studies. The curve is the regression line that summarizes the overall diagnostic accuracy.


Figure 6. Forest plots of the pooled sensitivity and specificity of PCR-RFLP for detection of EMB drug susceptibility.

Each solid circle indicates the point estimate of sensitivity and specificity from each study, the size of the solid circle indicates the size of each study. Both error bars and discontinuous lines indicate 95% confidence intervals. Diamond indicates the pooled sensitivity and specificity for all the studies. The curve is the regression line that summarizes the overall diagnostic accuracy.

A: sensitivity. B: specificity

Figure 7. Forest plots of the pooled sensitivity and specificity of other methods for detection of EMB drug susceptibility.

Each solid circle indicates the point estimate of sensitivity and specificity from each study, the size of the solid circle indicates the size of each study. Both error bars and discontinuous lines indicate 95% confidence intervals. Diamond indicates the pooled sensitivity and specificity for all the studies. The curve is the regression line that summarizes the overall diagnostic accuracy.

Table 1. Summary of the studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Authors (reference)</th>
<th>Year</th>
<th>Study settings</th>
<th>Country</th>
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<td>Peru</td>
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<td>L-JPM,BACTEC 460</td>
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<tr>
<td></td>
<td>Lee (16)</td>
<td>2004</td>
<td>UN</td>
<td>Singapore</td>
<td>isolated</td>
<td>BACTEC 460</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Ramaswamy (17)</td>
<td>2004</td>
<td>UN</td>
<td>Mexico</td>
<td>isolated</td>
<td>L-JPM</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Zhong (18)</td>
<td>2007</td>
<td>UN</td>
<td>China</td>
<td>isolated</td>
<td>L-JPM</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Sekiguchi (9)</td>
<td>2007</td>
<td>UN</td>
<td>Japan, Poland</td>
<td>isolated</td>
<td>AgarPM,L-JPM, MGIT 960</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Greer (19)</td>
<td>2008</td>
<td>UN</td>
<td>China</td>
<td>isolated</td>
<td>L-JPM</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Zhang (18)</td>
<td>2007</td>
<td>UN</td>
<td>China</td>
<td>isolated</td>
<td>L-JPM</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Guo (19)</td>
<td>2008</td>
<td>UN</td>
<td>China</td>
<td>isolated</td>
<td>L-JPM</td>
<td>66</td>
</tr>
<tr>
<td>GeneTypeMTBDRs</td>
<td>Huang (27)</td>
<td>2010</td>
<td>UN</td>
<td>France</td>
<td>isolated</td>
<td>L-JPM</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Hu (25)</td>
<td>2010</td>
<td>UN</td>
<td>France</td>
<td>isolated</td>
<td>L-JPM</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Kiet (30)</td>
<td>2011</td>
<td>UN</td>
<td>Vietnam</td>
<td>isolated</td>
<td>L-JPM</td>
<td>52</td>
</tr>
<tr>
<td>Other methods</td>
<td>Isola (37)</td>
<td>2005</td>
<td>UN</td>
<td>Abkhazia</td>
<td>isolated</td>
<td>L-JPM, BACTEC 460</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Ignatyeva (33)</td>
<td>2012</td>
<td>SRL</td>
<td>Estonia</td>
<td>isolated</td>
<td>L-JPM, BACTEC 460</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Johnson (40)</td>
<td>2006</td>
<td>UN</td>
<td>India</td>
<td>isolated</td>
<td>AgarPM, BACTEC 460</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Shen (41)</td>
<td>2009</td>
<td>UN</td>
<td>China</td>
<td>isolated</td>
<td>L-JPM</td>
<td>162</td>
</tr>
</tbody>
</table>

Abbreviations: SRL = Supranational Reference Laboratory, NRL = National Reference Laboratory, UN = Unknown, CDC = Center for disease control and prevention.
Table 2. All the pooled accuracy measures in the meta-analysis

Abbreviations: Se = Sensitivity, Sp = Specificity, PLR = Positive likelihood ratio; NLR = Negative likelihood ratio, PPV = Positive predictive value, NPV = Negative predictive value, DOR = Diagnostic odds ratio, CI = Confidence interval.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of studies (n)</th>
<th>Se (95% CI)</th>
<th>Sp (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-DNA sequence (306)</td>
<td>16 (2122)</td>
<td>0.57 (0.54-0.60)</td>
<td>0.93 (0.91-0.94)</td>
<td>10.19 (4.69-22.10)</td>
<td>0.48 (0.42-0.55)</td>
<td>0.85 (0.82-0.88)</td>
<td>0.75 (0.73-0.77)</td>
<td>21.28 (9.55-47.43)</td>
</tr>
<tr>
<td>PCR-DNA sequence (306, 406, 497)</td>
<td>3 (439)</td>
<td>0.76 (0.70-0.81)</td>
<td>0.89 (0.83-0.93)</td>
<td>10.18 (1.28-90.99)</td>
<td>0.27 (0.22-0.33)</td>
<td>0.92 (0.88-0.95)</td>
<td>0.69 (0.63-0.65)</td>
<td>33.69 (4.53-250.90)</td>
</tr>
<tr>
<td>GenoTypeMTBDRsl (isolates)</td>
<td>8 (1160)</td>
<td>0.64 (0.60-0.67)</td>
<td>0.70 (0.67-0.74)</td>
<td>5.17 (1.95-13.66)</td>
<td>0.46 (0.34-0.61)</td>
<td>0.69 (0.65-0.73)</td>
<td>0.65 (0.61-0.69)</td>
<td>12.85 (3.52-46.96)</td>
</tr>
<tr>
<td>GenoTypeMTBDRsl (pulmonary)</td>
<td>3 (266)</td>
<td>0.55 (0.47-0.63)</td>
<td>0.78 (0.69-0.85)</td>
<td>2.86 (0.98-8.29)</td>
<td>0.56 (0.47-0.68)</td>
<td>0.76 (0.67-0.84)</td>
<td>0.57 (0.49-0.65)</td>
<td>5.52 (2.07-14.71)</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>3 (343)</td>
<td>0.57 (0.49-0.65)</td>
<td>0.87 (0.82-0.92)</td>
<td>4.16 (2.80-6.19)</td>
<td>0.54 (0.40-0.72)</td>
<td>0.80 (0.71-0.87)</td>
<td>0.70 (0.63-0.76)</td>
<td>8.87 (5.16-15.30)</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>2 (222)</td>
<td>0.55 (0.44-0.66)</td>
<td>0.98 (0.94-1.00)</td>
<td>12.84 (4.29-38.46)</td>
<td>0.68 (0.57-0.80)</td>
<td>0.90 (0.74-0.98)</td>
<td>0.72 (0.65-0.78)</td>
<td>18.53 (5.68-60.45)</td>
</tr>
<tr>
<td>Other group</td>
<td>2 (541)</td>
<td>0.85 (0.75-0.92)</td>
<td>0.96 (0.92-0.96)</td>
<td>38.44 (17.44-95.37)</td>
<td>0.10 (0.06-0.95)</td>
<td>0.79 (0.69-0.88)</td>
<td>0.97 (0.90-0.99)</td>
<td>378.81 (62.27-22235.40)</td>
</tr>
</tbody>
</table>

Table 3. Group analyses different drug concentration and different countries

Abbreviations: Se = Sensitivity, Sp = Specificity, PLR = Positive likelihood ratio; NLR = Negative likelihood ratio, PPV = Positive predictive value, NPV = Negative predictive value, DOR = Diagnostic odds ratio, CI = Confidence interval.

<table>
<thead>
<tr>
<th>Group</th>
<th>Se (95% CI)</th>
<th>Sp (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low concentration</td>
<td>0.55 (0.51-0.60)</td>
<td>0.92 (0.89-0.94)</td>
<td>5.38 (4.18-6.92)</td>
<td>0.52 (0.47-0.57)</td>
<td>11.00 (7.87-15.36)</td>
</tr>
<tr>
<td>High concentration</td>
<td>0.67 (0.64-0.70)</td>
<td>0.73 (0.70-0.76)</td>
<td>2.96 (1.59-5.51)</td>
<td>0.56 (0.41-0.76)</td>
<td>5.42 (2.19-13.43)</td>
</tr>
<tr>
<td>Multiple reference stands</td>
<td>0.60 (0.56-0.65)</td>
<td>1.0 (0.99-1.0)</td>
<td>48.83 (16.15-147.65)</td>
<td>0.39 (0.32-0.49)</td>
<td>141.98 (46.56-432.96)</td>
</tr>
<tr>
<td>Europe</td>
<td>0.60 (0.63-0.75)</td>
<td>0.87 (0.81-0.93)</td>
<td>4.53 (2.83-7.26)</td>
<td>0.41 (0.37-0.46)</td>
<td>11.84 (7.17-24.55)</td>
</tr>
<tr>
<td>Asian</td>
<td>0.57 (0.53-0.61)</td>
<td>0.94 (0.92-0.96)</td>
<td>6.98 (3.20-12.41)</td>
<td>0.48 (0.41-0.56)</td>
<td>15.92 (7.84-32.29)</td>
</tr>
</tbody>
</table>
Table 4. All the heterogeneity test results of pooled accuracy measures

<table>
<thead>
<tr>
<th>Group</th>
<th>Se (I², P value)</th>
<th>Sp (I², P value)</th>
<th>PLR (I², P value)</th>
<th>NLR (I², P value)</th>
<th>DOR (I², P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-DNA sequencing (306)</td>
<td>35.8%, 0.0768</td>
<td>91.0%, &lt;0.05</td>
<td>89.1%, &lt;0.05</td>
<td>54.8%, 0.0035</td>
<td>80.9%, &lt;0.05</td>
</tr>
<tr>
<td>PCR-DNA sequencing (306, 406, 497)</td>
<td>0.0%, 0.6745</td>
<td>91.6%, &lt;0.05</td>
<td>86.4%, &lt;0.05</td>
<td>0.0%, 0.1940</td>
<td>78.2%, &lt;0.05</td>
</tr>
<tr>
<td>GenoTypeMTBDRsl (isolates)</td>
<td>66.7%, 0.002</td>
<td>95.1%, &lt;0.05</td>
<td>91.9%, &lt;0.05</td>
<td>83.0%, &lt;0.001</td>
<td>87.9%, &lt;0.05</td>
</tr>
<tr>
<td>GenoTypeMTBDRsl (sputum)</td>
<td>61.7%, 0.0734</td>
<td>93.1%, &lt;0.05</td>
<td>79.5%, 0.0075</td>
<td>0.0%, 0.7313</td>
<td>37.3%, 0.2030</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>57.9%, 0.1349</td>
<td>92.4%, &lt;0.05</td>
<td>91.9%, &lt;0.05</td>
<td>83.0%, &lt;0.05</td>
<td>87.9%, &lt;0.05</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>16.3%, 0.2744</td>
<td>0.0%, 0.3406</td>
<td>0.0%, 0.8218</td>
<td>10.1%, 0.2936</td>
<td>0.0%, 0.7402</td>
</tr>
<tr>
<td>Other group</td>
<td>94.3%, &lt;0.05</td>
<td>96.9%, &lt;0.05</td>
<td>96.4%, &lt;0.05</td>
<td>98.8%, &lt;0.05</td>
<td>95.2%, &lt;0.05</td>
</tr>
<tr>
<td>Low concentration</td>
<td>8.8%, 0.3576</td>
<td>90.7%, 0.0142</td>
<td>0.0%, 0.5546</td>
<td>4.0%, 0.4062</td>
<td>0.0%, 0.8091</td>
</tr>
<tr>
<td>High concentration</td>
<td>72.8%, &lt;0.05</td>
<td>94.2%, &lt;0.05</td>
<td>90.1%, &lt;0.001</td>
<td>81.9%, &lt;0.05</td>
<td>87.5%, &lt;0.05</td>
</tr>
<tr>
<td>Multiple reference stands</td>
<td>64.2%, &lt;0.05</td>
<td>21.5%, 0.2581</td>
<td>29.9%, 0.1897</td>
<td>69.3%, &lt;0.05</td>
<td>21.8%, 0.2558</td>
</tr>
<tr>
<td>Europe</td>
<td>76.8%, &lt;0.05</td>
<td>90.0%, 0.0075</td>
<td>90.5%, 0.0223</td>
<td>74.7%, &lt;0.001</td>
<td>21.0%, 0.2727</td>
</tr>
<tr>
<td>Asia</td>
<td>49.7%, 0.0503</td>
<td>79.3%, &lt;0.05</td>
<td>59.5%, 0.059</td>
<td>54.4%, 0.0156</td>
<td>57.6%, 0.0087</td>
</tr>
</tbody>
</table>

Abbreviations: Se = Sensitivity, Sp = Specificity, PLR = Positive likelihood ratio, NLR = Negative likelihood ratio, DOR = Diagnostic odds ratio, CI = Confidence interval.
Identification: 339 potentially relevant citations were identified from all searches → 104 duplicate citations were excluded

Screening: 235 articles were selected for more detailed evaluation → Articles excluded (n=145): Irrelevant (n=88); Review (n=27); Non-English language (n=30)

Eligibility: 90 articles were included to screen full text → Articles excluded (n=62): Samples didn’t meet eligibility criteria (n=9); Reference testing didn’t meet eligibility criteria (n=27); Couldn’t extract 2 by 2 tables (n=16); Without original data (n=10)

Included: 28 articles were included in the study → 34 studies* were included at last

*Different diagnostic tests or diagnostic tests performed with different types of specimens were defined as different studies

Figure 1. Flow chart of study selection
Figure 3. Forest plots of the pooled sensitivity and specificity and SROC curve of PCR-DNA sequencing for detection of EMB drug susceptibility.
Figure 4. Forest plots of the pooled sensitivity and specificity and SROC curve of GenoType MTBDRsl for detection of EMB drug susceptibility.
Figure 5. Forest plots of the pooled sensitivity and specificity and SROC curve of pyrosequencing for detection of EMB drug susceptibility.
Figure 6. Forest plots of the pooled sensitivity and specificity of PCR-RFLP for detection of EMB drug susceptibility.
Figure 7. Forest plots of the pooled sensitivity and specificity of other methods for detection of EMB drug susceptibility.