Rapid Diagnosis of Drug Resistance to Fluoroquinolones, Amikacin, Capreomycin, Kanamycin and Ethambutol Using Genotype MTBDRsl Assay: A Meta-Analysis

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Abstract

Background: There are urgent needs for rapid and accurate drug susceptibility testing of M. tuberculosis. GenoType MTBDRsl is a new molecular kit designed for rapid identification of the resistance to the second-line antituberculosis drugs with a single strip. In recent years, it has been evaluated in many settings, but with varied results. The aim of this meta-analysis was to synthesize the latest data on the diagnostic accuracy of GenoType MTBDRsl in detecting drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol, in comparison with the phenotypic drug susceptibility test.

Methods: This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. The search terms of “MTBDRsl” and “tuberculosis” were used on PubMed, EMBASE, and Web of Science. QUADAS-2 was used to assess the quality of included studies. Data were analyzed by Meta-Disc 1.4. We calculated the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and corresponding 95% confidence interval (CI) for each study. From these calculations, forest plots and summary receiver operating characteristic (SROC) curves were produced.

Results: Patient selection bias as well as flow and timing bias were observed in most studies. The summarized sensitivity (95% CI) was 0.874 (0.845–0.899), 0.826 (0.777–0.869), 0.820 (0.772–0.862), 0.444 (0.396–0.492), and 0.679 (0.652–0.706), for fluoroquinolones, amikacin, capreomycin, kanamycin, and ethambutol, respectively. The specificity (95% CI) was 0.971 (0.961–0.980), 0.995 (0.987–0.998), 0.973 (0.963–0.981), 0.993 (0.985–0.997), and 0.799 (0.773–0.823), respectively. The AUC (standard error) were 0.9754 (0.0203), 0.9300 (0.0598), 0.9885 (0.0038), 0.9689 (0.0359), and 0.6846 (0.0550), respectively.

Conclusion: Genotype MTBDRsl showed good accuracy for detecting drug resistance to fluoroquinolones, amikacin and capreomycin, but it may not be an appropriate choice for kanamycin and ethambutol. The lack of data did not allow for proper evaluation of the test on clinical specimens. Further systematic assessment of diagnostic performance should be carried out on direct clinical samples.

Introduction

Extensive drug resistant tuberculosis (XDR-TB) was first described in March 2006 by World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) of the United States [1], and has since been reported in more than 50 countries[2–4]. WHO has expressed concern over the emergence of XDR-TB and called for measures to prevent the spread of this type of deadly strain [5]. XDR-TB is a rare type of multidrug-resistant TB (MDR-TB) (i.e. resistant to isoniazid and rifampicin) and is resistant to the fluoroquinolones and at least one of three injectable second-line drugs (i.e. amikacin, kanamycin, or capreomycin) [6]. Drug resistance is a severe challenge to tuberculosis control, as it raises the possibility of a condition that can no longer effectively be treated with anti-tuberculosis drugs [7]. Threats of MDR-TB and XDR-TB highlight the urgent need for rapid and accurate drug susceptibility testing (DST) to optimize...
the treatment regimen and reduce the risk of acquired resistance [8].

Conventional DST for XDR strains is performed sequentially in a two-step procedure beginning with a culture and first-line drug testing, proceeding to further drug testing in the case of multidrug resistance. It takes more than 10 days for traditional culture-based drug resistance detection, even with the new automated liquid media culture systems. For example, the BACTEC MGIT 960 and BACTEC 460TB need 13.3 days and 10.6 days on average to report the drug resistance results, respectively [9].

A rapid, reliable, and accurate test is therefore necessary to avoid clinical deterioration, improve patient management, and prevent further transmissions [10]. During the last decade, a great deal of effort has gone into the development of the molecular-based rapid DST [11,12]. In 2008, WHO endorsed the line-probe assays (LPAs) for the rapid detection of drug resistance in low and middle income settings [13]. LPAs, in general, focus on detection of drug-resistance gene mutations [14]. The GenoType® MTBDRplus and MTBDRsl (Hain Lifescience, Nehren, Germany) are two types of LPAs designed for the detection of the first-line and second-line anti-tuberculosis drug resistance, respectively. Both rely on hybridization of amplified DNA fragments from Mycobacterium tuberculosis (M. tuberculosis) complex species to specific probes immobilized on nitrocellulose strips. In addition to GenoType® MTBDRplus which detects common mutations in katG gene, inhA promoter, and rpoB gene, GenoType MTBDRsl detects the most common mutations in gyrA gene for fluoroquinolones (FLQs) resistance, in rrs gene for amikacin (AM), capreomycin (CAP), and kanamycin (KAN) resistance, and in embB gene for ethambutol (EMB) resistance. GenoType® MTBDRsl contains 16 probes for mutation detection and 6 probes for quality control. Six control probes consist of a conjugate control (CC), an amplification control (AC), and three loci controls for gene amplification (gyrA, rrs, and embB) [15]. The remaining 16 probes include wild type gene probes and mutation probes: gyrA wild-type probes WT1 to WT3 (codons 85–90, 89–93

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**Figure 1. Flow chart of the meta analysis.**

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Table 1. Summary of included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Specimen type</th>
<th>Size</th>
<th>Gold standard</th>
<th>FLQs</th>
<th>AM</th>
<th>CAP</th>
<th>KAN</th>
<th>EMB</th>
<th>TR</th>
<th>FR</th>
<th>FS</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hillemann 2009</td>
<td>Germany</td>
<td>Clinical isolates</td>
<td>106</td>
<td>MGIT 960; L-J PM</td>
<td>29 0 3 74</td>
<td>39 0 7 60</td>
<td>39 1 6 60</td>
<td>NA  NA  NA  NA</td>
<td>36 0 16 54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hillemann 2009</td>
<td>Germany</td>
<td>Clinical specimens</td>
<td>64</td>
<td>MGIT 960; L-J PM</td>
<td>8 0 1 51</td>
<td>6 0 2 52</td>
<td>7 0 1 52</td>
<td>NA  NA  NA  NA</td>
<td>10 0 16 34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brossier 2010</td>
<td>France</td>
<td>Clinical isolates</td>
<td>52</td>
<td>L-J PM</td>
<td>21 1 3 27</td>
<td>10 0 0 10</td>
<td>9 1 2 40</td>
<td>13 0 3 39</td>
<td>16 2 12 22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiet 2010</td>
<td>Viet Nam</td>
<td>Clinical isolates</td>
<td>62</td>
<td>L-J PM</td>
<td>31 0 0 21</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>5 0 0 57</td>
<td>34 0 19 9</td>
<td></td>
<td></td>
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<tr>
<td>Van Ingen 2010</td>
<td>Netherlands</td>
<td>Clinical isolates</td>
<td>29</td>
<td>Agar PM</td>
<td>7 0 0 21</td>
<td>8 0 0 21</td>
<td>7 1 0 21</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
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<tr>
<td>Huang 2011</td>
<td>Taiwan</td>
<td>Clinical isolates</td>
<td>234</td>
<td>Agar PM; MGIT 960</td>
<td>63 0 11 160</td>
<td>16 0 3 215</td>
<td>10 6 4 214</td>
<td>16 0 21 197</td>
<td>91 0 71 72</td>
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<td>Kontsevaya 2011</td>
<td>Russian</td>
<td>Clinical isolates</td>
<td>51</td>
<td>L-J PM; MGIT 960</td>
<td>25 0 4 19</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
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<td>Ignatyeva 2012</td>
<td>Estonia</td>
<td>Clinical isolates</td>
<td>800</td>
<td>MGIT 960</td>
<td>288 21 24 367</td>
<td>147 0 34 526</td>
<td>135 15 14 542</td>
<td>147 0 197 363</td>
<td>479 19 141 142</td>
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<tr>
<td>Lacoma 2012</td>
<td>NA</td>
<td>Clinical isolates</td>
<td>34</td>
<td>Bactec 460</td>
<td>4 5 3 17</td>
<td>NA  NA  NA  NA</td>
<td>5 3 0 19</td>
<td>5 3 0 19</td>
<td>14 2 9 9</td>
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<tr>
<td>Lacoma 2012</td>
<td>NA</td>
<td>Clinical specimens</td>
<td>54</td>
<td>Bactec 460</td>
<td>3 2 5 42</td>
<td>NA  NA  NA  NA</td>
<td>23 4 0 25</td>
<td>23 4 0 25</td>
<td>22 4 18 6</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Miotto 2012</td>
<td>Italy</td>
<td>Clinical isolates</td>
<td>175</td>
<td>MGIT 960</td>
<td>42 1 15 116</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>85 2 37 50</td>
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<tr>
<td>Miotto 2012</td>
<td>Italy</td>
<td>Clinical specimens</td>
<td>59</td>
<td>MGIT 960</td>
<td>7 0 0 49</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>31 0 5 20</td>
<td></td>
<td></td>
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<tr>
<td>Said 2012</td>
<td>South Africa</td>
<td>Culture isolates</td>
<td>342</td>
<td>Agar PM</td>
<td>26 7 11 292</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>27 118 21 150</td>
<td></td>
<td></td>
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<tr>
<td>Tessema 2012</td>
<td>Northwest Ethiopia</td>
<td>Clinical isolates</td>
<td>260</td>
<td>Bactec/ALERT 3D</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>NA  NA  NA  NA</td>
<td>8 0 11 241</td>
<td></td>
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</tbody>
</table>

Abbreviations: TR = true resistance; FR = false resistance; FS = false susceptibility; TS = true susceptibility; FLQs = fluoroquinolones; AM = amikacin; CAP = capreomycin; KAN = Kanamycin; EMB = ethambutol; NA = not available.

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and 92–97; gyrA mutant probes MUT1, MUT2, MUT3A, MUT3B, MUT3C, and MUT3D for codons A90V, S91P, D94A, D94N/Y, D94G, and D94H, respectively; rrs wild-type probes WT1 (codons 1401 and 1402) and WT2 (codon 1484); rrs mutant probes MUT1 and MUT2, with A1401G and G1484T changes, respectively; embB wild-type probe WT1, covering codon 306; and embB probes MUT1A and MUT1B for the mutations of M306I and M306V, respectively [16].

To our knowledge, recent studies have conducted the diagnostic performance of GenoType MTBDRsl in many settings, but the results are inconsistent. The aim of this meta-analysis is to offer a systematic overview on the diagnostic accuracy of GenoType MTBDRsl in detecting drug resistance to FLQs, AM/CAP/KAN and EMB in comparison with phenotypic DST.

### Methods

#### Systematic Review

This systematic review was performed according to the guidelines of Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA) set by the PRISMA Group [17]. This review was registered (registration No: CRD42012002481) in PROSPERO (http://www.crd.york.ac.uk/prospero/), which is an international database of prospectively registered systematic reviews in health and social care.

#### Data Resource and Search Strategy

Two investigators independently performed a systematic search based on the PubMed, EMBASE and Web of Science database for original articles published before 1 June 2012. The search items “MTBDRsl” and “tuberculosis” were used. There were no language restrictions. In addition, the bibliographies of each article were reviewed carefully to identify additional relevant articles.

#### Inclusion and Exclusion Criteria

Studies that evaluated Genotype MTBDRsl for detection of drug resistance of M. tuberculosis to FLQs, AM, CAP, KAN, and EMB were included. Included studies should use the phenotypic DST as a gold standard. The exact number of true-resistance (drug resistance was correctly identified by MTBDRsl assay), false-resistance (drug resistance was falsely identified by MTBDRsl assay), false-susceptibility (drug resistant sample was falsely identified by MTBDRsl assay), and true-susceptibility (drug susceptible sample was correctly identified by MTBDRsl assay) should be available to reconstruct two by two tables. Relevant publications were excluded if they were duplicated articles, reviews (to avoid repeated data), or conference abstracts if the full texts were not available.

#### Quality of Studies

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) was used to assess the quality of each study (http://www.bris.ac.uk/quadas/). QUADAS-2 is the current version of QUADAS and the tool for use in systematic reviews to evaluate the risk of bias and applicability of diagnostic accuracy studies. It consists of four key domains: patient selection, index test, reference standard, and flow and timing. Each is assessed in terms of risk of bias and the first three in terms of concerns regarding applicability. Signalling questions are included to assist in judgments about the risk of bias [18]. Risk of bias was judged as “low” if all answers to all signal questions for a domain were “yes”, as “high” if any signal question in a domain was “no”, or as “unclear” if insufficient information was provided [18]. Concern about applicability was assigned as “low”, “high” or “unclear” with the similar criteria.

#### Data Extraction

Two investigators reviewed the articles independently. Information was extracted on author, publication year, country (where the specimen came from), specimen type, sample size, gold standard, the number of true-resistance, the number of false-
Meta Analysis

We used Meta-Disc 1.4 (http://www.hrc.es/investigacion/metadisc_en.htm) to analyze data [19]. Heterogeneity was identified by using chi-square test and $I^2$ ($P<0.05$ and $I^2>50\%$ indicated significant heterogeneity) [19–21]. According to the results of heterogeneity testing, we chose an appropriate statistic model (random or fixed model) to pool the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). Sensitivity and specificity and corresponding 95% confidence interval (CI) of each study were calculated according to the reconstructed two by two tables. Pooled sensitivity, specificity, PLR, NLR, and DOR were calculated. Additionally, summary receiver operating characteristic (SROC) curves were plotted. The area under the curve (AUC) and $Q^*$ index were also counted to evaluate the overall performance of the diagnostic test accuracy [19,22]. The AUC of an SROC is a measure of the overall performance of a
diagnostic test to accurately differentiate those with and those without the condition of interest. \(Q^*\) index is defined by the point where sensitivity and specificity are equal, which is closest to the ideal top-left corner of the SROC space. Both values range between 0 and 1, with higher values indicating better test performance. Moreover, in consideration of practical application, subgroup analysis was performed by considering specimen types (clinical specimen or clinical isolates) in this study.

Results

General Characteristics of Studies

A flow chart of inclusion and exclusion procedure of articles is illustrated in Figure 1. In brief, the PubMed search identified 13 articles; the EMBASE search identified 20 articles; and the Web of Science search identified 10 articles. A total of 24 articles was removed due to duplication. Based on the inclusion and exclusion criteria, additional 8 articles were excluded. Finally, 11 eligible articles were included in the meta-analysis and all of them were published in English\[15,16,23–31\]. As some articles evaluated more than one Genotype\(^*\) MTBDR\(sl\) diagnostic test using different specimen types, we defined 14 independent studies (including 2322 samples) from the 11 articles. Among these 14 studies, 3 studies tested clinical specimens, and others used clinical isolates. Among them, 2 studies were performed in Asia, 2 studies were performed in Africa, 8 studies were performed in Europe, and 2 studies didn’t clearly show the study area. Four types of culture media (L-J PM; agar PM; BACTEC MGIT 960; BACTEC 460TB) were used to perform DST in these studies. We summarized the diagnostic characteristics of these 14 studies in Table 1.

Quality Assessment

According to QUADAS-2 assess, only three (21\%) studies were at low risk of patient selection bias while nine (65\%) studies were at high risk of selection bias due to inconsecutive or nonrandom patient selection. The index test bias was minimal compared to patient selection bias. Although four (29\%) studies were lacking information to judge, the remaining ten (71\%) studies were all at low risk of index test bias. A similar situation was observed in the reference standard bias. Eight (57\%) studies were at high risk of flow and timing bias, resulting from the fact that not all selected patients were included in the diagnostic analysis. As for
applicability concerns, the overwhelming majority (86%) studies were at high risk of patient selection; however, all selected studies were at low risk of index test and the reference standard. In general, patient selection was the most high-risk bias and high-risk applicability concerns (Table 2).

Heterogeneity

Significant heterogeneity was observed when we pooled sensitivity, specificity, PLR, NLR, and DOR of selected studies, except for the sensitivity to AM. The heterogeneity test results of sensitivity and specificity are illustrated in the forest plots (Figure 2, 3, 4, 5, 6).

Diagnostic Accuracy

The pooled sensitivity, specificity, PLR, NLR, and DOR and their 95% CIs are listed in Table 3. The summarized sensitivity (95% CI) of GenoType® MTBDRsl was 0.874 (0.845–0.899), 0.826 (0.777–0.869), 0.820 (0.772–0.862), 0.444 (0.396–0.492), and 0.679 (0.652–0.706) for FLQs, AM, CAP, KAN, and EMB, respectively. The specificity (95% CI) was 0.971 (0.961–0.980), 0.995 (0.987–0.998), 0.973 (0.963–0.981), 0.993 (0.985–0.997), and 0.799 (0.773–0.823) for FLQs, AM, CAP, KAN, and EMB, respectively. The AUC (standard error) was 0.9754 (0.0203), 0.9300 (0.0598), 0.9885 (0.0038), 0.9689 (0.0359), and 0.6846 (0.0550) for FLQs, AM, CAP, KAN, and EMB, respectively. Additionally, Q* index (standard error) was 0.9288 (0.0353), 0.8651 (0.0718), 0.9550 (0.0089), 0.9181 (0.0573), and 0.6407 (0.0434) for FLQs, AM, CAP, KAN, and EMB, respectively. The SROC curves (pooled sensitivity against 1-pooled specificity) are shown in Figure 7. Figure 2, 3, 4, 5, 6 depicts the forest plots of sensitivity and specificity.

Subgroup Analysis

According to the type of specimen, 14 studies were classified into two groups for subgroup analysis. Pooled sensitivity, specificity, PLR, NLR and DOR for FLQs, AM, CAP, and EMB are presented in Table 4. As KAN resistance was only performed in the clinical isolates, subgroup analysis was not performed for KAN.
Discussion

In this study, we evaluated the diagnostic accuracy of Genotype MTBDRsl in order to identify whether it was a good tool for rapid drug resistance detection. Findings from this meta-analysis indicated that Genotype MTBDRsl had higher values in detecting drug resistance to FLQs, AM, and CAP by considering the diagnostic index.

Drug resistant tuberculosis has been a severe public health issue worldwide. About 440,000 MDR-TB cases and 25,000 XDR-TB cases are estimated to emerge annually, and 150,000 persons with MDR-TB die each year [32]. The 2009 world health assembly resolution has urged WHO member states “to achieve universal access to diagnosis and treatment of MDR-TB and XDR-TB” [32]. Challenges in standardization for conventional DST persist, especially detection time, inoculum size and dispersion of bacillary clumps, subculture bias, testing environment and critical concentration of second-line drug resistance testing [33]. Newer automated liquid media platforms, such as BACTEC system, may be prone to a higher risk of contamination [34]. Molecular DST mostly utilizes Polymerase Chain Reaction (PCR) to amplify mutation-related genes, and it could significantly shorten detection time. The benefits of rapid DST included increased cure rates, decreased mortality, reduced the development of additional drug resistance, and a reduced likelihood of treatment failure and relapse. The emergence of drug resistant tuberculosis has stimulated the development of molecular kits for rapid detection [35]. Since GenoType MTBC (differentiation of the M. tuberculosis complex from cultured material) was available in 2002–2003, GenoType MTBDR was developed in 2004 and then followed by GenoType MTBDR plus in 2007 and GenoType MTBDRsl in 2009. GenoType MTBDR plus was designed to identify the M. tuberculosis complex and its resistance to rifampicin and/or isoniazid from pulmonary clinical specimens or cultivated samples. The identification of rifampicin resistance is enabled by the detection of the most significant mutations of the rpoB gene (coding for the β-subunit of the RNA polymerase). For testing the high level isoniazid resistance, the katG gene (coding for the catalase peroxidase) is examined. For testing the low level isoniazid resistance, the promoter region of the inhA gene (coding for the NADH enoyl ACP reductase) is analyzed. The GenoType MTBDRsl gives the possibility to diagnose patients with MDR-TB to receive information on further antibiotic resistances to fluoroquinolones, aminoglycosides/cyclic peptides and ethambutol. The identification of drug resistance to fluoroquinolones is enabled by the detection of the mutations of the gyrA gene. For the detection of resistance to aminoglycosides/cyclic peptides, the 16S rRNA gene (rrs) is examined. For the detection of resistance to ethambutol, the embB gene (which, together with the genes embA and embC, codes for arabinosyl transferase) is examined.

In recent years, studies focusing on the diagnostic value of GenoType MTBDRsl were conducted in many settings, but with varied results. Thus, a systematic review is necessary to provide an
overall evaluation. Results from this meta-analysis showed that MTBDR<sub>sl</sub> test has a relatively high sensitivity for FLQs, AM and CAP, but not for KAN and EMB. Moreover, high specificity was observed except for EMB, which indicated that EMB susceptible strains or specimens would be identified as resistant ones with a low possibility. Significant heterogeneity was observed when we pooled sensitivity, specificity, PLR, NLR, and DOR of selected studies, except for the sensitivity to AM. Data were pooled by

![Forest plot of sensitivity for drug resistance to ethambutol.](image)

**Table 3.** Summarized diagnostic accuracy of Genotype MTBDR<sub>sl</sub>.

<table>
<thead>
<tr>
<th>Drug</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>FLQs</td>
<td>0.874 (0.845–0.899)</td>
<td>0.971 (0.961–0.980)</td>
<td>26.368 (12.851–54.102)</td>
<td>0.182 (0.109–0.303)</td>
<td>176.370 (69.230–449.330)</td>
</tr>
<tr>
<td>AM</td>
<td>0.826 (0.777–0.869)</td>
<td>0.995 (0.987–0.998)</td>
<td>68.851 (7.845–604.234)</td>
<td>0.192 (0.150–0.245)</td>
<td>446.130 (66.651–2986.200)</td>
</tr>
<tr>
<td>CAP</td>
<td>0.820 (0.772–0.862)</td>
<td>0.973 (0.963–0.981)</td>
<td>18.211 (9.964–33.285)</td>
<td>0.151 (0.037–0.609)</td>
<td>143.140 (56.896–360.120)</td>
</tr>
<tr>
<td>KAN</td>
<td>0.444 (0.396–0.492)</td>
<td>0.993 (0.985–0.997)</td>
<td>48.693 (7.289–325.260)</td>
<td>0.561 (0.430–0.732)</td>
<td>163.620 (29.812–898.090)</td>
</tr>
<tr>
<td>EMB</td>
<td>0.679 (0.652–0.706)</td>
<td>0.799 (0.773–0.823)</td>
<td>4.879 (2.250–10.581)</td>
<td>0.498 (0.383–0.648)</td>
<td>12.019 (4.189–34.481)</td>
</tr>
</tbody>
</table>

Abbreviations: Se = sensitivity; Sp = specificity; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; CI: confidence interval; FLQs = fluoroquinolones; AM = amikacin; CAP = capreomycin; KAN = kanamycin; EMB = ethambutol.

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proper models according to the heterogeneity results. To illustrate the overall significance of MTBDRsl test, we used multiple index such as AUC, Q* index, and DOR. AUC and Q* index in SROC curve were widely used as the summary index of overall test performance [36]. High AUC and Q* index of FLQs, AM, CAP and KAN except for EMB showed the high accuracy for detecting the resistance to these drugs. The DOR is defined as the ratio of the odds of the test being positive if the subject has a disease relative to the odds of the test being positive if the subject does not have the disease [37]. Higher values of DOR indicate better discriminatory test performance. In this meta-analysis, we observed that DOR of EMB was lower than that of FLQs, AM, CAP and KAN, which indicated that MTBDRsl test might not be a good choice for detecting EMB drug resistance. Although SROC curve and DOR could present the overall performance of the test, they are not easy to be used in clinical practice, and the likelihood ratios (LRs) are of more clinical significance [36]. The LRs combine the sensitivity and specificity into a summary index and indicate how much a given diagnostic test result will raise or lower the pretest probability of the target disease [38]. Although in the current analysis, index such as AUC, Q* index, DOR, and PLR showed good performance for KAN resistance detection, its sensitivity was much lower than FLQs, AM and CAP. In other words, more patients with drug resistance to KAN would be misdiagnosed.

Studies have shown that resistance to fluoroquinolones is associated with mutations in a quinolone resistance-determining region of gyrD and gyrB gene (coding A and B subunits of type II topoisomerase)[39–41]. Although Ala-90 and Asp-94 have been the most frequently mutated positions in gyrD, Gly-88, Ser-91 and Ala-74 were also reported as the possible mutation sites. However, these potential mutation positions were not all included in the Meta-Analysis on Genotype MTBDRsl. Moreover, FLQs stand for a series of antibiotics including ofloxacin, ciprofloxacin, moxifloxacin and gatifloxacin, etc. Moxifloxacin and ofloxacin were the most frequently used drugs in the studies that were involved in this series of antibiotics including ofloxacin, ciprofloxacin, moxifloxacin and gatifloxacin, etc. Moxifloxacin and ofloxacin were the most frequently used drugs in the studies that were involved in this meta-analysis. Mutations in rs gene alone were found to occur in up to 7% of CAP-susceptible strains [47]. Cross-resistance between KAN and AM or between KAN and CAP has been observed [45–48]. Mutations in eis promoter region of M. tuberculosis was also reported to be associated with KAN resistance but not being covered by MTBDRsl strip [49,50]. These facts may explain the discordant accuracy results among AM, CAP and KAN although they were tested by one strip with the same mutation positions.

While mutations in codon 306 of embB were recognized to be related to EMB resistance [51,52], the molecular basis of MTBDRsl for EMB was not sufficient. Previous studies showed that percentage of emb306 mutations in EMB resistant strains varied from 30% to 87.5% [15,23,24,53]. Furthermore, mutations at emb306 were reported to be associated with a broad antibiotic resistance rather than EMB resistance [54]. In addition, Huang and colleagues [2012] identified codon 319, codon 497 and other seven novel mutation positions of embB gene in the EMB-resistant strains [26]. These facts implied that emb306 mutation was not a stable and unique marker for detecting EMB drug resistance. Plinke et al. [2009] found that EMB resistant clinical isolates had an increased minimum inhibitory concentration (MIC) as compared to the susceptible ones; but the increase of the MIC was below the value of the critical concentration (2 mg/ml EMB) [55]. Therefore, these strains were regarded as susceptible to EMB by the conventional DST method on Lowenstein Jensen (LJ) media. Previous reports have highlighted the problems of the phenotypic DST on EMB carried out by MGIT [56,57]. Indeed, EMB testing by MGIT is more affected by lower sensitivity/specifcity, lower reproducibility and higher rate of false-positive in detecting resistant cases. In this regard, MGIT as the gold standard when comparing with MTBDRsl may under-evaluate the sensitivity and specificity for EMB resistance detection. One paper included in this meta-analysis clearly considered this point providing sensitivity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Specimen type</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>FLQs</td>
<td>Clinical isolates</td>
<td>0.879(0.850–0.904)</td>
<td>0.970(0.958–0.979)</td>
<td>26.399(11.610–60.027)</td>
<td>0.167(0.103–0.269)</td>
<td>192.690(71.070–522.420)</td>
</tr>
<tr>
<td></td>
<td>Clinical specimen</td>
<td>0.750(0.533–0.902)</td>
<td>0.986(0.951–0.998)</td>
<td>31.083(4.631–208.629)</td>
<td>0.225(0.031–1.653)</td>
<td>159.810(6.512–3921.400)</td>
</tr>
<tr>
<td>AM</td>
<td>Clinical isolates</td>
<td>0.833(0.783–0.876)</td>
<td>1.000(0.996–1.000)</td>
<td>120.34(28.834–502.202)</td>
<td>0.179(0.138–0.233)</td>
<td>1354.100(321.070–5710.500)</td>
</tr>
<tr>
<td></td>
<td>Clinical specimen</td>
<td>0.722(0.465–0.903)</td>
<td>0.949(0.885–0.983)</td>
<td>16.517(1.211–225.306)</td>
<td>0.310(0.153–0.628)</td>
<td>50.934(4.094–633.740)</td>
</tr>
<tr>
<td>CAP</td>
<td>Clinical isolates</td>
<td>0.803(0.750–0.849)</td>
<td>0.975(0.964–0.983)</td>
<td>20.535(11.418–36.933)</td>
<td>0.181(0.038–0.855)</td>
<td>124.310(42.688–361.990)</td>
</tr>
<tr>
<td></td>
<td>Clinical specimen</td>
<td>0.968(0.833–0.999)</td>
<td>0.951(0.878–0.986)</td>
<td>17.159(1.394–211.166)</td>
<td>0.089(0.013–0.589)</td>
<td>361.390(39.769–3284.000)</td>
</tr>
<tr>
<td>EMB</td>
<td>Clinical isolates</td>
<td>0.690(0.662–0.718)</td>
<td>0.805(0.778–0.830)</td>
<td>6.919(2.538–18.865)</td>
<td>0.467(0.345–0.632)</td>
<td>17.182(7.200–62.538)</td>
</tr>
<tr>
<td></td>
<td>Clinical specimen</td>
<td>0.536(0.424–0.645)</td>
<td>0.732(0.622–0.824)</td>
<td>1.935(0.743–5.038)</td>
<td>0.631(0.488–0.816)</td>
<td>3.839(0.960–15.355)</td>
</tr>
</tbody>
</table>

Abbreviations: Se = sensitivity; Sp = specificity; PLR = positive likelihood ratio; NLR = negative likelihood ratio; DOR = diagnostic odds ratio; CI = confidence interval; FLQs = fluoroquinolones; AM = amikacin; CAP = capreomycin; EMB = ethambutol.

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and specificity for EMB resistance adjusted for the results obtained retesting discrepant cases between MGIT and MTBDRsl [29].

There are several limitations of this study. While the bias of patient selection, index test, reference standard and flow and timing were all observed in this meta-analysis according to the QUADAS-2 assessment, most studies (79%) were at high-risk bias in patient selection. The lack of blinding resulted in unclear assessments of bias of index and reference test sections. In addition, regarding data analysis in each study, not all samples included were analyzed because of invalid results, leading to a high risk of flow and timing section bias. As for the review-level, four studies identified by the searching strategy were conference abstract and could not provide exact two by two tables, which affected the pooled data. Moreover, only 3 out of 14 studies tested clinical specimens, providing insufficient data for subgroup analysis for all five drugs.

Conclusions

Genotype MTBDRsl showed good accuracy for detecting drug resistance to FLQs, AM, and CAP of M. tuberculosis, but may not be an appropriate choice for KAN and EMB. The lack of data did not allow for proper evaluation of the test on clinical specimens. Further systematic assessment of diagnostic performances should be carried out on direct clinical samples.

Author Contributions

Conceived and designed the experiments: YF JW WL. Analyzed the data: YF SL QW LW ST JW WL. Wrote the paper: YF LW JW.

References

5. WHO (2006) Emergence of XDR-TB.