Rapid assays for fluoroquinolone resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis

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Objectives: Multidrug-resistant tuberculosis has emerged as a global health threat. Given poor treatment outcomes of fluoroquinolone-resistant multidrug-resistant tuberculosis, there is a pressing need for rapid drug susceptibility testing of multidrug-resistant Mycobacterium tuberculosis against fluoroquinolones. This review aims at evaluating these rapid assays.

Methods: PubMed and OvidSP were used to search MEDLINE and EMBASE for publications in English regarding rapid assays that tested ofloxacin, levofloxacin or moxifloxacin. Studies were included only in the concurrent presence of sensitivity and specificity data. Summary estimates of sensitivity and specificity were generated by the bivariate random effects model when there were at least three sets of data under the same assay category that tested the same fluoroquinolone with reference to a standard test.

Results: Of 108 articles identified, 24 articles were included in a meta-analysis of rapid assays that tested ofloxacin in culture isolates. Overall, rapid genotypic assays targeting gyrA only are significantly less specific (96% versus 99%) and non-significantly less sensitive (88% versus 94%) than rapid phenotypic assays. To test for the presence or absence of ofloxacin resistance to a certainty threshold of 90%, the required pre-test prevalence ranges of ofloxacin resistance for genotypic assays targeting gyrA only are 29%–47% overall, 36%–55% for PCR–DNA sequencing and 23%–44% for others. Corresponding ranges are 7%–65% for phenotypic assays overall and 3%–75% for Mycobacteria Growth Indicator Tube (MGIT).

Conclusions: Assuming that the mean pre-test prevalence of fluoroquinolone resistance in culture isolates of multidrug-resistant M. tuberculosis is ~20%, rapid genotypic assays other than PCR–DNA sequencing, targeting gyrA only, can reliably screen for ofloxacin resistance.

Keywords: drug susceptibility testing, fluoroquinolones, molecular, resistant, tuberculosis

Introduction

Multidrug-resistant tuberculosis (MDR-TB) has emerged as a global health threat. The global proportion of MDR-TB has been estimated to be 2.9% among new cases, 15.3% among retreatment cases and 5.3% among all cases.1 Fluoroquinolones play a pivotal role in the treatment of MDR-TB. It has been shown that fluoroquinolone resistance, a key defining condition of extensively drug-resistant tuberculosis (XDR-TB), is significantly associated with poor treatment outcomes, whereas the inclusion of fluoroquinolones in drug regimens significantly improves treatment outcomes of MDR-TB.2–4 Given the global emergence of MDR-TB and poor treatment outcomes of fluoroquinolone-resistant MDR-TB2 and XDR-TB,6 there is a pressing need for rapid drug susceptibility testing (DST) of MDR Mycobacterium tuberculosis against fluoroquinolones to improve clinical management.

Rapid DST of M. tuberculosis can be broadly classified as genotypic (or molecular) versus phenotypic assays. National tuberculosis (TB) programme managers are often faced with the difficulty of choosing an appropriate rapid assay. Besides field factors such as costs, sustainability and accessibility,7 test performance in terms of predictive values and turnaround time fundamentally influence the choice of rapid assays. Rapid genotypic tests can yield results within 1 day in either culture isolates or clinical specimens.8–11 Although the turnaround time of a rapid phenotypic assay is generally longer than that of a genotypic counterpart by 1–2 weeks in comparable samples,12–16 it is probably important to consider test reliability before turnaround time. A literature search through MEDLINE and EMBASE with key
phrases did not show any previous systematic review on the diagnostic performance of rapid assays for DST of M. tuberculosis against fluoroquinolones. This review aims at clarifying the clinical roles of these assays by comparing their test characteristics, with focus on likelihood ratios (LRs) and predictive values. As ofloxacin is predominantly used in DST of M. tuberculosis against fluoroquinolones, and levofloxacin and moxifloxacin are the major fluoroquinolones used in TB treatment, this review has examined rapid assays regarding these fluoroquinolones only.

Methods
PubMed and OvidSP were used to search for biomedical articles from MEDLINE, life science journals and EMBASE through 13 February 2010 for non-review and non-editorial publications in English regarding human subjects. The following key phrases containing Medical Subject Headings or keywords in titles or abstracts were used with the help of Boolean operators (‘and’, ‘or’) and wildcards: (i) tuberculosis; (ii) fluoroquinolone, levofloxacin, ofloxacin or moxifloxacin; (iii) resistance, susceptibility, test, assay or method; (iv) molecular, gyrase, genotype, mutation, hybridization, array, microarray, macroarray, probe, chip, biprobe or microchip; and (v) mycobacteria growth indicator tube, MGIT, microscopic observation drug susceptibility assay, MODS, slide culture, microcolonies, thin-layer agar, TLA, colorimetric, redox, indicator, dye, alamar, malachite, resazurin, tetrathionate, nitrate reductase assay, Griess, fastplaque, phage, mycobacteriophage, D29, luminometry, Bronx, luciferase, fluoromycoebacteriophage, fluorescent or flow cytometry. The search algorithm used in PubMed is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

Inclusion criteria
A study was included only in the concurrent presence of sensitivity and specificity data with reference to a standard method for DST of M. tuberculosis against ofloxacin, levofloxacin or moxifloxacin. There should be at least three subjects each for estimating sensitivity and specificity. Standard DST methods included the proportion method, the absolute concentration method, BACTEC 460 or BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960, which has been recommended by the WHO for drug resistance surveillance.7 Data were extracted by the first author in duplicates.

Exclusion criteria
Studies were grouped for meta-analysis by a combination of the assay category, the fluoroquinolone tested and the category of samples (culture isolates versus clinical specimens). A study would be excluded when there were fewer than three sets of data in a group after considering heterogeneity, which was resolved by classifying data under different groups. Sources of heterogeneity were examined by unweighted meta-regression analysis using the Moses–Shapiro–Littenberg method, which involves regression of the log diagnostic odds ratio against a measure of the diagnostic threshold.17–19 Significant heterogeneity for a covariate was considered present when the P value was <0.05. Covariates included the country of origin (for countries contributing at least two sets of data), the presence or absence of a defined study period and the breakpoint for resistance.

Summary estimates of sensitivity, specificity, positive LR and negative LR were generated by the bivariate random effects model using the SAS PROC MIXED procedure20 in the presence of at least three sets of data grouped under the same category of assays testing the same fluoroquinolone in the same category of samples. Funnel plot asymmetry was examined by a regression of the natural log diagnostic odds ratio against the standard error for each test method.21 Significant asymmetry was denoted by P values of ≤0.05.

Assuming that a rapid assay for DST is clinically useful when it can rule in or rule out resistance to ≥90% certainty threshold, the pre-test prevalence of fluoroquinolone resistance required for a test to attain predictive values of ≥90% was estimated with the following equations: (i) post-test odds=PPV/(1−PPV); (ii) post-test odds=1−NPV/NPV; (iii) pre-test odds=post-test odds/positive LR; (iv) pre-test odds=post-test odds/negative LR; and (v) pre-test prevalence=pre-test odds/(1+pre-test odds). NPV and PPV stand for negative and positive predictive values, respectively.

Meta-DiSc version 1.4,22 SAS Enterprise Guide 3.0, OpenOffice.org 3.0 and SPSS version 10 (Chicago, IL, USA) were used for statistical analysis.

Results
The literature search initially identified 108 articles. Figure 1 shows how 32 sets of data from 24 articles10–14,23–41 eventually were included in a meta-analysis of rapid assays for DST of M. tuberculosis against ofloxacin. Available data did not allow meta-analysis of the following groups of rapid assays applied in culture isolates, owing to inadequate data: genotypic assays regarding levofloxacin,25,42 (n=2); genotypic assays regarding moxifloxacin25,28 (n=2); MGIT 960 regarding levofloxacin43,44 (n=2); and the tetrathionate microplate assay regarding moxifloxacin.29 (n=1). Available data were also insufficient for meta-analysis of rapid genotypic assays applied in sputum specimens regarding ofloxacin10,11 (n=2) or levofloxacin15,25 (n=1). Table 1 summarizes 33 sets of data extracted from 25 articles10–14,23–41,46 initially included for meta-analysis. To reduce heterogeneity, the breakpoint for ofloxacin resistance was changed from 1 mg/L originally used by investigators to 2 mg/L in two studies that contained sufficient details for calculating sensitivity and specificity accordingly.15,46 One study on PCR–DNA sequencing that used 8 mg/L as the breakpoint for ofloxacin resistance was subsequently excluded, owing to significant heterogeneity due to the breakpoint and this study being the only study of its kind. No other significant sources of heterogeneity were identified.

Table 2 shows the test characteristics of rapid genotypic and phenotypic assays for resistance to ofloxacin in culture isolates of M. tuberculosis. Rapid genotypic assays are broadly classified by the gene target: gyrA versus gyrB and gyrB. Assays with the target at gyrA are further subdivided into PCR–DNA sequencing and other genotypic assays. Rapid phenotypic assays largely comprise MGIT and the resazurin assay. In the absence of significant heterogeneity, the nitrate reductase assay (NRA) and miscellaneous phenotypic assays are combined to facilitate statistical analysis. Overall, rapid genotypic assays targeting gyrA only are non-significantly less sensitive (88% versus 93%, P=0.36) and more specific (96% versus 91%, P=0.48) than those targeting gyrA and gyrB, and non-significantly less sensitive (88% versus 94%, P=0.08) and significantly less specific (96% versus 99%, P=0.03) than rapid phenotypic assays. Table 3 shows the P values of comparison between subgroups of rapid genotypic and phenotypic assays by sensitivity, specificity and diagnostic odds ratio. PCR–DNA sequencing with the target at gyrA only is more sensitive (92% versus 86%) and less specific (94% versus 97%) than other rapid genotypic
assays targeting gyrA only, but the difference is statistically non-significant. Among rapid phenotypic assays, MGIT is comparable to the resazurin assay by sensitivity (both 96%), more sensitive than NRA and miscellaneous (96% versus 90%), and more specific than the resazurin assay (100% versus 99%) and NRA and miscellaneous (100% versus 99%), but differences are again statistically non-significant. Compared with MGIT, PCR–DNA sequencing with the target at gyrA only is non-significantly less sensitive (92% versus 96%) but significantly less specific (94% versus 100%), whereas other rapid genotypic assays targeting gyrA only are non-significantly less sensitive (86% versus 96%) and non-significantly less specific (97% versus 100%).

Table 4 shows the pre-test prevalence of resistance to ofloxacin required for rapid assays to attain predictive values of ≥90%. To attain ≥90% for both PPV and NPV, the required pre-test prevalence ranges of ofloxacin resistance for rapid genotypic assays targeting gyrA only would be 29%–47% overall, 36%–55% for PCR–DNA sequencing and 23%–44% for other genotypic assays. Corresponding ranges would be 47%–59% for rapid genotypic assays targeting gyrA and gyrB, 7%–65% for phenotypic assays overall, 3%–75% for MGIT, 6%–75% for the resazurin assay, and 12%–52% for NRA and miscellaneous. Increasing critical predictive values to 95% reduces the corresponding pre-test prevalence ranges, and makes it unlikely for genotypic assays to achieve ≥95% for PPV and NPV concurrently.

Table 2 shows no evidence of significant funnel plot asymmetry in all assay subgroups, except for NRA and miscellaneous phenotypic assays. Overall, funnel plot asymmetry is significant for rapid phenotypic assays, but non-significant for rapid genotypic assays targeting gyrA only. Funnel plot asymmetry may suggest bias in meta-analysis due to publication bias for studies with positive findings or bias due to exaggerated estimates from smaller studies or studies of lower quality.

Sensitivity analyses restricted to studies of possibly higher quality, as denoted by the availability of the sampling method of cases,11 controls or both,19,24–26,28,35,36,38,40 were similar to the main findings (see Table 5).
Table 1. Thirty-three sets of data initially considered for meta-analysis of rapid assays for ofloxacin resistance in culture isolates of *M. tuberculosis*

<table>
<thead>
<tr>
<th>Assays</th>
<th>Country source of study subjects</th>
<th>Study period</th>
<th>Study design</th>
<th>Sampling method (cases)</th>
<th>Sampling method (controls)</th>
<th>Reference test</th>
<th>Breakpoint for resistance in reference test (phenotypic assay, if applicable), mg/L</th>
<th>R</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR–DNA sequencing 40</td>
<td>Shanghai, China</td>
<td>Mar 2004 – Nov 2007</td>
<td>XS</td>
<td>available MDR strains</td>
<td>available MDR strains</td>
<td>proportion method</td>
<td>2</td>
<td>54</td>
<td>121</td>
</tr>
<tr>
<td>PCR–DNA sequencing 28</td>
<td>Hong Kong</td>
<td>1999 – 2003</td>
<td>XS</td>
<td>ofloxacin-resistant strains with MIC ≥ 4 mg/L out of a collection of MDR strains, plus 11 non-MDR ofloxacin-resistant isolates</td>
<td>at random from ofloxacin-susceptible strains out of a collection of MDR strains</td>
<td>absolute concentration method in LJ, followed by MGIT</td>
<td>2</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>PCR–DNA sequencing, denaturing HPLC 16</td>
<td>Beijing, China</td>
<td>2002 – 03</td>
<td>XS</td>
<td>available patients with pulmonary TB</td>
<td>available patients with pulmonary TB</td>
<td>proportion method</td>
<td>2</td>
<td>68</td>
<td>41</td>
</tr>
<tr>
<td>PCR–DNA sequencing, resazurin assay 39</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>proportion method on 7H11 agar</td>
<td>2 (same)</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>PCR–DNA sequencing 46</td>
<td>various states in India</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>absolute concentration method on LJ</td>
<td>8</td>
<td>71</td>
<td>47</td>
</tr>
<tr>
<td>PCR–DNA sequencing 26</td>
<td>Karakalpakstan</td>
<td>Oct 2003 – Feb 2006</td>
<td>nested CC</td>
<td>MDR-TB patients of a regional TB programme</td>
<td>at random</td>
<td>proportion method in solid medium, BACTEC 460 or MGIT</td>
<td>2</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>PCR–DNA sequencing 14</td>
<td>Japan (largely); Poland Philippines</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>proportion method in 7H10 agar</td>
<td>2</td>
<td>3</td>
<td>135</td>
</tr>
<tr>
<td>Pyrosequencing 13</td>
<td>Philippines</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>submerged-disc proportion method</td>
<td>2</td>
<td>10</td>
<td>92</td>
</tr>
<tr>
<td>Microchip array 30</td>
<td>Moscow, Russian Federation</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>absolute concentration method</td>
<td>2</td>
<td>52</td>
<td>55</td>
</tr>
<tr>
<td>LNA-PCR 41</td>
<td>Ho Chi Minh City, Vietnam</td>
<td>Jul 2005 – Jul 2006</td>
<td>CC</td>
<td>all available FQ-resistant isolates received by a hospital</td>
<td>not mentioned</td>
<td>proportion method</td>
<td>2</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>A simplified non-radioactive PCR–SSCP 37</td>
<td>France (not stated explicitly)</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>proportion method, not explicitly mentioned, but compatible with 2 mg/L</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
PCR–SSCP/MPAC, then PCR–DNA sequencing

Hong Kong 1994–2004 CC all isolates collected from a hospital and with resistance to one or more of five TB drugs (HREZO) at random absolute concentration method

MTBDRsl11 Germany not mentioned CC not mentioned at random proportion method in LJ or MGIT

Reverse hybridization-based line probe assay77 Rome, Florence, Ancona, Milan, Siena, Italy not mentioned CC not mentioned proportion method in 7H11 agar

MPAC25 Hong Kong 1991–2000 CC ofloxacin-resistant strains among MDR strains largely from one hospital ofloxacin-susceptible MDR strains plus some selected at random absolute concentration method


MGIT13 Mumbai, India not mentioned CC not mentioned BACTEC 460 (same) 2 (same) 19 778

MGIT (manual)30 Institute of Tropical Medicine in Antwerp, Belgium not mentioned CC not mentioned proportion method in 7H11 agar (same) 2 (same) 41 147

IGIT15 Borstel, Germany; Cordoba, Spain; London, UK not mentioned CC MDR strains with high drug resistance as far as possible MDR strains with high drug resistance as far as possible BACTEC 460 (same) 2 (same) 9 83

Resazurin assay38 Rwanda; Benin; Bangladesh not mentioned CC ofloxacin-resistant strains among a collection of MDR isolates ofloxacin-susceptible strains among a collection of MDR isolates BACTEC 460 (same) 2 (same) 14 106

Resazurin assay, NRA12 Peru; Armenia; Azerbaijan; Georgia; Kazakhstan not mentioned CC not mentioned not mentioned proportion method in 7H11 agar, further confirmed by MGIT (same) 2 (same) 8 87

Resazurin assay2 Bolivian; Peru; Eastern European countries not mentioned CC not mentioned not mentioned proportion method in 7H11 agar (same) 2 (same) 8 142

NRA34 Honduras; Sweden largely 2002–06; unspecified for 40% CC not mentioned not mentioned BACTEC 460 (same) 2 (same) 45 43

NRA, MODS13 Nashville, TN, USA 2005–06 XS available isolates available isolates agar proportion method (same) 2 (same) 6 233

Continued
<table>
<thead>
<tr>
<th>Assays</th>
<th>Country source of study subjects</th>
<th>Study period</th>
<th>Study design&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sampling method (cases)</th>
<th>Sampling method (controls)</th>
<th>Reference test</th>
<th>Breakpoint for resistance in reference test (phenotypic assay, if applicable), mg/L</th>
<th>R</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLA&lt;sup&gt;31&lt;/sup&gt;</td>
<td>Institute of Tropical Medicine in Antwerp, Belgium</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>proportion method in 7H11 agar</td>
<td>2 (same)</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>Tetrazolium microtitre plate assay&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Samara Region of the Russian Federation; UK</td>
<td>not mentioned</td>
<td>CC</td>
<td>not mentioned</td>
<td>not mentioned</td>
<td>MGIT</td>
<td>1 (same)</td>
<td>34</td>
<td>97</td>
</tr>
</tbody>
</table>

CC, case–control; FQ, fluoroquinolone; HREZO, isoniazid, rifampicin, ethambutol, pyrazinamide and ofloxacin; LJ, Lowenstein–Jensen medium; LNA-PCR, locked nucleic acid probe real-time PCR; MDR, multidrug resistant; MGIT, Mycobacteria Growth Indicator Tube 960; MODS, microscopic observation drug susceptibility assay; MPAC, multiplex PCR amplimer conformation analysis; MTBDR<sup>sl</sup>, GenoType<sup>®</sup> M. tuberculosis drug resistance second line assay; R, ofloxacin-resistant cases; S, ofloxacin-susceptible controls; SSCP, single-stranded conformation polymorphism; TB, tuberculosis; TLA, thin-layer agar; XS, cross-sectional; NRA, nitrate reductase assay.

<sup>a</sup>None of the studies involved blinding during testing, except for two<sup>31,38</sup> with full blinding and one<sup>13</sup> with limited blinding in six samples for quality control.

<sup>b</sup>Using criteria originally defined by Angeby et al.<sup>60</sup>
### Table 2. Test characteristics of rapid assays for ofloxacin resistance in culture isolates of *M. tuberculosis*

<table>
<thead>
<tr>
<th>Assay category (gene target for genotypic assays)</th>
<th>Sets of data</th>
<th>Resistant strains</th>
<th>Susceptible strains</th>
<th>Mean sensitivity, % (95% CI)</th>
<th>Mean specificity, % (95% CI)</th>
<th>Mean positive LR (95% CI)</th>
<th>Mean negative LR (95% CI)</th>
<th>Mean diagnostic odds ratio (95% CI)</th>
<th>Funnel plot (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypic assays <em>(gyrA + gyrB)</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>70</td>
<td>73</td>
<td>93 (81–98)</td>
<td>91 (51–99)</td>
<td>10.1 (1.1–94.3)</td>
<td>0.08 (0.02–0.25)</td>
<td>131 (10–1649)</td>
<td>0.39</td>
</tr>
<tr>
<td>Genotypic assays <em>(gyrA)</em></td>
<td>15</td>
<td>543</td>
<td>977</td>
<td>88 (83–92)</td>
<td>96 (90–98)</td>
<td>22.0 (8.1–59.2)</td>
<td>0.12 (0.08–0.19)</td>
<td>177 (60–523)</td>
<td>0.92</td>
</tr>
<tr>
<td>PCR–DNA sequencing <em>(gyrA)</em></td>
<td>6</td>
<td>211</td>
<td>381</td>
<td>92 (84–96)</td>
<td>94 (78–99)</td>
<td>16.0 (3.4–75.7)</td>
<td>0.09 (0.04–0.19)</td>
<td>179 (32–1009)</td>
<td>0.07</td>
</tr>
<tr>
<td>Others <em>(gyrA)</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>332</td>
<td>596</td>
<td>86 (79–91)</td>
<td>97 (90–99)</td>
<td>29.7 (8.1–109.2)</td>
<td>0.14 (0.08–0.24)</td>
<td>209 (51–851)</td>
<td>0.17</td>
</tr>
<tr>
<td>Phenotypic assays</td>
<td>14</td>
<td>265</td>
<td>2216</td>
<td>94 (88–97)</td>
<td>99 (98–100)</td>
<td>118.6 (39.9–352.5)</td>
<td>0.06 (0.03–0.12)</td>
<td>1955 (531–7193)</td>
<td>0.003</td>
</tr>
<tr>
<td>MGIT&lt;sup&gt;13,30,33,35&lt;/sup&gt;</td>
<td>4</td>
<td>72</td>
<td>1078</td>
<td>96 (85–99)</td>
<td>100 (97–100)</td>
<td>312.2 (36.6–2663.5)</td>
<td>0.04 (0.01–0.17)</td>
<td>8633 (616–120962)</td>
<td>0.11</td>
</tr>
<tr>
<td>resazurin assay&lt;sup&gt;12,32,38,39&lt;/sup&gt;</td>
<td>4</td>
<td>55</td>
<td>350</td>
<td>96 (85–99)</td>
<td>99 (94–100)</td>
<td>131.1 (15.3–1122.1)</td>
<td>0.04 (0.01–0.17)</td>
<td>3473 (248–48559)</td>
<td>0.72</td>
</tr>
<tr>
<td>nitrate reductase assay&lt;sup&gt;12,33,34&lt;/sup&gt; and miscellaneous&lt;sup&gt;13,29,31&lt;/sup&gt;</td>
<td>6</td>
<td>138</td>
<td>788</td>
<td>90 (78–96)</td>
<td>99 (93–100)</td>
<td>64.0 (12.9–316.2)</td>
<td>0.10 (0.04–0.26)</td>
<td>626 (99–3943)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CI, confidence interval; LR, likelihood ratio; MGIT, Mycobacteria Growth Indicator Tube 960.

<sup>a</sup>Comprised PCR–DNA sequencing and a reverse hybridization-based line probe assay.

<sup>b</sup>Comprised pyrosequencing, microchip array, locked nucleic acid probe real-time PCR, reverse hybridization-based line probe assay, simplified non-radioactive PCR–single-stranded conformation polymorphism (SSCP) analysis, multiplex PCR amplimer conformation (MPAC) analysis, SSCP/MPAC analysis, denaturing HPLC and GenoType® *M. tuberculosis* drug resistance second line assay.

<sup>c</sup>Comprised thin-layer agar, microscopic observation drug susceptibility assay and tetrazolium microplate assay.
Diagnostic odds ratio

<table>
<thead>
<tr>
<th>Assay category [gene(s) for genotypic assays]</th>
<th>Genotypic assays (gyrA + gyrB)</th>
<th>Genotypic assays (gyrA)</th>
<th>MGIT</th>
<th>Resazurin assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence levels for attaining</td>
<td>PPV</td>
<td>NPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>other genotypic assays (gyrA)</td>
<td>0.86</td>
<td>0.03</td>
<td>0.004</td>
<td>0.03</td>
</tr>
<tr>
<td>genotypic assays (gyrA+gyrB)</td>
<td>0.80</td>
<td>0.03</td>
<td>0.003</td>
<td>0.03</td>
</tr>
<tr>
<td>MGIT</td>
<td>0.004</td>
<td>0.006</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>resazurin assay</td>
<td>0.03</td>
<td>0.03</td>
<td>0.032</td>
<td>0.032</td>
</tr>
<tr>
<td>NRA and miscellaneous</td>
<td>0.20</td>
<td>0.21</td>
<td>0.205</td>
<td>0.205</td>
</tr>
</tbody>
</table>

Table 3. Comparing the test characteristics of rapid assays for ofloxacin resistance in culture isolates of M. tuberculosis

MGIT, Mycobacteria Growth Indicator Tube 960; NRA, nitrate reductase assay.

Table 4. Pre-test prevalence of ofloxacin resistance required of rapid assays for DST of M. tuberculosis to attain predictive values ≥90%

<table>
<thead>
<tr>
<th>Assay category [gene(s) for genotypic assays]</th>
<th>Prevalence levels for attaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypic assays (gyrA + gyrB)</td>
<td>PPV</td>
</tr>
<tr>
<td>≥47%</td>
<td>≥65%</td>
</tr>
<tr>
<td>Genotypic assays (gyrA)</td>
<td>≥29%</td>
</tr>
<tr>
<td>PCR–DNA sequencing (gyrA)</td>
<td>≥36%</td>
</tr>
<tr>
<td>other genotypic assays (gyrA)</td>
<td>≥23%</td>
</tr>
<tr>
<td>Phenotypic assays</td>
<td>≥7%</td>
</tr>
<tr>
<td>MGIT</td>
<td>≥3%</td>
</tr>
<tr>
<td>resazurin assay</td>
<td>≥6%</td>
</tr>
<tr>
<td>NRA and miscellaneous</td>
<td>≥12%</td>
</tr>
</tbody>
</table>

DST, drug susceptibility testing; MGIT, Mycobacteria Growth Indicator Tube 960; NPV, negative predictive value; NRA, nitrate reductase assay; PPV, positive predictive value.

Discussion

To our knowledge, this is probably the first systematic review and meta-analysis of the test characteristics of rapid genotypic and phenotypic assays for ofloxacin resistance in culture isolates of M. tuberculosis. Available data were insufficient for evaluating the test performance of rapid assays applied directly in clinical specimens. Targeting gyrB in addition to gyrA enhances the sensitivity and reduces the specificity of rapid genotypic assays, but the difference failed to reach statistical significance. In general, rapid genotypic assays targeting gyrA only are non-significantly less sensitive but significantly less specific than rapid phenotypic assays.

Notwithstanding lower sensitivity and specificity, rapid genotypic assays other than PCR–DNA sequencing, targeting gyrA only, are probably reliable for detecting ofloxacin resistance to a certainty threshold of 90% when the prevalence of ofloxacin resistance is ≥23%. A number of studies suggest that this prevalence level probably approximates the best-estimated or mean prevalence of fluoroquinolone resistance in MDR-TB patients or other at-risk groups. The prevalence levels of fluoroquinolone resistance generally associated with antimicrobial use in the treatment of MDR-TB and other bacterial sepsis were ~20%. The mean prevalence level of resistance to ofloxacin among 267 MDR-TB strains in Hong Kong from 1999 to 2005 also amounted to this value. Higher prevalence has been reported: 50% of the bacilli harboured by MDR-TB patients enrolled in the Green Light Programme initiated by the WHO were resistant to a fluoroquinolone. Unless the prevalence of resistance to ofloxacin exceeds 44% (see Table 4), rapid genotypic assays other than PCR–DNA sequencing, targeting gyrA only, are also reliable for ruling out ofloxacin resistance at a certainty threshold of 90%. Thus, these rapid genotypic assays can reliably test for the presence or absence of ofloxacin resistance among MDR-TB patients, especially in national TB programme settings that permit the use of molecular-based methods for the diagnosis of MDR-TB. In the absence of risk factors, when the prevalence

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Table 5. Sensitivity analysis restricted to studies of possibly higher quality

<table>
<thead>
<tr>
<th>Assay category (gene target for genotypic assays)</th>
<th>Sets of data</th>
<th>Resistant strains</th>
<th>Susceptible strains</th>
<th>Mean sensitivity, % (95% CI)</th>
<th>Mean specificity, % (95% CI)</th>
<th>Mean positive LR (95% CI)</th>
<th>Mean negative LR (95% CI)</th>
<th>Mean diagnostic odds ratio (95% CI)</th>
<th>Funnel plot P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypic assays (gyrA) PCR–DNA sequencing</td>
<td>9</td>
<td>431</td>
<td>668</td>
<td>88% (81%–93%)</td>
<td>96% (88%–99%)</td>
<td>24.0 (6.6–87.3)</td>
<td>0.12 (0.07–0.21)</td>
<td>199 (49–811)</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>183</td>
<td>231</td>
<td>91% (81%–96%)</td>
<td>95% (71%–99%)</td>
<td>17.3 (2.4–123.2)</td>
<td>0.09 (0.04–0.22)</td>
<td>189 (22–1630)</td>
<td>0.72</td>
</tr>
<tr>
<td>others (gyrA)</td>
<td>5</td>
<td>248</td>
<td>437</td>
<td>86% (75%–93%)</td>
<td>97% (86%–100%)</td>
<td>32.0 (5.5–184.7)</td>
<td>0.14 (0.07–0.29)</td>
<td>223 (34–1479)</td>
<td>0.61</td>
</tr>
<tr>
<td>Phenotypic assays</td>
<td>5</td>
<td>54</td>
<td>1433</td>
<td>95% (84%–99%)</td>
<td>100% (97%–100%)</td>
<td>226.0 (35.5–1438.8)</td>
<td>0.05 (0.01–0.19)</td>
<td>4890 (483–49539)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

CI, confidence interval; LR, likelihood ratio.

To attain >90% for both positive and negative predictive values, the required pretest prevalence ranges of ofloxacin resistance for rapid genotypic assays targeting gyrA only are 27%–48% overall, 34%–55% for PCR–DNA sequencing and 22%–44% for other genotypic assays. The corresponding range for phenotypic assays is 4%–71%.
or similar cohorts. Estimates based on case–control studies are often exaggerated when cases and controls belong to different cohorts. Nonetheless, sensitivity analysis restricted to studies of possibly higher quality has corroborated the main findings of the current review. Third, the number of studies used for meta-analysis in individual assay subgroups was relatively small. This could have reduced the statistical power for detecting funnel plot asymmetry and showing differences between assay subgroups. Fourth, the presence of significant funnel plot asymmetry among all rapid phenotypic assays might suggest an overestimation of their overall test performance. Although this might lend support to a preference for rapid genotypic over phenotypic assays among MDR-TB and other high-risk patients, it should be noted that funnel plot asymmetry was non-significant for MGIT and resazurin assays. Fifth, heterogeneity exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true effect heterogeneity exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true effect variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. The estimate obtained from a random effects model refers to a mean effect about which true variability exists between included studies (data not shown). Although the random effects model allows for the presence of heterogeneity, there may still be some controversy about combining study estimates in its presence. 

In conclusion, the current review suggests that rapid genotypic assays other than PCR–DNA sequencing, targeting gyrA only, can reliably test for the presence or absence of ofloxacin resistance in culture isolates of MDR M. tuberculosis.

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**Transparency declarations**

None to declare.

**Supplementary data**

The search algorithm used in PubMed is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


