Journal of Antimicrobial Chemotherapy Advance Access published June 11, 2010

J Antimicrob Chemother
doi:10.1093/jac/dkq202

Journal of Antimicrobial Chemotherapy

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

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Received 7 March 2010; returned 20 April 2010; revised 6 May 2010; accepted 10 May 2010

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-TB[5] 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

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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, microcolony, thin-layer agar, TLA, colorimetric, redox, indicator, dye, alamar, malachite, resazurin, tetrazolium, nitrate reductase assay, Griess, fastplaque, phage, mycobacteriophage, D29, luminometry, Bronx, luciferase, fluoromycobacteriophage, 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 $\leq$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 procedure[20] 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 $\leq$0.05.

Assuming that a rapid assay for DST is clinically useful when it can rule in or rule out resistance to $\geq$90% certainty threshold, the pre-test prevalence of fluoroquinolone resistance required for a test to attain predictive values of $\geq$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 articles[10–14,23–41] were eventually 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 moxifloxacin[25,28] (n = 2); MGIT 960 regarding levofloxacin[43,44] (n = 2); and the tetrazolium microplate assay regarding moxifloxacin[29] (n = 1). Available data were also insufficient for meta-analysis of rapid genotypic assays applied in sputum specimens regarding ofloxacin[10,11] (n = 2) or levofloxacin[45] (n = 1).

Table 1 summarizes 33 sets of data extracted from 25 articles[10–14,23–41,46] 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.[25,36] 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[46] 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 *gyrA* 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

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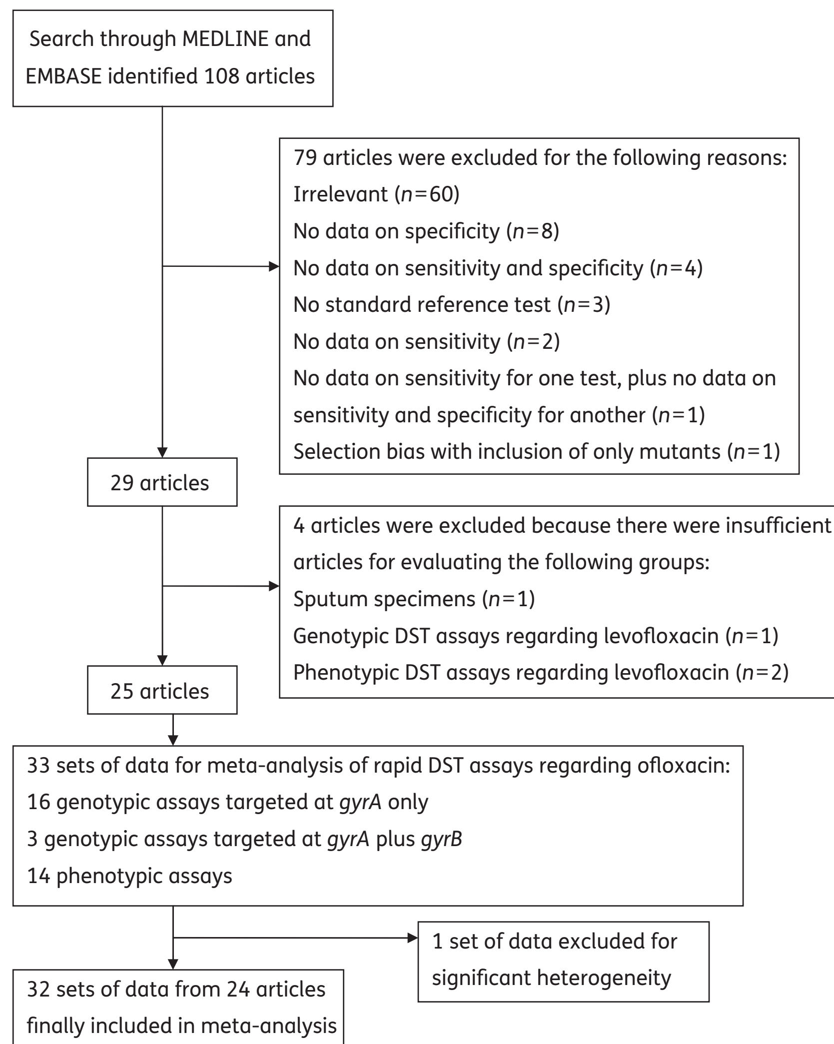

Figure 1. Flow diagram of reviewed articles. DST, drug susceptibility testing.

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,[41] controls[11] or both,[13,24–26,28,35,36,38,40] were similar to the main findings (see Table 5).

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Table 1. Thirty-three sets of data initially considered for meta-analysis of rapid assays for ofloxacin resistance in culture isolates of *M. tuberculosis*

Assays	Country source of study subjects	Study period	Study design[a]	Sampling method (cases)	Sampling method (controls)	Reference test	Breakpoint for resistance in reference test (phenotypic assay, if applicable), mg/L	R	S
PCR–DNA sequencing[40]	Shanghai, China	Mar 2004–Nov 2007	XS	available MDR strains	available MDR strains	proportion method	2	54	121
PCR–DNA sequencing[28]	Hong Kong	1999–2003	XS	ofloxacin-resistant strains with MIC ≥4 mg/L out of a collection of MDR strains, plus 11 non-MDR ofloxacin-resistant isolates	at random from ofloxacin-susceptible strains out of a collection of MDR strains	absolute concentration method in LJ, followed by MGIT	2	35	20
PCR–DNA sequencing, denaturing HPLC[36]	Beijing, China	2002–03	XS	available patients with pulmonary TB	available patients with pulmonary TB	proportion method	2	68	41
PCR–DNA sequencing, resazurin assay[39]	not mentioned	not mentioned	CC	not mentioned	not mentioned	proportion method on 7H11 agar	2 (same)	25	15
PCR–DNA sequencing[46]	various states in India	not mentioned	CC	not mentioned	not mentioned	absolute concentration method on LJ	8	71	47
PCR–DNA sequencing[26]	Karakalpakstan	Oct 2003–Feb 2006	nested CC	MDR-TB patients of a regional TB programme	at random	proportion method in solid medium, BACTEC 460 or MGIT	2	26	49
PCR–DNA sequencing[14]	Japan (largely); Poland	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H10 agar	2	3	135
Pyrosequencing[23]	Philippines	not mentioned	CC	not mentioned	not mentioned	submerged-disc proportion method	2	10	92
Microchip array[10]	Moscow, Russian Federation	not mentioned	CC	not mentioned	not mentioned	absolute concentration method	2	52	55
LNA-PCR[41]	Ho Chi Minh City, Vietnam	Jul 2005–Jul 2006	CC	all available FQ-resistant isolates received by a hospital	not mentioned	proportion method	2	42	40
A simplified non-radioactive PCR–SSCP[37]	France (not stated explicitly)	not mentioned	CC	not mentioned	not mentioned	proportion method	not explicitly mentioned, but compatible with 2 mg/L	3	3

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PCR–SSCP/MPAC, then PCR–DNA sequencing[24]	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	2.4	71	179
MTBDRsl[11]	Germany	not mentioned	CC	not mentioned	at random	proportion method in LJ or MGIT	2	32	74
Reverse hybridization-based line probe assay[27]	Rome, Florence, Ancona, Milan, Siena, Italy	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H11 agar	2	19	9
MPAC[25]	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 in LJ, followed by broth macrodilution method	2	35	103
MGIT[13]	Nashville, TN, USA	Jan 2002–Dec 2007	XS	available isolates	available isolates	agar proportion method	2 (same)	19	778
MGIT[33]	Mumbai, India	not mentioned	CC	not mentioned	not mentioned	BACTEC 460	2 (same)	3	70
MGIT (manual)[30]	Institute of Tropical Medicine in Antwerp, Belgium	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H11 agar	2 (same)	41	147
MGIT[35]	Borstel, Germany; Cordoba, Spain; London, UK	not mentioned	CC	MDR strains and with high drug resistance as far as possible	MDR strains and with high drug resistance as far as possible	BACTEC 460	2 (same)	9	83
Resazurin assay[38]	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	2 (same)	14	106
Resazurin assay, NRA[12]	Peru; Armenia; Azerbaijan; Georgia; Kazakhstan	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H11 agar, further confirmed by MGIT	2 (same)	8	87
Resazurin assay[32]	Bolivia; Peru; Eastern European countries	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H11 agar	2 (same)	8	142
NRA[b][34]	Honduras; Sweden	largely 2002–06; unspecified for 40%	CC	not mentioned	not mentioned	BACTEC 460	2 (same)	45	43
NRA, MODS[13]	Nashville, TN, USA	2005–06	XS	available isolates	available isolates	agar proportion method	2 (same)	6	233

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Table 1. *Continued*

Assays	Country source of study subjects	Study period	Study design[a]	Sampling method (cases)	Sampling method (controls)	Reference test	Breakpoint for resistance in reference test (phenotypic assay, if applicable), mg/L	R	S
TLA[31]	Institute of Tropical Medicine in Antwerp, Belgium	not mentioned	CC	not mentioned	not mentioned	proportion method in 7H11 agar	2 (same)	39	95
Tetrazolium microtitre plate assay[29]	Samara Region of the Russian Federation; UK	not mentioned	CC	not mentioned	not mentioned	MGIT	1 (same)	34	97

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*sl*, GenoType® *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.

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

[b]Using criteria originally defined by Angeby *et al*.[60]

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Table 2. Test characteristics of rapid assays for ofloxacin resistance in culture isolates of *M. tuberculosis*

Assay category (gene target for genotypic assays)	Sets of data	Resistant strains	Susceptible strains	Mean sensitivity, % (95% CI)	Mean specificity, % (95% CI)	Mean positive LR (95% CI)	Mean negative LR (95% CI)	Mean diagnostic odds ratio (95% CI)	Funnel plot (*P* value)
Genotypic assays (*gyrA*+*gyrB*)[a][26,27,39]	3	70	73	93 (81–98)	91 (51–99)	10.1 (1.1–94.3)	0.08 (0.02–0.25)	131 (10–1649)	0.39
Genotypic assays (*gyrA*)	15	543	977	88 (83–92)	96 (90–98)	22.0 (8.1–59.2)	0.12 (0.08–0.19)	177 (60–523)	0.92
PCR–DNA sequencing (*gyrA*)[14,26,28,36,39,40,46]	6	211	381	92 (84–96)	94 (78–99)	16.0 (3.4–75.7)	0.09 (0.04–0.19)	179 (32–1009)	0.07
others (*gyrA*)[b][10,11,23–25,27,36,37,41]	9	332	596	86 (79–91)	97 (90–99)	29.7 (8.1–109.2)	0.14 (0.08–0.24)	209 (51–851)	0.17
Phenotypic assays	14	265	2216	94 (88–97)	99 (98–100)	118.6 (39.9–352.5)	0.06 (0.03–0.12)	1955 (531–7193)	0.003
MGIT[13,30,33,35]	4	72	1078	96 (85–99)	100 (97–100)	312.2 (36.6–2663.5)	0.04 (0.01–0.17)	8633 (616–120962)	0.11
resazurin assay[12,32,38,39]	4	55	350	96 (85–99)	99 (94–100)	131.1 (15.3–1122.1)	0.04 (0.01–0.17)	3473 (248–48559)	0.72
nitrate reductase assay[12,13,34] and miscellaneous[c][13,29,31]	6	138	788	90 (78–96)	99 (93–100)	64.0 (12.9–316.2)	0.10 (0.04–0.26)	626 (99–3943)	0.01

CI, confidence interval; LR, likelihood ratio; MGIT, Mycobacteria Growth Indicator Tube 960.
[a]Comprised PCR–DNA sequencing and a reverse hybridization-based line probe assay.
[b]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.
[c]Comprised thin-layer agar, microscopic observation drug susceptibility assay and tetrazolium microplate assay.

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

	PCR–DNA sequencing (*gyrA*)	Other genotypic assays (*gyrA*)	Genotypic assays (*gyrA*+*gyrB*)	MGIT	Resazurin assay
Sensitivity					
other genotypic assays (*gyrA*)	0.23				
genotypic assays (*gyrA*+*gyrB*)	0.78	0.24			
MGIT	0.30	0.08	0.48		
resazurin assay	0.32	0.09	0.50	0.97	
NRA and miscellaneous	0.74	0.51	0.60	0.23	0.25
Specificity					
other genotypic assays (*gyrA*)	0.49				
genotypic assays (*gyrA*+*gyrB*)	0.71	0.36			
MGIT	**0.03**	0.08	**0.03**		
resazurin assay	0.12	0.28	0.10	0.57	
NRA and miscellaneous	0.20	0.48	0.16	0.26	0.63
Diagnostic odds ratio					
other genotypic assays (*gyrA*)	0.86				
genotypic assays (*gyrA*+*gyrB*)	0.80	0.69			
MGIT	**0.004**	**0.003**	**0.006**		
resazurin assay	**0.03**	**0.03**	**0.032**	0.57	
NRA and miscellaneous	0.20	0.21	0.205	0.051	0.20

MGIT, Mycobacteria Growth Indicator Tube 960; NRA, nitrate reductase assay.
[a]Data are *P* values, which are shown in bold if the value is ≤0.05.

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

Assay category [gene(s) for genotypic assays]	Prevalence levels for attaining PPV ≥90%	Prevalence levels for attaining NPV ≥90%	Prevalence levels for attaining PPV ≥95%	Prevalence levels for attaining NPV ≥95%
Genotypic assays (*gyrA*+*gyrB*)	≥47%	≤59%	≥65%	≤41%
Genotypic assays (*gyrA*)	≥29%	≤47%	≥46%	≤30%
PCR–DNA sequencing (*gyrA*)	≥36%	≤55%	≥54%	≤37%
other genotypic assays (*gyrA*)	≥23%	≤44%	≥39%	≤27%
Phenotypic assays	≥7%	≤65%	≥14%	≤46%
MGIT	≥3%	≤75%	≥6%	≤59%
resazurin assay	≥6%	≤75%	≥13%	≤58%
NRA and miscellaneous	≥12%	≤52%	≥23%	≤34%

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.[47–49] The prevalence levels of fluoroquinolone resistance generally associated with antimicrobial use in the treatment of MDR-TB and other bacterial sepsis were ∼20%.[47,48] 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.[49] 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.[1] 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.[50] In the absence of risk factors, when the prevalence

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

Assay category (gene target for genotypic assays)	Sets of data	Resistant strains	Susceptible strains	Mean sensitivity, % (95% CI)	Mean specificity, % (95% CI)	Mean positive LR (95% CI)	Mean negative LR (95% CI)	Mean diagnostic odds ratio (95% CI)	Funnel plot (*P* value)
Genotypic assays (*gyrA*)	9	431	668	88% (81%–93%)	96% (88%–99%)	24.0 (6.6–87.3)	0.12 (0.07–0.21)	199 (49–811)	0.71
PCR–DNA sequencing (*gyrA*)[26,28,36,40]	4	183	231	91% (81%–96%)	95% (71%–99%)	17.3 (2.4–123.2)	0.09 (0.04–0.22)	189 (22–1630)	0.72
others (*gyrA*)[11,24,25,36,41]	5	248	437	86% (75%–93%)	97% (86%–100%)	32.0 (5.5–184.7)	0.14 (0.07–0.29)	223 (34–1479)	0.61
Phenotypic assays[13,35,38]	5	54	1433	95% (84%–99%)	100% (97%–100%)	226.0 (35.5–1438.8)	0.05 (0.01–0.19)	4890 (483–49539)	0.22

CI, confidence interval; LR, likelihood ratio.
To attain ≥90% for both positive and negative predictive values, the required pre-test 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%.

of ofloxacin resistance may be <3% among non-MDR strains,[49] MGIT is probably more reliable for detecting resistance to ofloxacin. Thus, the current review corroborates the recommendation by the WHO of using MGIT for drug resistance surveillance.[7] Nonetheless, drug susceptibility testing for patient management can be different from that for drug resistance surveillance. Broth-based media, especially MGIT, can miss rifampicin-resistant strains that are detectable by agar-based media and molecular assays.

Besides the ability of assessing non-viable bacilli with a shorter turnaround time relative to rapid phenotypic assays, rapid genotypic assays applied directly in clinical specimens do not risk missing MDR *M. tuberculosis* strains that may grow poorly.[7] A few studies have demonstrated the feasibility of directly applying rapid genotypic assays in sputum specimens with high sensitivity and specificity.[10,11] As bacterial growth is generally sufficient under optimal laboratory conditions, the current review regarding the test performance of rapid assays for ofloxacin resistance in culture isolates of *M. tuberculosis* may provide a yardstick for evaluating rapid genotypic as well as phenotypic assays for ofloxacin resistance in clinical specimens.

The sensitivity of rapid genotypic assays is expected to be lower than that of phenotypic assays, because mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, which largely cluster at codons 90, 91 and 94,[25,51–54] only partly account for the genetic mechanism of fluoroquinolone resistance. The inclusion of *gyrB* in addition to *gyrA* in rapid genotypic assays probably increases sensitivity at the expense of specificity. However, as mutations in the QRDR of *gyrB* are much rarer in culture isolates,[52,53,55] the inclusion of *gyrB* may not considerably change the test performance, as shown by the current review. Other underlying mechanisms that may give rise to fluoroquinolone resistance include decreased cell wall permeability, drug efflux pump, drug sequestration and drug inactivation.[56] The failure of genotypic assays in attaining 100% specificity is attributable to silent mutations, which also likely accounts for the lower positive LR and higher pre-test prevalence required of PCR–DNA sequencing for attaining PPV of ≥90% in comparison with other genotypic assays. Mutation at codon 95, a polymorphism unrelated to quinolone resistance,[57] has been excluded from false-positive findings in this review.

Using a random effects model allows for the presence of heterogeneity between studies. The bivariate random effects model, rather than the summary receiver operating characteristic approach, was used in the current review for meta-analysis to remove the effect of a possible diagnostic threshold and preserve relevant clinical information about test performance that would otherwise be lost in the latter approach.[17,20,58] It is controversial whether a univariate random effects model by the DerSimonian–Laird approach[59] may be used to pool LRs for estimating predictive values. Even if this approach is valid, it would be precluded by a significant threshold effect.

There are a number of limitations in the current review. First, although the literature search among publications in English may have been reasonably thorough, as suggested by the lack of significant funnel plot asymmetry in all assay subgroups except for NRA and miscellaneous phenotypic assays, exclusion of publications in other languages from the current review could have introduced publication bias. Second, most of the included studies were case–control by design and it was difficult to tell whether cases and controls had been selected from the same

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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[21] 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 study effects vary, rather than a universally true study effect that varies between studies due to sampling error. Lastly, the performance of rapid assays included in the current review has been evaluated by conventional assays with no reference to clinical treatment outcomes or assurance of unique isolates by genotyping. This could have introduced bias in validation.

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*.

Funding

This study was not supported by any specific funding.

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/).

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