

## Original Article

## Rapid Diagnosis of XDR and Pre-XDR TB: A Systematic Review of Available Tools



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## ARTICLE INFO

## Article history:

Received 4 July 2022

Accepted 15 July 2022

Available online 28 July 2022

## Keywords:

Tuberculosis

MDR-TB

XDR-TB

Pre-XDR

Drug susceptibility test

Xpert MTB/XDR

GenoType MTBDRsl

Line probe assay

Sensitivity

Specificity

## ABSTRACT

**Introduction:** No previous systematic reviews have comprehensively investigated the features of Xpert MTB/XDR and other rapid tests to diagnose pre-XDR/XDR-TB. The aim of this systematic review is to assess existing rapid diagnostics for pre-XDR/XDR-TB from a point-of-care perspective and describe their technical characteristics (i.e., sensitivity, specificity, positive and negative predictive values).

**Methods:** Embase, PubMed, Scopus, and Web of Science were searched to detect the articles focused on the accuracy of commercially available rapid molecular diagnostic tests for XDR-TB according to PRISMA guidelines. The analysis compared the diagnostic techniques and approaches in terms of sensitivity, specificity, laboratory complexity, time to confirmed diagnosis.

**Results:** Of 1298 records identified, after valuating article titles and abstracts, 97 (7.5%) records underwent full-text evaluation and 38 records met the inclusion criteria.

Two rapid World Health Organization (WHO)-endorsed tests are available: Xpert MTB/XDR and GenoType MTBDRsl (VER1.0 and VER 2.0). Both tests had similar performance, slightly favouring Xpert, although only 2 studies were available (sensitivity 91.4–94; specificity 98.5–99; accuracy 97.2–97.7; PPV 88.9–99.1; NPV 95.8–98.9).

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**Conclusions:** Xpert MTB/XDR could be suggested at near-point-of-care settings to be used primarily as a follow-on test for laboratory-confirmed TB, complementing existing rapid tests detecting at least rifampicin-resistance.

Both Xpert MTB/XDR and GenoType MTBDRsl are presently diagnosing what WHO defined, in 2021, as pre-XDR-TB.

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## Introduction

The appearance of drug-resistance has followed in the wake of extensive programmatic use of anti-tuberculosis (TB) drugs.<sup>1</sup> The increasing problem of drug resistance poses different levels of diagnostic challenge: to identify the presence of drug resistance, define the extent of resistance present; and support the design of an effective anti-TB regimen.<sup>2</sup>

**Defining the drug resistance profile and its severity.** The appearance of strains of *Mycobacterium tuberculosis* resistant to both isoniazid and rifampicin, the two core anti-TB drugs, has defined multidrug-resistant tuberculosis (MDR-TB) from the outset. Later in 2006, when more resistant forms of TB appeared, the definition of extensively drug-resistant tuberculosis (XDR-TB) was agreed, referring to MDR-TB strains of *M. tuberculosis* with additional resistance to any fluoroquinolone and at least one of the three injectable anti-TB drugs (capreomycin, kanamycin and amikacin). The XDR-TB definition was made on the assumption that these classes of drugs were essential to successfully treat a patient with MDR-TB.<sup>3–5</sup>

More recent evidence demonstrated that XDR-TB patients had worse outcomes than MDR-TB patients with susceptibility to these drugs (those with resistance to fluoroquinolones faring worse than those resistant to injectables).<sup>6</sup> The scientific community also considered codification of patterns of resistance beyond XDR<sup>7</sup> and the possibility of defining so-called “total drug-resistance”, although no agreement was found on this.<sup>8,9</sup>

Although not formally recognized by the World Health Organization (WHO), clinicians widely adopted the term pre-XDR to indicate MDR-TB patients with additional resistance to either fluoroquinolones or injectables.<sup>9,10</sup>

In 2021 WHO introduced the definition pre-XDR-TB (e.g., MDR-TB plus resistance to fluoroquinolones) and modified the definition of XDR-TB, now being MDR-TB plus additional resistance to any fluoroquinolone and at least one WHO Group A drug (bedaquiline, linezolid).<sup>5,11,12</sup>

**Diagnosing and treating pre- and XDR-TB.** Considering the difficulty of treating XDR-TB, typically with costly and often toxic second-line anti-TB drugs, the importance of achieving rapid and effective diagnosis to design an effective regimen is obvious.

Of the 4.8 million people diagnosed with pulmonary TB worldwide in 2020, only 59% were bacteriologically confirmed and 71% of them were tested for rifampicin resistance. Notably, a WHO-recommended rapid molecular test was used as the initial diagnostic test for only 33% of new TB diagnoses, contributing to reasons why only about one third of the 157,903 MDR/rifampicin-resistant TB cases detected in 2020 had access to treatment.<sup>12</sup>

In a recent rapid communication,<sup>13,14</sup> WHO recommended the use of shorter regimens on a programmatic basis. The pivotal initial tests which are used globally to classify patients as drug-susceptible or drug-resistant are the Xpert MTB/RIF and Xpert MTB/RIF Ultra, which allow detection of resistance to rifampicin only, and other new low-complexity molecular assays (Truenat MTB-RIF Dx) or moderate complexity nucleic acid amplification tests (NAATs) systems (Abbott Molecular RealTime MTB RIF/INH; Becton Dickinson BD MAX MDR-TB; Bruker-Hain Diagnostics FluoroType MTBDR; Roche Diagnostics Cobas MTB-RIF/INH) have been recently conditionally recommended as well.<sup>15</sup> As the shorter MDR/RR-TB

regimens are based on WHO Group A drugs, and no rapid WHO-approved tests are available for bedaquiline and linezolid, it is crucial that at least resistance to fluoroquinolones is rapidly detected, together with resistance to isoniazid.<sup>16</sup> In case longer regimens are used, rapid detection of resistance to other drugs could be important (e.g., to injectables and ethionamide). Xpert MTB/XDR (isoniazid, ethionamide, fluoroquinolones, amikacin, capreomycin and kanamycin), first-line Line Probe Assays (LPAs; rifampicin and isoniazid) and second-line LPAs (fluoroquinolones, amikacin, capreomycin and kanamycin), plus other moderate complexity automated NAATs and high complexity reverse hybridization-based NAATs (Nipro Genoscholar PZA-TB) are the follow on rapid diagnostic tests currently recommended for the detection of additional drug-resistance beyond rifampicin.<sup>15,17</sup>

Despite advances, treatment success of MDR-TB remains low (globally at 59%); being lower for pre-XDR and XDR-TB patients and higher in settings where all oral regimens including new drugs are used.<sup>12,18,19</sup> Improving treatment success will require access to rapid diagnostic tests able to reliably detect and classify drug resistance, and inform clinicians designing individual regimens.<sup>20</sup>

In 2021–2022 a Cochrane Review<sup>21,22</sup> investigated the efficacy of Xpert MTB/XDR to rapidly detect resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin (but not to the other injectable drugs kanamycin and capreomycin). However, no previous systematic reviews have comprehensively investigated the features of tests available to diagnose pre-XDR/XDR-TB. The aim of this systematic review is to assess all the tools able to rapidly diagnose pre-XDR/XDR-TB (including LPAs, and the Xpert XDR assay) under a point-of-care perspective and describe their technical characteristics (i.e., sensitivity, specificity, positive and negative predictive values).

## Methods

### Search strategy

Four electronic search engines repositories (i.e., Embase, PubMed, Scopus, and Web of Science) were searched for the detection of articles focused on the accuracy of rapid molecular diagnostic tests in the diagnosis of extensively drug-resistant tuberculosis (XDR-TB) cases.

The following keywords, combined in different strings, depending on the electronic database, were used to search articles published until February 2022: “Xpert MTB/XDR”, “drug susceptibility test”, “XDR-TB”, “sensitivity”, “specificity”, “tuberculosis”, “TB”, “*Mycobacterium tuberculosis*”, “GenoType MTBDRsl”, and “line probe assay (LPA)”.

Reports published in the grey literature or in the social and conventional media were excluded following the risk of unreliable and poor scientific information on the adopted methodology. Reports that were not written in English language were excluded.

### Study selection

Only manuscripts describing the diagnostic accuracy of Xpert MTB/XDR compared to the drug susceptibility test (DST) were initially included.

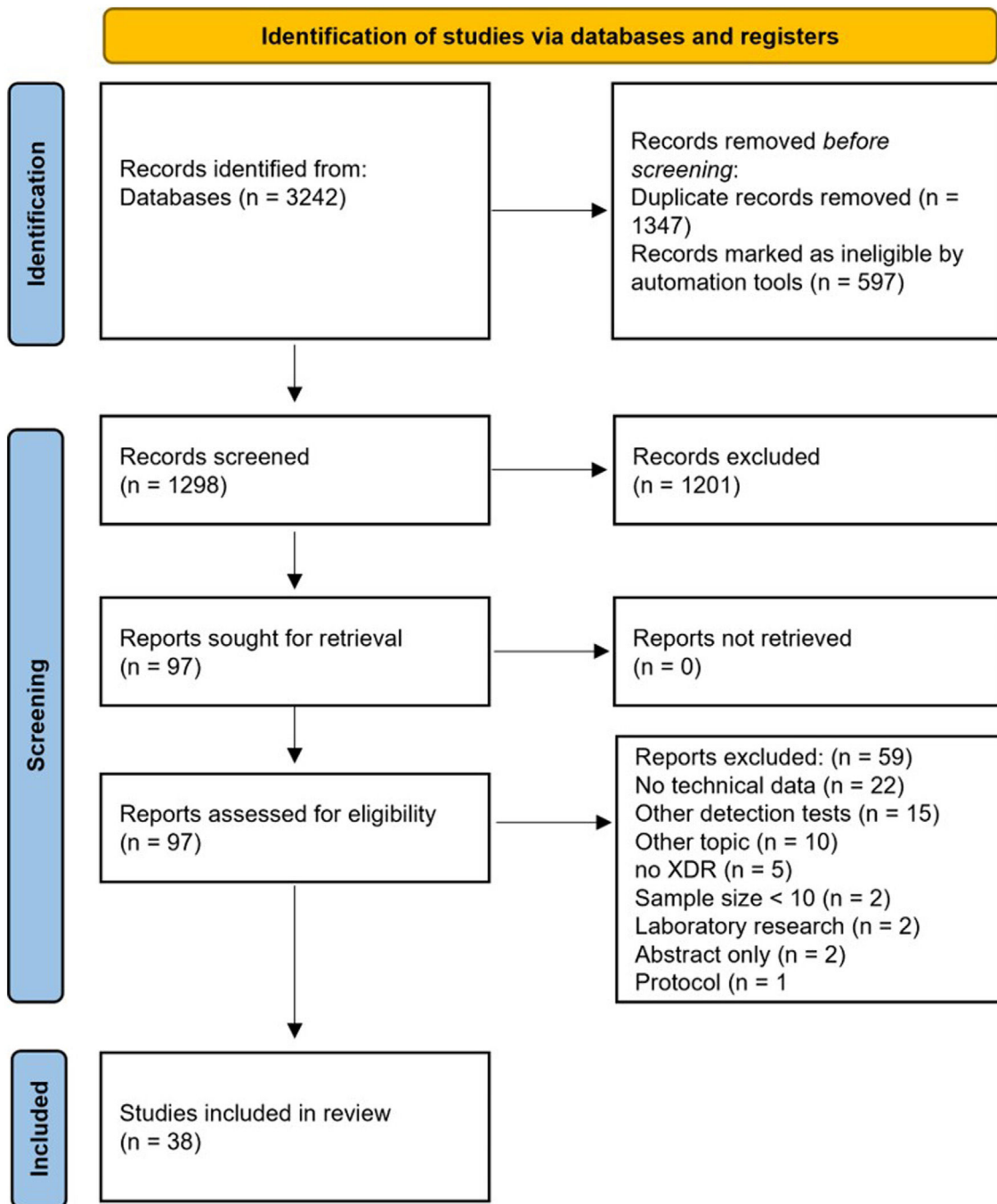


Fig. 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases.

All the studies with an experimental and observational design were selected with the exclusion of editorials, narrative reviews, case-reports or -series, laboratory studies, letters, study protocols, or correspondences.

Titles and abstracts were screened to evaluate the suitability of the manuscripts based on the above-mentioned inclusion and exclusion criteria.

The first assessment, carried out by two investigators (BDL and MVP), was supervised by a third investigator (GS). Full-texts were independently assessed by the same investigators and potential disagreement in the article selection process was resolved by consensus of the third investigator.

### Study quality assessment

This study was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.<sup>23</sup>

The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist was used to evaluate the methodological quality and applicability of the included studies.<sup>24</sup> The following items were assessed: patient selection methods; index text description, conduction, and interpretation; reference standard (standard gold test) description, conduction, and interpretation.

## Statistical analysis

Descriptive statistics were used to summarize data. Meta-analytic estimates were computed and described with pooled and heterogeneity indicators. Forest plots were used to represent study variability with 95% confidence intervals (CI) for prevalence of drug resistance and for diagnostic accuracy of molecular techniques in the resistance identification. To assess the heterogeneity among studies the inconsistency indicator ( $I_2$ ) was calculated, where an  $I_2$  value > 50% indicated substantial heterogeneity. Thus, fixed- and random-effects models were computed keeping into consideration the expected between-study heterogeneity. Bias assessment plots and Egger weighted regression test methods were used to assess the publication bias. A two-tailed  $p$ -value less than 0.05 was considered statistically significant. Data analyses were performed with the statistical softwares StatsDirect version 3.1.12 (StatsDirect Ltd.) and STATA version 17 (StatsCorp, College Station, Texas, USA).

## Results

### Study characteristics

The search strategy identified 1298 records. After the evaluation of article titles and abstracts, 97 (7.5%) records underwent to the full-text evaluation. 38<sup>25–62</sup> records met the inclusion criteria, whereas 59 (60.8%) were excluded for the following reasons: no technical data available ( $n = 22$ ), other detection tests ( $n = 15$ ), other topics ( $n = 10$ ), no XDR-TB isolates/samples included ( $n = 5$ ), sample size < 10 ( $n = 2$ ), laboratory research ( $n = 2$ ), only abstract available ( $n = 2$ ), and only research protocol description ( $n = 1$ ) (Fig. 1).

Publication year ranged from 2009<sup>62</sup> to 2022<sup>25</sup> (Table 1). All the included studies had an observational design, and the minority were multicentric (8, 21.1%).<sup>25,28,30,32,42,43,47,48</sup> The patient recruitment period ranged from 1992<sup>54</sup> to 2020<sup>25</sup> with a sample size ranging from 17<sup>53</sup> to 2649<sup>49</sup> (Table 1). Following the list of WHO regions,<sup>63</sup> 20 reference centres enrolled patients from the European region,<sup>25,28,41,42,44,47–49,52,54,55,57,59,62</sup> 10 from the Western Pacific Region,<sup>28,32,34,36,38,45,46,51,60,61</sup> 8 from the African Region,<sup>25,30,39,40,43,48,56,58</sup> 6 from the South-East Asian Region,<sup>25,29,31,33,35,48,53</sup> 3 from the Eastern Mediterranean Region,<sup>26,27,37</sup> 3 from the Region of the Americas.<sup>28,50</sup>

Among the included studies, two (5.3%),<sup>25,28</sup> 10 (26.3%),<sup>26,29,34,35,39,40,42,47,48,54</sup> and 26 (68.4%)<sup>30–33,35–38,41,43–46,49–57,59–62</sup> articles described the application of Xpert MTB/XDR, GenoType MTBDRsl VER 2.0, and GenoType MTBDRsl VER 1.0, respectively, for the detection of XDR-TB in strains or clinical isolates in comparison with the DST. The definition of XDR-TB used in the evaluated studies was the 2006 one.<sup>64</sup>

### Resistance patterns

The overall pooled prevalence of XDR-TB pattern estimated by the DST was assessed in 28 (73.7%) studies<sup>26,27,29,32–52,56,58,59,61</sup> and was 15% (95% CI = 11–20;  $I_2 = 95.6\%$ ). The pooled prevalence of resistance was higher for fluoroquinolones (FQ) (34%; 95% CI = 27–41;  $I_2 = 97.6\%$ ), followed by kanamycin (KAN) (28%; 95% CI = 21–36;  $I_2 = 97\%$ ), amikacin (AMK) (18%; 95% CI = 14–23;  $I_2 = 93.6\%$ ), and capreomycin (CM) (17%; 95% CI = 13–21;  $I_2 = 92\%$ ) (Fig. 2).

### Xpert MTB/XDR

Two studies evaluated the diagnostic accuracy of the Xpert MTB/XDR test.<sup>25,28</sup> Both assessed the resistance genes to FQ, KAN, AMK, and CM. Cao and colleagues estimated a sensitivity of 91.4%, 98.1%, 91.0%, and 70.0% for FQ, KAN, AMK, CM,

respectively, whereas Penn-Nicholson and colleagues assessed a sensitivity of 94.0%, 86.0%, 73.0%, and 61.0% for FQ, KAN, AMK, CM (Table 2). Specificity and the other diagnostic characteristics for Xpert MTB/XDR were included in Table 2.

### GenoType MTBDRsl VER 2.0

The pooled proportion of diagnostic sensitivity and specificity of GenoType MTBDRsl VER 2.0 in detecting XDR-TB were evaluated by 9 out of 10 (90%) studies,<sup>26,27,29,34,35,39,40,42,47</sup> and were 85% (95% CI = 72–94;  $I_2 = 75.6\%$ ) and 98% (95% CI = 95–100;  $I_2 = 88.3\%$ ), respectively (Figs. 3 and 4).

The pooled sensitivity and specificity for FQ resistance were high, reaching 95% (95% CI = 91–98;  $I_2 = 81.4\%$ ) and 98% (95% CI = 97–99;  $I_2 = 55.1\%$ ). When the test was used for the detection of second line injectable drugs (SLIDs), the pooled sensitivity and specificity estimates were: 89% (95% CI = 72–99;  $I_2 = 92.1\%$ ) and 98% (95% CI = 96–100;  $I_2 = 83.9\%$ ) for KAN; 90% (95% CI = 84–95;  $I_2 = 0\%$ ) and 100% (95% CI = 98–100;  $I_2 = 62.2\%$ ) for AMK; and 89% (95% CI = 76–97;  $I_2 = 73\%$ ) and 99% (95% CI = 97–100;  $I_2 = 77.4\%$ ) for CM. The diagnostic variables, including accuracy, PPV, and NPV, for XDR-TB detection and for resistance to FQ, KAN, AMK, and CM were described in Table S1.

### GenoType MTBDRsl VER 1.0

The GenoType MTBDRsl VER 1.0 showed a pooled sensitivity and specificity for XDR-TB detection of 75% (95% CI = 56–90;  $I_2 = 91.5\%$ ) and 99% (95% CI = 99–100;  $I_2 = 21.7\%$ ), respectively, based on the evaluation of 11 studies.<sup>31–33,36,41,43,44,46,49,50,52</sup> The pooled proportions of diagnostic sensitivity for resistance to FQ, KAN, AMK, and CM were 84% (95% CI = 79–88;  $I_2 = 80\%$ ), 64% (95% CI = 46–80;  $I_2 = 94\%$ ), 87% (95% CI = 79–94;  $I_2 = 79.9\%$ ), and 73% (95% CI = 59–85;  $I_2 = 85\%$ ), respectively. Furthermore, the pooled specificity for resistance to all tested drugs was higher than 95%: 98% (95% CI = 97–99;  $I_2 = 69\%$ ), 99% (95% CI = 98–99;  $I_2 = 8.6\%$ ), 99% (95% CI = 99–100;  $I_2 = 26.2\%$ ), and 97% (95% CI = 94–99;  $I_2 = 82.6\%$ ) for resistance to FQ, KAN, AMK, and CM, respectively (Figs. 5 and 6). Additional diagnostic performance variables of GenoType MTBDRsl VER 1.0 were summarized in Table S2.

### Quality assessment for the included studies

The quality of included studies was assessed using the QUADAS-2 tool.<sup>24</sup> Within the patient selection domain, 7 (18.4%) and 8 (21.1%) studies were evaluated as at high and unclear risk of bias, respectively. A high risk of bias was found in 4 studies in relation to the index and reference standard test domains (blinding evaluation). Moreover, 65.8% of the selected studies did not clarify if index test results were interpreted without previous knowledge of the reference standard results. All studies included a reference standard and were judged to have a low risk of bias in the flow and timing domains. No concerns emerged on the applicability of the study domains (Fig. S1).

## Discussion

The aim of our study was to assess all the tools able to rapidly diagnose pre-XDR/XDR-TB (and describe their technical characteristics), as well as to compare the diagnostic techniques and approaches in terms of sensitivity, specificity, laboratory complexity, time to confirmed diagnosis (Table 3).

The results of our study demonstrated that two rapid WHO-endorsed tests are available for these purposes, namely the Xpert MTB/XDR and GenoType MTBDRsl (VER1.0 and VER 2.0).

**Table 1**  
Summary of the included studies.

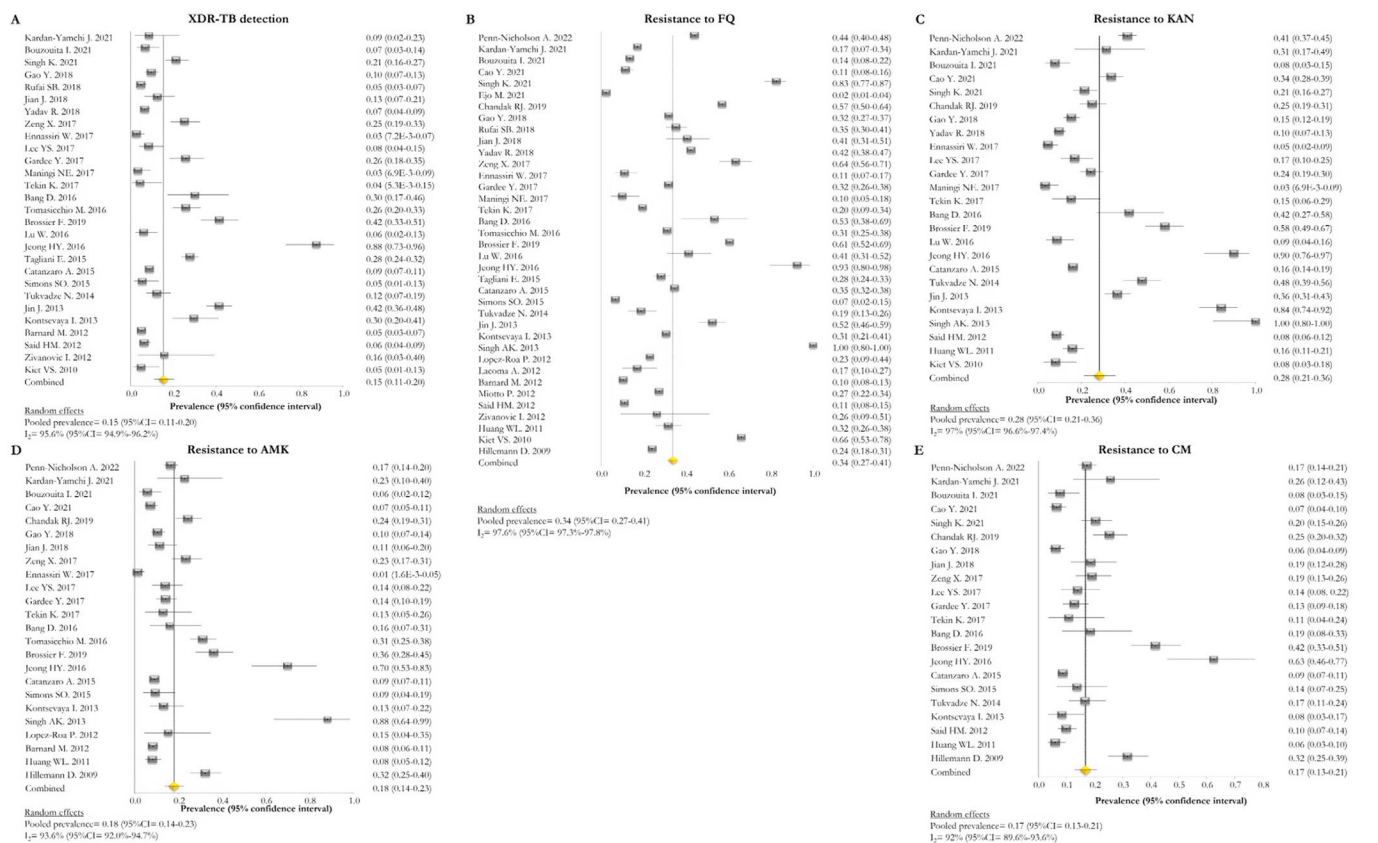
First author	Title	Year	Country	Mono-/multi-centre study	Study period	Sample size, <i>n</i>
Penn-Nicholson A	Detection of isoniazid, fluoroquinolone, ethionamide, amikacin, kanamycin, and capreomycin resistance by the Xpert MTB/XDR assay: a cross-sectional multicentre diagnostic accuracy study	2022	India, Moldova, South Africa	Multicenter	Jul 2019–Mar 2020	611
Kardan-Yamchi J	Assessment of the GenoType MTBDRsl VER 2.0 compared to the phenotypic drug susceptibility testing and whole genome sequencing for the rapid detection of resistance to fluoroquinolone and second-line injectable drugs among rifampicin-resistant Mycobacterium tuberculosis isolates	2021	Iran	Monocenter		35
Bouzouita I	Performance of the GenoType MTBDRsl V 2.0 for detecting second-line drugs resistance of Mycobacterium tuberculosis isolates in Tunisia	2021	Tunisia	Monocenter	May 2015–Dec 2019	103
Cao Y	Xpert MTB/XDR: a 10-Colour Reflex Assay Suitable for Point-of-Care Settings To Detect Isoniazid, Fluoroquinolone, and Second-Line-Injectable-Drug Resistance Directly from Mycobacterium tuberculosis-Positive Sputum	2021	US, Georgia, Moldova, Peru, Vietnam, Italy	Multicenter		314
Singh K	Direct detection of resistance to fluoroquinolones/SLIDs in sputum specimen by GenoType MTBDRsl v.2.0 assay A study from Eastern Uttar Pradesh, India	2021	India	Monocenter	Jan 2019–Dec 2019	225
Ejo M	Effectiveness of GenoType MTBDRsl in excluding TB drug resistance in a clinical trial	2021	Africa	Multicenter	Jul 2012–Jun 2015	413
Chandak RJ	Evaluation of MTBDRsl for detecting resistance in Mycobacterium tuberculosis to second-line drugs	2019	India	Monocenter		205
Gao Y	Multi-centre evaluation of GenoType MTBDRsl line probe assay for rapid detection of pre-XDR and XDR Mycobacterium tuberculosis in China	2018	Province of China	Multicenter	2015–2017	353
Rufai SB	Association of gyrA and rrs gene mutations detected by MTBDRsl V1 on Mycobacterium tuberculosis strains of diverse genetic background from India	2018	India	Monocenter		359
Jian J	Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, China	2018	China	Monocenter	2015–2016	96
Yadav R	Diagnostic accuracy of GenoType(®) MTBDRsl VER 2.0 in detecting second-line drug resistance to M. tuberculosis	2018	India	Monocenter		415
Zeng X	Performance of the MTBDRsl Line probe assay for rapid detection of resistance to second-line anti-tuberculosis drugs and ethambutol in China	2017	China	Monocenter		162
Ennassiri W	Extensively drug-resistant tuberculosis (XDR-TB) in Morocco	2017	Morocco	Monocenter	2015	155
Lee YS	Performance of the GenoType MTBDRsl assay for the detection second-line anti-tuberculosis drug resistance	2017	South Korea	Monocenter	Dec 2011–Feb 2017	107
Gardee Y	Evaluation of the GenoType MTBDRsl Version 2.0 Assay for Second-Line Drug Resistance Detection of Mycobacterium tuberculosis Isolates in South Africa	2017	South Africa	Monocenter	2012–2014	268
Maningi NE	Comparison of line probe assay to BACTEC MGIT 960 system for susceptibility testing of first and second-line anti-tuberculosis drugs in a referral laboratory in South Africa	2017	South Africa	Monocenter	Jan 2014–Jun 2014	97
Tekin K	Evaluation of the BACTEC MGIT 960 SL DST Kit and the GenoType MTBDRsl Test for Detecting Extensively Drug-resistant Tuberculosis Cases	2017	Turkey	Monocenter		46
Bang D	Performance of the GenoType MTBDRplus assay (v2.0) and a new extended GenoType MTBDRsl assay (v2.0) for the molecular detection of multi- and extensively drug-resistant Mycobacterium tuberculosis on isolates primarily from Lithuania	2016	Lithuania, Denmark	Multicenter	2009–2010	43
Tomasicchio M	The diagnostic accuracy of the MTBDRplus and MTBDRsl assays for drug-resistant TB detection when performed on sputum and culture isolates	2016	South Africa	Multicenter		234

Table 1 (Continued)

First author	Title	Year	Country	Mono-/multi-centre study	Study period	Sample size, n
Brossier F	Performance of the New Version (v2.0) of the GenoType MTBDRsl Test for Detection of Resistance to Second-Line Drugs in Multidrug-Resistant Mycobacterium tuberculosis Complex Strains	2016	France	Monocenter	2005–2015	127
Lu W	Evaluation of MTBDRplus and MTBDRsl in Detecting Drug-Resistant Tuberculosis in a Chinese Population	2016	China	Monocenter	May 2008–Dec 2008	99
Jeong HY	Evaluation of the GenoType <sup>®</sup> MTBDRsl assay in Korean patients with MDR or XDR tuberculosis	2016	Korea	Monocenter	Apr 2008–Jun 2008	40
Tagliani E	Diagnostic Performance of the New Version (v2.0) of GenoType MTBDRsl Assay for Detection of Resistance to Fluoroquinolones and Second-Line Injectable Drugs: a Multicenter Study	2015	Italy, Sweden, Germany, Moldova	Multicenter	2009–2013	459
Catanzaro A	Performance Comparison of Three Rapid Tests for the Diagnosis of Drug-Resistant Tuberculosis	2015	India, Moldova, South Africa	Multicenter		914
Simons SO	Molecular drug susceptibility testing in the Netherlands: performance of the MTBDRplus and MTBDRsl assays	2015	Netherlands	Monocenter	2007–2012	2649
Tukvadze N	Performance of the MTBDRsl assay in Georgia	2014	Georgia (USA)	Monocenter	Nov 2011–Apr 2012	138
Jin J	Underestimation of the resistance of Mycobacterium tuberculosis to second-line drugs by the new GenoType MTBDRsl test	2013	China	Monocenter	2006–2009	261
Kontsevaya I	Diagnostic accuracy of the genotype MTBDRsl assay for rapid diagnosis of extensively drug-resistant tuberculosis in HIV-coinfected patients	2013	Russian	Monocenter	2008–2010	90
Singh AK	Rapid detection of drug resistance and mutational patterns of extensively drug-resistant strains by a novel GenoType <sup>®</sup> MTBDRsl assay	2013	India	Monocenter	Jan 2011–Aug 2012	17
Lopez-Roa P	Susceptibility testing to second-line drugs and ethambutol by GenoType MTBDRsl and Bactec MGIT 960 comparing with agar proportion method	2012	Spain	Monocenter	1992–2010	26
Lacoma A	GenoType MTBDRsl for molecular detection of second-line-drug and ethambutol resistance in Mycobacterium tuberculosis strains and clinical samples	2012	Spain	Monocenter		88
Barnard M	Genotype MTBDRsl line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory	2012	South Africa	Monocenter	Mar 2010–Sep 2011	516
Miotto P	GenoType MTBDRsl performance on clinical samples with diverse genetic background	2012	Italy	Monocenter		234
Said HM	Evaluation of the GenoType <sup>®</sup> MTBDRsl assay for susceptibility testing of second-line anti-tuberculosis drugs	2012	South Africa	Monocenter		336
Zivanovic I	Detection of drug-resistant Mycobacterium tuberculosis strains isolated in Serbia by the genotype MTBDRsl assay	2012	Serbia	Monocenter	2011	19
Huang WL	Performance assessment of the GenoType MTBDRsl test and DNA sequencing for detection of second-line and ethambutol drug resistance among patients infected with multidrug-resistant Mycobacterium tuberculosis	2011	Taiwan	Monocenter	Jan 2008–Feb 2009	234
Kiet VS	Evaluation of the MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis	2010	Vietnam	Monocenter	Jan 2005–Jul 2006	62
Hillemann D	Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens	2009	Germany	Monocenter		170

Xpert MTB/XDR test is a rapid, automated NAAT test at low complexity.<sup>21,22</sup> It does not require any sophisticated laboratory infrastructure. The molecular amplification of small quantity of genetic materials takes place inside the cartridge and require less than 2 h to provide results. The main advantages over the LPAs are the higher sensitivity for detecting *Mycobacterium tuberculosis* (it is also recommended in persons with a sputum smear-negative specimen), faster time to response (90 min vs. 48–72 h), simple handling, reduced number of invalid results for direct testing,

its potential implementation outside a sophisticated laboratory, and its safety, as the cartridge represents ‘the laboratory’ where molecular amplification takes place. The Xpert MTB/XDR test can be done taking advantage of existing GeneXpert platforms equipped with 10-colour modules for Xpert MTB/RIF Ultra testing, or through system upgrade from 6-colour modules, with technical capacity to run the Xpert technology available yet on very large scale as infrastructure and procedures are equal. As technology upgrade may be logistically challenging and somehow expensive,



**Fig. 2.** Forest plots of pooled prevalence of XDR-TB detection (A), and for resistance to FQ (B), KAN (C), AMK (D), and CM (E). The point estimates of prevalence for each study are indicated as a square and a 95% confidence interval is shown with a horizontal line; the yellow diamond is representative for the combined prevalence.

**Table 2**  
 Diagnostic performance indicators of the Xpert MTB/XDR test.

Study	TP	FP	FN	TN	Sensitivity, %	Specificity, %	Accuracy, %	PPV, %	NPV, %
<b>Fluoroquinolones</b>									
Penn-Nicholson A, 2022	222	2	13	295	94.0	99.0	97.2	99.1	95.8
Cao Y, 2021	32	4	3	266	91.4	98.5	97.7	88.9	98.9
<b>Kanamycin</b>									
Penn-Nicholson A, 2022	181	5	29	300	86.0	98.0	93.4	97.3	91.2
Cao Y, 2021	101	6	2	197	98.1	97.4	97.4	94.4	99.0
<b>Amikacin</b>									
Penn-Nicholson A, 2022	60	2	22	427	73.0	100.0	95.3	96.8	95.1
Cao Y, 2021	20	3	2	278	91.0	98.9	98.3	87.0	99.3
<b>Capreomycin</b>									
Penn-Nicholson A, 2022	53	1	34	425	61.0	100.0	93.2	98.1	92.6
Cao Y, 2021	14	1	6	284	70.0	99.7	97.7	93.3	97.9

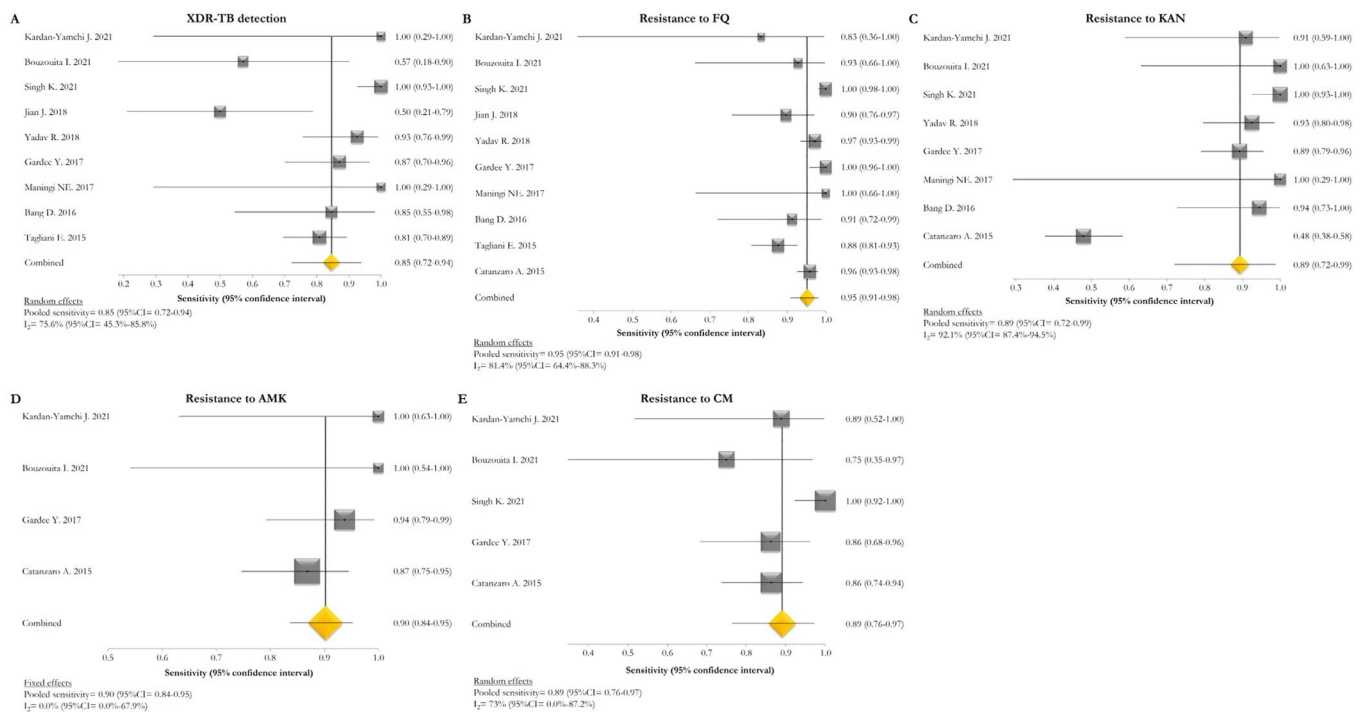
**Table 3**  
 Summary of the main indicators of diagnostic accuracy of the selected molecular techniques.

	Xpert MTB/XDR					GenoType MTBDRsl VER 2.0					GenoType MTBDRsl VER 1.0				
	XDR	FQ	KAN	AMK	CM	XDR	FQ	KAN	AMK	CM	XDR	FQ	KAN	AMK	CM
Sensitivity (%)	–	94.0	86.0	73.0	61.0	85.0	95.0	89.0	90.0	89.0	75.0	84.0	64.0	87.0	73.0
Specificity (%)	–	99.0	98.0	100.0	100.0	98.0	98.0	98.0	100.0	99.0	99.0	98.0	99.0	99.0	97.0

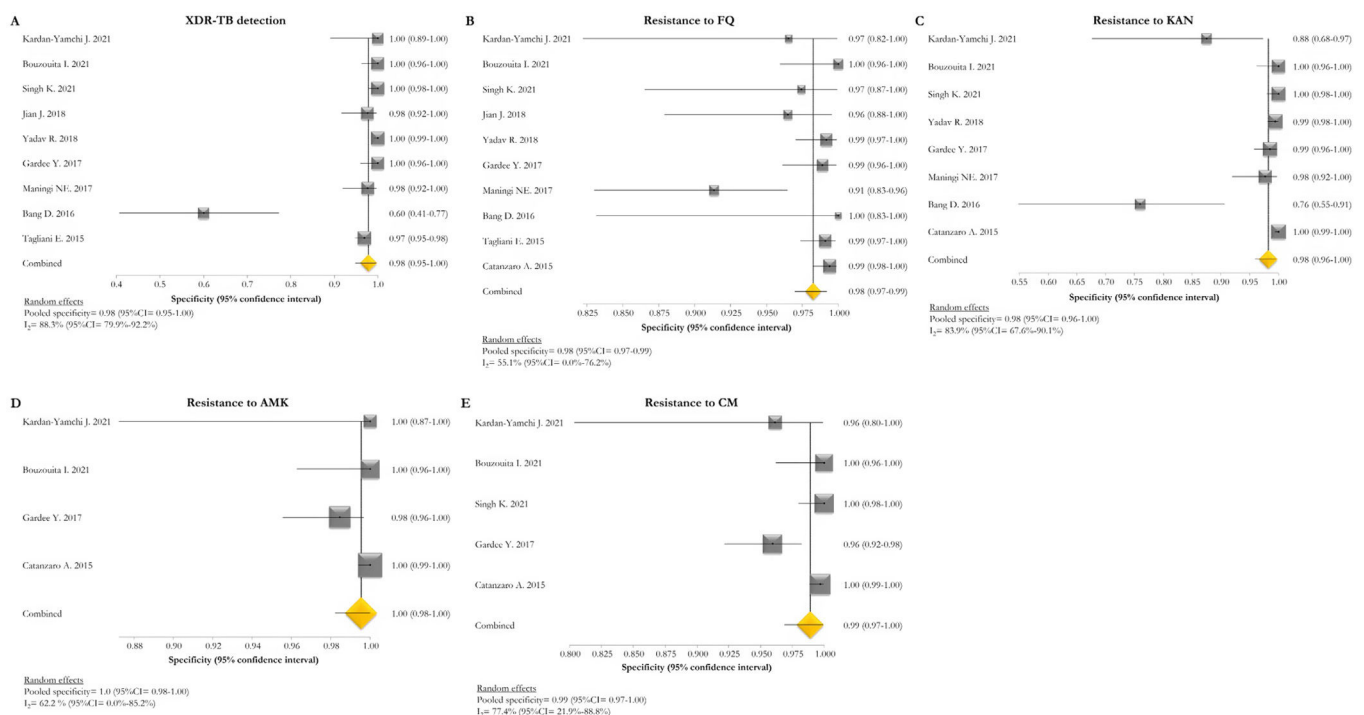
different solutions have been conceived, including procurement of new 10-colour GeneXpert modules, new full systems, satellites (instrument only) to connect to existing GeneXpert system or converting an existing system with 6 colours modules to 10 colour modules. In terms of performance of the tests, they are very similar: in our study, it seems to slightly favour Xpert, although

only 2 studies were available (sensitivity 91.4–94; specificity 98.5–99; accuracy 97.2–97.7; PPV 88.9–99.1; NPV 95.8–98.9).

The Xpert MTB/XDR is intended as a follow-on test after Xpert MTB/RIF or Xpert MTB/RIF Ultra testing, using left over sample reagent treated specimen, thus not necessarily requesting a second sample or culture to be collected. This approach is attractive with



**Fig. 3.** Forest plots of pooled sensitivity of XDR-TB detection (A), and for resistance to FQ (B), KAN (C), AMK (D), and CM (E) of GenoType MTBDRsl VER 2.0. The point estimates of sensitivity from each study are indicated as a square and a 95% confidence interval is shown with a horizontal line; the yellow diamond is representative for the combined sensitivity.



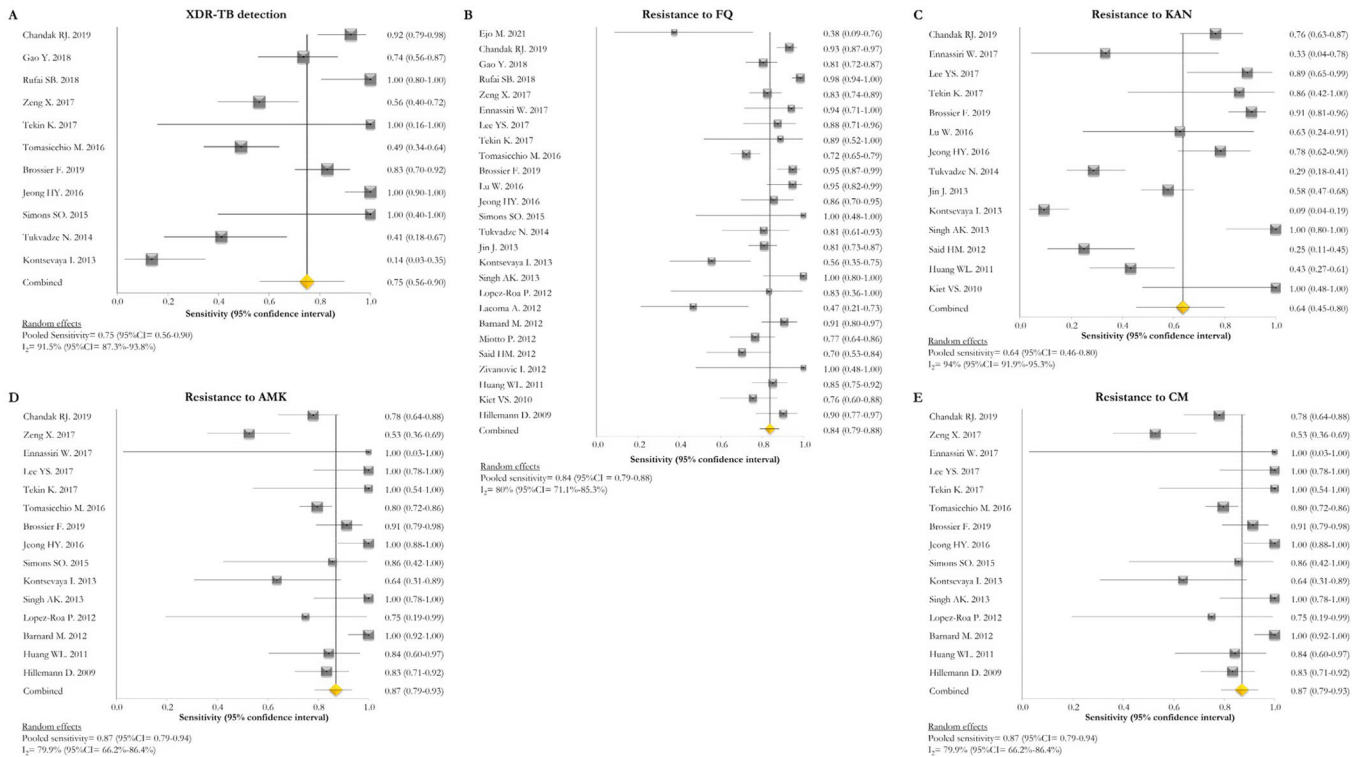
**Fig. 4.** Forest plots of pooled specificity of XDR-TB detection (A), and for resistance to FQ (B), KAN (C), AMK (D), and CM (E) of GenoType MTBDRsl VER 2.0. The point estimates of specificity from each study are indicated as a square and a 95% confidence interval is shown with a horizontal line; the yellow diamond is representative for the combined specificity.

benefits for access to rapid DST, diagnostic laboratory workflow and turnaround time, or after other WHO-endorsed bacteriological tests confirming the presence of pulmonary TB. However, the performance of any rapid assay used as reflex test for a specimen previously determined to be *M. tuberculosis* positive and harbouring other resistance (e.g., rifampicin) will need to be carefully assessed

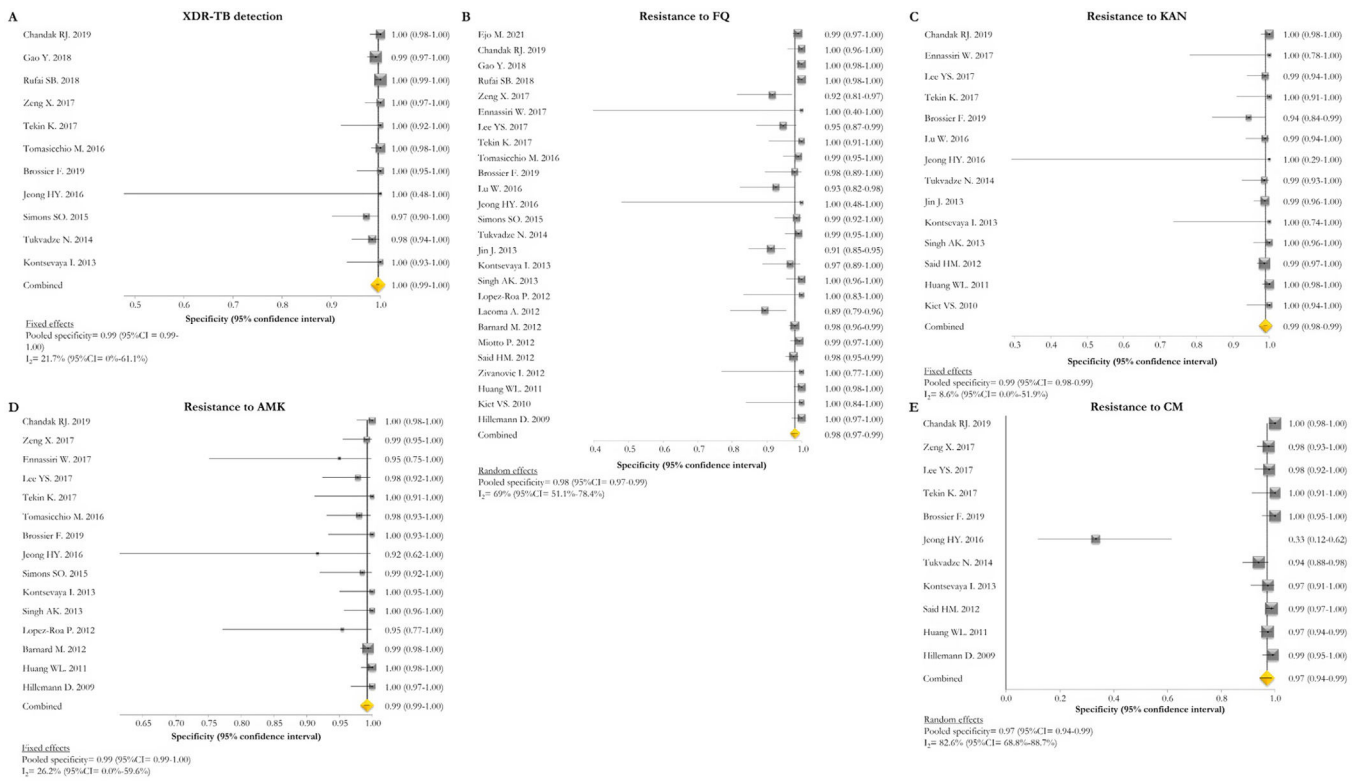
in future studies, for if the initial rapid test is more sensitive than its reflex assay it may lead to invalid or non-actionable results and challenges with final interpretation of laboratory results.

A second set of considerations relates to the capacity of these tests to reliably diagnose XDR-TB, as newly defined by WHO in 2021.<sup>11</sup> Xpert MTB/XDR detects *M. tuberculosis* complex DNA and





**Fig. 5.** Forest plots of pooled sensitivity of XDR-TB detection (A), and for resistance to FQ (B), KAN (C), AMK (D), and CM (E) of GenoType MTBDRsl VER 1.0. The point estimates of sensitivity from each study are indicated as a square and a 95% confidence interval is shown with a horizontal line; the yellow diamond is representative for the combined sensitivity.



**Fig. 6.** Forest plots of pooled specificity of XDR-TB detection (A), and for resistance to FQ (B), KAN (C), AMK (D), and CM (E) of GenoType MTBDRsl VER 1.0. The point estimates of specificity from each study are indicated as a square and a 95% confidence interval is shown with a horizontal line; the yellow diamond is representative for the combined specificity.

mutations associated with resistance to isoniazid, fluoroquinolones (ofloxacin, moxifloxacin, levofloxacin, gatifloxacin), second-line injectable drugs (amikacin, kanamycin, capreomycin), and ethionamide in a single test, whereas GenoType MTBDR<sub>plus</sub> detects resistance to isoniazid and rifampicin, and GenoType MTBDR<sub>sl</sub> to fluoroquinolones (ofloxacin and moxifloxacin) and aminoglycosides/cyclic peptides (injectable antibiotics such as kanamycin, amikacin, capreomycin, and viomycin) in its 2 versions. Unfortunately, these tests are testing the drugs defining XDR-TB according to the previous 2006 WHO definition,<sup>64</sup> which now reflects pre-XDR due to their capacity to detect resistance to fluoroquinolones, and not XDR-TB as neither these tests nor other genotypic DST assays can rapidly diagnose resistance to linezolid and bedaquiline. This is an important gap that will become more important with time; currently bedaquiline resistance is being detected in over 40% of cases failing the all oral regimen in South Africa, prevalence of bedaquiline resistance is currently 4% in South Africa and 15% in Moldova.<sup>65,66</sup> Higher MICs to pretomanid have been detected in Lineage 1 strains.<sup>67</sup> A 3 drug BPaL (bedaquiline, pretomanid, linezolid) regimen for pre-XDR with no rapid resistance test beforehand may therefore not be without risk and a shorter duration of lower dose linezolid may select for resistance to linezolid and thwart future salvage regimens. Targeted Next Generation Sequencing solutions are available but there is insufficient understanding of the role of resistance-conferring mutations to these drugs and such technologies are expensive and not implemented yet in many settings.<sup>68,69</sup> Nonetheless, the two rapid assays provide valuable information at least on resistance to isoniazid and fluoroquinolones with now also the Xpert MTB/XDR technology and not only LPAs enabling to distinguish low- versus high-level resistance to these drugs.<sup>28</sup> The detection of specific mutations is not implemented in the GeneXpert software automated interpretation. This information is nevertheless very relevant for clinicians who can rapidly design the best regimen with high precision guided by the knowledge of the association between specific genetic mutations and phenotypic resistance levels.<sup>70</sup> For example, in the case where *gyrA* A90V, S91P, and D94A mutations associated with low-level fluoroquinolone resistance are identified by specific melting temperature window patterns, high dose moxifloxacin could still work. The detection of resistance to ethionamide and second-line injectables seems currently less clinically relevant, although these medicines can still be added to complete MDR regimens when other options are not available.

Considering the TB diagnostic algorithms and diagnostic characteristics, Xpert MTB/XDR could be placed at or near point-of-care settings to be used primarily as a follow-on test for laboratory-confirmed TB, complementing existing rapid tests that detect only rifampicin, or rifampicin/isoniazid resistance. Detection of fluoroquinolone resistance is essential to inform the use of all-oral 6–12 month standardized shorter regimen for MDR/rifampicin-resistant TB. The assay is conditionally recommended for the initial detection of resistance to isoniazid and fluoroquinolones, rather than culture-based phenotypic DST in people with bacteriologically confirmed pulmonary TB, and for the initial detection of resistance to these drugs and ethionamide and amikacin in people with bacteriologically confirmed pulmonary TB and resistance to rifampicin.<sup>17</sup> Xpert MTB/RIF or Xpert Ultra and Xpert MTB/XDR could also be used as the initial diagnostic tests to detect TB, rifampicin, isoniazid and fluoroquinolone resistance in order to achieve universal DST; this algorithm may be preferable in settings with a high MDR-TB burden and also in those with high risk of isoniazid resistance (algorithm 1<sup>17</sup>). If not feasible, it may be used for further evaluation of patients with rifampicin-resistant or MDR-TB, for patients with rifampicin-susceptible TB but no results available for isoniazid but at a high risk for isoniazid resistance (algorithms 3–4).

Our review is limited by high heterogeneity of the population targets and limited range of laboratory tools available. We also note limitations in the study designs of published work identified, including little clinical outcome data following treatment based on test findings.

Finally, rapid diagnostic tests are not only helpful in existing algorithms for guiding drug selection but may also guide dose selection (higher dose) in case of low-level resistance when limited alternative treatment options exist.<sup>20</sup> Further testing options when a patient is demonstrating slower response to TB treatment than expected, despite proven drug susceptibility would be valuable, such as supplementary rapid tests for drug exposure. We foresee that the introduction of saliva- and urine-based point-of-care testing as well as microsampling techniques could be provided in conjunction with rapid DST as they are becoming more affordable for resource-limited settings, and may support laboratory testing efforts towards better outcomes for people affected by TB around the world.

## Funding

The systematic review was partially funded via an unrestricted grant by Cepheid Europe SAS to the Public Health Consulting Group, Lugano, Switzerland. The donor had no role in conducting the systematic review, as well as analysing and interpreting the results and writing the manuscript.

## Conflict of interest

The authors declare to have no conflict of interest directly or indirectly related to the manuscript contents.

## Acknowledgements

The study is part of the scientific activities of the GTN (Global Tuberculosis Network).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.arbres.2022.07.012](https://doi.org/10.1016/j.arbres.2022.07.012).

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