Targeted next-generation sequencing to diagnose drug-resistant tuberculosis: a systematic review and meta-analysis

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Summary

Background Targeted next-generation sequencing (NGS) can rapidly and simultaneously detect mutations associated with resistance to tuberculosis drugs across multiple gene targets. The use of targeted NGS to diagnose drug-resistant tuberculosis, as described in publicly available data, has not been comprehensively reviewed. We aimed to identify targeted NGS assays that diagnose drug-resistant tuberculosis, determine how widely this technology has been used, and assess the diagnostic accuracy of these assays.

Methods In this systematic review and meta-analysis, we searched MEDLINE, Embase, Cochrane Library, Web of Science Core Collection, Global Index Medicus, Google Scholar, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform for published and unpublished reports on targeted NGS for drug-resistant tuberculosis from Jan 1, 2005, to Oct 14, 2022, with updates to our search in Embase and Google Scholar until Feb 13, 2024. Studies eligible for the systematic review described targeted NGS approaches to predict drug resistance in *Mycobacterium tuberculosis* infections using primary samples, reference strain collections, or cultured isolates from individuals with presumed or confirmed tuberculosis. Our search had no limitations on study type or language, although only reports in English, German, and French were screened for eligibility. For the meta-analysis, we included test accuracy studies that used any reference standard, and we assessed risk of bias using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. The primary outcomes for the meta-analysis were sensitivity and specificity of targeted NGS to diagnose drug-resistant tuberculosis compared to phenotypic and genotypic drug susceptibility testing. We used a Bayesian bivariate model to generate summary receiver operating characteristic plots and diagnostic accuracy measures, overall and stratified by drug and sample type. This study is registered with PROSPERO, CRD42022368707.

Findings We identified and screened 2920 reports, of which 124 were eligible for our systematic review, including 37 review articles and 87 reports of studies collecting samples for targeted NGS. Sequencing was mainly done in the USA (14 [16%] of 87), western Europe (ten [11%]), India (ten [11%]), and China (nine [10%]). We included 24 test accuracy studies in the meta-analysis, in which 23 different tuberculosis drugs or drug groups were assessed, covering first-line drugs, injectable drugs, and fluoroquinolones and predominantly comparing targeted NGS with phenotypic drug susceptibility testing. The combined sensitivity of targeted NGS across all drugs was 94·1% (95% credible interval [CrI] 90·9–96·3) and specificity was 98·1% (97·0–98·9). Sensitivity for individual drugs ranged from 76·5% (52·5–92·3) for capreomycin to 99·1% (98·3–99·7) for rifampicin; specificity ranged from 93·1% (88·0–96·3) for ethambutol to 99·4% (98·3–99·8) for amikacin. Diagnostic accuracy was similar for primary clinical samples and culture isolates overall and for rifampicin, isoniazid, ethambutol, streptomycin, and fluoroquinolones, and similar after excluding studies at high risk of bias (overall sensitivity 95·2% [95% CrI 91·7–97·1] and specificity 98·6% [97·4–99·3]).

Interpretation Targeted NGS is highly sensitive and specific for detecting drug resistance across panels of tuberculosis drugs and can be performed directly on clinical samples. There is a paucity of data on performance for some currently recommended drugs. The barriers preventing the use of targeted NGS to diagnose drug-resistant tuberculosis in high-burden countries need to be addressed.

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Introduction

Rapidly determining the drug-resistance status of *Mycobacterium tuberculosis*, the causative bacterium of tuberculosis, is essential at the individual-patient level for the selection of appropriate treatment regimens, and accurate drug susceptibility testing is crucial in controlling drug-resistant tuberculosis.[1,2] Culture-based, phenotypic drug susceptibility testing is the standard for detecting drug resistance. However, the stringent laboratory conditions required for culturing *M tuberculosis*

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Research in context

Evidence before this study

We searched MEDLINE, Embase, Cochrane, Web of Science, Global Index Medicus, Google Scholar, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform for any publications on targeted next-generation sequencing (NGS) for drug-resistance detection in *Mycobacterium tuberculosis* published between Jan 1, 2005, and Feb 13, 2024, with no language restrictions. We identified 37 review articles published in this period. Previous reviews were mostly narrative and did not include a formal diagnostic meta-analysis; two were described as mini-reviews, and two were published as abstracts only. Five systematic reviews and diagnostic meta-analyses on pyrosequencing were published between 2010 and 2021. They examined just one drug or drug class, such as ethambutol or fluoroquinolones. In March, 2024, WHO included targeted NGS in its guidelines on rapid diagnostics for tuberculosis detection.

Added value of this study

To our knowledge, this is the first comprehensive systematic review and meta-analysis on the use of newer targeted NGS methods, other than pyrosequencing, for genotypic drug susceptibility testing in tuberculosis. We identified nine commercial assays and numerous non-commercial, in-house assays that used four different NGS technologies. The diagnostic accuracy of targeted NGS to diagnose drug-resistant tuberculosis was high for first-line drugs, injectable drugs, and fluoroquinolones. There were insufficient data to compare the test accuracy of targeted NGS for bedaquiline, linezolid, clofazimine, pretomanid, and delamanid. Importantly, test accuracy of targeted NGS assays was similar for primary clinical samples and culture isolates. Samples were collected in many countries, half of which were on WHO's list of nations with a high burden of tuberculosis. The primary locations for sequencing these samples were the USA, western Europe, India, and China.

Implications of all the available evidence

Targeted NGS is an accurate and rapid approach for comprehensive genotypic drug-resistance profiling of *M tuberculosis*. This approach is sensitive and specific in detecting drug resistance across panels of tuberculosis drugs simultaneously, even when performed on primary clinical samples. Although targeted NGS assays can be selected on the basis of, or adapted to, setting-specific needs and sequencing capacities, factors preventing sequencing in limited-resource settings need to be addressed. For instance, simplifying operational procedures, establishing supply chains, and building local sequencing capacities might improve access and uptake. More studies are needed to investigate the clinical impact of these assays and the use of different sample types on test accuracy.

and the slow growth of the bacteria make phenotypic testing both time intensive and resource intensive, prohibiting its use in many resource-constrained settings. Additionally, phenotypic drug susceptibility testing is suboptimal for some drugs.[3]

Genotypic drug susceptibility testing methods, which detect mutations in the *M tuberculosis* genome that could be associated with drug resistance, can be a rapid and cost-effective alternative to phenotypic drug susceptibility testing.[4–6] The WHO-endorsed catalogue of mutations in *M tuberculosis* complex summarises the evidence for resistance-associated mutations, supporting the interpretation of genotypic drug susceptibility testing.[7] Commercial nucleic acid amplification tests (NAATs) detect such mutations, are simple to use, produce results within hours, and improve access to drug susceptibility testing in settings where phenotypic drug susceptibility testing is unavailable. However, NAATs only target a small number of known resistance-conferring mutations and are restricted to a limited set of drugs. By contrast, next-generation sequencing (NGS) of *M tuberculosis* genomes provides a comprehensive approach to genotypic drug susceptibility testing and drug-resistance surveillance in select health systems.[8,9] However, uptake of whole-genome sequencing (WGS) is low in high-burden, low-resource settings due to the sometimes prohibitive investment and running costs and specialised technical and bioinformatic expertise needed for its implementation.[10,11]

Targeted NGS offers an attractive intermediary approach between NAATs and WGS. By amplifying multiple regions of the *M tuberculosis* genome, targeted NGS can be performed directly on clinical samples and can predict drug resistance to many tuberculosis drugs simultaneously. Targeted NGS can address the need for rapid resistance prediction for both first-line and second-line tuberculosis drugs. Recognising its potential, WHO endorsed targeted NGS for genotypic drug susceptibility testing for drug-resistant tuberculosis in March, 2024, and named it one of its research and development priorities for tuberculosis diagnostic tools.[12,13]

We systematically reviewed the literature to examine the use and performance of available targeted NGS assays for predicting *M tuberculosis* drug resistance. We reviewed commercial and non-commercial in-house assays, determined how widely and in which settings these assays have been used, and assessed the diagnostic accuracy of these assays against culture-based phenotypic drug susceptibility testing and WGS.

Methods

Search strategy and selection criteria

In this systematic review and meta-analysis, we performed a comprehensive literature search in MEDLINE (Ovid), Embase, Cochrane Library, Web of Science Core Collection, Global Index Medicus, Google Scholar, ClinicalTrials.gov, and the WHO International

Clinical Trials Registry Platform. The search strategy combined thesaurus or free-text terms in title, abstract, or author fields related to tuberculosis, targeted NGS, and their synonyms; a full list of search terms is in the appendix (pp 4–5). NGS methods became available in the mid-2000s, and so we restricted our searches to items published between Jan 1, 2005, and Oct 14, 2022. We searched for published and unpublished studies with no restrictions to study type, including, but not limited to, reviews, observational studies, methodological studies, published study protocols, and interventional studies. We applied no language restrictions to the search; however, we only screened reports in English, German, and French for eligibility. We imported identified records to EndNote (version 20). We removed duplicate records using Deduklick[14] and hand-searched reference lists of included reviews and studies to identify additional eligible studies. We set search alerts for Embase and Google Scholar to monitor newly published research articles until Feb 13, 2024.

We defined inclusion criteria according to the PIRT (participants, index test, reference standard, and target condition) format. Studies were eligible for the systematic review if they used or described targeted NGS approaches (index test) to predict drug resistance in *M tuberculosis* infections (target condition) from primary samples, reference strain collections, or cultured isolates from individuals with presumed or confirmed tuberculosis (participants). We did not specify a reference standard for the systematic review. We excluded studies that only did WGS, used Sanger sequencing (ie, not NGS), used targeted NGS to detect heteroresistance (ie, the simultaneous presence of drug-susceptible and drug-resistant organisms) or mixed infections (ie, infections with multiple genetically distinct bacterial strains), or used targeted NGS for microbial diagnosis of *M tuberculosis* without evaluating drug resistance. For the meta-analysis, we included test accuracy studies in individuals with presumed or confirmed tuberculosis (participants) that reported results from targeted NGS assays (index test) to diagnose drug-resistant tuberculosis for any tuberculosis drug or drug groups (target condition) and the results of any phenotypic or genotypic drug susceptibility testing as the reference standard. Since the diagnostic accuracy of pyrosequencing for drug-resistant tuberculosis detection has been comprehensively reviewed, we excluded studies reporting on pyrosequencing from the meta-analysis.[15–19]

Two reviewers (APL and PCG) independently screened titles and abstracts for eligibility using Rayyan.[20] From screened studies, two reviewers (PCG and TCS) independently selected studies for inclusion by screening the full texts of possibly eligible studies. Disagreements between reviewers were resolved through discussion (PCG, TCS, and LF).

We registered the protocol with PROSPERO on Nov 5, 2022, CRD42022368707, where the protocol is also available. We report the study according to the PRISMA guidelines.[21]

Data extraction

We developed a standardised questionnaire, which was pilot tested by two extractors (LP and TCS) on 16 studies that were representative of the study designs identified in the search (appendix p 6). TCS, LP, and FFGDlH extracted study-level data on where samples were collected, where sequencing and analysis were done, what sample types were used for targeted NGS assays, and what reference test the targeted NGS assay was compared with. For each dataset described by the studies included in the meta-analysis, we recorded the reported counts of calls that were true and false positive and negative per drug tested (drug-level data), compared with the study-specific reference standard (by use of contingency tables). Resistance calls were recorded as reported on the basis of resistance-conferring variants and cutoffs defined in each study. We excluded uncharacterised variants (ie, non-synonymous variants detected by targeted NGS but with no known associated resistance). We included hetero-resistant calls as resistant calls. If the counts of true and false positives and negatives were not reported, we calculated the number of true and false positive calls from reported sensitivities, specificities, or other information.

See Online for appendix

Risk-of-bias assessment

We evaluated risk of bias and applicability of test accuracy studies with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool.[22] Case–control studies and studies using convenience sampling for patient selection were classified as high risk. Regarding the index test, studies were considered high risk if the variants conferring drug resistance were not prespecified, the allele frequency threshold at which resistance was called was not prespecified, or variants were interpreted without masking of the reference test result. Studies using phenotypic drug susceptibility testing as the reference test for ethambutol or pyrazinamide were considered high risk because phenotypic drug susceptibility testing is generally considered unreliable for these drugs.[3] For flow and timing, studies were considered high risk if a different clinical sample was used for targeted NGS and the reference test, or if more than 50% of samples were excluded from the final analysis. To combine the risk-of-bias assessment across the QUADAS-2 domains, we classified studies with a high risk of bias in the patient selection, index test, or flow and timing domains as having an overall high risk of bias. We classified studies that specifically recruited patients with drug-resistant tuberculosis with a high concern for applicability.

Statistical analysis

Our primary outcomes of interest for the meta-analysis were the sensitivity and specificity of targeted NGS

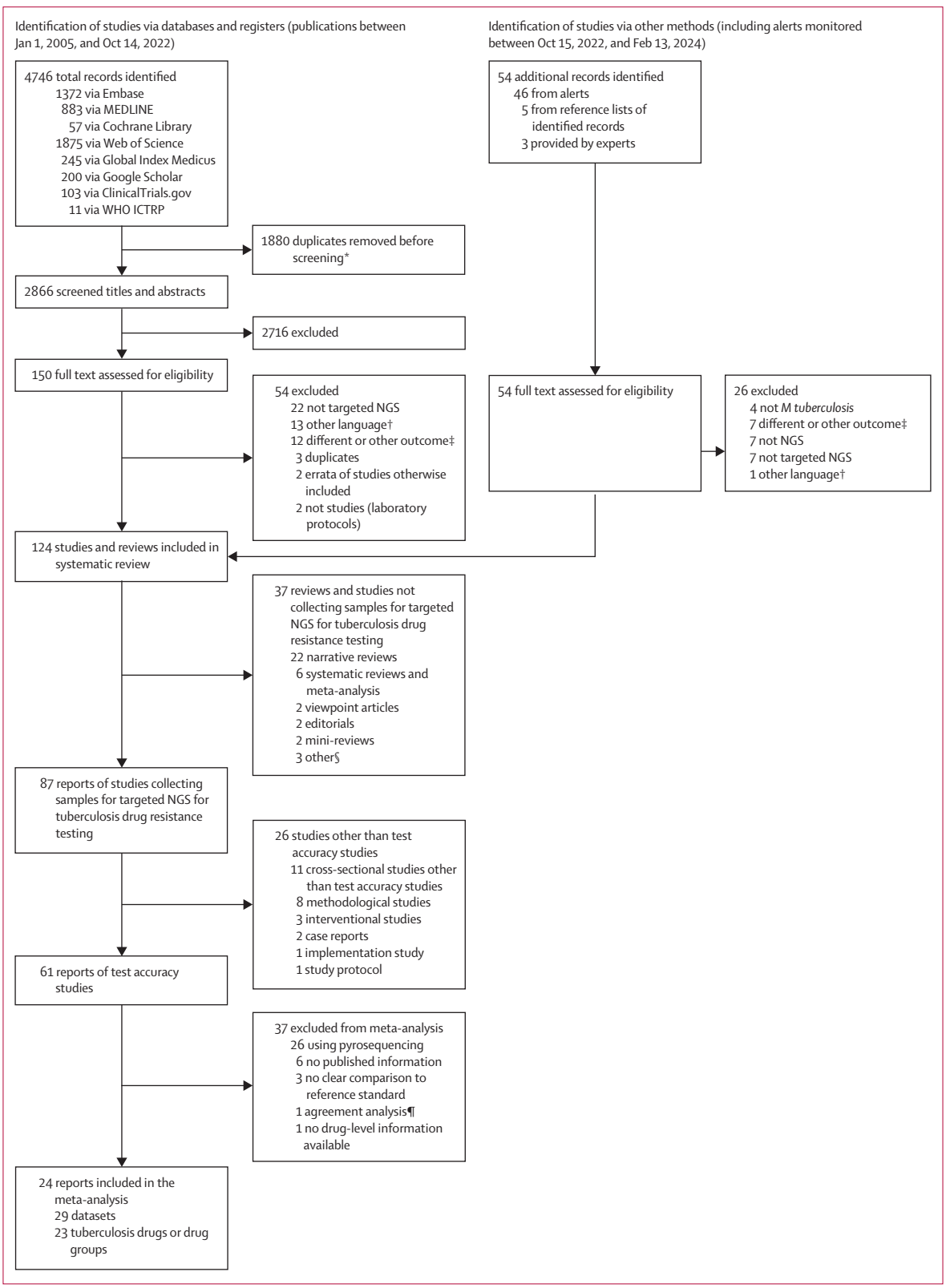

Figure 1: **Study selection**
ICTRP=International Clinical Trials Registry Platform. *M tuberculosis=Mycobacterium tuberculosis*. NGS=next-generation sequencing. *Automated de-duplication using Deduklick.[14] †Studies published in Spanish, Chinese, Korean, and Russian. ‡Studies investigating outcomes other than drug-resistance detection in *M tuberculosis* by targeted NGS. §Pipeline report, technical guide, and a WHO rapid communication. ¶As opposed to a complete test accuracy study.

	Manufacturer	NGS technology	Number of gene targets (number of tuberculosis drugs covered)	Number of test accuracy studies	Number of studies that collected samples
Deeplex Myc-TB	GenoScreen (Lille, France)	Illumina (adapted for Oxford Nanopore Technologies in Cabibbe et al [2020][25])	18 (15)	9[25–31]*	7[11,32–36]†
Next Gen-RDST assay	Translational Genomics Research Institute (Phoenix, AZ, USA)	Illumina	6 (7)	4[5,37–39]	0
Ion Ampliseq TB Research Panel	ThermoFisher Scientific (Waltham, MA, USA)	Ion Torrent	8 (10)	5[40–44]	0
Tuberculini	Clemedi AG (Schlieren, Switzerland)	Ion Torrent	NA (12)	0	0
DeepChek-TB	ABL Diagnostics (Paris, France)	NA	13 (13)	0	0
tNGS by Hugobiotech	Hugobiotech (Beijing, China)	Illumina	NA	3[45–47]	0
Ampliseq for Illumina TB Research Panel	Illumina (San Diego, CA, USA)	Illumina	NA	0	0
CleanPlex	Paragon Genomics (Fremont, CA, USA)	NA	NA	0	0
NanoTB	Oxford Nanopore Technologies (Oxford, UK)	Oxford Nanopore Technologies	NA	0	0
TBSeq	ShengTing Biotech (Hangzhou, China)	Oxford Nanopore Technologies	NA (16)	0	0
Non-pyrosequencing in-house assays	In-house	Illumina, IonTorrent, Oxford Nanopore Technologies	1–73 (1–12)	10[48–57]	8[58–65]
Pyrosequencing in-house assays	In-house	Pyromark, PSQ 96	1–8 (1–7)	26[66–91]	8[92–99]
Not reported	NA	NA	NA	4‡	3[100]§

NA=not available. NGS=next-generation sequencing. *Total includes two studies on clinical trial registries (NCT03303963 and NCT04397536) with no published data. †Total includes one study on a clinical trial registry (NCT05007795) with no published data. ‡Total comprises four test accuracy studies that were registered on clinical trial registries (NCT04923958, NCT04239326, ChiCTR2300078691, and CTRI/2019/11/021973) with no published data and no additional information on the assay specified. §Total includes two studies on clinical trial registries (NCT05553236, and NCT03604848) with no published data and no additional information on the assay specified.

Table 1: **Characteristics of targeted NGS assays for diagnosis of drug-resistance tuberculosis identified in the systematic review**

assays that diagnose drug-resistant tuberculosis compared with commonly used phenotypic and genotypic drug susceptibility testing. We defined sensitivity as the proportion of samples classified as drug-resistant by the reference standard in which a resistance-conferring variant was detected by the targeted NGS assay. We defined specificity as the proportion of samples identified as drug-susceptible by the reference test in which no resistance-conferring variant was detected by the targeted NGS assay. We used a Bayesian bivariate model to generate summary receiver operating characteristic (ROC) plots and combined sensitivity and specificity estimates for individual drugs and drug groups. We also summed the values of the contingency tables reported for each drug to obtain one contingency table for each test accuracy study and calculated combined estimates for these pooled tables using the same model. We used the MetaBayesDTA web application (version 1.5.1), which implements a bivariate random-effects model with exact binomial likelihoods.[23] We used the default weakly informative priors for all parameters (appendix p 7) and ran four chains of 2500 iterations, after a burn-in of 500 iterations to calculate the combined sensitivity and specificity estimates and their 95% credibility intervals (CrIs). We assessed convergence of the model by checking R-hat values (values >1·05 indicating potential convergence issues) and visually inspecting posterior density plots and trace plots provided by MetaBayesDTA. For drugs with insufficient data to meaningfully meta-analyse, we describe the sensitivity and specificity estimates reported by the studies.

Since the bacterial load of the sample might influence whether targeted NGS is successful and drug resistance can be determined, we categorised studies by their use of culture isolates or primary clinical samples in a subgroup analysis. We also compared studies that tested all patients with tuberculosis, regardless of their resistance status, with studies that specifically recruited patients who had drug-resistant tuberculosis. In sensitivity analyses, we excluded studies that we classified as having an overall high risk of bias and we used a frequentist version of the model.[24]

To assess heterogeneity between studies, we visually inspected the prediction regions in each ROC plot.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We identified and screened 2920 reports (2866 identified via databases and registers; 54 via other methods) and included 124 in the systematic review (87 reports of studies collecting samples for targeted NGS for tuberculosis drug-resistance testing, including 61 test accuracy studies, and 37 review articles; figure 1). The characteristics of studies included in the systematic review are provided in the appendix (pp 14–33).

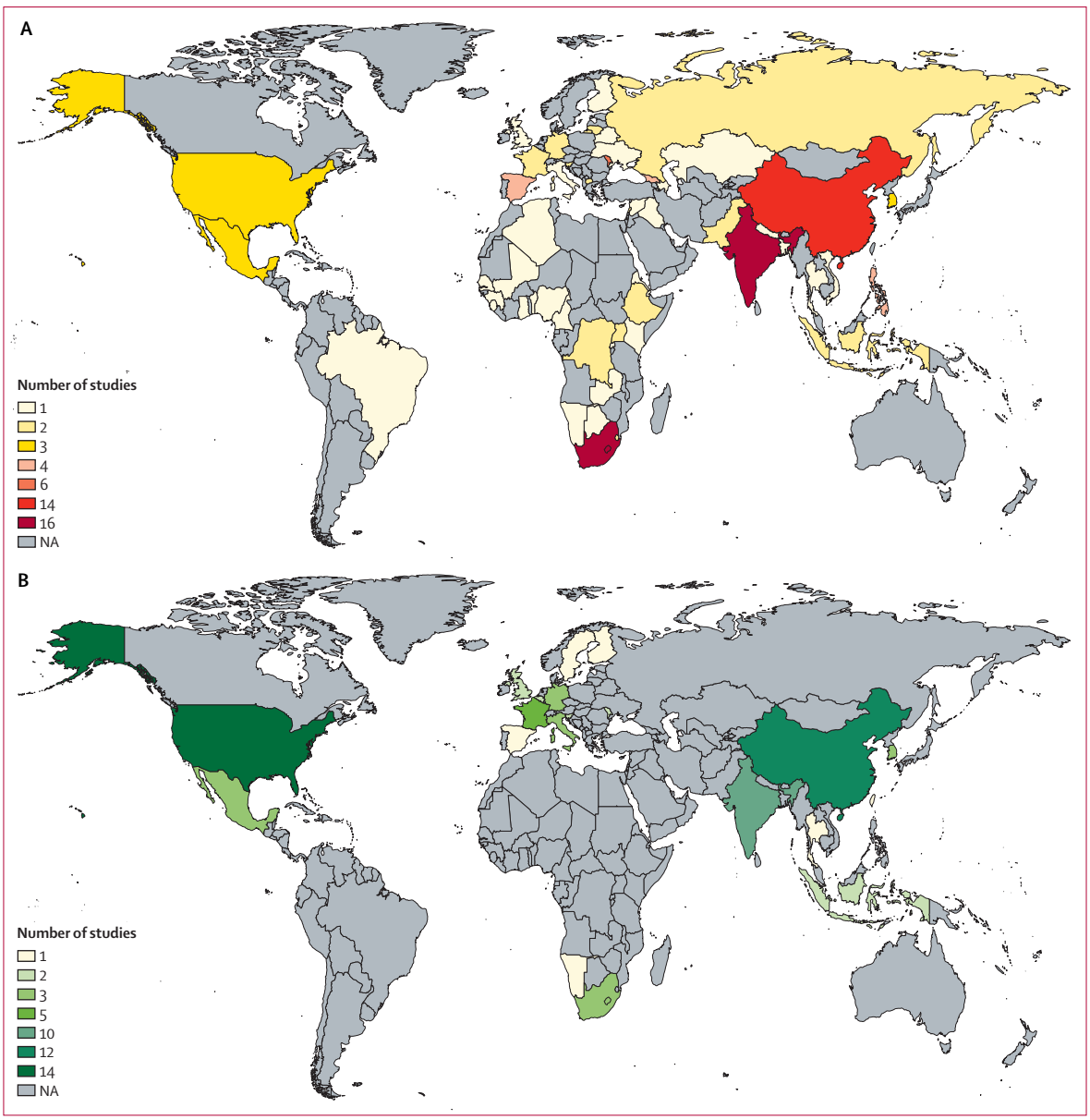

Figure 2: **Countries where samples were collected (A; 79 studies) and where sequencing and analysis were conducted (B; 68 studies)**
Of 87 studies that collected samples, two did not report where samples were collected, four used archive or reference isolates, one used samples from another study already included in the review, and one used reference isolates and sequencing data from other studies also included in the review. Furthermore, 17 of 87 studies in which samples were collected did not clearly report where sequencing was done and two used sequencing data produced in a previous study (and so were removed as duplicates). Grey areas indicate countries where no samples were collected and no targeted NGS for predicting drug-resistant tuberculosis was done. NA=not applicable. NGS=next generation sequencing.

The identified targeted NGS assays use various sequencing technologies and investigate multiple targets simultaneously (table 1). Deeplex Myc-TB (GenoScreen, Lille, France) was the commercial assay that was most frequently reported, included in nine test accuracy studies. Some commercial tests, such as the Tuberculini (Clemedi, Schlieren, Switzerland) or DeepChek-TB (ABL Diagnostics, Paris, France), were mentioned in reviews or reports, but we found no studies using or evaluating the accuracy of these tests. Information on the technical specifications of these tests, such as sequencing technology or targeted genes, might be available through other sources. Several studies reported on custom in-house target panels, using diverse sequencing platforms (table 1). Upon assessing reports for our systematic review, we grouped together studies that did and did not use pyrosequencing platforms. Pyrosequencing was the leading sequencing technology before 2015 that has since been superseded by NGS techniques, which can analyse more targets simultaneously.

Samples for testing were collected across the world, but sequencing was generally not done locally. Samples originated from 53 countries, including 27 countries on WHO's tuberculosis high-burden country lists.[101] Most studies were conducted in India and South Africa (16 studies each), both high tuberculosis-burden countries with high incidence rates of multidrug-resistant tuberculosis (figure 2A). Samples were also collected in low and intermediate prevalence settings, such as Slovenia and Eritrea, and settings with low prevalence of multidrug-resistant tuberculosis, such as Algeria. Studies conducted in south Asia, east Asia, and Latin America largely sequenced samples locally, whereas little local sequencing was done across Africa (figure 2B). In 13 (76%) of 17 studies conducted in sub-Saharan Africa, the collected samples were shipped to North America or Europe for sequencing (appendix p 8). Overall, sequencing was mainly done in the USA (14 [16%] of 87 sites), western Europe (ten [11%]), India (ten [11%]), and China (nine [10%]).

For the meta-analysis, we included 24 test accuracy studies, providing contingency tables for 29 datasets (figure 1; appendix pp 35–38). These studies evaluated 23 different drugs or drug groups, ranging from one to 14 per study, on 2866 samples, resulting in 189 tables of pairwise comparisons of 13 639 observations. The number of comparisons per drug in the meta-analysis ranged from one (bedaquiline and clofazimine;[30] ethionamide;[26] ofloxacin and levofloxacin[51]) to 26 (rifampicin;[26–31,37,39,41–44,49,50–52,54–57] isoniazid[26–31,37,39,41–45,49–52,54–57]). We excluded 19 comparisons with no resistant (true positive and false negative) or susceptible (true negative and false positive) samples. There were sufficient data to meaningfully meta-analyse the diagnostic accuracy of targeted NGS for rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin, the injectable drugs (amikacin, capreomycin, and kanamycin), moxifloxacin, and fluoroquinolones, a class of antibiotics including moxifloxacin and levofloxacin. 17 studies used culture isolates for targeted NGS and 14 studies used primary clinical samples; the specific type of samples used for sequencing are listed in the appendix (pp 34–38).

Main sources of potential bias in the 24 studies included in the meta-analysis related to patient selection (figure 3). Many studies either used a case–control study design (five [17%] of 29 datasets), selected stored isolates or samples by convenience sampling (four [14%]), or did not clearly report their selection criteria (seven [24%]). 59% of the study datasets (17 of 29) included patients with tuberculosis who were recruited with an unknown resistance status, while the remaining datasets (12 [41%]) specifically recruited patients with drug-resistant tuberculosis. In most comparisons, targeted NGS assays were compared with phenotypic drug susceptibility testing (171 [90%] of 189 pairwise comparisons), either on solid or liquid media (appendix pp 35–38). One study[29] used WGS as the reference standard (13 [7%] of 189 pairwise comparisons). In three pairwise comparisons (three [2%] of 189), targeted NGS was compared with the pyrazinamidase activity test. In two comparisons (two [1%] of 189) NAATs were used as the reference standard. 16 (55%) of 29 datasets used culture or reference isolates, while 13 (45%) used a variety of primary clinical samples (appendix p 34).

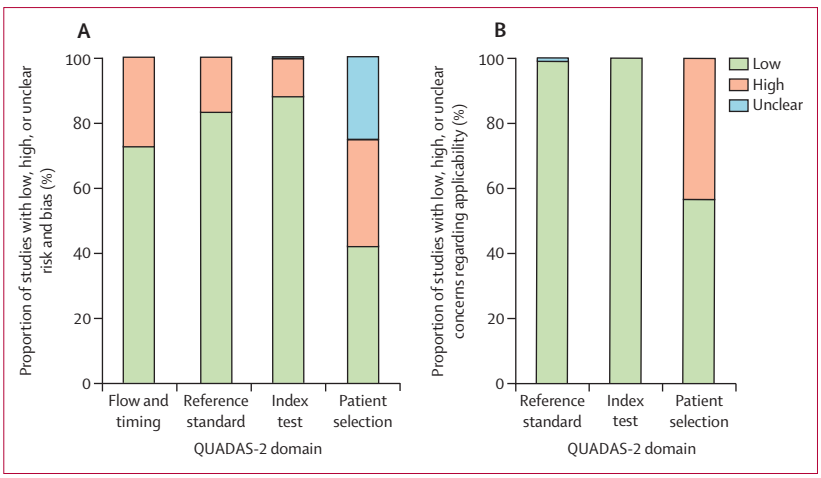

Figure 3: **Assessment of risk of bias (A) and concerns regarding applicability (B) of the 24 test accuracy studies included in the meta-analysis, using the QUADAS-2 tool**

Concerns regarding applicability address whether there are concerns that the included patients do not match the review question (patient selection); that the index test, its conduct, or interpretation differ from the review question (index test); or that there are concerns that the target condition, as defined by the reference standard, does not match the review question (reference standard). QUADAS-2=Quality Assessment of Diagnostic Accuracy Studies-2.

The overall sensitivity of targeted NGS for drug-resistant tuberculosis testing was 94·1% (95% CrI 90·9–96·3) and specificity was 98·1% (97·0–98·9; table 2). The point estimate for sensitivity was above 95% for isoniazid, rifampicin, and ethambutol, with a combined sensitivity estimate for rifampicin resistance of 99·1% (98·3–99·7). The point estimate for specificity was above 95% for all drugs except ethambutol (93·1% [88·0–96·3]). The sensitivities across studies varied more than the specificities for most drugs, except for rifampicin and ethambutol, for which we observed a higher incidence of false positive calls and a wider range of specificities across studies (figure 4; appendix pp 9–10). We saw no clear trend of a better or worse performance across drugs for any specific targeted NGS assay (appendix pp 9–10). Study-level sensitivity and specificity estimates for ethionamide ranged from 43% to 100% and 68% to 100%, respectively. For bedaquiline, no or one drug-resistant sample was reported, resulting in wide confidence intervals for study-level sensitivity estimates (21–100%). Study-level specificity for bedaquiline was high (100%), with a wide range of lower boundaries for 95% confidence intervals (34–91%). No delamanid-resistant samples were reported (appendix pp 39–40).

We found no evidence of a difference in diagnostic accuracy between targeted NGS assays conducted on

culture isolates and those conducted on primary clinical samples for rifampicin, isoniazid, ethambutol, streptomycin, and fluoroquinolones; for the other drugs, the study-level estimates were too variable, with large and overlapping credible intervals, to draw any conclusions (table 2, figure 4). The estimated combined sensitivity for pyrazinamide and the injectable drugs (amikacin, capreomycin, and kanamycin) was lower for primary samples than for culture isolates; however, the sensitivity estimates across studies for these drugs varied substantially (0–100%) for the injectable drugs and by 50–100% for pyrazinamide (figure 4; appendix pp 9–12, 35–38). A subgroup analysis showed no substantial difference in diagnostic performance in studies that included patients with tuberculosis regardless of resistance status compared with studies that specifically recruited patients with drug-resistant tuberculosis (table 2). Excluding studies considered to have a high risk of bias did not change the combined estimates considerably (table 2). All models showed good convergence on the basis of their trace plots and had R-hat values of ≤1·05 (data not shown). The frequentist analysis produced similar results for combined estimates as the Bayesian analysis (appendix pp 41–43).

Discussion

In this systematic review and meta-analysis, we found that the diagnostic accuracy of targeted NGS assays to diagnose drug-resistant tuberculosis was good, particularly for first-line drugs and fluoroquinolones. These assays make use of various sequencing platforms and can assess numerous gene targets simultaneously, offering comprehensive genotypic drug susceptibility testing directly from primary clinical samples. Studies on targeted NGS assays for drug-resistant tuberculosis have been conducted globally; however, few studies in lower-income, high-burden settings in sub-Saharan Africa did sequencing locally.

Our systematic review has several strengths and limitations. We comprehensively reviewed available

	All samples	Subgroup and sensitivity analyses				
		Excluding studies at high risk of bias	Any patients with tuberculosis enrolled	Only patients with drug-resistant tuberculosis enrolled	Primary samples	Culture isolates
Overall						
Datasets	29	15	16	13	13	16
Observations	13 639	7749	8906	4733	5223	6574
Sensitivity	94·1% (90·9–96·3)	95·2% (91·7–97·1)	91·5% (88·3–93·9)	95·4% (92·7–97·3)	90·5% (86·2–93·9)	95·2% (93·0–96·9)
Specificity	98·1% (97·0–98·9)	98·6% (97·4–99·3)	97·6% (96·5–98·5)	98·0% (96·7–98·8)	97·6% (96·6–98·3)	98·2% (97·2–99·0)
Isoniazid						
Datasets	25	12	15	10	12	11
Observations	2065	1137	1504	561	742	1052
Sensitivity	95·6% (92·4–97·8)	96·8% (93·4–98·7)	91·6% (87·1–94·8)	97·8% (95·9–98·9)	93·8% (90·0–96·4)	94·0% (89·4–96·9)
Specificity	98·9% (97·8–99·6)	99·0% (97·2–99·7)	98·5% (97·4–99·2)	98·2% (95·1–99·5)	97·5% (95·7–98·8)	99·1% (98·1–99·7)
Rifampicin						
Datasets	24	12	14	10	12	12
Observations	2054	946	1469	585	743	1163
Sensitivity	99·1% (98·3–99·7)	98·9% (97·5–99·6)	98·4% (97·0–99·3)	99·3% (98·1–99·8)	97·5% (95·1–98·9)	99·2% (98·3–99·7)
Specificity	97·6% (94·4–99·0)	96·2% (89·2–98·8)	98·2% (96·5–99·0)	92·9% (86·0–96·9)	97·8% (95·8–98·9)	97·9% (95·5–99·0)
Ethambutol						
Datasets	16	7	10	6	7	8
Observations	1332	521	1030	302	417	782
Sensitivity	96·2% (92·3–98·6)	97·3% (91·1–99·4)	93·2% (88·8–96·3)	97·5% (93·3–99·2)	92·9% (84·0–97·6)	93·9% (89·8–96·9)
Specificity	93·1% (88·0–96·3)	91·0% (75·9–96·8)	93·9% (91·4–96·0)	86·8% (74·9–94·0)	94·2% (90·7–96·5)	93·1% (89·4–96·1)
Pyrazinamide						
Datasets	18	7	10	8	6	11
Observations	1346	796	578	768	228	974
Sensitivity	90·0% (82·1–94·6)	91·6% (78·2–96·9)	88·5% (82·2–93·2)	89·0% (78·8–94·4)	76·2% (60·2–88·3)	92·3% (86·7–95·5)
Specificity	97·2% (95·2–98·5)	96·7% (93·2–98·3)	97·8% (96·0–98·9)	95·9% (93·1–97·7)	95·6% (91·6–98·0)	97·4% (95·6–98·7)
Streptomycin						
Datasets	13	7	7	6	5	7
Observations	933	469	608	325	313	474
Sensitivity	89·8% (78·8–95·6)	89·4% (69·5–96·3)	90·4% (84·2–94·3)	86·1% (70·5–94·1)	83% (68·2–91·9)	88·4% (78·8–93·9)
Specificity	97·7% (95·5–99·1)	97·7% (92·9–99·3)	97·1% (94·5–98·6)	96·8% (93·0–98·8)	95·5% (91·2–97·9)	98·7% (96·8–99·6)

(Table 2 continues on next page)

	All samples	Subgroup and sensitivity analyses				
		Excluding studies at high risk of bias	Any patients with tuberculosis enrolled	Only patients with drug-resistant tuberculosis enrolled	Primary samples	Culture isolates
(Continued from previous page)						
Kanamycin						
Datasets	12	7	7	5	5	6
Observations	918	581	634	284	467	309
Sensitivity	87·2% (69·5–95·3)	91·8% (76·8–97·6)	87·4% (72·8–94·5)	77·2% (60·1–91·2)	80·6% (56·8–92·9)	93·4% (84·7–97·8)
Specificity	98·6% (96·7–99·5)	97·6% (94·2–99·1)	98·6% (96·8–99·5)	97·4% (94·4–98·9)	98·1% (95·5–99·4)	98·5% (96·4–99·5)
Amikacin						
Datasets	9	5	6	3	4	4
Observations	812	519	591	221	435	232
Sensitivity	86·3% (65·6–95·6)	86·8% (57·1–96·8)	83·5% (64·4–93·5)	88·1% (67·8–96·9)	61·4% (36·4–82·3)	96·9% (88·8–99·5)
Specificity	99·4% (98·3–99·8)	98·9% (95·0–99·7)	99·1% (98·0–99·7)	98·9% (95·6–99·8)	98·8% (96·7–99·6)	99·0% (96·6–99·8)
Capreomycin						
Datasets	11	7	6	5	5	5
Observations	929	576	647	282	459	330
Sensitivity	76·5% (52·5–92·3)	82·8% (54·9–94·9)	76·2% (50·5–90·7)	67·8% (47–84·7)	55·7% (28·9–80·3)	90·3% (76·8–96·6)
Specificity	98·2% (96·6–99·2)	97·7% (94·4–99·2)	98·3% (96·7–99·2)	96·9% (93·6–98·9)	98·9% (97·3–99·6)	97·7% (95·1–99·1)
Fluoroquinolones						
Datasets	10	6	4	6	3	6
Observations	524	310	198	326	80	296
Sensitivity	92·3% (81·8–96·4)	93·0% (79·5–97·0)	93·2% (82·0–98·0)	91·5% (80·5–95·9)	88·4% (65·1–97·1)	90·4% (81·2–95·9)
Specificity	99·0% (97·2–99·7)	98·4% (94·8–99·6)	98·2% (94·0–99·5)	98·6% (96·1–99·6)	97·2% (88·8–99·5)	98·8% (96·6–99·7)
Moxifloxacin						
Datasets	6	3	4	2	3	3
Observations	532	312	453	79	361	171
Sensitivity	87·6% (74·0–94·9)	87·9% (65·3–95·6)	85·5% (70·6–93·8)	88·3% (60·5–97·7)	80·6% (58·4–93·1)	90·2% (75·4–96·8)
Specificity	98·0% (93·1–99·4)	98·4% (84·9–99·6)	97·3% (93·0–99·0)	97·2% (83·8–99·5)	96·7% (86·8–98·9)	98·0% (93·3–99·5)

Data in parentheses are 95% credible intervals.

Table 2: **Sensitivity and specificity estimates from Bayesian bivariate models, combined for all drugs in a given study (overall) and by drug, in the main analysis and in subgroup and sensitivity analyses**

technologies based on the published literature and public registries, including in-house targeted NGS assays. We used a complex search strategy, extracted and evaluated data using standardised processes, and followed the PRISMA reporting guidelines. However, assessing the quality of the studies was challenging because of the heterogeneous methodologies used. We adopted a lenient study selection approach, including studies with diverse patient selection criteria and that used various reference methods for their evaluations, spanning phenotypic and genotypic drug susceptibility testing. Studies were often done retrospectively and in settings with a high burden of tuberculosis or drug-resistant tuberculosis, possibly limiting the generalisability of our findings. We used study-specific reference standards to determine true positive or true negative calls, which was phenotypic drug susceptibility testing for most included studies. The inaccuracy of phenotypic drug susceptibility testing for some drugs might lead to an overestimation of diagnostic accuracy if targeted NGS and phenotypic drug susceptibility testing have correlated errors.[3] Similarly, cases of errors by phenotypic drug susceptibility testing that are correctly classified by targeted NGS would underestimate targeted NGS test accuracy. Contingency tables reported by the studies only included samples with analysable results from both the targeted NGS assay and the reference test, often excluding many collected samples from the accuracy calculations. Unfortunately, we had insufficient data to meta-analyse the diagnostic performance of targeted NGS assays to diagnose resistance to bedaquiline, pretomanid, and linezolid, which form part of the WHO-recommended BPaL regimen for treating multidrug-resistant tuberculosis.[102] Additionally, we excluded several Chinese, Russian, Korean, and Spanish reports due to reviewer language constraints. If these studies made use of targeted NGS to diagnose drug-resistant tuberculosis in local settings, we may have underestimated the use of these assays in these settings.

Some of the targeted NGS assays identified in this systematic review have been examined in previous reports and reviews, but the reviews did not include the wide range of in-house targeted NGS assays for

drug-resistant tuberculosis reported in the published literature.[48–54,58–64,103,104] Here we included all studies as of Feb 13, 2024, to evaluate where commercial and in-house targeted NGS assays have been used and their performance. WHO published a diagnostic accuracy evaluation of targeted NGS based on unpublished and

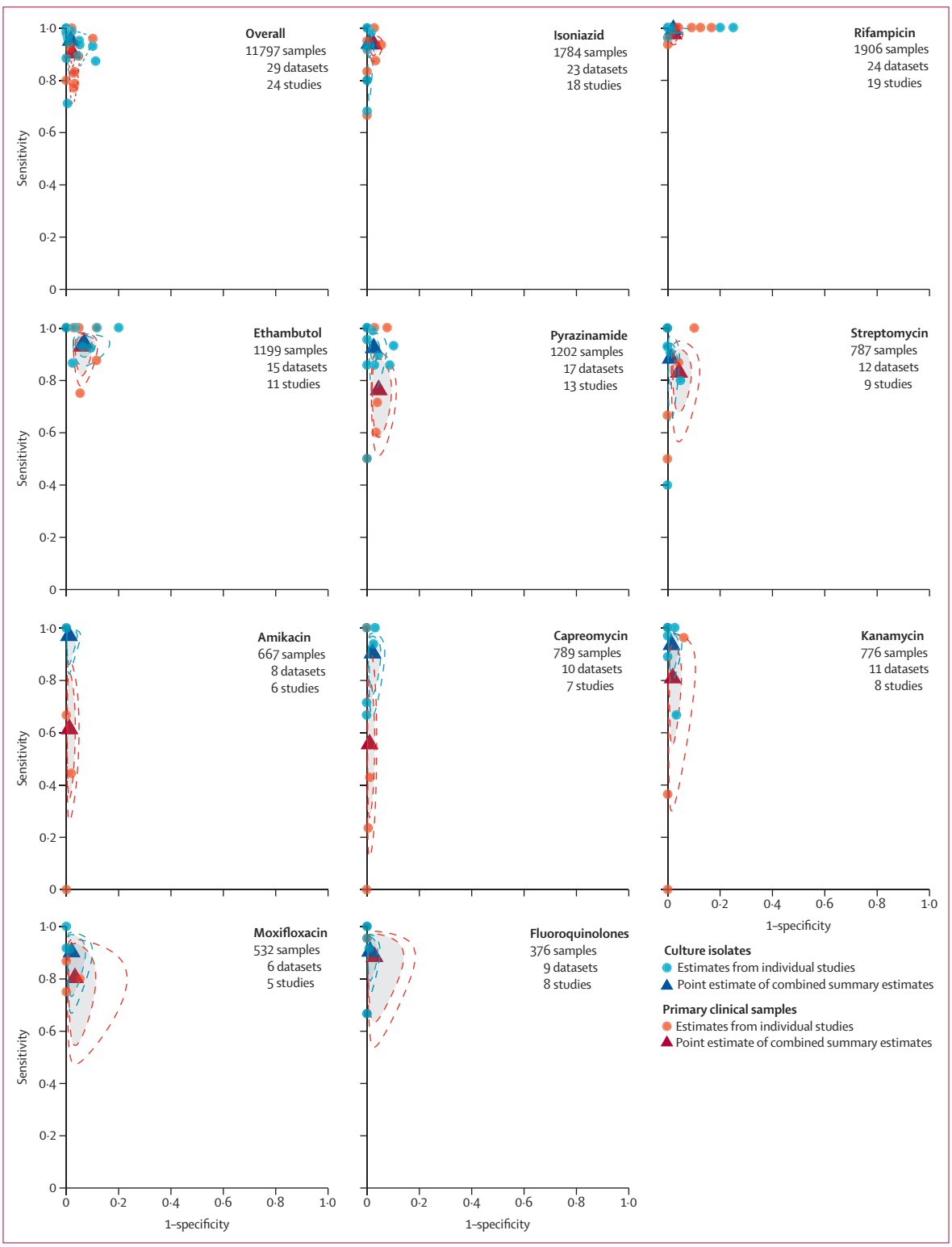

Figure 4: **Summary receiver operating characteristic plots derived from a meta-analysis of targeted next generation sequencing assays to diagnose drug-resistant tuberculosis versus phenotypic or genotypic drug susceptibility testing**
Circles indicate estimates from the individual diagnostic accuracy studies; triangles indicate point estimates of combined summary estimates, generated using a Bayesian bivariate random-effects model. Dotted lines indicate the 95% prediction region from the bivariate model; the greyed-out areas indicate the 95% credible region from the bivariate model. Overall indicates data combined for all drugs in a given study. Notably, not all studies reported sample-level information on sample type, and so these studies were not included in the meta-analysis for this subgroup analysis.

published data in March, 2024.[12] The report showed similar diagnostic accuracy estimates to our findings.

The overall performance of targeted NGS assays for drug-resistant tuberculosis was high for samples that produced an analysable result. For rifampicin resistance, the 99·1% sensitivity estimate was higher than the reported sensitivity of 95% for Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) and the specificities were similar (97·6% for targeted NGS and 98% for Xpert MTB/RIF Ultra).[105,106] Combined sensitivity and specificity estimates for rifampicin and isoniazid were similar to those reported for WGS in a systematic review assessing the diagnostic accuracy of WGS for drug-resistance detection.[107] The ranges of sensitivities we found for other drugs were similar to those reported for WGS, whereas the ranges of specificities were higher and narrower for targeted NGS assays than the ranges reported previously for WGS[107] For isoniazid, fluoroquinolones, and the injectable drugs, our combined sensitivity and specificity estimates for targeted NGS were similar to diagnostic accuracy measures reported for the Xpert MTB/XDR assay (Cepheid).[108]

As with WGS, the analytical performance of targeted NGS for drug-resistant tuberculosis was variable across studies.[107] The variability in the specificity of targeted NGS across studies for rifampicin and ethambutol might reflect discrepancies between targeted NGS and phenotypic testing in liquid media due to the occurrence of so-called low-level resistance, and the ability of targeted NGS to detect minor variants and heteroresistance. The substantial variation in sensitivity of targeted NGS assays across studies for the injectable drugs and streptomycin might in part be attributed to a paucity of available data, a more limited understanding of the genetic variants associated with phenotypic resistance to these drugs, and reduced scientific interest because the routine use of these drugs is discouraged.[109,110] For pyrazinamide, phenotypic drug susceptibility testing is complex, requiring specific media and pyrazinamide preparation kits. The WHO-recommended reference method for pyrazinamide is sequencing the *pncA* gene; however, the molecular mechanisms involved in the development of resistance are complicated.[3,111] In this meta-analysis, uncharacterised mutations were excluded, possibly leading to an underestimation of sensitivity of targeted NGS, but more research is needed to establish the association between these mutations and drug resistance. Additionally, differences in test accuracy between different targeted NGS assays need to be investigated. Furthermore, composite reference standards combining phenotypic and genotypic drug susceptibility testing might be a more appropriate comparator to evaluate test accuracy, especially for newer tuberculosis drugs. However, composite reference standards have their own limitations.[112]

Targeted NGS appears to be an accurate and rapid sequencing approach for comprehensive genotypic drug-resistance profiling. WHO has endorsed this method as a complementary tool for genotypic drug susceptibility testing and published conditional recommendations on its use.[12] By contrast with the Xpert MTB/RIF Ultra assay, which only investigates resistance to rifampicin, targeted NGS has the potential to simultaneously provide genotypic drug susceptibility testing for multiple drugs, including those contained in the BPaL regimen and future regimens. Amplicon sequencing increases sequence coverage, enabling the investigation of heteroresistance and mixed infections.[113,114] Moreover, whereas WGS requires *M tuberculosis* cultures to provide sufficient DNA for testing, targeted NGS assays can be performed directly on clinical samples, with no evidence of a reduced or improved performance compared with testing on culture isolates. The studies included in our systematic review reported that sequencing results were obtained within several hours, while the total turnaround time from primary sample collection to result ranged from 1 day to 10 days.[53,54,64,73,74,84] Sputum was the predominant primary sample type used in studies of targeted NGS to diagnose drug-resistant tuberculosis, but stool and cerebrospinal fluid have also been used.[26,66] Commercial assays, the WHO-endorsed mutation catalogue, and automated pipelines for data analysis support standardised methods for targeted NGS, while the customisability of targeted NGS assays should allow them to be applicable across different settings. These assays can be selected on the basis of available sequencing capacities, tailored to the throughput required, and target genes can be updated according to new evidence or setting-specific differences, with minor changes necessary to the available infrastructure and testing procedures. Targeted NGS can be a rapid, complementary diagnostic tool in the context of shortened drug-resistant tuberculosis treatment regimens.

Although our review of published literature is inherently limited in terms of comprehensively evaluating the true sequencing capacity and use of these assays globally, our findings do reiterate the disparity in sequencing capacities between high-income and low-income and middle-income countries, particularly in Africa.[115] To improve the use of targeted NGS assays to diagnose drug-resistant tuberculosis, technical and logistical challenges need to be overcome, possibly by simplifying operational procedures through automation, establishing supply chains, and improving local technical skills.[11] Additionally, the infrastructural and operational costs remain prohibitive in many settings, despite the decreasing cost of NGS.[116] Increased use and dissemination of data will provide valuable insights on the performance of targeted NGS to diagnose drug-resistant tuberculosis in specific settings and patient populations because sensitivity and specificity are not fixed properties of a test and can vary between populations. Investigation of the performance of targeted NGS to diagnose drug-resistant tuberculosis in

populations with paucibacillary tuberculosis, such as children and people with HIV, is important. Further research is also needed to assess the effects of different sample types and bacterial load on targeted NGS assay performance. Some data indicate that targeted NGS is not as successful as other rapid molecular diagnostic methods in paucibacillary samples, presenting an opportunity for further technical optimisation.[53,116]

In summary, targeted NGS is an exciting addition to current, routine drug-resistant tuberculosis diagnostics that can be performed directly on primary clinical samples, rapidly providing resistance profiles to several drugs, thus supporting improved clinical management. More research is needed to evaluate assay performance on newer, second-line tuberculosis drugs, and the factors prohibiting use of targeted NGS assays locally in high-burden settings need to be addressed.

Contributors

LF, ME, APL, and TCS designed the study. BM and APL developed the search strategy with feedback from LF and TCS. APL, PCG, and TCS screened and selected studies. TCS, LP, and FFGDlH extracted the data and prepared the data for analysis. TCS and FFGDlH verified the study data. TCS and OE analysed the data. TCS wrote the first draft of the manuscript and all authors critically revised the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The study protocol is available at PROSPERO (CRD42022368707). The datasets generated and analysed in this meta-analysis are published in the appendix. Additional information collected from studies is available from the corresponding author on reasonable request.

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