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Diagnostic accuracy of nanopore sequencing for detecting *Mycobacterium tuberculosis* and drug-resistant strains: a systematic review and meta-analysis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) infection, remains a significant public health threat. The timeliness, portability, and capacity of nanopore sequencing for diagnostics can aid in early detection and drug susceptibility testing (DST), which is crucial for effective TB control. This study synthesized current evidence on the diagnostic accuracy of the nanopore sequencing technology in detecting MTB and its DST profile. A comprehensive literature search in PubMed, Scopus, MEDLINE, Cochrane, EMBASE, Web of Science, AIM, IMEMR, IMSEAR, LILACS, WPRO, HERDIN Plus, MedRxiv, and BioRxiv was performed. Quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. Pooled sensitivity, specificity, predictive values (PV), diagnostic odds ratio (DOR), and area under the curve (AUC) were calculated. Thirty-two studies were included; 13 addressed MTB detection only, 15 focused on DST only, and 4 examined both MTB detection and DST. No study used Flongle or PromethION. Seven studies were eligible for meta-analysis on MTB detection and five for DST; studies for MTB detection used GridION only while those for DST profile used MinION only. Our results indicate that GridION device has high sensitivity [88.61%; 95% CI (83.81–92.12%)] and specificity [93.18%; 95% CI (85.32–96.98%)], high positive predictive value [94.71%; 95% CI (89.99–97.27%)], moderately high negative predictive value [84.33%; 95% CI (72.02–91.84%)], and excellent DOR [107.23; 95% CI (35.15–327.15)] and AUC (0.932) in detecting MTB. Based on DOR and AUC, the MinION excelled in detecting pyrazinamide and rifampicin resistance; however, it underperformed in detecting isoniazid and ethambutol resistance. Additional studies will be needed to provide more precise estimates for MinION's sensitivity in detecting drug-resistance, as well as DOR in detecting resistance to pyrazinamide, streptomycin, and ofloxacin. Studies on detecting resistance to bedaquiline, pretomanid, and linezolid are lacking. Subgroup analyses suggest that overall accuracy of MTB detection tends to be higher with prospective study design and use of standards other than CSTB (Chinese national standard for diagnosing TB). Sensitivity analyses reveal that retrospective study design, use of GridION, and use of Illumina whole-genome sequencing (WGS) decrease overall accuracy in detecting any drug-resistant MTB. Findings from both types of analyses, however, should be interpreted with caution because of the low number of studies and uneven distribution of studies in each subgroup.

Keywords Tuberculosis, Detection, Drug resistance, Third-generation sequencing, GridION, MinION

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) infection, remains a significant public health threat, with an estimated 10 million new infections annually[1]. In 2022, it was the second leading cause of mortality among infectious diseases after COVID-19[2]. Though affecting all countries and age groups, TB is most prevalent in low- and middle-income countries (LMICs), with almost half of the cases occurring in mainly eight countries (Bangladesh, China, India, Indonesia, Nigeria, Pakistan, Philippines, and South Africa)[2]. Early detection and diagnosis can dramatically affect treatment outcomes and prognosis, preventing the development of drug resistance. Yet, some diagnostic challenges present, such as lack of point-of-care (POC) tests that can be accessed in remote areas and the long turnaround time of the culture, which to this date remains the gold standard for diagnosis[3].

The gold standard for determining TB drug susceptibility testing (TB-DST) is culture-based methods (e.g., liquid, MGIT-DST or solid, LJ-DST). These methods are reliable for determining drug resistance; however, these are time-consuming, often taking weeks to yield results in resource-constrained settings. In addition, nanopore sequencing simultaneously screens for mutations associated with DST against multiple antibiotics (instead of selected options), provides high sensitivity and is not prone to contamination, compared to culture-based phenotypic DST like the MGIT- or LJ-DST. Later on, nucleic acid amplification tests (NAAT), such as GeneXpert® MTB/RIF (Xpert), have improved the detection and management of TB. Due to its timeliness, and high sensitivity and specificity compared to acid-fast bacilli (AFB) smears and cultures, Xpert has gained widespread use, particularly in diagnosing multi-drug resistant (MDR) TB[5,6]. While Xpert offers rapid diagnosis, its high laboratory cost, encompassing consumables, annual calibrations, and maintenance, restricts its use in resource-limited settings where access to NAAT technologies might be inconsistent[4,7,8].

Recent developments in third-generation sequencing technology, such as Oxford Nanopore Technologies sequencing platforms, have shown several advantages over other diagnostic tools used for TB. Nanopore sequencing, a versatile third-generation sequencing technology, comes in various types such as MinION, GridION, and PromethION, each offering different capacities and throughput for genome analysis. In this context, nanopore sequencing is being applied in full-length transcript detection and base modification detection focused on rapid clinical diagnoses. MTB is an ideal candidate for analysis since nanopore sequencing excels in the investigation of complex genomic loci and large repetitive elements[8]. The ability of nanopore sequencing to better detect structural variations and enhance the resolution of repetitive regions, provides deeper understanding of *M. tuberculosis* virulence and pathogenesis. Furthermore, real-time analysis and portability of nanopore sequencing enable rapid detection of drug resistance mutations, facilitating prompt treatment decisions and potentially improving patient outcomes, particularly in resource-limited clinical settings dealing with acute and severe pulmonary infections[9]. In addition to its utility within healthcare facilities, nanopore's portability and rapid turnaround time enables field testing in epidemiological and infectious disease outbreak investigations[9]. Compared to other sequencing techniques such as Illumina, nanopore sequencing offers portability and real-time results due to its capacity to directly sequence nucleic acid molecules. Conversely, Illumina provides higher accuracy but with longer wait times and at higher costs, both in equipment and training of personnel. This favors nanopore sequencing in resource-limited settings such as in LMICs where tuberculosis is prevalent[10]. This technology depends on nanoscale protein pores working as biosensors of changes in ionic current as the DNA or RNA is translocated into each pore. Each DNA or RNA base has a fixed size that blocks current flow as the strand is translocated, registering a unique and interpretable alteration in current.

Nanopore sequencing technology continues to evolve to this day, leading to increased accuracy, speed, and a wider range of applications in various fields, including clinical diagnostics and metagenomics. In its early days, nanopore sequencing technology had an error rate as high as 15%.[10] However, recent advancements in pore design such as the ONT R10 have shown comparable results to Illumina short-read sequencing[11]. As the nanopore technology continues progressing, the need to investigate its utility becomes timelier in the face of modern-day healthcare challenges, particularly due to the increase of drug-resistance in TB, including focusing on newer WHO approved drugs that have just recently entered clinical use[12].

Despite the advancements in TB diagnostics, there still is a paucity of systematic reviews and meta-analyses evaluating the diagnostic accuracy of nanopore sequencing for drug susceptible and drug resistant (DR) MTB strains. Our study addresses this gap by providing a comprehensive synthesis of available data, thereby informing clinical practice and guiding future research. This study aims to appraise and synthesize current evidence on the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), diagnostic odds ratio (DOR) and area under the curve (AUC) of nanopore sequencing technology in detecting MTB and its drug-resistant patterns[13].

Methods

Study protocol and registration

The study protocol was submitted to The International Prospective Register of Systematic Reviews (PROSPERO) (ID: CRD42024518764) and reported in compliance with the PRISMA 2020 guidelines[14].

Search strategy

Literature searches were independently conducted by two different co-authors using the following databases: PubMed, Scopus, MEDLINE, Cochrane, EMBASE, Web of Science, AIM, IMEMR, IMSEAR, LILACS, WPRO, HERDIN Plus, MedRxiv, and BioRxiv. Separate comprehensive searches were done for studies about MTB detection and drug susceptibility. As the first nanopore sequencing platform, namely minION, was first commercialized in 2014, our search range was narrowed down to articles published from January 2014 until

February 2024[15]. Furthermore, aside from feasibility, the search was limited to English articles since restricting systematic review search to English-language has no to little impact on estimates and conclusions[16,17]. The selected reference list of each article was also screened to identify studies relevant to this review. The following search terms were used: "*Mycobacterium tuberculosis*" OR "*M. tuberculosis*" AND "nanopore sequencing" OR "nanopore-based sequencing" OR "Oxford nanopore" OR "nanopore technology" OR "Flongle" OR "minION" OR "gridION" OR "promethION". The combination of these search terms is detailed in the supplementary materials.

Study selection

All studies that compare nanopore sequencing technology to any conventional diagnostic test or reference standard in MTB detection and/or DST on clinical specimens were included except reviews, meta-analyses, commentary papers, case reports, conference summaries, animal experiments, and non-English literature. Two researchers independently screened and deduplicated the titles and abstracts and collated a list of points of disagreement. A third researcher then independently in a blinded manner resolved the disagreements by rechecking if the study design, bacteria of study, presence of comparator, and nature of samples (if human or animal, if directly sampled from humans or not) matched the inclusion criteria.

Data extraction

Basic diagnostic accuracy parameters such as true positive, false positive, true negative, and false negative were extracted using a 2×2 table. Other relevant data included the following: year, country, research type, sample size, type of nanopore sequencing technology, comparator group, reference standard, sample type, and average age of patients. Research type was determined using the classification algorithm by Mathes & Pieper[18]. Data extraction was carried out independently by two researchers.

Risk of bias assessment

The risk of bias in diagnostic accuracy was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool[19]. This tool contains signaling questions on four domains: patient selection, index test, reference standard, and flow and timing[19]. The risk of bias is evaluated in each domain, while concerns regarding applicability are assessed in the first three domains[19]. The risk of bias and concern on applicability in each domain are rated as high, low, or unclear[19]. Risk of bias assessment was carried out independently by two researchers.

Statistical analysis

Pooled sensitivity, specificity, NPV, PPV, and DOR were reported with 95% confidence interval (CI). Heterogeneity was quantified using the inconsistency value (I^2); where I^2>50% is considered as a remarkable heterogeneity, which warranted subgroup analysis or meta-regression to identify sources of heterogeneity. All pooled effect sizes were determined using a random effects model to account for both within- and between-studies variance. Receiver operating characteristic (ROC) curves were plotted to assess the overall accuracy of nanopore sequencing; there was a high diagnostic value if the AUC values exceeded 0.9. Sensitivity analyses were performed for meta-analyses with at least three studies to examine the robustness of results. Subgroup analyses were done given that potential subgroups have at least two studies. Both subgroup and sensitivity analyses were done based on the study design, nanopore sequencing platform, and reference standard. Deek's test for publication bias was not performed since there were < 10 eligible studies with computable diagnostic odds ratios[20]. Statistical significance was set at $p < 0.05$. Analyses were performed using the "*mada*" and "*meta*" packages of R statistical software vr. 4.3.3 and IBM SPSS vr. 26[21].

Results

Characteristics of included studies

Comprehensive search across multiple databases yielded a total of 1,073 records about MTB detection and 226 records about MTB drug susceptibility. The full search strategies were summarized in Supplementary Tables 1 and 2. Following a thorough screening process, 17 studies about MTB detection were included while 19 studies about MTB drug susceptibility were selected (Fig. 1).

To facilitate our analysis, studies were classified into two categories: those focusing on the detection of MTB (Table 1) and those focusing on the detection of DR-MTB (Table 2). Among the 32 articles, 13 articles related to MTB detection only[22–34],15 articles focused on DR-MTB detection only[34–49], while 4 articles examined both[50–53]. Among the four studies which analyzed both MTB detection and DST[50–53], only the study by Sun et al. was included in the meta-analyses on MTB detection because this is the only study which has results for diagnostic accuracy measures[51]. In the meta-analyses on DST, only the study by Nilgiriwala et al. was included among the four studies, since it has data on diagnostic accuracy measures for DST, specifically in detecting isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, amikacin, and moxifloxacin resistance[50].

Detection of MTB

Out of the 17 articles, nine were retrospective cross-sectional while eight were prospective cross-sectional. GridION was predominantly used in these studies (12 out of 17), followed by minION alone (4 out of 17), then the combination of both sequencing platforms (1 out of 17). No study used Flongle or PromethION. Included studies were published between 2017 and 2024 (Table 1).

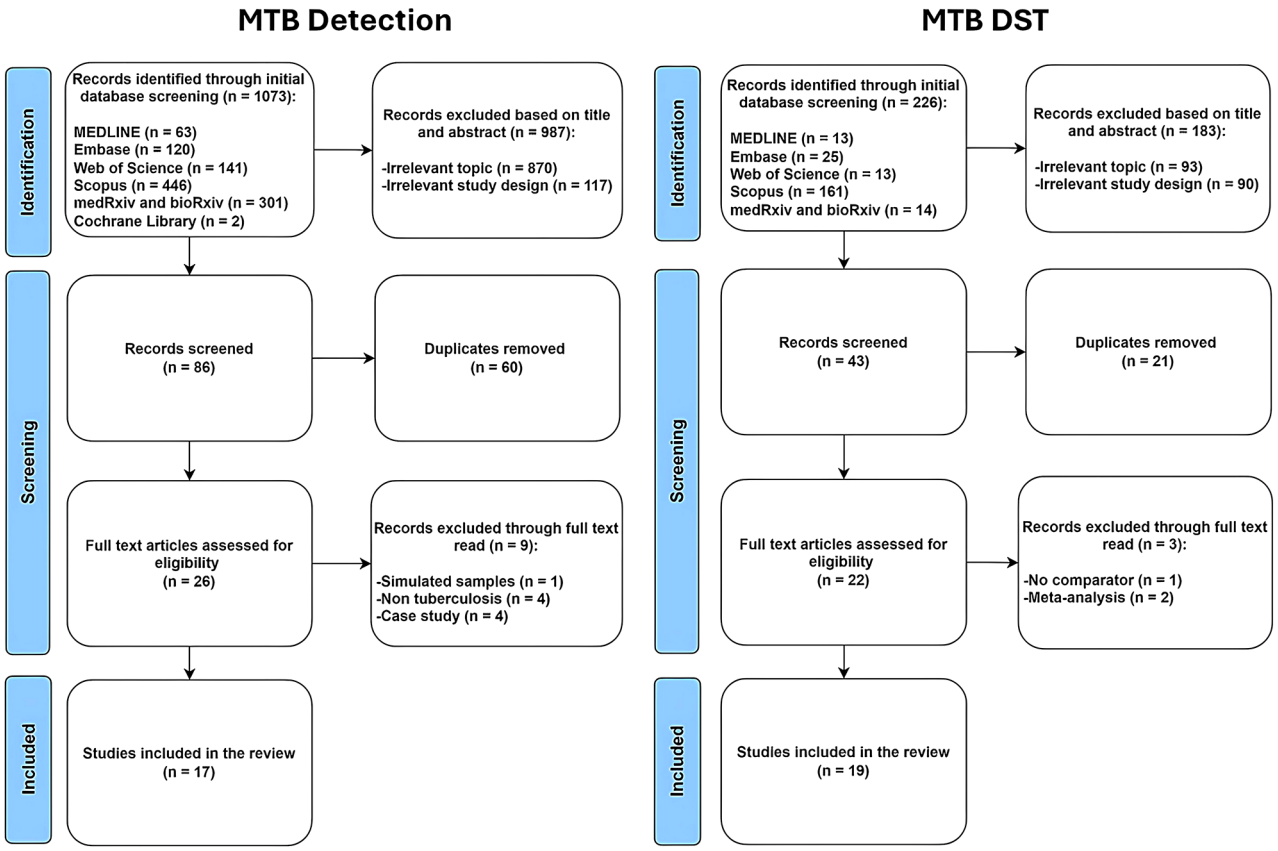

Fig. 1. PRISMA flow diagram of this study selection process.

Risk of bias and applicability assessment for detection of MTB

Separate assessments of the risk of bias and applicability concerns using the QUADAS-2 tool were performed for studies on MTB detection (Fig. 2) and drug susceptibility (Fig. 3). Mainly, patient selection is the top source of bias and applicability concerns. In the 17 MTB detection studies selected, only one explicitly described the sampling strategy, consisting of consecutive enrollment[51] (Table 1). Further, nine of the selected studies focused on general infections, lower respiratory tract infections, and pneumonia; thus, their MTB results derived from secondary analyses; hence, the heightened applicability concerns. Additionally, among the 17 MTB detection studies, nine adopted a retrospective cross-sectional design (Fig. 2), increasing the risk of bias concerning the evaluated index test, reference test, and the flow of timing in these studies.

Meta-analysis of MTB detection studies

Heterogeneity test. Our results showed that the logit-transformed sensitivity did not correlate with logit transformed false positive rate (1-specificity) (Spearman correlation −0.486 ($p = 0.356$)). Our analysis revealed no threshold effect among the included studies. Higgin's I^2 of the diagnostic measures ranged from 63 to 88%.

Evaluation of diagnostic accuracy. The meta-analysis for detecting MTB included seven studies, with 1,741 specimens from 1,709 adult participants. All of the included studies to diagnose MTB utilized the GridION platform. Our results indicate that the diagnostic sensitivity of nanopore sequencing in detecting MTB ranged from 75 to 95%, while its specificity varied between 81% and 100%. Nanopore sequencing had a pooled sensitivity of 88.61% (95% CI 83.81–92.12%, $I^2 = 63\%$), and a pooled specificity of 93.18% (95% CI 85.32–96.98%, $I^2 = 77\%$) (Fig. 4). Its PPV ranged from 81 to 100%, while its NPV varied between 67% and 99%. The pooled PPV value was 94.71% (95% CI 89.99–97.27%, $I^2 = 65\%$), while its pooled NPV was computed to be 84.33% (95% CI: 72.02–91.84%, $I^2 = 88\%$) (Fig. 4). Our results also indicated that AUC was excellent [AUC = 0.932 (SE = 0.03)], with a remarkable wide DOR range, where values varied from 13 to 1,311. Pooled DOR was determined to be 107.23 (95% CI 35.15–327.15), with moderate heterogeneity ($I^2 = 65\%$) (Fig. 4). The summary receiver operating characteristic (SROC) curve for nanopore sequencing in MTB detection is shown in Fig. 4. Youden's J index was calculated to be 0.82 (Fig. 4).

Detection of DR-MTB

Among 19 studies selected, 12 studies adopted a prospective cross-sectional design, while 7 used a retrospective cross-sectional approach (Table 2). MinION alone was predominantly used in these studies (14 out of 19), followed by GridION alone (3 out of 19), then the combination of both sequencing platforms (2 out of 19). These studies span publication years from 2017 to 2024. No study used Flongle or PromethION.

Author (Year)	Country	Research Type*	Type of Nanopore	Reference Standard	Comparator	Sample Size**	Sample Type	Age (years)
Votintseva et al. (2017)[52]	UK	Retrospective	MinION	Illumina WGS	Illumina WGS	67	BALF, sputum	-
Wang et al. (2020)[29]	China	Retrospective	MinION GridION	Culture^{G}	Culture^{G}	1,312 from 1,257 patients	BALF, sputum	-
Deng et al. (2020)[22]	China	Prospective	MinION	Culture^{G}	Culture^{G}	56	BALF, sputum, Clinical specimens[a]	61 ± 14
Guo et al. (2021)[23]	China	Retrospective	GridION	All microbial tests, pathological, clinical	Illumina WGS, Culture^{G}	133	Lung biopsy	54 ± 19
Zhang J et al. (2022)[33]	China	Prospective	GridION	Clinical diagnosis	Illumina mNGS, Culture^{G}	66	BALF	Md = 68 (IQR 58, 72)
Yu et al. (2022)[31]	China	Prospective	GridION	CSTB	Culture^{L}, Xpert MTB/RIF	164	BALF, sputum	51 ± 18
Zhang H et al. (2022)[53]	China	Prospective	GridION	Culture^{G}	Culture^{G}	146	BALF, sputum, Clinical specimens[b]	16–87
Fu et al. (2022)[23]	China	Retrospective	GridION	Culture^{G}	Culture^{G}, PCR and Sanger sequencing	472	BALF, Clinical specimens[c]	58 ± 17
Zhou et al. (2024)[34]	China	Prospective**	GridION	CSTB	Culture^{L}, Xpert MTB/RIF, RT-PCR	195	BALF	52 ± 33
Liu et al. (2023)[27]	China	Retrospective	GridION	CSTB	Culture^{L}, Xpert MTB/RIF	55	BALF, sputum	39 ± 16
Yang et al. (2023)[30]	China	Retrospective	GridION	CSTB	Culture^{L}, Xpert MTB/RIF	172	BALF, sputum, pleural fluid	45 ± 14
Sun et al. (2023)[51]	China	Prospective	GridION	Xpert MTB/RIF, Culture^{L}	Culture^{L}, Xpert MTB/RIF, DST	58	BALF	45 ± 19
Guo et al. (2023)[25]	China	Retrospective	GridION	All microbial tests, pathological, clinical	Culture^{G}, clinical judgment	450 from 418 patients	BALF, sputum	Md = 62 (IQR 52, 69)
Luo et al. (2023)[28]	China	Prospective**	MinION	Culture^{G}, qPCR	Culture^{G}, TB-DNA, Xpert MTB/RIF	70	BALF	53 ± 13
Nilgiriwala et al. (2023)[50]	India, MDG	Retrospective	MinION	Illumina	Illumina WGS	60	Sputum	-
Lin et al. (2023)[26]	China	Prospective	GridION	mNGS, NTS, Culture^{G}, pathological, clinical	Culture^{G}, mNGS	218	BALF	56 ± 15
Yu et al. (2024)[32]	China	Retrospective	GridION	CSTB	Culture^{L}	647	BALF, sputum	50 ± 19

Table 1. Characteristics of included studies on the detection of MTB. *All studies are cross sectional. **The number of specimens samples is equal to the number of participants unless stated otherwise. a = blood, urine, pleural fluid, peritoneal fluid, abscess. b = blood, puncture fluid, transbronchial lung biopsy. c = blood, urine, pleural fluid, ascitic fluid, cerebrospinal fluid, wound drainage. Culture^{G} = culture using 7H10 or another agar such as MacConkey, chocolate, or blood agar. Culture^{L} = culture using Mycobacteria Growth Indicator Tube (MGIT) or another liquid medium. *MDG* Madagascar, *BALF* Bronchoalveolar lavage fluid, *CSTB* Chinese national standard for diagnosing TB (official name: WS 288–2017); *mNGS* Metagenomic next generation sequencing, *TB-DNA* Tuberculosis DNA, *Culture* onfirms the presence of the organism without drug susceptibility profile, *pDST* phenotypic drug susceptibility testing, *qPCR* quantitative polymerase chain reaction, *NTS* Nanopore target sequencing. Age expressed as either mean ± standard deviation (SD) or median (Md) and Interquartile range (IQR). Numerical superscripts indicate reference numbers.

Risk of bias and applicability assessment for DR-MTB

Among the 19 DR-MTB studies, two studies followed STARD recommendations[54]; one study stated the use of consecutive sampling[51] and one study mentioned use of random sampling[42]. Further lack of clinical information about the samples contributed to the applicability concerns in drug resistance studies. Additionally, 6 out of the 19 DR-MTB studies employed a case-control design, amplifying the risk of bias related to the index test, reference test, and the sequencing of events in these investigations (Fig. 3).

Meta-analysis of studies on DR-MTB

Heterogeneity test. Our results showed that the logit-transformed sensitivity did not correlate with the logit-transformed false positive rate (1-specificity) across all drug resistance groups [Spearman coefficient range: -0.5 to 0.5 (*p-value range* = 0.5 to 1)]. Higgin's I^2 of the diagnostic measures ranged from 0 to 87%.

Evaluation of diagnostic accuracy. The meta-analysis on detecting DR-MTB comprised 917 adult participants from five studies, all of which utilized the MinION platform. Our results indicated that pooled sensitivity of nanopore sequencing was lower than its specificity in detecting any drug resistance; pooled sensitivity ranged from 77.99 to 93.66%, while the pooled specificity varied between 94.84% and 99.47% (Table 3). The pooled PPV ranged from 87.57 to 100% while the pooled NPV varied between 91.27% and 99.46% (Table 3).

Our results indicate that among first-line DST, accurate detection of pyrazinamide-resistance was most challenging in terms of sensitivity and PPV. Conversely, detection of rifampicin resistance was easiest for nanopore sequencing among first-line DST in terms of sensitivity and PPV. Pooled specificity of nanopore

Author (Year)	Country	Research Type*	Type of Nanopore	Reference Standard	Comparator	Sample Size	Sample Type	Age (years)
Votintseva et al. (2017)[52]	UK	Retrospective	MinION	pDST	Illumina WGS	67	BALF, sputum	-
Smith et al. (2020)[43]	USA	Prospective	MinION	pDST	Illumina, pDST	431	DNA extracts^	-
Cervantes et al. (2020)[36]	USA	Retrospective	MinION	Illumina WGS	Illumina WGS	6	Sputum, DNA extracts^	-
Chan et al. (2020)[37]	South Africa	Prospective	MinION	Illumina WGS	Illumina WGS	23	Sputum, bronchial aspirate, biopsy[a]	-
Tafess et al. (2020)[44]	Hongkong Ethiopia	Prospective	MinION	pDST	Illumina WGS	163	DNA extracts^	-
Cabibbe et al. (2020)[35]	Italy	Retrospective	MinION	Illumina WGS	Illumina WGS	104	DNA extracts^	-
Gliddon et al. (2021)[38]	United Kingdom	Retrospective	MinION	pDST	Illumina WGS, pDST	34	Sputum	Md = 36 (IQR, 31–42)
Mariner-Llicer et al. (2021)[41]	Spain	Prospective	MinION	Illumina WGS	Illumina WGS	15	Sputum, culture	-
Yu et al. (2021)[48]	Taiwan	Retrospective	MinION	pDST	-	50	DNA extracts^	-
Zhang H et al. (2022)[53]	China	Prospective	GridION	Culture[G]	Culture[G]	146	BALF, sputum, Clinical specimens[b]	16–87
Gómez-González et al. (2022)[39]	UK	Retrospective	MinION	pDST	Illumina WGS	10	DNA extracts^	-
Yu et al. (2022)[49]	Taiwan	Retrospective	MinION	pDST	-	50	DNA extracts^	-
Zhao et al. (2022)[45]	China	Prospective	MinION	Culture[G]	PCR with Sanger sequencing	20	Sputum	40 ± 11
Sun et al. (2023)[51]	China	Prospective	GridION	pDST	Culture, Xpert MTB/RIF, pDST	58	BALF	45 ± 19
Hall et al. (2023)[40]	MDG, South Africa, England	Prospective	MinION, GridION	pDST	Culture[G], Illumina WGS	208	DNA extracts^	-
Nilgiriwala et al. (2023)[50]	India, MDG	Prospective	MinION	Illumina WGS	Illumina WGS	60	Sputum	-
Murphy et al. (2023)[42]	USA	Prospective	MinION, GridION	pDST	RT-PCR, Illumina WGS	127	BALF, sputum, biopsy[a]	-
Zhao et al. (2024)[46]	Taiwan	Prospective	GridION	pDST	Culture[G]	297	BALF, sputum, clinical specimens[c]	55 ± 18
Liu et al. (2024)[47]	China	Prospective	GridION	pDST + Xpert MTB/RIF	-	184	BALF, sputum, hydrothorax, ascites, pus	Md = 48 (IQR, 37–57)

Table 2. Characteristics of included studies on detection of DR-MTB. *All studies are cross-sectional. ^DNA extracts refer to DNA extractions from MTB-positive clinical isolates with unspecified source. a = lymph node biopsy, lung biopsy. b = blood, puncture fluid, transbronchial lung biopsy. c = plasma, cerebrospinal fluids, abscess, serous cavity effusions, and urine. Culture[G] = culture using 7H10 or another agar such as MacConkey, chocolate, or blood agar; Culture[L] = culture using Mycobacteria Growth Indicator Tube (MGIT) or another liquid medium. *MDG* Madagascar, *BALF* Bronchoalveolar lavage fluid, *mNGS* Metagenomic next generation sequencing, *Culture* confirms the presence of the organism without drug susceptibility profile, *pDST* Phenotypic drug susceptibility testing, *RT-PCR* reverse-transcriptase polymerase chain reaction; *WGS* whole genome sequencing. Age expressed as either mean ± standard deviation (SD) or median (Md) and Interquartile range (IQR). Numerical superscripts indicate reference numbers.

sequencing was highest in detecting ethambutol-resistance and lowest in isoniazid resistance. Pooled NPV, on the other hand, was highest in detecting pyrazinamide-resistance and lowest in rifampicin-resistance. Meanwhile, among second-line DST, the pooled sensitivity and NPV of nanopore sequencing were the highest but PPV was the lowest in detecting kanamycin-resistance. The highest specificity was noted in detecting ofloxacin resistance. Detection of streptomycin-resistance appeared to be more challenging; pooled specificity and NPV were at its lowest for MTB resistant to this aminoglycoside. Interestingly, pooled sensitivity was lowest, but pooled PPV was the highest for moxifloxacin-resistance. Resistance to ethionamide[43], ciprofloxacin[40], capreomycin[40], and bedaquiline[47] were each investigated by only one study; diagnostic accuracy measures are shown in Table 4. However, only one bedaquiline-resistant sample was detected in the sole study it was mentioned, making calculation of diagnostic accuracy measures impossible. Furthermore, no study mentioned about pretomanid or linezolid resistance. Among these three drugs, nanopore sequencing had the lowest diagnostic accuracy measures in detecting ethionamide resistance.

The AUC indicated clinically acceptable performance across all DST studies, with values ranging from 0.85 to 0.990 (Table 5), observed for both first-line and second-line DST (Fig. 5)[55]. AUC was excellent in most of the DSTs investigated; it was lower than 0.9 only in detection of ethambutol and streptomycin resistance. The range of pooled DOR was remarkably wide; values varied from 106.94 to 8943.00 (Table 5). DOR was the lowest in detecting isoniazid and streptomycin resistance, and the highest in detecting pyrazinamide and moxifloxacin resistance.

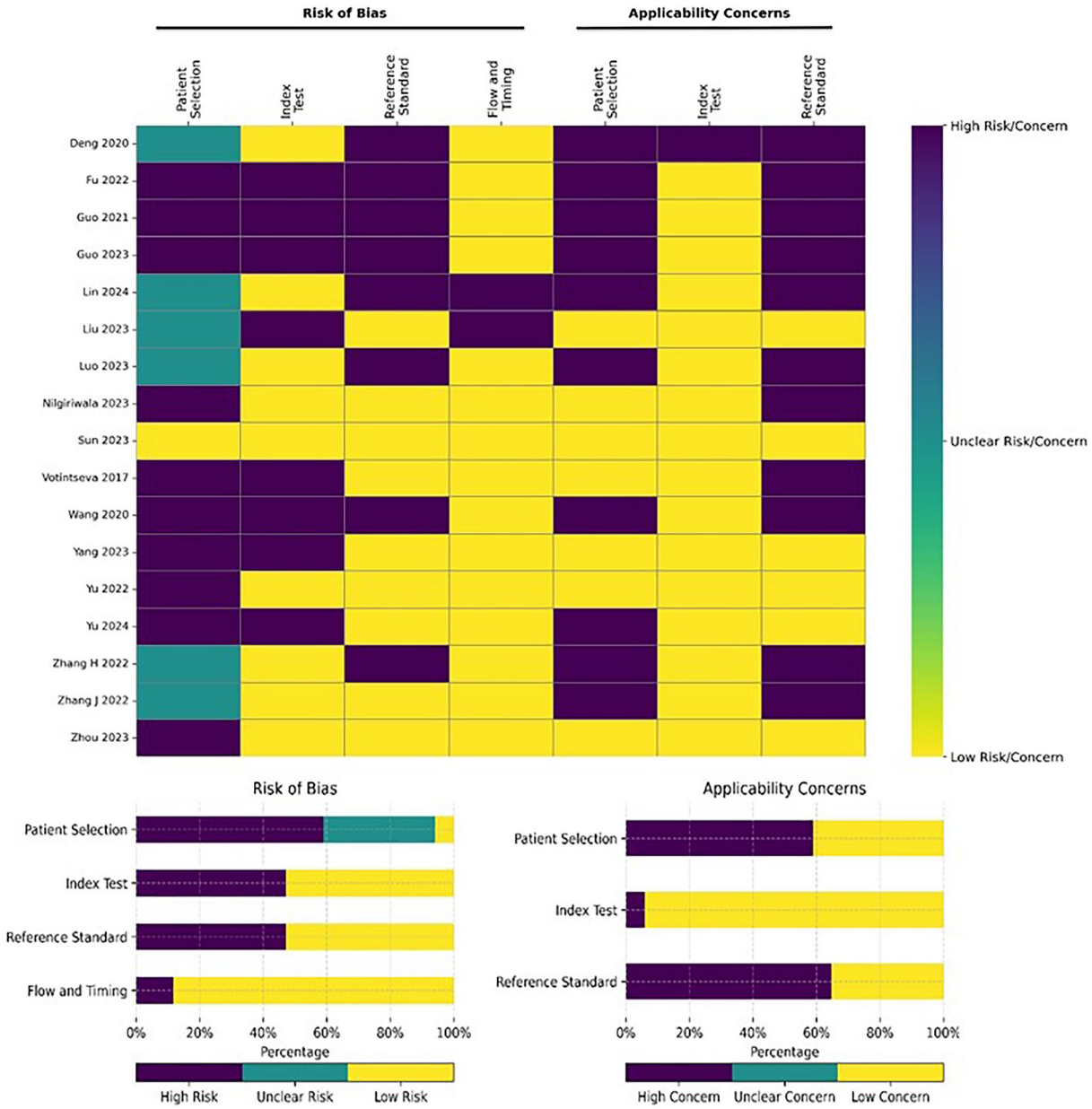

Fig. 2. Heat map showing risk of bias and applicability concerns among the selected 17 studies focusing on the detection of MTB.

Subgroup analyses

Subgroup analyses for accuracy in MTB detection were performed based on study design, nanopore sequencing platform, and reference standard. No subgroup analysis was done to investigate drug resistance because of the low number of studies and potential subgroups composed of only one study.

Retrospective studies are more heterogeneous in all diagnostic accuracy measures ($I^2 = 73–93\%$). Studies which used CSTB are more heterogeneous in more diagnostic accuracy measures, specifically in sensitivity, PPV, and DOR. Heterogeneity due to the type of nanopore sequencing cannot be analyzed; no study using MinION was included in the quantitative synthesis. Most of the subgroup analyses performed indicated no statistically significant subgroup effect ($p > 0.1$). For brevity, individual analyses were only shown for statistically significant subgroup effects (Table 6).

Subgroup Analysis: Effect of Study Design on Sensitivity and AUC for MTB detection. Study design significantly affects the sensitivity of nanopore sequencing in MTB detection, as shown by a statistically significant subgroup effect ($p = 0.08$). Prospective studies have higher sensitivity ($p = 0.08$) than retrospective ones (Table 6). However, since fewer studies and participants contributed data to the prospective subgroup (3 studies) than to the retrospective subgroup (4 studies), evidence is not enough to confidently conclude that there is a true subgroup effect. Furthermore, there is substantial unexplained heterogeneity between the studies within the retrospective subgroup (retrospective: $I^2 = 76\%$), making the validity of the sensitivity estimate for

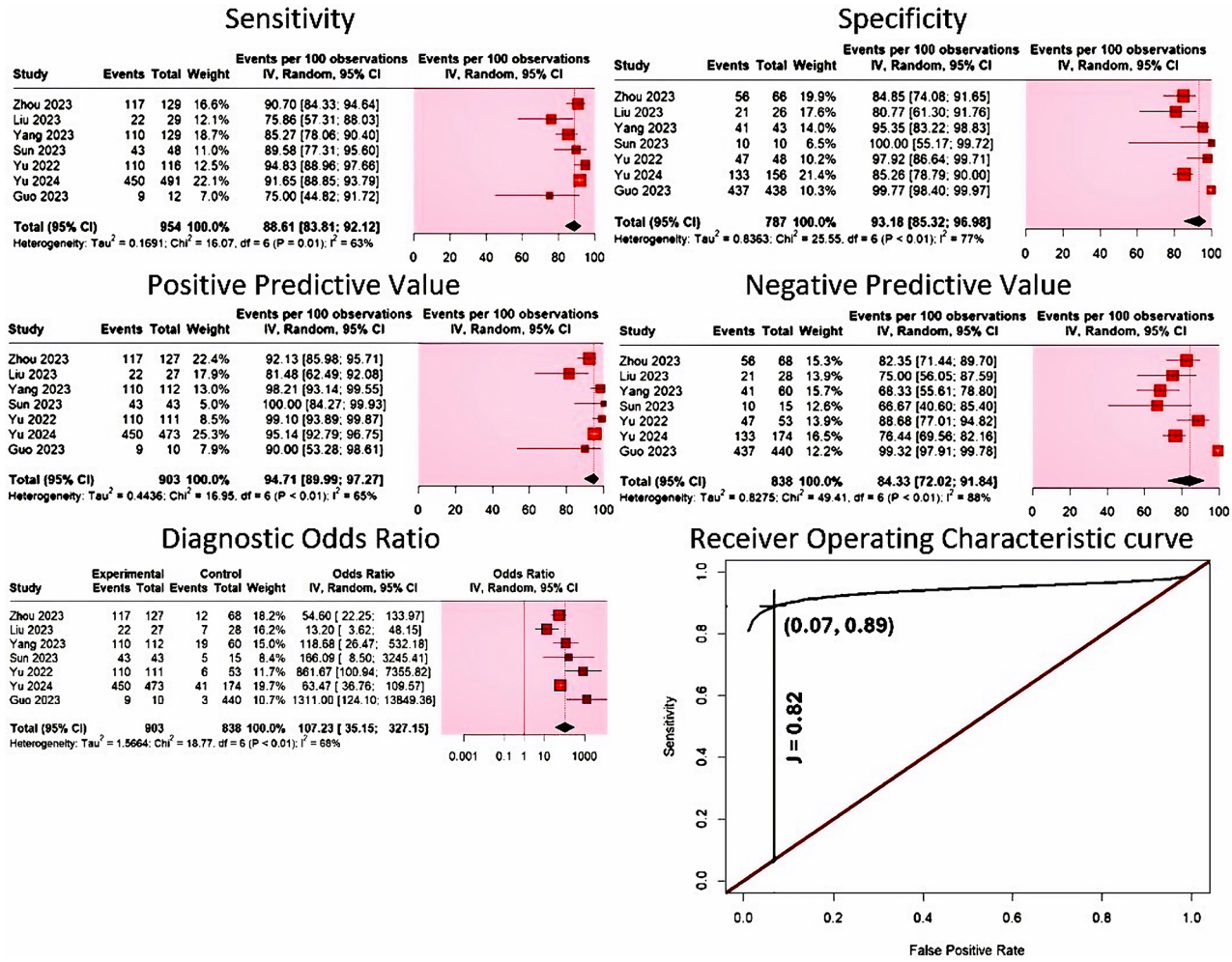

Fig. 3. Heat map showing risk of bias and applicability concerns among studies focusing on the detection of DR-MTB.

retrospective studies uncertain, as individual study results are inconsistent. The estimate for prospective study design is most likely valid because of the homogeneity of studies (prospective: $I^2 = 0\%$). The AUC in MTB detection is significantly higher in studies using prospective design (0.94 vs. 0.91, $p = 0.03$).

Subgroup Analysis: Effect of Reference Standard on Specificity for MTB detection. Reference standard significantly affects the specificity of nanopore sequencing in MTB detection, as shown by a statistically significant subgroup effect ($p = 0.07$). Studies which used a reference standard other than CSTB have higher specificity than those which used CSTB (Table 6). However, since fewer studies contributed data to the non-CSTB subgroup (2 studies), evidence is not enough to confidently conclude that there is a true subgroup effect. Furthermore, there is substantial unexplained heterogeneity between the trials within each of these subgroups (CSTB: $I^2 = 47\%$; non-CSTB: $I^2 = 66\%$). Therefore, the validity of the specificity estimate for each subgroup is uncertain, as individual study results are inconsistent.

Subgroup Analysis: Effect of Reference Standard on DOR and AUC for MTB detection. Reference standard significantly affects the DOR of nanopore sequencing in MTB detection, as shown by a statistically significant subgroup effect ($p = 0.07$). Studies which used a reference standard other than CSTB have higher DOR ($p = 0.07$) than those which used CSTB (Table 6). However, since fewer studies contributed data to the non-CSTB subgroup (2 studies), evidence is not enough to confidently conclude that there is a true subgroup effect. Furthermore, there is substantial unexplained heterogeneity between the studies within the CSTB subgroup (retrospective: $I^2 = 67\%$), making the validity of the DOR estimate for studies which used CSTB uncertain, as individual study results are inconsistent. The DOR estimate for studies which used a reference standard other than CSTB is most likely valid because of the unremarkable heterogeneity of studies (non-CSTB: $I^2 = 12\%$). The AUC in MTB detection is significantly higher in studies which used non-CSTB reference standard (0.94 vs. 0.90, $p = 0.08$).

Sensitivity analyses

Sensitivity analyses were performed based on study design, platform, and reference standard and were conducted to investigate the robustness of diagnostic accuracy measures for DR-MTB detection.

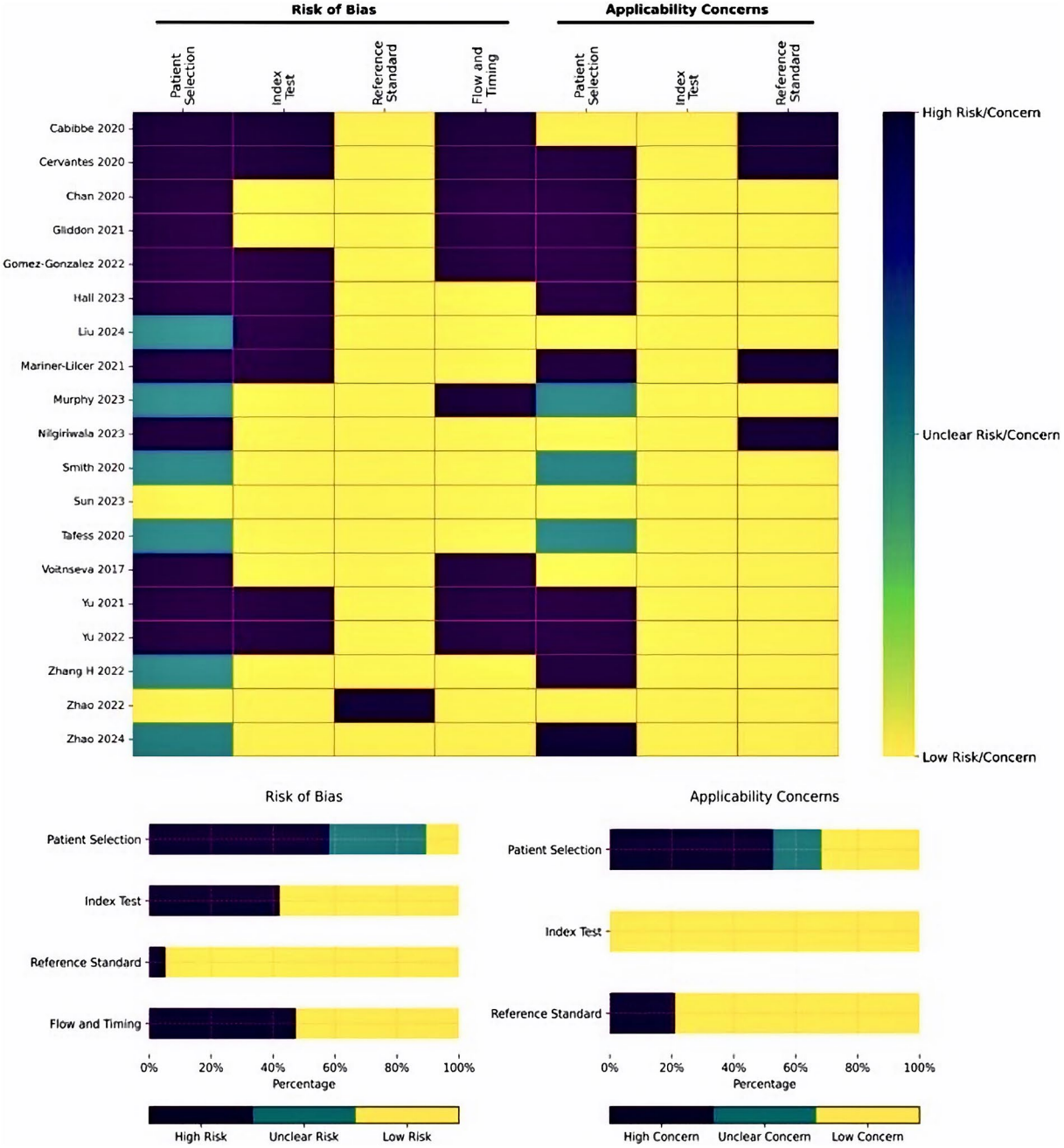

Fig. 4. Diagnostic accuracy measures of nanopore sequencing in MTB detection.

Sensitivity analyses for Isoniazid Resistance Detection. In the detection of isoniazid resistance (Table 7), the omission of a study that used retrospective design decreased the overall DOR and AUC; the retrospective study cited has higher sensitivity. On the contrary, omission of a study that used GridION increased the overall DOR and AUC; the study which used GridION has remarkably lower specificity and NPV. Likewise, exclusion of the study which used Illumina WGS increased the overall DOR and AUC; the study which used Illumina WGS as reference standard has remarkably lower sensitivity and PPV. The heterogeneity of specificity and NPV decreased upon elimination of the study which used GridION while the heterogeneity of sensitivity decreased after removal of the study which used Illumina WGS. The heterogeneity of NPV and DOR is stable despite the removal of the study which used Illumina WGS; the heterogeneity of PPV also remained the same after removal of the study which used GridION. For the rest of accuracy measures, the heterogeneity increased after removal of the study which used either GridION or Illumina WGS.

Sensitivity analyses for Rifampicin Resistance Detection. In the detection of rifampicin resistance (Table 8), the omission of a study using retrospective design increased the overall DOR and AUC; the retrospective study cited has remarkably lower NPV and smaller 95% confidence interval lower limit for specificity. On the other hand, omission of a study that used GridION or Illumina WGS increased only the overall DOR; AUC is unchanged.

Drug	Number of studies	Pooled sample size	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled PPV (95% CI)	Pooled NPV (95% CI)
INH	5[38,40,43,47,50]	605	90.67 (73.30; 97.18)	95.82 (82.57; 99.11)	95.14 (83.23; 98.72)	93.23 (77.44; 98.22)
RIF	5[38,40,43,47,50]	622	93.43 (77.42; 98.33)	97.82 (95.21; 99.02)	96.43 (76.75; 99.55)	92.35 (55.49; 99.15)
PZA	3[40,43,50]	423	77.99 (22.52; 97.74)	97.20 (93.64; 98.80)	89.40 (23.59; 99.57)	97.16 (88.29; 99.36)
EMB	4[40,43,47,50]	555	83.78 (46.76; 96.81)	98.22 (86.70; 99.79)	92.02 (63.12; 98.73)	97.06 (87.87; 99.34)
STR	4[40,43,47,50]	399	79.63 (59.76; 91.14)	94.84 (89.23; 97.60)	88.09 (74.85; 94.84)	91.27 (82.00; 96.00)
AMI	3[40,43,50]	237	83.71 (16.89; 99.24)	99.00 (96.09; 99.75)	89.50 (60.50; 97.94)	98.79 (95.30; 99.70)
KAN	2[40,43]	218	93.66 (65.69; 99.13)	98.92 (95.80; 99.73)	87.57 (60.88; 96.96)	99.46 (96.28; 99.92)
OFX	2[40,43]	222	80.54 (7.95; 99.50)	99.47 (96.37; 99.93)	93.45 (64.44; 99.12)	98.22 (83.83; 99.83)
MOX	2[40,50]	170	77.99 (3.78; 99.69)	99.02 (93.36; 99.86)	100.00 (66.44; 99.82)	98.32 (80.78; 99.88)

Table 3. Summary of pooled diagnostic indices for nanopore sequencing in the detection of DR-MTB. Legend: *INH* Isoniazid, *RIF* Rifampicin, *PZA* Pyrazinamide, *EMB* Ethambutol, *STR* Streptomycin, *AMI* Amikacin, *KAN* Kanamycin, *OFX* – Ofloxacin, *MOX* Moxifloxacin, *PPV* positive predictive value, *NPV* negative predictive value. Numerical superscripts indicate reference numbers.

Drug	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	DOR (95% CI)	AUC
ETH[43]	50.00 (31.67; 68.33)	97.30 (89.83; 99.32)	86.67 (59.46; 96.64)	84.71 (75.42; 90.91)	36.00 (7.26; 178.59)	0.99
CIP[40]	100.00 (66.44; 99.82)	100.00 (94.40; 99.98)	100.00 (66.44; 99.82)	100.00 (94.40; 99.98)	8943.00 (171.66; 465900.13)	0.99
CAP[40]	100.00 (61.61; 99.78)	100.00 (94.52; 99.98)	100.00 (61.61; 99.78)	100.00 (94.52; 99.98)	7479.00 (142.64; 392156.18)	0.99

Table 4. Summary of diagnostic indices of nanopore sequencing in detecting DR-MTB resistant to drugs mentioned in only one study[40,43]. Legend: *ETH* ethionamide, *CIP* ciprofloxacin,*CAP* capreomycin, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve.

Antibiotic	DOR (95% CI)	AUC
INH	265.30 (17.80; 3953.16)	0.91
RIF	386.97 (44.41; 3371.57)	0.98
PZA	806.33 (5.67; 114649.22)	0.98
EMB	744.07 (21.46; 25799.73)	0.85
STR	106.94 (12.47; 917.27)	0.85
AMI	625.53 (34.83; 11234.81)	0.99
KAN	916.31 (88.25; 9514.06)	0.99
OFX	815.34 (9.10; 73012.26)	0.99
MOX	8943.00 (171.66; 465900.13)	0.99

Table 5. Summary of pooled DOR and AUC for nanopore sequencing in the detection of DR-MTB.

The study which used GridION has higher PPV but remarkably lower NPV. Remarkably lower sensitivity is observed in the use of Illumina WGS. The heterogeneity of PPV and NPV decreased upon elimination of the study which used GridION while the heterogeneity of sensitivity and DOR decreased after removal of the study which used Illumina WGS. Specificity remained completely homogenous even after removal of the study which used retrospective design or GridION. For the rest of accuracy measures, the heterogeneity increased after removal of the study which used either GridION or Illumina WGS.

Sensitivity analyses for Ethambutol Resistance Detection. In the detection of ethambutol resistance (Table 9), the omission of a study that used GridION increased the overall DOR and AUC; the study which used GridION has remarkably lower specificity, PPV and NPV. Similarly, the omission of a study that used Illumina WGS increased the overall AUC; the study which used Illumina WGS has remarkably lower sensitivity and NPV. The analyses for PPV and DOR were not done due to absence of false positives. Sensitivity analyses based on study design were not performed due to the lack of retrospective study. The heterogeneity of specificity and PPV decreased after the elimination of the study which used GridION while that of sensitivity decreased upon removal of the study which used Illumina WGS. For the rest of accuracy measures, the heterogeneity increased after removal of the study which used either GridION or Illumina WGS.

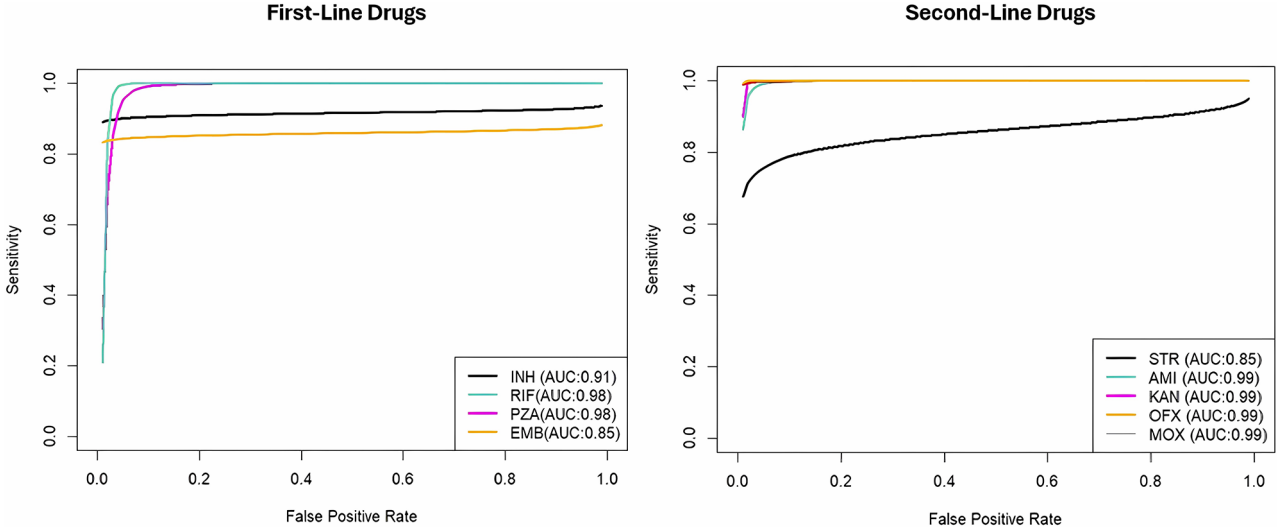

Fig. 5. Summary ROC curve of nanopore sequencing in detection of DR-MTB resistant to first-line drugs. *INH* isoniazid, *RIF* rifampicin, *PZA* pyrazinamide, *EMB* ethambutol; and second-line drugs. *STR* streptomycin, *AMI* amikacin, *KAN* kanamycin, *OFX* ofloxacin, *MOX* moxifloxacin, *CAP* capreomycin.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	7	88.61 (83.81–92.12); 63	93.18 (85.32–96.98); 77	94.71 (89.99–97.27);65	84.33 (72.02–91.84); 88	107.23 (35.15–327.15); 68	0.93
Design							
Prosp	3	91.84 (88.02–94.52); 0	92.97 (74.94–98.32); 56	97.29(86.55–99.50);67	81.76(69.82–89.67); 48	161.49 (26.88–970.30); 64	0.94
Retrosp	4	85.12 (75.37–91.45); 76	94.24 (80.40-98.49); 86	93.52(84.09–97.52);73	86.99 (66.43–95.76) 93	87.40 (16.88-452.66); 76	0.91
p-value		0.08	0.84	0.37	0.57	0.62	0.03
Platform							
GridION	7	88.61 (83.81–92.12)	93.18 (85.32–96.98)	94.71 (89.99–97.27)	84.33 (72.02–91.84)	107.23 (35.15–327.15)	0.93
MinION	0	N/A	N/A	N/A	N/A	N/A	N/A
p-value		N/A	N/A	N/A	N/A	N/A	N/A
Ref. Std.							
CSTB	5	89.27 (83.95–92.97); 70	87.71 (80.52, 92.50); 47	94.6(89.10, 97.43);74	77.93(70.98, 83.61);47	70.51 (24.64, 201.79); 67	0.90
Non-CSTB	2	84.94(67.24, 93.94); 40	99.14 (85.68, 99.95); 66	95.8(71.96, 99.51); 39	94.44(20.27, 99.91);97	573.67 (78.91-4170.68); 12	0.94
p-value		0.49	0.07	0.82	0.47	0.07	0.08

Table 6. Subgroup analyses of the diagnostic accuracy measures in MTB detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std.* reference standard, *CSTB* Chinese national standard for diagnosing TB (official name: WS 288-2017), *N/A* not available, cannot be calculated.

Sensitivity analyses for Streptomycin Resistance Detection. In the detection of streptomycin resistance (Table 10), the omission of a study that used GridION increased the overall DOR and AUC; the study which used GridION has remarkably lower values in all accuracy measures. Likewise, the exclusion of the study which used Illumina WGS increased the overall DOR and AUC; the study which used Illumina WGS as reference standard has remarkably lower sensitivity. Sensitivity analyses based on study design were not performed due to the lack of retrospective study. The heterogeneity of PPV did not change after the elimination of the study which used GridION. For the rest of accuracy measures, the heterogeneity increased after removal of the study which used either GridION or Illumina WGS.

Sensitivity analyses for Pyrazinamide Resistance Detection. In the detection of pyrazinamide resistance (Table 11), the omission of a study that used Illumina WGS increased the overall AUC; the study which used Illumina WGS has remarkably lower sensitivity and NPV. The analyses for PPV and DOR were not done due to absence of false positives. Sensitivity analyses based on study design and type of nanopore sequencing were not performed due to the lack of retrospective study. The heterogeneity of sensitivity barely changed; the heterogeneity of specificity increased while that of NPV decreased.

Sensitivity analyses for Amikacin Resistance Detection. In the detection of amikacin resistance (Table 12), the omission of a study that used Illumina WGS did not change the overall AUC and the heterogeneity of specificity

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	5	90.67 (73.30-97.18); 71	95.82 (82.57–99.11); 72	95.14(83.23–98.72);58	93.23(77.44–98.22); 82	265.30 (17.80; 3953.16);80	0.91
Design							
Prosp	4	88.43 (65.95-96.79); 76	96.61 (81.32–99.47); 79	94.34(77.14–98.80);65	93.71(75.79-98.61); 86	246.07 (8.59-7046.70); 84	0.90
Retrosp	1	98.08 (75.64-99.88)	90.00 (32.64–99.41)	98.08 (75.64–99.88)	90.00 (32.64-99.41)	459.00 (8.03-26240.33)	0.98
Platform							
MinION	4	91.91(54.38–99.08); 78	97.66 (90.08–99.48); 40	96.30(77.80;99.48);59	95.58(80.97–99.07);72	487.6 (16.71;14232.03);78	0.98
GridION	1	89.72 (82.38–94.22)	83.33 (70.97–91.09)	91.43 (84.34–95.48)	80.36 (67.91–88.78)	43.64 (16.89- 112.77)	0.93
Ref. Std.							
pDST	4	93.29 (84.97–97.15);	96.56 (78.05–99.55);78	96.77(89.06–99.10);49	95.20(76.13–99.19);	654.66(47.68- 8988.66); 81	0.92
Illumina WGS	1	44 25.00 (3.35–76.22)	93.33 (64.80- 99.07)	50.00 (5.89–94.11)	95.20(76.13–99.19); 82 82.35 (57.29–94.20)	4.67 (0.22–97.50)	0.74

Table 7. Sensitivity analyses of the diagnostic accuracy measures in isoniazid resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	5	93.43(77.42; 98.33); 63	97.82 (95.21; 99.02); 0	96.43(76.75;99.55);74	92.35(55.49; 99.15);87	386.97(44.41; 3371.57); 53	0.98
Design							
Prosp	4	92.49 (64.87; 98.80);72	98.15 (95.81; 99.20);0	95.96(65.21;99.67);79	95.11(54.45; 99.69);90	561.80 (38.10; 8283.61);61	0.99
Retrosp	1	96.30 (77.92; 99.48)	100.00 (19.36; 99.05)	100.00 (76.36; 99.89)	66.67 (15.35; 95.66)	88.33 (2.79; 2791.79)	0.96
Platform							
MinION	4	93.53(58.02; 99.36); 73	98.00 (95.44; 99.13); 0	93.52(65.45;99.10);66	96.47(73.93; 99.62);75	455.32(24.63; 8416.36); 64	0.98
GridION	1	93.53 (88.69; 96.38)	100.00 (49.53; 99.66)	100.00 (95.21; 99.98)	42.11 (22.63; 64.39)	235.78 (12.79; 4346.74)	0.98
Ref. Std.							
pDST	4	94.54 (90.63–96.87);1	97.73 (93.97, 99.16); 11	97.80(78.03,99.82);80	93.73(37.63, 99.73);90	757.84(93.66, 6131.65); 42	0.98
Illumin WGS	1	33.33 (4.34, 84.65)	100.00 (66.44, 99.82)	100.00 (10.89, 98.66)	88.89 (64.78, 97.21)	19.80 (0.62-633.78)	0.88

Table 8. Sensitivity analyses of the diagnostic accuracy measures in rifampicin resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	**4**	83.78 (46.76; 96.81);67	98.22 (86.70; 99.79);75	92.02(63.12;98.73);61	97.06(87.87; 99.34);74	744.07(21.46-25799.73);82	0.85
Design							
Prosp	4	83.78 (46.76; 96.81);67	98.22 (86.70; 99.79);75	92.02(63.12;98.73);61	97.06(87.87; 99.34);74	744.07(21.46-25799.73);82	0.85
Retrosp	0	N/A	N/A	N/A	N/A	N/A	N/A
Platform							
MinION	3	82.20(15.00; 99.18); 78	99.32 (96.70; 99.86); 0	97.03(73.93;99.73);32	98.41(85.03; 99.85);76	4330.8(294.4;63600.94);10	0.99
GridION	1	85.45 (73.52; 92.56)	89.42 (81.90; 94.05)	81.03 (68.91; 89.17)	92.08 (84.95; 95.99)	49.67 (18.72; 131.81)	0.94
Ref. Std.							
pDST	3	90.44 (67.71; 97.71);52	98.59 (79.98; 99.92);83	92.02(63.12;98.73);61	98.46(86.36; 99.85);81	744.07(21.46-25799.73);82	0.91
IlluminaWGS	1	0.00 (0.95; 80.64)	100.00 (67.79; 99.83)	N/A	89.47 (66.26; 97.35)	N/A	0.79

Table 9. Sensitivity analyses of the diagnostic accuracy measures in ethambutol resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	4	79.63(59.76; 91.14); 54	94.84 (89.23; 97.60); 29	88.09(74.85;94.84);38	91.27(82.00; 96.00);50	106.94 (12.47; 917.27); 68	0.85
Design							
Prosp	4	79.63 (59.76; 91.14)	94.84 (89.23; 97.60)	88.09 (74.85; 94.84)	91.27 (82.00; 96.00)	106.94 (12.47; 917.27)	0.85
Retrosp	0	N/A	N/A	N/A	N/A	N/A	N/A
Platform							
MinION	3	85.84(42.21; 98.05); 67	96.68 (88.27; 99.12); 38	91.82(73.13;97.88);38	95.07(76.60; 99.13);66	194.90 (7.90; 4809.18); 75	0.97
GridION	1	74.00 (60.21; 84.26)	92.73 (86.13; 96.32)	82.22 (68.29; 90.85)	88.70 (81.50; 93.32)	36.29 (13.93; 94.56)	0.92
Ref. Std.							
pDST	3	82.47 (61.42; 93.29);66	94.87 (87.67; 97.96); 49	89.40(74.16;96.12);57	91.11(79.13; 96.52);64	157.64 (8.26; 3007.17);78	0.97
IlluminaWGS	1	50.00 (5.89; 94.11)	100.00 (67.79; 99.83)	100.00 (10.89; 98.66)	94.44 (69.35; 99.22)	35.00 (0.95; 1292.43)	0.92

Table 10. Sensitivity analyses of the diagnostic accuracy measures in streptomycin resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	3	77.99 (22.52; 97.74);73	97.20 (93.64; 98.80);9	89.40(23.59;99.57);81	97.16 (88.29;99.36);65	806.33 (5.67;114649.22);83	0.98
Design							
Prosp	3	77.99 (22.52; 97.74);73	97.20 (93.64; 98.80);9	89.40(23.59;99.57);81	97.16 (88.29;99.36);65	806.33 (5.67;114649.22);83	0.98
Retrosp	0	N/A	N/A	N/A	N/A	N/A	N/A
Platform							
MinION	3	77.99 (22.52; 97.74);73	97.20 (93.64; 98.80);9	89.40(23.59;99.57);81	97.16 (88.29;99.36);65	806.33 (5.67;114649.22);83	0.98
GridION	0	N/A	N/A	N/A	N/A	N/A	N/A
Ref. Std.							
pDST	2	91.19 (37.96; 99.43);74	98.16 (88.49; 99.73);54	89.40(23.59;99.57);81	98.39 (94.42;99.55);23	806.33 (5.67;114649.22);83	0.99
IlluminaWGS	1	0.00 (0.95; 80.64)	100.00 (67.79; 99.83)	N/A	89.47 (66.26; 97.35)	N/A	0.79

Table 11. Sensitivity analyses of the diagnostic accuracy measures in pyrazinamide resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

Subgroups	*n*	Sn	Sp	PPV	NPV	DOR	AUC
Overall	3	83.71 (16.89; 99.24);63	99.00 (96.09; 99.75);0	89.50 (60.50; 97.94);0	98.79 (95.30; 99.70);0	625.53 (34.83;11234.81);30	0.99
Design							
Prosp	3	83.71 (16.89; 99.24);63	99.00 (96.09; 99.75);0	89.50 (60.50; 97.94);0	98.79 (95.30; 99.70);0	625.53 (34.83;11234.81);30	0.99
Retrosp	0	N/A	N/A	N/A	N/A	N/A	N/A
Platform							
MinION	3	83.71 (16.89; 99.24);63	99.00 (96.09; 99.75);0	89.50 (60.50; 97.94);0	98.79 (95.30; 99.70);0	625.53 (34.83; 11234.81); 30	0.99
GridION	0	N/A	N/A	N/A	N/A	N/A	N/A
Ref. Std.							
pDST	2	83.71 (16.89; 99.24);63	99.26 (96.42; 99.85);0	89.50 (60.50; 97.94);0	99.05 (95.43; 99.81);0	625.53 (34.83;11234.81);30	0.99
IlluminaWGS	1	N/A	100.00 (70.19; 99.85)	N/A	100.00 (70.19; 99.85)	N/A	0.79

Table 12. Sensitivity analyses of the diagnostic accuracy measures in amikacin resistance detection. Data presented as point estimate (95% CI) with inconsistency value (I^2). *n* number of studies, *Sn* sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *DOR* diagnostic odds ratio, *AUC* area under the curve, *Prosp* prospective, *Retrosp* retrospective, *Ref. Std*. reference standard, *pDST* phenotypic drug susceptibility testing, *WGS* whole genome sequencing.

and NPV. The analyses for sensitivity, PPV, and DOR were not done due to absence of false negatives and false positives. Sensitivity analyses based on study design and type of nanopore sequencing were not performed due to the lack of retrospective study.

Sensitivity analyses were not conducted for kanamycin, ofloxacin, and moxifloxacin resistance since only two studies investigate each antibiotic resistance.

Discussion

Most of the studies aimed at evaluating only MTB detection by nanopore sequencing used GridION. In fact, GridION was used in all the studies included in this meta-analysis. Our study shows that GridION has high sensitivity [88.61%; 95% CI (83.81–92.12%)] and specificity [93.18%; 95% CI (85.32–96.98%)] in detecting MTB, indicating that this technology is accurate enough when compared to reference genotypic or phenotypic methods[56]. Its high PPV [94.71%; 95% CI (89.99–97.27%)] and moderately high NPV [84.31%; 95% CI (72.02–91.84%)] suggest its practical usefulness in clinical practice[56]. GridION also has an excellent discriminatory performance, as reflected by its large DOR [107.23; 95% CI (35.15–327.15)], which far exceeded the minimum standard for a very good test, which is a DOR of 10. Its AUC (0.932) also exceeded 0.9, and its Youden index ($J = 0.82$) exceeded 0.50[57–60]. The high specificity of nanopore sequencing in detecting drug susceptible and DR-MTB strains indicate that it can be used as a confirmatory test. The accuracy and practical usefulness in clinical practice of nanopore sequencing can enable timely and targeted diagnosis and treatment, which is particularly crucial in high-burden settings where delays in diagnosis can exacerbate transmission and worsen patient outcomes[59,60].

Most of the studies investigating DST by nanopore sequencing, including those considered in our meta-analysis, used minION. Among first-line DST, nanopore sequencing performed best in detecting pyrazinamide and rifampicin resistance. Pyrazinamide had the highest DOR (806.33) and AUC (0.98) despite having the lowest sensitivity (77.99) and PPV (89.40). Although pyrazinamide had the highest DOR (806.33) and AUC (0.98) despite having the lowest sensitivity (77.99) and PPV (89.40), accurate detection of pyrazinamide-resistance is still challenging in culture due to drug sensitivity to pH variations and degradation in agar, thus, nanopore results need to be cautiously considered as it may not have added value in pyrazinamide DST testing[61]. Rifampicin also had the highest AUC, tied with pyrazinamide, due to its sensitivity (93.43) and PPV (96.43) which were highest among first-line drugs. Isoniazid resistance has been a challenge, with the lowest DOR (265.30) and second to the lowest AUC (0.91), primarily due to its specificity (95.82), which was lowest. Similarly, ethambutol had the lowest AUC (0.85) despite having the highest specificity (98.22).

On the other hand, among second-line DST, nanopore sequencing excelled in detecting resistance to most second-line drugs, especially moxifloxacin. Moxifloxacin resistance had the highest DOR (8943.00), AUC (0.99) and PPV (100) despite having the lowest sensitivity (77.99). Notably, AUC was at its highest value (0.99) for all second-line drugs, except streptomycin. Streptomycin resistance detection had the lowest performance in DOR (106.94) and AUC (0.85) mainly due to its specificity (94.84) and NPV (91.27), which were also lowest. This may be attributed to still unknown resistance mechanisms, a finding consistent with previously reported conflicts with DST[43]. Furthermore, streptomycin resistance involves several genes (e.g., *rpsL*, *rrs*); the presence of multiple potential sites for mutations makes comprehensive detection more challenging. While streptomycin is a valuable option for the treatment of DR-TB, its usage is in decline due to the expanded acquisition and maintenance of resistant MTB isolates[60].

Mathematically, DOR is directly related to AUC; the higher the DOR, the higher the AUC[62]. However, it has been shown that AUC is maximized when the study diagnostic odds ratios are homogeneous[58]. This explains why even though among first-line drugs, the DOR of ethambutol resistance detection is second to the highest [DOR 744.07; 95% CI (21.46; 25799.73)], it has the lowest AUC (0.85). Furthermore, the DOR of isoniazid is lowest [DOR 265.30; 95% CI (17.80; 3953.16)] but it has the second highest AUC (0.91). Indeed, the precision and not only the point estimate of DOR affects AUC. It is also notable that the heterogeneity of most diagnostic measures is remarkable ($I^2 > 50\%$); however, this is common with meta-analyses of diagnostic accuracy research[63].

Subgroup analyses indicate that estimates of PPV and NPV for MTB detection are robust despite different study designs, nanopore sequencing platforms, and reference standards. Surprisingly, prospective studies have higher sensitivity than retrospective ones since overestimation of accuracy measures are typically observed in retrospective studies. However, underestimation could also occur in retrospective studies because of the lack of consistent reference standard; routine clinical practice dictates that patients do not receive the reference standard. Instead, the clinical diagnosis within a specified period of time from the index test being conducted is used as the reference standard for retrospective studies. Disease could not be present at the time of test, but is subsequently diagnosed within specified interval; the study mislabels this true negative as false negative, which results in underestimation of accuracy. Another scenario is that the test identifies the disease but is diagnosed outside the specified interval; the study miscategorized this true positive result as a false positive one, leading also to underestimation of accuracy[64]. Decreased specificity, DOR, and AUC may indicate that using CSTB as reference standard may increase false positives due to a broad definition of clinical diagnosis of TB, which includes both confirmed and probable TB cases[29–32,34].

Sensitivity analyses indicate that DOR and AUC estimates for drug resistant MTB detection are not robust to changes in study design, nanopore sequencing platform, and reference standard. In almost all instances, either the DOR or AUC increased after elimination of a study which used retrospective design, GridION, or Illumina WGS, implying that these characteristics decrease overall accuracy in detecting any drug-resistant MTB. The decrease in these global accuracy measures can be attributed to distinctively lower basic accuracy measures; the study which used GridION generally has lower specificity and NPV while the study which used Illumina WGS typically has lower sensitivity in detecting any drug-resistant MTB. In most instances, heterogeneity increased after removal of a study which used retrospective design, GridION, or Illumina WGS. However, in cases where

heterogeneity decreased after the removal of a study, a similar trend with changes in accuracy measures was observed. The removal of a study which used GridION generally lowered heterogeneity in specificity and NPV while the elimination of a study which used Illumina WGS typically decreased the heterogeneity in sensitivity in detecting any drug-resistant MTB.

Sensitivity analyses also show that removal of a study does not necessarily result in similar changes in both measures. Though mathematically, DOR is directly related to AUC, varying prevalence in each study setting could affect these global accuracy measures. AUC is prevalence-independent while diagnostic odds ratio sometimes shows spikes in increase at low prevalence[65].

Aside from its accuracy, the advantages of using nanopore sequencing is its rapid turnaround time and relatively lower operating cost in detecting drug susceptibility to tuberculosis. In two studies, the turnaround time of nanopore sequencing, specifically minION, is 1 day shorter than that of Illumina[43,44]. The shorter turnaround time of nanopore sequencing is more suitable for clinical decisions requiring rapid diagnostics, while Illumina's longer processing time is more appropriate for non-urgent applications. Furthermore, the per sample sequencing cost of MinION was 50% lower ($63 for minION versus $130 for Illumina MiSeq); Illumina MiSeq also requires more expenses for reagents and maintenance[43]. The lower sequencing cost and expenses for maintenance and reagents make nanopore sequencing more accessible for smaller-scale and decentralized applications, particularly for resource-constrained environments and point-of-care diagnostic settings, whereas Illumina is better suited for high-throughput, centralized laboratories. In contrast, one of the primary weaknesses of nanopore sequencing technology in its early use, is its suboptimal accuracy[66] due to a high error rate (20–35%)[69]. However, the change of assay chemistry from R7 to R9 by using an *Escherichia coli* pore protein CsgG, the shift from HMM-based base-caller to neural network-based base-caller, the refinement of statistical techniques to reduce structural noise, and the use of post-sequencing correction tools greatly improved the performance of nanopore sequencing technology, which is partly evidenced by the results of our study[67,68]. On the other hand, Illumina sequencing maintains an error rate of < 1%, making it the gold standard for applications requiring high precision, such as detecting specific mutations. Illumina sequencing is the preferred choice for applications requiring high accuracy, while nanopore sequencing is acceptable for diagnostics where speed and cost are prioritized over absolute precision. Indeed, nanopore sequencing has a great potential for impact on tuberculosis diagnostics but limited data are available on its application in the tuberculosis field [69].

The maximum run time of both MinION and GridION is 72 h but GridION has five times the available channels and theoretical 1D maximum yield as MinION (Hall et al., 2020). The meta-analyses in this study suggest that GridION is used more in general MTB detection while MinION is used more in detecting resistance to specific drugs. Samples submitted for drug resistance detection have already been narrowed down, making MinION an economically sound choice for drug resistance detection[70].

This study has several limitations, all stemming from the small number of studies included in the actual meta-analysis. Accuracy measures reflected in the subgroup analyses should be interpreted with caution; most of the subgroup analyses performed indicated no statistically significant subgroup effect ($p > 0.1$), with uneven distribution and small number of studies, and substantial heterogeneity within each subgroup, rendering these analyses unlikely to produce findings with low validity[71]. Furthermore, the extent of the sensitivity analyses is limited since the possible effects of study design on accuracy measures and their respective heterogeneity levels were not fully assessed. Sensitivity analyses were also not conducted for kanamycin, ofloxacin, and moxifloxacin resistance since only two studies investigated each antibiotic resistance. Publication bias was also not investigated due to the same reason the limited number of studies (i.e., less than 10 studies)[72]. The low statistical power due to the small number of studies (7 studies for MTB detection and 5 for DST), in addition to the above mentioned limitations, impacts generalizability; additional studies will be needed to provide more reliable estimates of the diagnostic accuracy of nanopore sequencing.

The lower 95% Cl estimates of DOR in detecting resistance to pyrazinamide (5.67), streptomycin (7.90), and ofloxacin (9.10) were found to be < 10, which is the minimum DOR for a very good test. Even though this imprecision may raise concerns about the reliability of nanopore sequencing in detecting MTB isolates resistant to these three drugs, the use of nanopore sequencing for MTB DST is still emerging. More studies will also provide more reliable estimates of the diagnostic accuracy of nanopore sequencing in detecting resistance to ethionamide, ciprofloxacin, and capreomycin. In this context, nanopore sequencing, similar to other sequencing platforms, fares relatively poorly in detecting ethionamide resistance, which could be explained by the presence of unknown resistance mechanisms for ethionamide, which is also one of the primary reasons for overall conflicts with DST[43]. Furthermore, diagnostic accuracy studies on bedaquiline, pretomanid, and linezolid should be of top priority since statistical studies on these drugs are lacking. To address this research gap, future research should prioritize large-scale genomic studies to identify and validate resistance-conferring mutations for these drugs. Research should also focus on improving nanopore sequencing accuracy for detecting low-frequency mutations and analyzing complex resistance mechanisms.

Real-world settings pose limitations in diagnostic performance. The quality of results obtained from genetic analysis can be significantly affected by the specific software tools used for processing raw sequencing data, such as those for base-calling, alignment, and variant calling, because different tools have varying levels of accuracy and computational demands. These differences then can lead to potentially inconsistent diagnostic findings across different studies using different tool combinations. Future research may work on standardizing bioinformatics pipelines and exploring the impact of different sample types on sequencing accuracy to enhance the reliability of nanopore sequencing as a diagnostic tool for MTB detection[66]. Future research may also need to focus on longitudinal studies to assess the impact of nanopore sequencing on treatment outcomes and transmission dynamics. Additionally, studies exploring the integration of nanopore sequencing into existing diagnostic workflows and its cost-effectiveness in various healthcare settings could be valuable[55,66]. Ideally, the high specificity of nanopore in both MTB detection and DST makes it a good fit as a confirmatory test in

diagnostic algorithms for tuberculosis, though in low-resource settings, it can be used as a first-line test for both instances. Addressing these challenges will improve the consistency and applicability of diagnostic performance assessments across diverse clinical settings.

Conclusion

Nanopore sequencing provides a comparable advantage over both metagenomic next-generation sequencing (mNGS) and traditional methods in diagnosing MTB, with high sensitivity [88.61% (95% CI 83.81–92.12%)] and high specificity [93.18% (95% CI: 85.32–96.98%)]. As the included studies in diagnosing MTB utilized GridION, the platform demonstrated high sensitivity and specificity, strong positive predictive value (PPV), moderately high negative predictive value (NPV), and excellent diagnostic odds ratio (DOR) and area under the curve (AUC) in detecting MTB.

This meta-analysis also demonstrates that nanopore sequencing achieves a pooled sensitivity ranging from 77.99 to 93.66% and pooled specificity between 94.84% and 99.47% in diagnosing drug-resistant (DR)-MTB, depending on the specific drug tested. All included studies in diagnosing DR-MTB utilized the MinION platform. Findings reveal that MinION excels in detecting pyrazinamide and rifampicin resistance and underperforms in detecting isoniazid and ethambutol resistance when compared to current phenotypic DST methods, though nanopore results in detecting pyrazinamide-resistance need to be cautiously considered since accurate detection of pyrazinamide-resistance is still challenging in culture. Furthermore, additional studies are required to provide more precise estimates for MinION's sensitivity in detecting drug-resistance and DOR in detecting resistance to pyrazinamide, streptomycin, and ofloxacin.

Accuracy estimates for general and DR-MTB detection are not robust to changes in study design, nanopore sequencing platform, and reference standard. Subgroup analyses generally suggest that overall accuracy of MTB detection tends to be higher with prospective study design and use of reference standards other than CSTB. Conversely, sensitivity analyses reveal that in most cases, retrospective study design, use of GridION, and use of Illumina WGS as reference standard decrease overall accuracy in detecting any drug-resistant MTB. Findings from both types of analyses, however, should be interpreted with caution because of the low number of studies and uneven distribution of studies in each subgroup.

Overall, nanopore's portability and low cost compared to phenotypic DST may still provide a promising solution for remote areas. The decision to use which sequencing modality will ultimately depend on many factors, including the context in which it will be applied and the available resources. Furthermore, studies on detecting resistance to current drugs to treat DR-MTB such as the WHO's approved BPaL treatment (bedaquiline, pretomanid, and linezolid) are still lacking despite the emergence of drug-resistant strains to these newer drugs. Future studies will require the development of new strategies to address this gap, including isolating and identifying BPaL resistant strains and identifying the gene mutations driving this resistance, which will allow the development of PCR-based true POC diagnostic tools for their quick identification in the future.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

All authors meet the journal's criteria for authorship. THDCC did conceptualization, visualization (all figures and tables), methodology, statistical analysis, validation, data curation, writing-original draft, writing-review and editing. DJC did visualization (Tables 1 and 2), methodology, validation, data curation, writing-original draft, writing-review and editing. GSC did visualization (Tables 1 and 2) methodology, validation, data curation, writing-original draft, writing-review and editing. JDP did visualization (Figs. 2 and 4), methodology, validation, data curation, writing-review and editing. JIG, BIR, MY, JBT did writing-review and editing. KIN did conceptualization, visualization (Figs. 2, 3 and 4), methodology, validation, data curation, writing-review and editing.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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