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Supplementary appendix 1

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Evaluating twelve automated *Mycobacterium tuberculosis* complex whole genome sequencing analysis pipelines: a comparative study

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Supplementary appendix

19	Table of Contents	
20	Supplementary methods	4
21	Supplementary results	8
22	Supplementary tables	9
23	Table S1: Drugs for which genotypic resistance prediction was performed for 11 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines	9
24		
25	Table S2: M.tuberculosis whole genome sequencing analysis pipelines excluded from evaluation in this study with reason for exclusion.....	10
26		
27	Table S3: Sequencing platform compatibility and methods applied to lineage classification, resistance prediction and genomic relatedness inference by twelve automated M.tuberculosis whole genome sequencing analysis pipelines	11
28		
29		
30	Table S4: Additional features of automated M.tuberculosis whole genome sequencing analysis pipelines	13
31		
32	Table S5: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for 12 antituberculosis drugs for 7 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines using the 1,000 Illumina sequences dataset.	14
33		
34		
35	Table S6: Summarised phenotypic drug susceptibility results for isolates included in 100 Illumina sequences and 1,000 Illumina sequences datasets	15
36		
37	Table S7: Pooled sensitivity and specificity of genotypic drug susceptibility testing for 6 M.tuberculosis whole genome sequencing analysis pipelines using the 1,000 Illumina sequences dataset. Included drugs are rifampicin, isoniazid, ethambutol, moxifloxacin, levofloxacin, amikacin, kanamycin, ethionamide, delamanid and linezolid.	15
38		
39		
40		
41	Table S8: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for 8 antituberculosis drugs for 11 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines using the 100 Illumina sequences dataset.	16
42		
43		
44	Table S9: False negative rates and false positive rates (%) of genotypic drug susceptibility testing for bedaquiline, clofazimine, delamanid and linezolid for 10 M.tuberculosis whole genome sequencing analysis pipelines using the 100 Illumina sequences dataset	17
45		
46		
47	Table S10: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for rifampicin, isoniazid, ethambutol and streptomycin for 4 Nanopore-compatible and 3 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines using the 90 sequences dataset	18
48		
49		
50		
51	Table S11: Summarised phenotypic drug susceptibility results for isolates included in the 90 Nanopore- and Illumina sequences dataset	19
52		
53	Table S12: Distribution of main lineages, as determined using TBProfiler, of isolates included in test datasets.....	19
54		
55	Supplementary figures	20
56	Figure S1: Proportion of all genotypic drug susceptibility predictions that were errors for 7 M.tuberculosis whole genome sequencing analysis pipelines, stratified by error type, using the 1,000 Illumina sequences dataset.	20
57		
58		

59	Figure S2: False negative predictions for 7 M.tuberculosis whole genome sequencing	
60	analysis pipelines, stratified by error type, using the 1,000 Illumina sequences dataset.	
61	*Excludes isolates for which all pipeline predictions were discordant with phenotype	21
62	Figure S3: False positive predictions for 7 M.tuberculosis whole genome sequencing analysis	
63	pipelines, stratified by WHO mutation catalogue grade and presence of heteroresistance,	
64	using the 1,000 Illumina sequences dataset. *Excludes isolates for which all pipeline	
65	predictions were discordant with phenotype. WHO catalogue, 2 nd ed.....	22
66	Figure S4: Proportion of all genotypic drug susceptibility predictions that were errors for 11	
67	M.tuberculosis whole genome sequencing analysis pipelines, stratified by error type, using	
68	the 100 Illumina sequences dataset.	23
69	Figure S5: False negative rates (above) and false positives rates (below) of genotypic drug	
70	susceptibility testing for rifampicin, isoniazid, ethambutol and streptomycin for 4 Nanopore-	
71	compatible and 3 Illumina-compatible M.tuberculosis whole genome sequencing analysis	
72	pipelines using the 90 sequences dataset. Values above bars denote absolute number of	
73	false negative (above) and false positive (below) predictions.	24
74	Figure S6: Proportion of all genotypic drug susceptibility predictions that were errors for 4	
75	M.tuberculosis whole genome sequencing analysis pipelines, stratified by error type, using	
76	the 90 Nanopore (left) and Illumina (right) sequences dataset.	25
77	Figure S7: Time, in hours, taken by GPAS, MAGMA, MTBseq, Mykrobe, TBProfiler, TBSeqPipe	
78	and tbAMR to process 1,000 M.tuberculosis whole genome sequences.	26
79	References	27
80		
81		

Supplementary methods

Pipeline search

The PubMed search was first performed on 04/03/2024 using the search terms presented in Box 1. The search was most recently repeated on 31/08/2024. GitHub was searched on the same dates using the terms “mycobacterium tuberculosis”. We reviewed both included and excluded pipelines for updates on 31/11/2024.

Search	Query	Results
#1	“Mycobacterium tuberculosis” [Mesh] OR “Mycobacterium tuberculosis” [tiab]	78,199
#2	“Whole Genome Sequencing” [Mesh] OR “Whole genome sequencing” [tiab]	43,370
#3	“Software” [Mesh] OR “software” [tiab]	402,056
#4	#1 AND #2 AND #3	51

Box 1: Search terms and number of results returned for most recent PubMed search.

Illumina-sequenced isolates test dataset curation

The Illumina test datasets were curated using isolates obtained from the CRyPTIC data compendium, a dataset including 12,289 *M.tuberculosis* genomes and pDST results for 13 anti-TB drugs¹. Specifically, “[CRyPTIC_reuse_table_20231208.csv](https://ftp.ebi.ac.uk/pub/databases/cryptic/release_june2022/reuse/)”, available at https://ftp.ebi.ac.uk/pub/databases/cryptic/release_june2022/reuse/ was used to identify isolates. In the CRyPTIC study, whole genome sequencing was performed on 14-day subcultures from clinical isolates, either using Lowenstein-Jensen tubes, 7H10 agar plates or Mycobacteria Growth Indicator Tubes (MGIT).¹ Phenotypic drug-susceptibility testing (pDST) was performed using UKMY5/6 96-well broth microdilution plates, onto which 14-day-old subcultures of clinical samples were inoculated.¹ To minimise potential phenotypic error, we only included isolates designated high-quality phenotypes in the CRyPTIC dataset.¹ We enriched the 1,000 Illumina-sequenced isolate test dataset for drug-resistant isolates by first including all available isolates with phenotypic resistance to bedaquiline (n=4), delamanid (n=13), linezolid (n=16) or clofazimine (n=16), drugs with the fewest number resistant isolates in the CRyPTIC data compendium.¹ We then randomly selected rifampicin-susceptible and resistant isolates to construct a final dataset where approximately 50% of isolates were rifampicin resistant. The 100 Illumina-sequenced isolates dataset also included all available isolates with phenotypic resistance to bedaquiline, clofazimine, delamanid and linezolid and included a similar proportion of isolates with rifampicin susceptibility and resistance. All sequences analysed in this study were downloaded with fastq-dl (v2.0.4; <https://github.com/rpetit3/fastq-dl>).

Processing time

We measured the wall-clock time required by each pipeline to process 1,000 Illumina-sequenced isolates using the ‘time’ command on the Linux command-line-interface. All pipelines were executed separately, within the same computational environment: a virtual machine running Ubuntu 20.04.6 LTS, configured with 32 CPU and 62 GB RAM. To optimise performance, we utilised the maximum possible level of multi-threading for each pipeline, leveraging all available CPU cores when supported. Additionally, the most comprehensive versions of the pipelines were executed, meaning all available modules were selected for analysis.

Resistance prediction for Nanopore-compatible pipelines

We curated a third test dataset of 90 isolates sequenced using both Nanopore and Illumina technologies, previously compiled by Hall et al.² These had pDST results for rifampicin, isoniazid, ethambutol and streptomycin. Data were processed using three pipelines compatible with both Illumina and Nanopore sequences (GPAS, Mykrobe and TBProfiler), and one Nanopore-specific pipeline (tbpore). For GPAS, this analysis was conducted using an unreleased beta version of the Nanopore pipeline which has subsequently been made publicly available. Pipelines were executed with default settings where applicable. Pipeline- and platform-specific gDST false negative rates (FNRs) and false positive rates (FPRs) were calculated using the reported binary pDST results as the reference standard. 95% confidence intervals (CIs) for these estimates were calculated using Wilson score intervals with the *binom* package in R.³

Discordance adjudication

Errors in gDST in *M.tuberculosis* WGS analysis pipelines may result from variant identification errors or from the reference catalogues used for variant annotation. To determine the most likely sources of gDST errors, we reviewed the variant call format (VCF) files for pipelines that generate these outputs (GPAS, MAGMA, MTBseq, TBProfiler, TBSeqPipe and tbAMR) and the resistance mutation catalogue for Mykrobe which does not generate VCF files. We excluded isolates where predictions across all pipelines were discordant with the pDST result (e.g., where all pipelines reported susceptibility to a drug while pDST reported resistance), attributing such cases to either phenotypic errors or uncharacterised mutations.

False negatives

Where the gDST prediction for at least one pipeline, but not all, was discordant with a resistant pDST result, we considered the mutation(s) reported by the majority of pipelines with concordant gDST predictions as likely responsible for the resistant phenotype. For GPAS, MAGMA, MTBseq, TBProfiler, TBSeqPipe and tbtAMR, if this mutation was present in the sequence VCF file, we classified the error as a catalogue error. If absent, we attributed it to a variant calling error. For Mykrobe, where VCF files were not generated, we searched the resistance mutation catalogue for the mutation. If the mutation appeared in the catalogue, the error was labelled as a variant calling error; if not, we were unable to adjudicate the error type. In cases where resistance was linked to large insertions or deletions (indels) at a specific genomic location, we accepted any identified large indels within the candidate gene as a correctly identified variant. Heteroresistance was defined by the presence of a resistance-associated mutation at an allele frequency <1 . We summarised the number of false negative predictions, stratified by error type, for each pipeline.

False positives

Where the gDST prediction for at least one pipeline, but not all, was discordant with a susceptible pDST result, we identified the mutation(s) reported by the majority of pipelines with discordant gDST predictions. We then assessed the grading of these mutations in the WHO catalogue, 2nd edition⁴ and if there was any evidence of heteroresistance. For each pipeline, we summarised the number of false positive prediction, stratified by the grading of the implicated mutation in the WHO catalogue. This included “uncertain significance” and “associated with resistance” (includes associated with resistance interim). We further stratified the “associated with resistance” category by whether the implicated mutation was identified at an allele frequency <1 (heteroresistance).

Species identification

We processed 10 Illumina-sequenced non-tuberculous mycobacteria (NTM) isolates with each of the Illumina-compatible pipelines to screen if any incorrectly reported these as *M.tuberculosis*. The NTM sequences included 2 *Mycobacterium avium*⁵, 2 *Mycobacterium intracellulare*⁵, 2 *Mycobacterium intracellulare subsp. chimaera*⁵, 2 *Mycobacterium abscessus*⁶ and 2 *Mycobacterium kansasii*⁷ isolates.

Lineage classification

Where more than 1 lineage or sub-lineage was reported for a sample (mixed infections), lineage and sub-lineage was set to “mixed”. Reported lineages that were not *M.tuberculosis* were set to NA. GenTB and MTBseq were excluded from the lineage classification analysis as they reported results from multiple classification schemas for each sample. PhyResSE was excluded from our assessment of sub-lineage agreement as its non-numerical outputs could only reliably be translated to main lineages.

Genomic relatedness

We used runListCompare v0.3.8 (<https://github.com/davideyre/runListCompare>) on default settings to concatenate the individual-isolate FASTA files produced by GPAS into an MSA consisting solely of variant sites, masking resistance-associated and repetitive regions of the genome. We used snp-dists v0.8.2 (<https://github.com/tseemann/snp-dists>) with default settings to derive pairwise SNP distances from MSAs for each pipeline.

Note on transparency

During the course of this study, preliminary findings and technical issues identified with GAPS and MAGMA were informally communicated to the respective pipeline administrators. For GPAS, this led to a minor adjustment in the filter settings. Feedback was not provided systematically across all evaluated pipelines but was instead offered opportunistically, typically when we encountered issues that prevented the pipelines from running.

We also contacted the administrators of COMBAT-TB, UVP, simpITB, geno2phenoTB, TransFlow, and NCHHSTP-DTBE-Varpipe-WGS to inform them of problems with pipeline execution. However, we did not receive any responses, and the issues remained unresolved. These pipelines were therefore excluded from the analysis. For Lodestone, we reported a performance issue and received a response, but the problem could not be resolved in time for inclusion.

Additionally, preliminary results were publicly presented at The Union World Conference on Lung Health (15 November 2024). These communications and disclosures may have influenced subsequent pipeline updates, potentially affecting performance in the final analysis, which was conducted after the conference. Despite any

influence we may have had on individual pipelines, the results presented in this study reflect the performance of publicly available, referenced versions at the time of final analysis.

Supplementary results

90 Nanopore- and Illumina-sequenced isolates

gDST accuracies were similar across Nanopore-compatible pipelines. Additionally, in most cases, gDST accuracy was similar across sequencing platforms (Illumina vs Nanopore) for the same pipeline (Figure S6, Table S10). TBProfiler's Nanopore module was an outlier, exhibiting a high FPR for isoniazid (66%, 51%-78%), a discrepancy not observed in its corresponding Illumina module (FPR=2%, 0-13%). These false positives were due to the reporting of low allele frequency deletions in the *katG* gene. Approximately 5% of predictions on both Nanopore and Illumina data were false negatives (Figure S7). See Table S11 for pDST results for these 90 isolates.

213 **Supplementary tables**

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215 *Table S1: Drugs for which genotypic resistance prediction was performed for 11 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines*

Drug	GPAS	Gen-TB	GenoMycAnalyzer	MAGMA	MTBseq	Mykrobe	PhyResSE	SAM-TB	TBProfiler	TBSeqPipe	tbtAMR
Rifampicin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Isoniazid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethambutol	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Moxifloxacin	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓
Levofloxacin	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Amikacin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kanamycin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethionamide	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Bedaquiline	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✓
Linezolid	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clofazimine	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✓
Delamanid	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓

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✓ – drug included, ✗ – drug excluded

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219 **Table S2: *M.tuberculosis* whole genome sequencing analysis pipelines excluded from evaluation in this study**
220 **with reason for exclusion**

Pipeline	Reason for exclusion
SNP-TB (https://github.com/aditi9783/SNPTB) ⁸	Requires high-performance computing cluster and installation of multiple programs. Does not provide lineage classification, resistance prediction, relatedness or phylogeny as outputs.
MycoVarP ⁹	No publicly accessible repository or website identified.
TGS-TB ¹⁰	Website not functional.
BraSeqTB (https://github.com/LaPAM-USP/BraSeqTB)	Still under development. No instructions to execute presently.
Resistance Sniffer ¹¹	Does not allow for batch upload. Users need to upload and process each sequence individually. Did not generate any outputs.
CASTB ¹²	Website not functional .
TB-Annotator (https://github.com/avkitex/tbAnnotator) ⁵	Does not accept raw fastq files as input, does not provide lineage classification, resistance prediction, relatedness or phylogeny as outputs.
COMBAT-TB (https://github.com/COMBAT-TB/irida-galaxy-deploy) ¹³	Unsolvable error when trying to execute pipeline – query not addressed by repository owner.
UVP (https://github.com/CPTR-ReSeqTB/UDP) ¹⁴ 23/09/2025 18:16:00	Unsolvable error when trying to execute pipeline – query not addressed by repository owner.
simpITB (https://github.com/dcouvin/simpITB) ¹⁵	Unsolvable error when trying to execute pipeline. Reply by repository owner but no solution at time of writing.
geno2phenoTB (https://github.com/msmdev/geno2phenoTB)	Unsolvable error when trying to execute pipeline – query not addressed by repository owner.
TransFlow: Tuberculosis Transmission Analysis Workflow (https://github.com/cvn001/transflow)	Unsolvable error when trying to execute pipeline – query not addressed by repository owner.
SNP-IT(https://github.com/philipwflower/snpit) ¹⁶	Does not perform genotypic resistance prediction
KvarQ (https://github.com/kvarq/kvarq) ¹⁷	Complex installation process, not user-friendly. Repository appears unmaintained with no activity in >10 years.
NCHHSTP-DTBE-Varpipes-WGS (https://github.com/CDCgov/NCHHSTP-DTBE-Varpipes-WGS)	Unsolvable error when trying to execute pipeline – query not addressed by repository owner.
Lodestone (https://github.com/Pathogen-Genomics-Cymru/lodestone)	Generated spurious results. Contacted author who reported these may be due to a bug. Unresolved at the time of writing,

221 *Last updated 31/11/2024

222 **Table S3: Sequencing platform compatibility and methods applied to lineage classification, resistance prediction and genomic relatedness inference by twelve automated**
223 ***M.tuberculosis* whole genome sequencing analysis pipelines**

Pipeline	Sequencing platforms	Lineage classification	Resistance prediction	Relatedness
GPAS	Illumina, Nanopore	Mykrobe v0.13	Custom interpretation of WHO catalogue 2 nd edition ⁴ . (https://github.com/oxfordmmm/tuberculosis_amr_catalogues)	Interactive web interface displays sequences within 20 SNPs of index sequence. Generates pairwise SNP difference matrix for sequences within 20 SNPs. Produces fixed-length FASTA file for each sequence, aligned to H37Rv reference genome.
GenTB	Illumina	Coll et al ¹⁸ , Freschi et al ¹⁹ , Lipworth et al ¹⁶ , Shitikov et al ²⁰ , Stucki et al ²¹	GenTB Random Forest model ²² GenTB Wide and Deep Neural Network ²³	Not performed
GenoMycAnalyser	Illumina	Coll et al ¹⁸ and Napier et al ²⁴ 23/09/2025 18:16:00	Custom interpretation of WHO catalogue 1 st edition ²⁵ .	Not performed
MAGMA	Illumina	TBProfiler v6.2.0	TBProfiler v6.2.0 (https://github.com/jodyphelan/TBProfiler/releases/tag/v6.2.0)	Produces MSA of variant sites, pairwise SNP difference matrix and identifies clusters at 5 and 12 SNP thresholds using Cluster Picker ²⁶ . Phylogenetic tree constructed using IQ-TREE with options to specify parameters. ²⁷ Sites masked: variants present in <95% of samples, common drug-resistance variants and variants in rRNA genes.
MTBseq	Illumina, Ion Torrent	Coll et al ¹⁸ , Homolka et al ²⁸ , Merker et al ²⁹	Custom catalogue including WHO catalogue 1st edition ²⁵ (https://github.com/ngs-fzb/MTBseq_source/tree/master/var/res)	Produces MSA of variant sites, pairwise SNP difference matrix and identifies sequences clustered at user-specified thresholds. Sites masked: resistance associated genes and repetitive regions.
Mykrobe	Illumina, Nanopore	Chiner-Oms 2020 lineage schema ³⁰	Custom catalogue including CRYPTIC ³¹ , Walker 2015 ³² , WHO catalogue 1 st edition ²⁵ and Bradley 2015 ³³ (https://figshare.com/ndownloader/files/42494211)	Not performed
PhyResSE	Illumina, Ion Torrent	Feuerriegel et al ³⁴	Custom catalogue.	Produces MSA of variant sites (but not in a format amenable to downstream analysis). Maximum likelihood tree generated using FastTree. ³⁵
SAM-TB	Illumina	Napier et al ²⁴	Custom catalogue including CRYPTIC ³¹ , and TBProfiler database (version unspecified).	Produces MSA of variant sites, SNP difference matrix and identifies clusters at user-specified thresholds. Phylogenetic tree built with RAXML-NG. ³⁶ Sites masked: resistance associated genes and repetitive regions.

tbpore	Nanopore	Mykrobe v0.12	Mykrobe v0.12 (https://ndownloader.figshare.com/files/36197349)	Produces fixed-length fasta file aligned to H37Rv reference genome and identifies clusters at user-specified thresholds. Sites masked: Repetitive regions.
TBProfiler	Illumina, Nanopore, Ion Torrent	Coll et al ¹⁸ , Napier et al ²⁴ and Zwyer et al ³⁷ .23/09/2025 18:16:00	Custom catalogue including WHO catalogue 2 nd edition ⁴ (https://github.com/jodyphelan/TBProfiler/tree/master/db)	Not performed
TBSeqPipe	Illumina	TBProfiler v4.1.1	TBProfiler v4.1.1 (https://github.com/jodyphelan/TBProfiler/releases/tag/v4.1.1)	Produces MSA of variant sites and SNP difference matrix. Phylogenetic tree built with RAXML-NG. ³⁶ Sites masked: resistance associated genes and repetitive regions.
tbtAMR	Illumina	pathogen-profiler (https://github.com/jodyphelan/pathogen-profiler)	WHO catalogue 2 nd edition ⁴ (https://github.com/MDU-PHL/tbtamr/tree/master/tbtamr/db)	Not performed

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235 **Table S4: Additional features of automated *M.tuberculosis* whole genome sequencing analysis pipelines**

Pipeline	Additional features
GPAS	Cloud-based processing. Removes human reads from sequences with Hostile ³⁸ before upload to cloud. Performs non-tuberculous mycobacteria (NTM) species identification. Allows users to share results and intermediate files with one another through web platform.
GenoMycAnalyzer	Performs NTM species identification. Generates analysis report summary for each isolate with patient meta-data and sequencing results.
MAGMA	Performs NTM species identification with NTM-Profiler (https://github.com/jodyphelan/NTM-Profiler). Provides output to infer genomic relatedness and phylogeny from MSAs both excluding and including complex regions of the genome. Calibrated to analyse sequences generated directly from clinical samples.
MTBseq	Allows user to exchange the reference genome and specify custom resistance prediction mutation catalogue.
Mykrobe	Allows users to specify custom resistance prediction mutation catalogue. Additionally performs antibiotic resistance prediction for <i>Staphylococcus aureus</i> , <i>Shigella sonnei</i> , <i>Salmonella typhi</i> and <i>Salmonella enterica</i> serotype Paratyphi B.
SAM-TB	Performs NTM species identification. Generates analysis report summary for each isolate with patient meta-data and sequencing results. Identifies mixed infections (mixed MTBC or MTBC/NTM mixtures). Allows users to share results and intermediate files with one another through web platform. Interface available in English and Chinese.
tbAMR	Allows users to specify custom resistance prediction mutation catalogue and custom interpretive criteria.
TBProfiler	Allows users to specify custom resistance prediction mutation catalogue.
TBSeqPipe	Identifies mixed infections with MixInfect (https://github.com/bensobkowiak/MixInfect) ³⁹ . Generates batch summary report.

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244 **Table S5: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for 12 antituberculosis drugs for 7 Illumina-compatible *M.tuberculosis***
245 **whole genome sequencing analysis pipelines using the 1,000 Illumina sequences dataset.**

Drug	GPAS		MAGMA		MTBseq		Mykrobe		TBProfiler		TBSeqPipe		tbtAMR	
	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR
Rifampicin	1.1 (0.4–2.4)	4.2 (2.8–6.3)	1.1 (0.5–2.5)	4.6 (3.1–6.8)	3.4 (2.1–5.4)	3.7 (2.4–5.6)	2.1 (1.1–3.8)	4.4 (2.9–6.5)	1.3 (0.6–2.7)	4.6 (3.1–6.7)	0.8 (0.3–2.1)	4.6 (3.1–6.8)	1.7 (0.9–3.3)	4.2 (2.8–6.3)
Isoniazid	4.3 (2.8–6.4)	2.9 (1.7–4.8)	4.1 (2.7–6.2)	4.3 (2.8–6.6)	7.9 (5.9–10.6)	2.3 (1.3–4.1)	4.0 (2.7–6.1)	3.1 (1.9–5.1)	3.3 (2.1–5.2)	4.0 (2.5–6.1)	2.5 (1.5–4.3)	4.0 (2.6–6.1)	4.4 (3.0–6.6)	2.9 (1.7–4.8)
Ethambutol	6.6 (4.5–9.7)	6.8 (5.1–9.0)	7.0 (4.8–10.1)	7.0 (5.2–9.3)	2.5 (1.3–4.7)	7.9 (6.0–10.2)	3.3 (1.9–5.7)	7.7 (5.9–10.0)	1.7 (0.8–3.6)	9.1 (7.1–11.6)	1.4 (0.6–3.2)	9.3 (7.3–11.8)	7.2 (5.0–10.3)	6.8 (5.1–9.0)
Moxifloxacin	7.0 (4.2–11.7)	4.7 (3.4–6.4)	3.4 (1.5–7.1)	4.8 (3.5–6.5)	13.0 (8.9–18.7)	4.1 (2.9–5.7)	5.9 (3.4–10.3)	4.5 (3.3–6.2)	5.4 (3.0–9.7)	4.8 (3.5–6.5)	7.1 (4.2–11.7)	4.8 (3.5–6.5)	4.9 (2.6–9.0)	4.6 (3.3–6.2)
Levofloxacin	8.5 (5.5–13.1)	2.2 (1.4–3.4)	5.4 (3.0–9.4)	2.2 (1.4–3.5)	14.3 (10.2–19.7)	1.8 (1.1–3.0)	7.6 (4.7–12.0)	2.0 (1.3–3.3)	7.1 (4.4–11.4)	2.3 (1.4–3.6)	8.6 (5.5–13.1)	2.3 (1.5–3.6)	7.1 (4.4–11.4)	2.2 (1.4–3.4)
Amikacin	10.7 (6.1–18.1)	1.0 (0.5–1.9)	11.9 (6.9–19.6)	0.9 (0.5–1.8)	15.7 (9.9–24.0)	0.8 (0.4–1.6)	13.6 (8.3–21.5)	0.8 (0.4–1.6)	12.6 (7.5–20.4)	1.0 (0.5–1.9)	10.8 (6.1–18.3)	1.0 (0.5–1.9)	12.7 (7.6–20.6)	1.0 (0.5–1.9)
Kanamycin	11.7 (7.0–19.0)	2.6 (1.7–3.9)	12.8 (7.8–20.4)	2.2 (1.4–3.4)	16.4 (10.6–24.3)	2.5 (1.6–3.6)	14.4 (9.1–22.1)	2.4 (1.6–3.6)	12.6 (7.7–20.1)	2.8 (1.9–4.1)	10.9 (6.4–18.1)	2.8 (1.9–4.1)	13.6 (8.4–21.3)	2.5 (1.6–3.7)
Ethionamide	17.7 (13.1–23.4)	4.1 (2.9–5.7)	10.7 (7.2–15.7)	5.7 (4.3–7.6)	44.0 (37.5–50.8)	4.1 (2.9–5.7)	21.5 (16.5–27.6)	5.2 (3.8–7.0)	10.5 (7.1–15.4)	8.1 (6.4–10.2)	16.7 (12.3–22.4)	7.6 (6.0–9.7)	12.0 (8.2–17.1)	4.6 (3.3–6.3)
Bedaquiline	75.0 (40.9–92.9)	1.2 (0.7–2.1)	50.0 (21.5–78.5)	2.9 (2.0–4.2)	75.0 (40.9–92.9)	0.5 (0.2–1.2)	NA	NA	50.0 (21.5–78.5)	1.7 (1.1–2.7)	100.0 (67.6–100.0)	0.3 (0.1–0.9)	50.0 (21.5–78.5)	1.7 (1.1–2.7)
Clofazimine	94.6 (82.3–98.5)	1.1 (0.6–2.0)	75.0 (58.9–86.2)	2.3 (1.6–3.5)	94.6 (82.3–98.5)	0.2 (0.5–0.7)	NA	NA	81.1 (65.8–90.5)	1.3 (0.8–2.3)	100.0 (90.6–100.0)	0.3 (0.1–0.9)	81.1 (65.8–90.5)	1.4 (0.8–2.3)
Delamanid	50.0 (26.8–73.2)	0.0 (0.0–0.4)	42.9 (21.4–67.4)	3.9 (2.8–5.3)	71.4 (45.4–88.3)	0.3 (0.1–0.9)	78.6 (52.4–92.4)	0.0 (0.0–0.4)	50.0 (26.8–73.2)	0.0 (0.0–0.4)	71.4 (45.4–88.3)	0.0 (0.0–0.4)	50.0 (26.8–73.2)	0.0 (0.0–0.4)
Linezolid	44.4 (24.6–66.3)	0.0 (0.0–0.4)	37.5 (18.5–61.4)	0.0 (-0.0–0.4)	44.4 (24.6–66.3)	0.0 (0.0–0.4)	50.0 (29.0–71.0)	0.0 (0.0–0.4)	44.4 (24.6–66.3)	0.0 (0.0–0.4)	44.4 (24.6–66.3)	0.0 (0.0–0.4)	44.4 (24.6–66.3)	0.0 (0.0–0.4)

246 *FNR – false negative rate, FPR – false positive rate*

Table S6: Summarised phenotypic drug susceptibility results for isolates included in 100 Illumina sequences and 1,000 Illumina sequences datasets

Drug	Phenotypic DST result			
	100 Illumina sequences dataset		1,000 Illumina sequences dataset	
	S (%)	R (%)	S (%)	R (%)
Rifampicin	40 (40)	60 (60)	523 (52.3)	477 (47.7)
Isoniazid	42 (42)	58 (38)	519 (51.9)	481 (48.1)
Ethambutol	49 (49)	35 (35)	638 (63.8)	362 (36.2)
Moxifloxacin	75 (75)	25 (25)	815 (81.5)	185 (18.5)
Levofloxacin	72 (72)	28 (28)	789 (78.9)	211 (21.1)
Amikacin	77 (77)	23 (23)	897 (89.7)	103 (10.3)
Kanamycin	78 (78)	22 (22)	889 (88.9)	111 (11.1)
Ethionamide	70 (70)	20 (20)	791 (79.1)	209 (20.9)
Bedaquiline	96 (96)	4 (4)	996 (99.6)	4 (0.4)
Clofazimine	84 (84)	16 (16)	984 (98.4)	16 (1.6)
Linezolid	84 (84)	16 (16)	984 (98.4)	16 (1.6)
Delamanid	87 (87)	13 (13)	987 (98.7)	13 (1.3)

Values represent counts and proportions. DST – drug susceptibility testing, S – susceptible, R – resistant

Table S7: Pooled sensitivity and specificity of genotypic drug susceptibility testing for 6 *M.tuberculosis* whole genome sequencing analysis pipelines using the 1,000 Illumina sequences dataset. Included drugs are rifampicin, isoniazid, ethambutol, moxifloxacin, levofloxacin, amikacin, kanamycin, ethionamide, delamanid and linezolid.

Pipeline	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
GPAS	88.8 (78.5-94.5)	97.9 (95.7-99.0)
MAGMA	91.0 (83.0-95.5)	96.8 (95.0-97.9)
MTBseq	82.9 (67.2-91.9)	98.2 (96.4-99.1)
Mykrobe	87.3 (72.8-94.7)	98.1 (95.5-99.2)
TBProfiler	90.9 (80.81-96.0)	97.7 (94.6-99.0)
TBSeqPipe	90.2 (76.8-96.3)	97.7 (94.6-99)
tbAMR	89.1 (79.4-94.5)	98 (95.7-99.1)

257 *Table S8: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for 8 antituberculosis drugs for 11 Illumina-compatible M.tuberculosis*
258 *whole genome sequencing analysis pipelines using the 100 Illumina sequences dataset.*

Pipeline	Rifampicin		Isoniazid		Ethambutol		Levofloxacin		Moxifloxacin		Kanamycin		Amikacin		Ethionamide	
	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR
GPAS	3.3 (0.9–11.4)	2.6 (0.5–13.2)	6.9 (2.7–16.4)	2.4 (0.4–12.6)	5.7 (1.6–18.6)	6.2 (2.1–16.8)	14.3 (5.7–31.5)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.4 (0.2–7.3)	13.6 (4.7–33.3)	2.6 (0.7–9.0)	21.7 (9.7–41.9)	2.6 (0.7–9.1)	15.0 (5.2–36.0)	7.2 (3.1–15.9)
Gen-TB	5.4 (1.8–14.6)	0.0 (0.0–10.7)	7.4 (2.9–17.6)	2.9 (0.5–14.9)	9.1 (3.1–23.6)	9.8 (3.9–22.5)	18.5 (8.2–36.7)	0.0 (-0.0–5.9)	NA	NA	23.8 (10.6–45.1)	0.0 (0.0–5.4)	27.3 (13.2–48.2)	0.0 (0.0–5.5)	36.8 (19.1–59.0)	3.4 (0.9–11.5)
GenoMycAnalyzer	1.7 (0.3–8.9)	27.5 (16.1–42.8)	6.9 (2.7–16.4)	26.2 (15.3–41.1)	2.9 (0.5–14.5)	12.2 (5.7–24.2)	14.3 (5.7–31.5)	5.6 (2.2–13.4)	8.0 (2.2–25.0)	6.7 (2.9–14.7)	13.6 (4.7–33.3)	5.1 (2.0–12.5)	21.7 (9.7–41.9)	3.9 (1.3–10.8)	35.0 (18.1–56.7)	20.0 (12.3–30.8)
MAGMA	3.3 (0.9–11.4)	2.7 (0.5–13.8)	6.9 (2.7–16.4)	2.6 (0.5–13.2)	5.7 (1.6–18.6)	6.5 (2.2–17.5)	14.3 (5.7–31.5)	0.0 (0.0–5.3)	8.0 (2.2–25.0)	1.4 (0.2–7.5)	13.6 (4.7–33.3)	2.7 (0.7–9.2)	21.7 (9.7–41.9)	2.7 (0.7–9.3)	10.0 (2.8–30.1)	9.0 (4.2–18.2)
MTBseq	5.0 (1.7–13.7)	2.5 (0.4–12.9)	10.3 (4.8–20.8)	2.4 (0.4–12.3)	11.4 (4.5–26.0)	6.1 (2.1–16.5)	14.3 (5.7–31.5)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.3 (0.2–7.2)	22.7 (10.1–43.4)	2.6 (0.7–8.9)	26.1 (12.5–46.5)	2.6 (0.7–9.0)	60.0 (38.7–78.1)	7.1 (3.1–15.7)
Mykrobe	3.3 (0.9–11.4)	2.5 (0.4–12.9)	6.9 (2.7–16.4)	2.4 (0.4–12.3)	8.6 (3.0–22.4)	6.1 (2.1–16.5)	14.3 (5.7–31.5)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.3 (0.2–7.2)	22.7 (10.1–43.4)	1.3 (0.2–6.9)	30.4 (15.6–50.9)	1.3 (0.2–7.0)	40.0 (21.9–61.3)	2.9 (0.8–9.8)
PhyResSE	3.3 (0.9–11.4)	2.6 (0.5–13.2)	8.6 (3.7–18.6)	2.4 (0.4–12.6)	5.7 (1.6–18.6)	6.2 (2.1–16.8)	14.3 (5.7–31.5)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.4 (0.2–7.3)	13.6 (4.7–33.3)	2.6 (0.7–9.0)	21.7 (9.7–41.9)	2.6 (0.7–9.1)	70.0 (48.1–85.5)	7.2 (3.1–15.9)
SAM-TB	3.3 (0.9–11.4)	2.5 (0.4–12.9)	6.9 (2.7–16.4)	2.4 (0.4–12.3)	5.7 (1.6–18.6)	6.1 (2.1–16.5)	NA	NA	8.0 (2.2–25.0)	1.3 (0.2–7.2)	13.6 (4.7–33.3)	3.8 (1.3–10.7)	21.7 (9.7–41.9)	2.6 (0.7–9.0)	10.0 (2.8–30.1)	10.0 (4.9–19.2)
TBProfiler	3.4 (0.9–11.5)	2.5 (0.4–12.9)	7.0 (2.8–16.7)	2.4 (0.4–12.3)	5.7 (1.6–18.6)	6.1 (2.1–16.5)	14.8 (5.9–32.5)	0.0 (0.0–5.1)	8.3 (2.3–25.8)	1.3 (0.2–7.2)	14.3 (5.0–34.6)	3.8 (1.3–10.7)	22.7 (10.1–43.4)	2.6 (0.7–9.0)	15.0 (5.2–36.0)	10.1 (5.0–19.5)
TBSeqPipe	3.3 (0.9–11.4)	2.6 (0.5–13.2)	5.3 (1.8–14.4)	2.4 (0.4–12.3)	2.9 (0.5–14.9)	6.1 (2.1–16.5)	11.1 (3.9–28.1)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.4 (0.2–7.3)	13.6 (4.7–33.3)	3.9 (1.3–10.8)	18.2 (7.3–38.5)	2.6 (0.7–9.0)	5.3 (0.9–24.6)	8.6 (4.0–17.5)
tbAMR	5.0 (1.7–13.7)	2.5 (0.4–12.9)	6.9 (2.7–16.4)	2.4 (0.4–12.3)	5.7 (1.6–18.6)	6.1 (2.1–16.5)	14.3 (5.7–31.5)	0.0 (0.0–5.1)	8.0 (2.2–25.0)	1.3 (0.2–7.2)	13.6 (4.7–33.3)	2.6 (0.7–8.9)	21.7 (9.7–41.9)	2.6 (0.7–9.0)	15.0 (5.2–36.0)	5.7 (2.2–13.8)

259 *Table S9: False negative rates and false positive rates (%) of genotypic drug susceptibility testing for bedaquiline, clofazimine, delamanid and linezolid for 10*
260 *M.tuberculosis whole genome sequencing analysis pipelines using the 100 Illumina sequences dataset*

Pipeline	Bedaquiline		Linezolid		Clofazimine		Delamanid	
	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR
GPAS	50.0 (15.0–85.0)	7.4 (3.6–14.4)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	87.5 (64.0–96.5)	7.2 (3.4–14.9)	46.2 (23.2–70.9)	0.0 (0.0–4.3)
GenoMycAnalyzer	100.0 (51.0–100.0)	1.0 (0.2–5.7)	93.8 (71.7–98.9)	0.0 (0.0–4.4)	100.0 (80.6–100.0)	1.2 (0.2–6.4)	76.9 (49.7–91.8)	1.1 (0.2–6.2)
MAGMA	50.0 (15.0–85.0)	9.7 (5.2–17.4)	42.9 (21.4–67.4)	0.0 (0.0–4.4)	68.8 (44.4–85.8)	6.2 (2.7–13.6)	38.5 (17.7–64.5)	6.0 (2.6–13.2)
MTBseq	75.0 (30.1-95.4)	1.0 (0.2-5.7)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	93.8 (71.7-98.9)	0.0 (0.0–4.4)	69.2 (42.4–87.3)	0.0 (0.0–4.2)
Mykrobe	NA	NA	56.2 (33.2–76.9)	0.0 (0.0–4.4)	NA	NA	76.9 (49.7–91.8)	0.0 (0.0–4.2)
PhyResSE	75.0 (30.1–95.4)	2.1 (0.6–7.4)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	93.8 (71.7–98.9)	0.0 (0.0–4.4)	66.7 (39.1–86.2)	0.0 (0.0–4.2)
SAM-TB	50.0 (15.0–85.0)	9.4 (5.0–16.9)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	75.0 (50.5–89.8)	7.1 (3.3–14.7)	46.2 (23.2–70.9)	0.0 (0.0–4.2)
TBProfiler	50.0 (15.0–85.0)	9.5 (5.1–17.0)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	75.0 (50.5–89.8)	7.2 (3.4–14.9)	46.2 (23.2–70.9)	0.0 (0.0–4.3)
TBSeqPipe	100.0 (43.9–100.0)	1.0 (0.2–5.7)	46.7 (24.8–69.9)	0.0 (0.0–4.4)	100.0 (79.6–100.0)	1.2 (0.2–6.4)	66.7 (39.1–86.2)	0.0 (0.0–4.2)
tbtAMR	50.0 (15.0–85.0)	9.4 (5.0–16.9)	50.0 (28.0–72.0)	0.0 (0.0–4.4)	75.0 (50.5–89.8)	7.1 (3.3–14.7)	46.2 (23.2–70.9)	0.0 (0.0–4.2)

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268 *Table S10: False negative rates and false positives rates (%) of genotypic drug susceptibility testing for rifampicin, isoniazid, ethambutol and streptomycin for 4*
269 *Nanopore-compatible and 3 Illumina-compatible M.tuberculosis whole genome sequencing analysis pipelines using the 90 sequences dataset*

Drug	GPAS				Mykrobe				TBProfiler				tbpore	
	Nanopore		Illumina		Nanopore		Illumina		Nanopore		Illumina		Nanopore	
	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR	FNR	FPR
Rifampicin	10.6 (4.6–22.6)	0.0 (0.0–8.2)	8.5 (3.4–19.9)	0.0 (0.0–8.2)	10.6 (4.6–22.6)	0.0 (0.0–8.2)	8.5 (3.4–19.9)	0.0 (0.0–8.2)	12.8 (6.0–25.2)	0.0 (0.0–8.2)	10.6 (4.6–22.6)	0.0 (0.0–8.2)	10.6 (4.6–22.6)	0.0 (0.0–8.2)
Isoniazid	18.4 (10.0–31.4)	2.4 (0.4–12.6)	18.4 (10.0–31.4)	2.4 (0.4–12.6)	18.4 (10.0–31.4)	4.9 (1.3–16.1)	18.4 (10.0–31.4)	2.4 (0.4–12.6)	8.2 (3.2–19.2)	65.9 (50.5–78.4)	16.3 (8.5–29.0)	2.4 (0.4–12.6)	18.4 (10.0–31.4)	4.9 (1.3–16.1)
Ethambutol	28.6 (11.7–54.6)	21.1 (13.4–31.5)	28.6 (11.7–54.6)	21.1 (13.4–31.5)	28.6 (11.7–54.6)	19.7 (12.3–30.0)	28.6 (11.7–54.6)	22.4 (14.5–32.9)	21.4 (7.6–47.6)	22.4 (14.5–32.9)	21.4 (7.6–47.6)	22.4 (14.5–32.9)	28.6 (11.7–54.6)	19.7 (12.3–30.0)
Streptomycin	25.0 (7.1–59.1)	9.9 (5.1–18.3)	25.0 (7.1–59.1)	9.5 (4.7–18.3)	25.0 (7.1–59.1)	32.9 (23.7–43.7)	25.0 (7.1–59.1)	15.9 (9.5–25.3)	25.0 (7.1–59.1)	17.1 (10.5–26.6)	12.5 (2.2–47.1)	12.2 (6.8–21.0)	25.0 (7.1–59.1)	32.9 (23.7–43.7)

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275 **Table S11: Summarised phenotypic drug susceptibility results for isolates included in the 90 Nanopore- and Illumina sequences dataset**

Drug	Susceptible (%)	Resistant (%)
Rifampicin	43 (48)	47 (52)
Isoniazid	41 (46)	49 (54)
Ethambutol	76 (84)	14 (16)
Streptomycin	82 (91)	8 (9)

276 *Values represent counts and percentages*

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279 **Table S12: Distribution of main lineages, as determined using TBProfiler, of isolates included in test datasets**

Dataset	Lineage 1	Lineage 2	Lineage 3	Lineage 4	Mixed/unknown
100 Illumina sequences	1 (1)	56 (56)	1 (1)	39 (39)	3 (3)
1,000 Illumina sequences	50 (5)	414 (41)	63 (6)	451 (45)	22 (2)
90 Nanopore sequences	30 (30)	10 (11)	9 (10)	41 (46)	0

280 *Values represent counts and percentages*

Supplementary figures

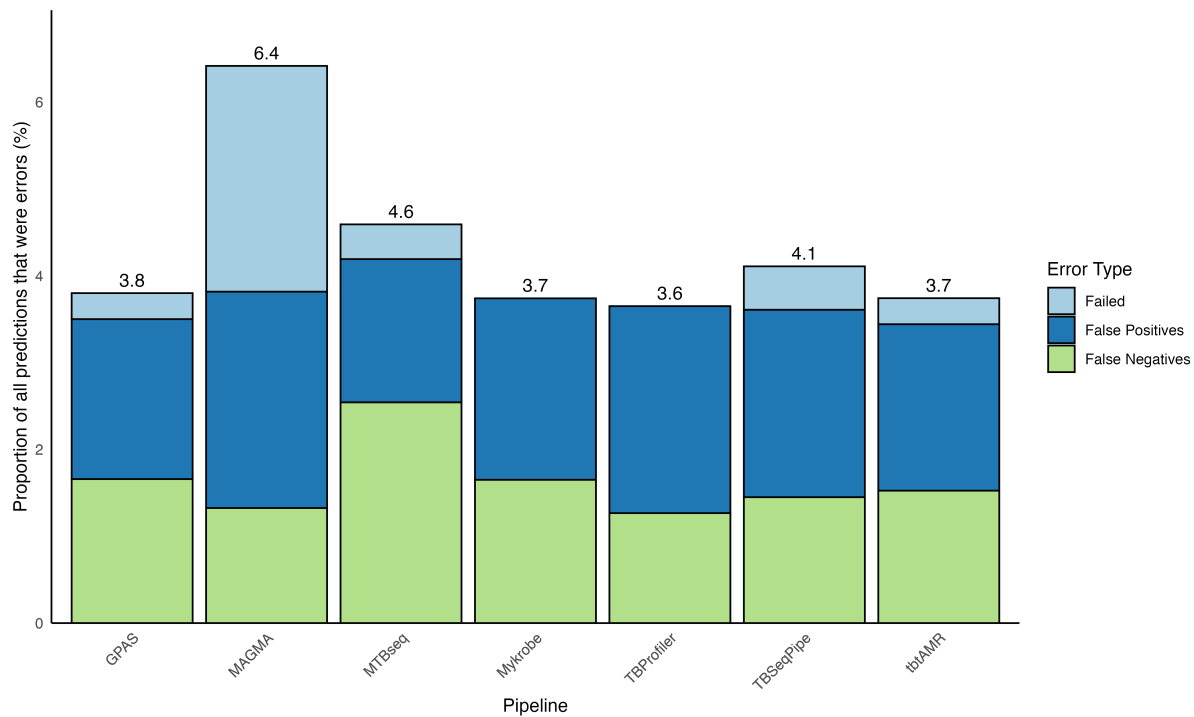
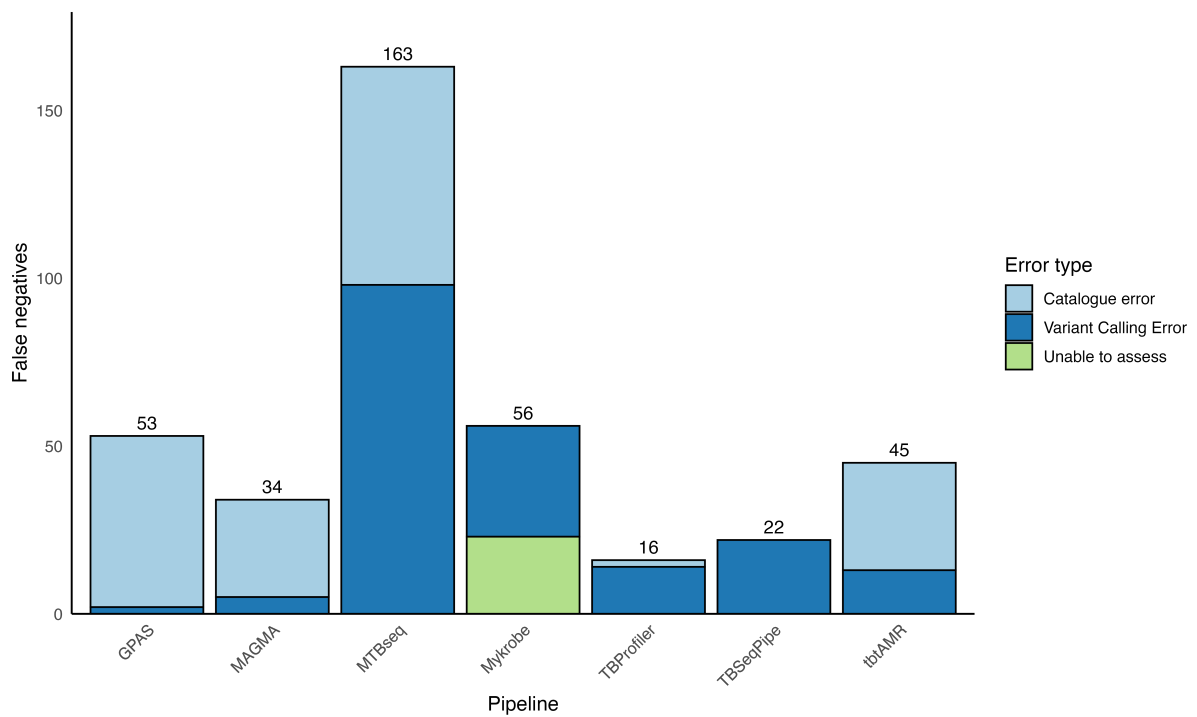


Figure S1: Proportion of all genotypic drug susceptibility predictions that were errors for 7 *M.tuberculosis* whole genome sequencing analysis pipelines, stratified by error type, using the 1,000 Illumina sequences dataset.



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Figure S2: False negative predictions for 7 *M.tuberculosis* whole genome sequencing analysis pipelines, stratified by error type, using the 1,000 Illumina sequences dataset.
**Excludes isolates for which all pipeline predictions were discordant with phenotype*

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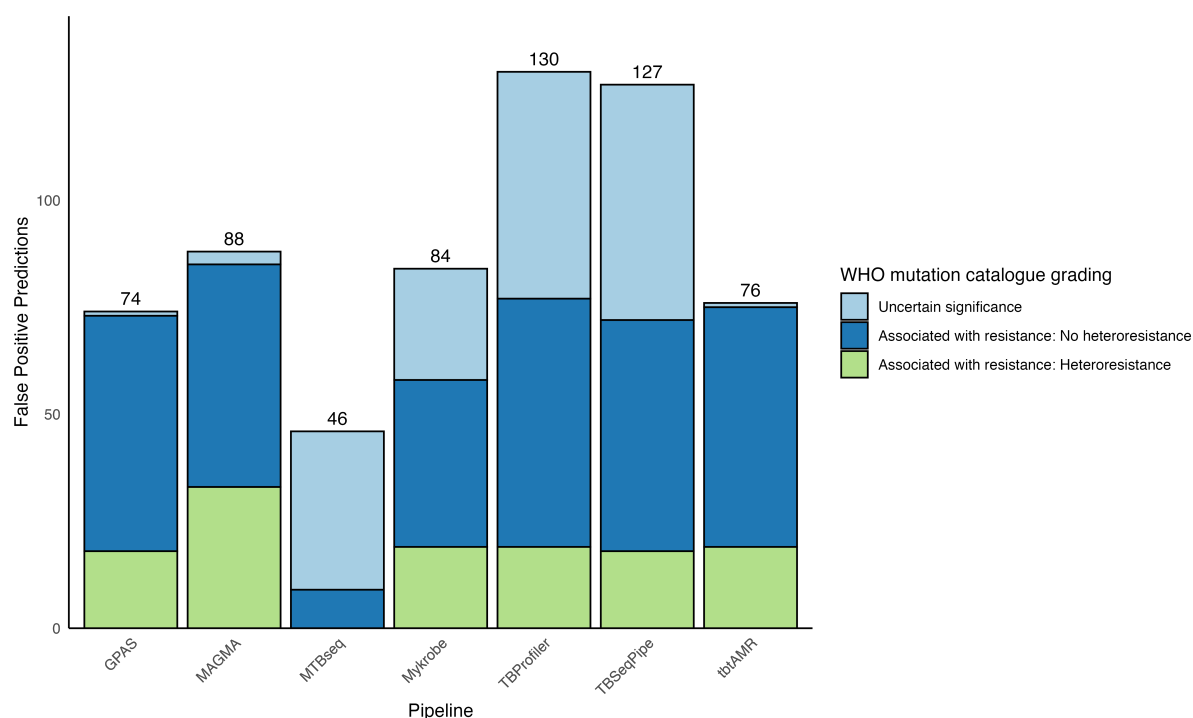
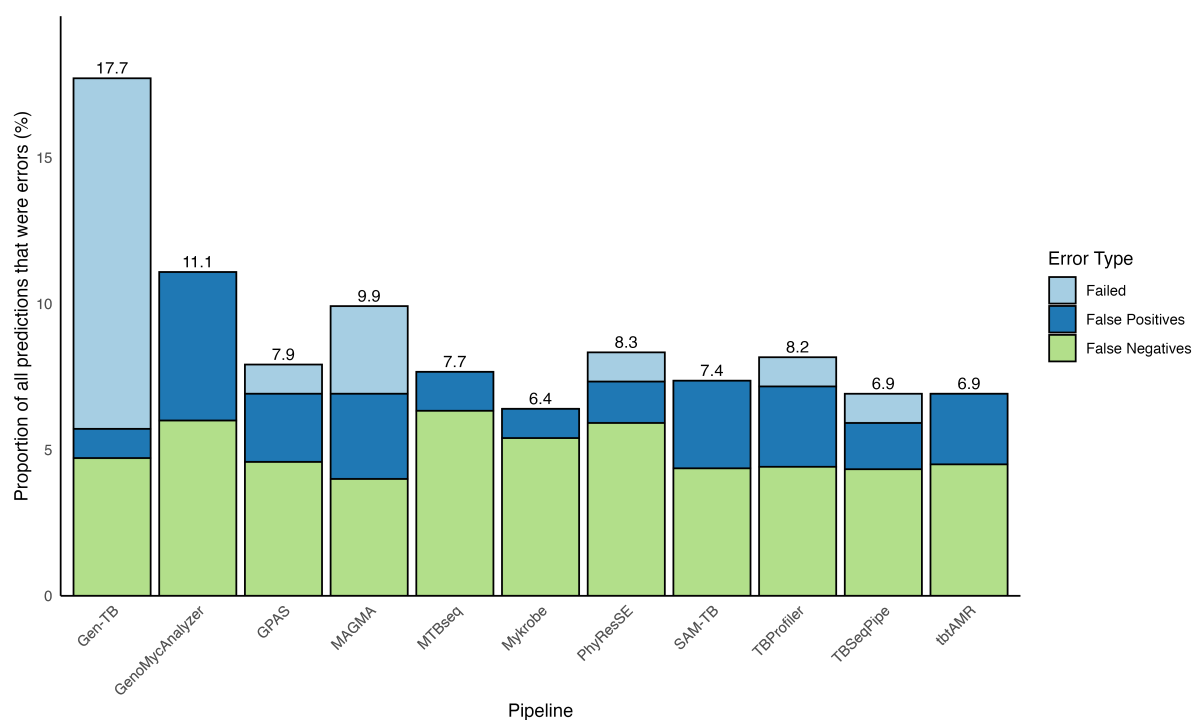


Figure S3: False positive predictions for 7 *M.tuberculosis* whole genome sequencing analysis pipelines, stratified by WHO mutation catalogue grade and presence of heteroresistance, using the 1,000 Illumina sequences dataset.

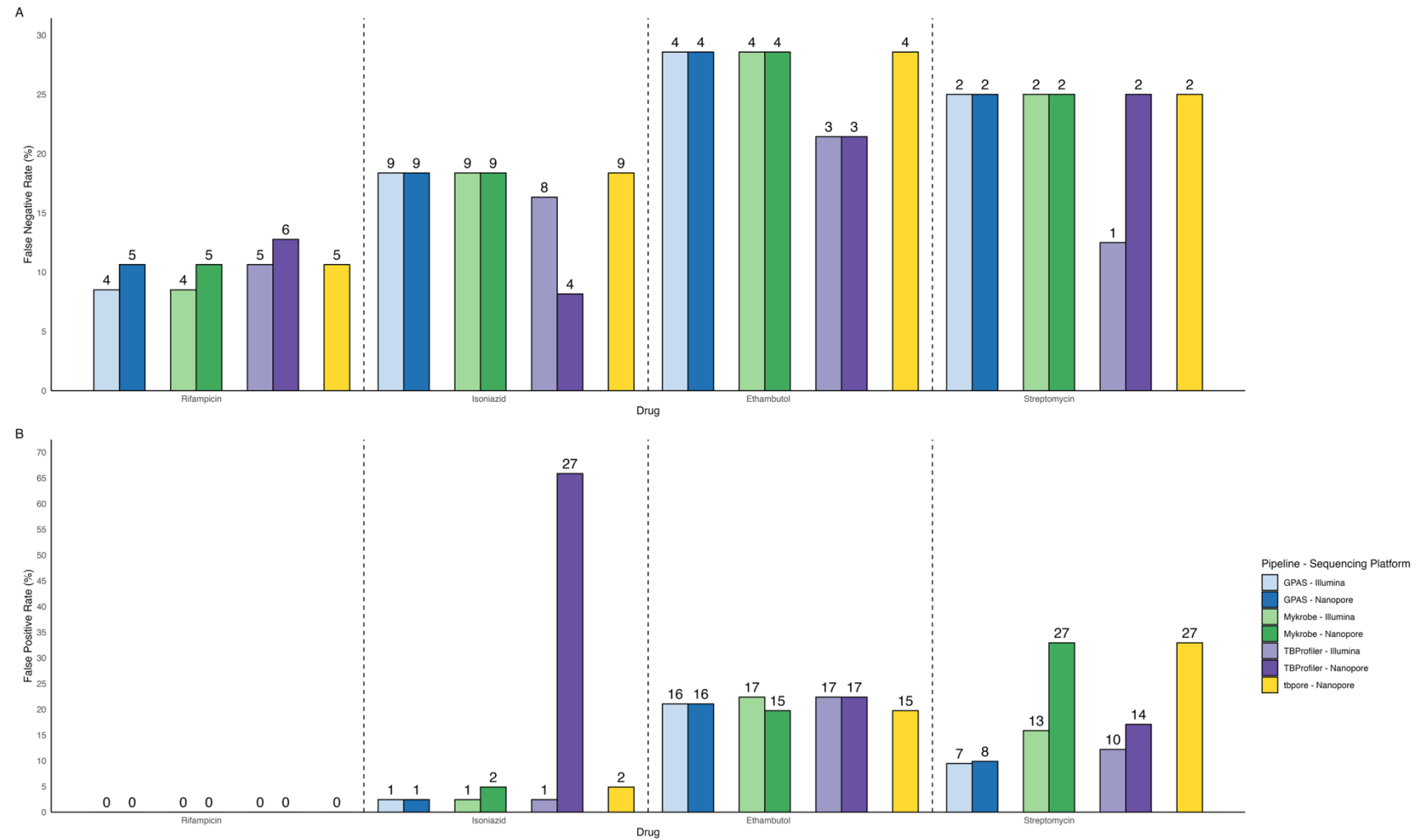
**Excludes isolates for which all pipeline predictions were discordant with phenotype. WHO catalogue, 2nd ed.*

False positives may be due to false positive variant calling, the annotation of “uncertain significance” mutations as resistant or phenotypic error. For example, ERR2510366 is reported resistant by TBProfiler and TBSeqPipe on the basis of identifying the *rpoB* p.Phe433Leu mutation. The canonical rifampicin-resistance conferring mutation is not identified by any other pipelines. This sequence has relatively low coverage (24X) and this potentially represents false positive variant calling. Conversely, sample ERR483180 contains the *embA* c.-16C>G mutation which has “uncertain significance” and results in false positive predictions for MTBseq, Mykrobe, TBProfiler and TBSeqPipe. Sample ERR2510315 contains the *gyrA* p.Ala90Val mutation resulting in a false positive moxifloxacin resistance predictions for all pipelines except tbtAMR. However, this likely reflects phenotypic error in this isolate with tbtAMR coincidentally obtaining the “correct” gDST result despite a likely variant calling error. See supplementary files for greater detail.



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Figure S4: Proportion of all genotypic drug susceptibility predictions that were errors for 11 *M.tuberculosis* whole genome sequencing analysis pipelines, stratified by error type, using the 100 Illumina sequences dataset.



330 **Figure S5: False negative rates (above) and false positives rates (below) of genotypic drug susceptibility testing for rifampicin, isoniazid, ethambutol and streptomycin for 4 Nanopore-compatible and 3 Illumina-compatible *M.tuberculosis* whole genome sequencing analysis pipelines using the 90 sequences dataset.** Values above bars denote absolute number of false negative (above) and false positive (below) predictions.

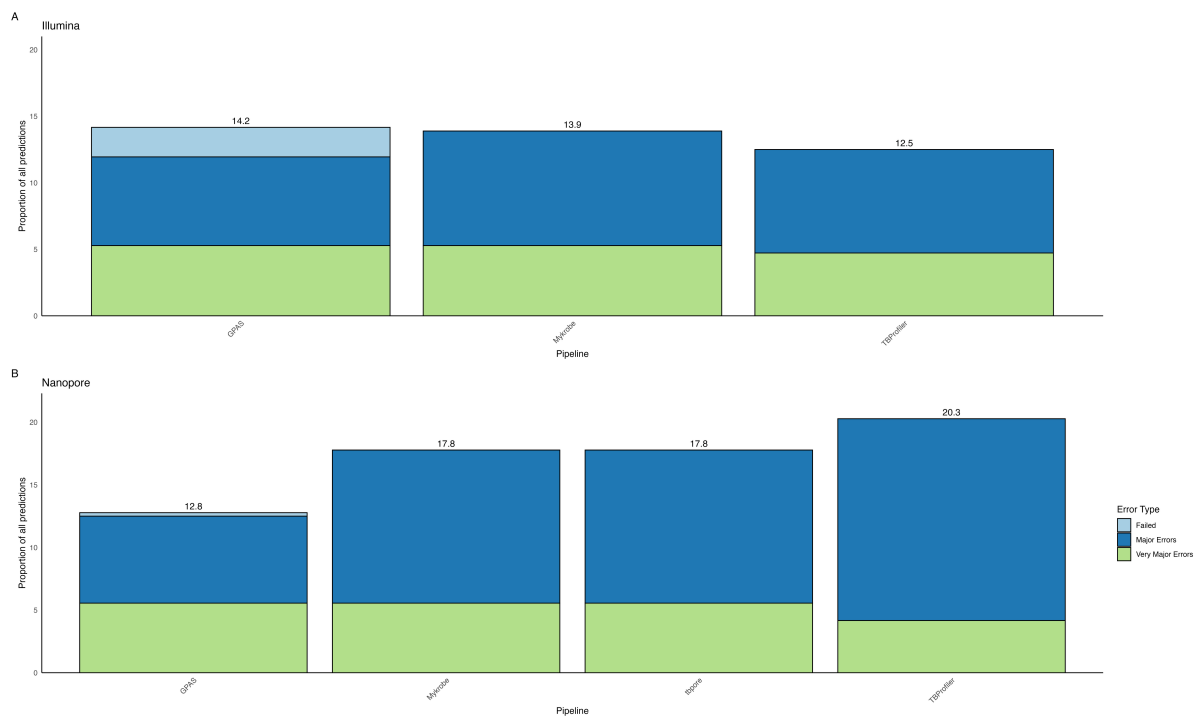
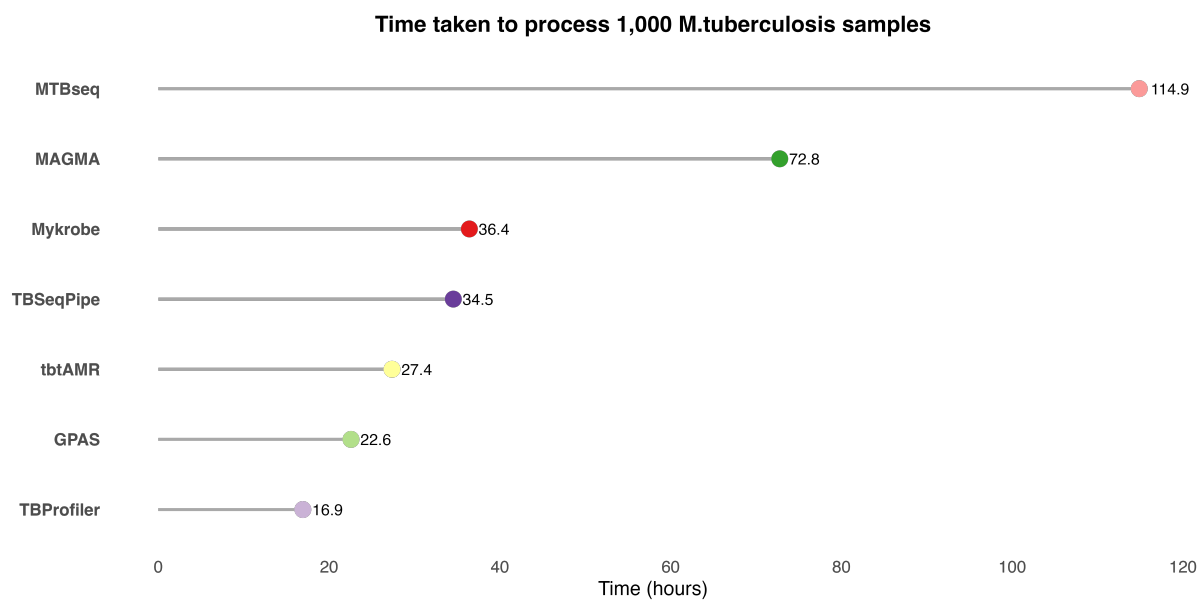


Figure S6: Proportion of all genotypic drug susceptibility predictions that were errors for 4 *M.tuberculosis* whole genome sequencing analysis pipelines, stratified by error type, using the 90 Nanopore (left) and Illumina (right) sequences dataset.

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Figure S7: Time, in hours, taken by GPAS, MAGMA, MTBseq, Mykrobe, TBProfiler, TBSeqPipe and tbtAMR to process 1,000 M.tuberculosis whole genome sequences.

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