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MECHANISMS OF RESISTANCE

Melting the *eis*: Nondetection of Kanamycin Resistance Markers by Routine Diagnostic Tests and Identification of New *eis* Promoter Variants

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ABSTRACT *Eis* promoter mutations can confer reduced *Mycobacterium tuberculosis* kanamycin susceptibility. GenoType MTBDR*sl,* a widely used assay evaluating this region, wrongly classified 17/410 isolates as *eis* promoter wild type. Six out of seventeen isolates harbored mutations known to confer kanamycin resistance, and the remainder harbored either novel *eis* promoter mutations (7/11) or disputed mutations (4/11). GenoType MTBDR*sl* can miss established and new variants that cause reduced susceptibility. These data highlight the importance of reflex phenotypic kanamycin testing.

KEYWORDS *Mycobacterium tuberculosis*, extensive drug resistance, second-line injectables

The drugs amikacin (AMK), kanamycin (KAN), and capreomycin (CAP) have been part of the recommended second-line antituberculosis treatment since the 1970s. The most common genetic resistance marker for these drugs is a single-nucleotide variant (SNV) at position 1401 of the rRNA 16S encoding gene, *rrs* (1, 2). An alternative mechanism conferring (low-level) resistance to KAN includes SNVs in the promoter region of *eis* (Rv2416c) (Fig. S1 in the supplemental material) (3). Amikacin is often used as a surrogate for KAN phenotypic drug-susceptibility testing (pDST) based on the assumption of complete cross-resistance. Similarly, if the strain was susceptible to AMK, KAN susceptibility was assumed, and low-level KAN resistance was potentially overlooked. Until 2017, *eis* promoter mutations were not routinely tested for in South Africa, leading to undetected resistance and less effective treatment.

This study investigated the presence, type, and detection of *eis* promoter mutations in clinical *Mycobacterium tuberculosis* isolates collected in South Africa using the line probe assay GenoType MTBDR*sl* VER 2.0 (MTBDR*sl*; Hain Lifescience, Germany), Sanger sequencing, and whole-genome sequencing (WGS).

Two unique sample sets were analyzed. Sample set 1 consisted of 951 *M. tuberculosis* isolates from Xpert MTB/RIF (Cepheid) rifampin (RIF)-resistant specimens from South Africa that were collected between June 2016 and June 2017 as part of routine diagnostics by the National Health Laboratory Services, Cape Town. These isolates

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were analyzed using the GenoType MTBDR*plus* assay (detecting resistance against RIF and/or isoniazid [INH]) and MTBDR*sl* (4). To determine the number of *eis* promoter mutations missed by MTBDR*sl*, isolates that were phenotypically susceptible to AMK, wild type (WT) for *eis* promoter and *rrs* by MTBDR*sl*, and available in the Stellenbosch University biobank ($n = 398$) were Sanger sequenced (i.e., the region covering 222 bp upstream of the transcriptional start site of the *eis* gene, subsequently referred to as "*eis* promoter region"; Fig. S1). Sample set 2 consisted of a convenience sample of 2,863 whole-genome sequences of clinical *M. tuberculosis* isolates derived from sputum samples collected between 1993 and 2018 and sequenced as part of different research projects (5–9). These sequences were screened *in silico* for *eis* promoter mutations (genome positions 2715332 to 2715582 of *M. tuberculosis* H37Rv; GenBank accession no. AL123456). Of those isolates with *eis* promoter mutations, representatives for each (combination of) mutation(s) were selected for further analyses with targeted Sanger sequencing, MTBDR*sl*, and pDST. An overview of the study workflow for both sample sets is given in Fig. 1.

For isolates of sample set 1, PCR amplification—and subsequent Sanger sequencing—was conducted on thermal lysates, whereas purified DNA was used for sample set 2. Briefly, the PCR mixture contained the following final concentrations: 1× HotStartTaq Plus master mix (Qiagen, San Diego, CA, USA), 500 nM each primer (forward, 5′-CCATGGGACCGGTACTTGCT-3′; reverse, 5′-ACTTCACCAGGCACCGTCAA-3′), and 1× SYTO 9 green fluorescent nucleic acid stain (Thermo Fisher Scientific). As a template, 1 μl of thermal lysate (sample set 1) or purified DNA (sample set 2) was added to the reaction mixture. Amplification of the *eis* promoter region of the selected isolates was carried out using a CFX96TM real-time system C1000 Touch thermal cycler (Bio-Rad) running the following thermocycling protocol: initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, annealing at 62°C for 1 min, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 10 min. Successful amplification was confirmed by a high-resolution melt from 80°C to 95°C with an increment of 0.5°C, each increment temperature held for 5 s. Successfully amplified PCR products were sent to the Central DNA Sequencing Facilities of Stellenbosch University for targeted Sanger sequencing using the forward PCR primer. The MTBDR*sl* assay was conducted according to the manufacturer's protocol using the same DNA used for WGS. The assay defines specific banding patterns (i.e., presence or absence of WT and MUT bands) for the following most common *eis* promoter mutations: −37 G > T, −14 T > C, −12 T > C, −10 G > A, and −2 A > C. In this study, these mutations were therefore defined as "detectable by MTBDR*sl*." However, only the mutation −14 T > C is explicitly detected by a MUT probe (4). Other known *eis* promoter mutations (Fig. S1) may also cause one of the WT bands to fail but appear not to have been validated by the manufacturer. In this study, these mutations were therefore defined as "not included in MTBDR*sl*." Phenotypic DST was performed on all isolates using solid Löwenstein-Jensen medium according to the 1% proportion method at clinical breakpoints of 0.2 μg/ml for INH, 40.0 μg/ml for RIF, 30 μg/ml for AMK, and 2 μg/ml for ofloxacin (10, 11). MICs for KAN were subsequently determined for isolates with an *eis* promoter mutation missed by the MTBDR*sl* (sample set 1) and for representatives of each additional (combination of) *eis* promoter mutation(s) (sample set 2). These MICs were done using 2-fold serial dilutions ranging from 10.0 μg/ml to 1.25 μg/ml using the Bactec MGIT 960 system with the TB eXiST module of the EpiCentre software (12). Susceptibility to KAN was determined using the 1% proportion method based on a clinical breakpoint of 2.5 μg/ml. For WGS, each isolate was recultured from culture stocks, and DNA was extracted as previously described (13). Whole-genome sequencing libraries were prepared according to the manufacturer's protocol (Illumina, Inc, San Diego, CA), and sequenced on an Illumina HiSeq or Illumina NextGen Seq platform. The resulting sequencing reads were mapped to the *M. tuberculosis* H37*Rv* reference strain (GenBank accession no. AL123456). Variant calling and annotation were conducted using a within-house pipeline as previously described (6). The genotypic

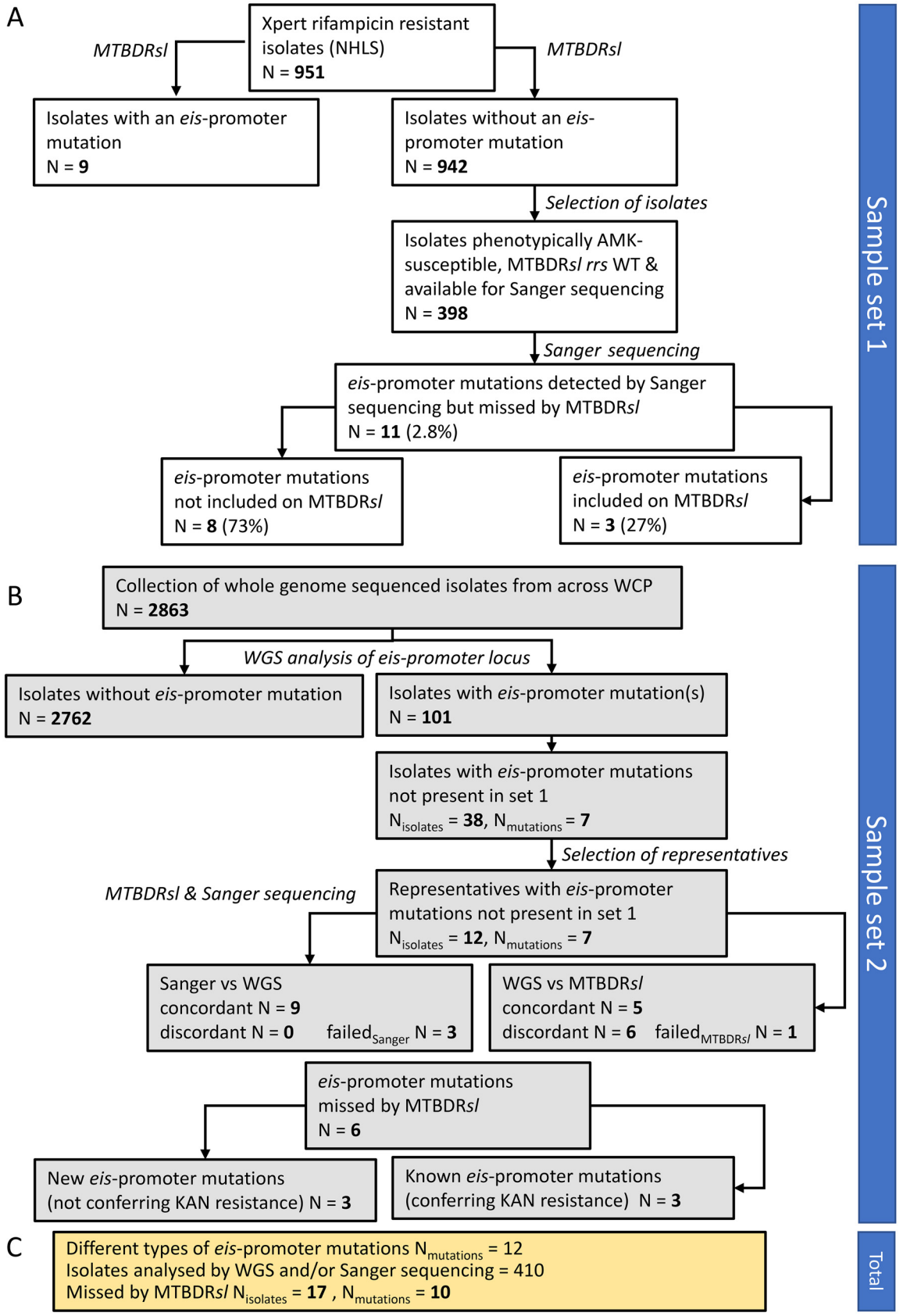

FIG 1 Workflow diagram. (A) Workflow and number of isolates included in each step for sample set 1. (B) Workflow and number of isolates included in each step for sample set 2. (C) Total number of *eis* promoter mutations detected and missed by routine MTBDR*sl* across both sample sets. NHLS, National Health Laboratory Services; WCP, Western Cape Province; WGS, whole-genome sequencing.

drug resistance profile of each isolate was determined using markers defined by Miotto et al. and Coll et al. (14, 15). Raw sequencing reads of the isolates listed in Tables 1 and 2 have been deposited at the European Nucleotide Archive (ENA accession no. PRJEB41458). Additional details for all methods are described in Supplemental File 1.

In sample set 1, *eis* promoter mutations were detected in 9/951 (0.95%) isolates by MTBDR*sl*. These isolates were phenotypically AMK susceptible with no *rrs* 1401 mutation (Table 3). From the 951 isolates, 398 were phenotypically AMK susceptible, *eis* promoter, and *rrs* WT, based on the MTBDR*sl*, and available in the biobank (Fig. 2). Sanger sequencing revealed that 11/398 (2.8%) isolates classified as *eis* promoter and *rrs* WT by routine diagnostics harbored at least one *eis* promoter mutation (Table 4). Three of those 11 carried the known KAN resistance markers 12 C > T and −10 G > A and should have been detected by the MTBDR*sl*. As Sanger sequencing revealed no heteroresistance for these isolates, it is unlikely that MTBDR*sl* missed this mutation because of the detection limit. Two of the three were phenotypically resistant to KAN (Table 4). The failure to detect these mutations therefore falsely classified the isolates as KAN susceptible, impacting the patient's treatment options. The third isolate was phenotypically susceptible to KAN despite carrying an *eis* promoter mutation, −12 C > T. Previous studies also reported variable KAN pDST results for this mutation, including KAN susceptibility (2, 16–19). The *eis* promoter mutations of the remaining eight isolates could potentially have been detected through failing WT bands but were missed by the MTBDR*sl*. These mutations are either considered not to confer KAN resistance ($n = 4$; *eis* promoter mutation, −10 G > C) or undescribed ($n = 4$; *eis* promoter mutations, −50 T > C and −100 C > T) (Table 4). The latter are unlikely to affect the transcription of the *eis* gene, as they are located upstream of the usual promoter area. Since none of these mutations elevated the KAN MIC, patient treatment should not have been affected despite undetected mutations.

The screening of 2,863 WGS of clinical *M. tuberculosis* isolates (sample set 2) identified 101 isolates from 69 patients that carried at least one mutation in the *eis* promoter region (Tables S1 and S2). Seven mutations (−6 G > A, −8 C > A, −14 C > T, −15 G > A, −32 C > T, −37 G > T, −104 G > A) were not present in sample set 1. The mutations −6 G > A, −32 C > T, and −104 G > A were previously undescribed. More in-depth analyses of 12 representative isolates revealed that 6 (50%) were wrongly classified as *eis* promoter WT by the MTBDR*sl*, 4 with *eis* promoter mutations not included in the MTBDR*sl* and 2 isolates with mutations detectable by MTBDR*sl* (−37 G > T; −10 G > A and −15 C > G) (Tables 1 and 2). The reasons for the failure of detecting these mutations remain unclear. However, the assay failed to detect the −10 G > A mutation when in combination with −15 C > G in all four isolates with that *eis* promoter combination (Table S2), even when the majority of the WGS reads belonged to the *M. tuberculosis* subpopulation with the −10 G > A mutation (i.e., 63% of reads versus 36%; Tables 1 and 2). It is therefore unlikely that the mutant subpopulation was missed due to the detection limit of the assay. As all other isolates with different combinations of *eis* promoter mutations were correctly identified as mutant, the presence of more than one SNV in the same isolate does not generally seem to affect the assay's performance. For one isolate with three *eis* promoter mutations (−12 C > T, −14 C > T, and −37 G > T), MTBDR*sl* correctly identified all mutations, but the result would not have been properly interpretable without the additional information of WGS and Sanger sequencing.

The phenotypic and genotypic results were partially discrepant (Tables 1 and 2): three of six isolates misclassified as WT carried an *eis* promoter mutation known to confer low-level KAN resistance (−8 C > A, −37 G > T, and −10 G > A) and were thereby falsely classified as KAN susceptible. At the time these isolates were collected, the routine diagnostic algorithm did not yet include MTBDR*sl* but only pDST. All three isolates were phenotypically AMK resistant, which, following the national treatment guidelines, would have led to the exclusion of KAN from the treatment regimen for those patients. An isolate with *eis* promoter mutation −32 C > T was phenotypically KAN susceptible, yet intermediate growth (<1%) was observed at all drug concentrations measured (1.25, 2.5, 5.0, and 10.0 μg/ml). The latter is usually an indication of heteroresistance with an underlying resistant *M. tuberculosis*

TABLE 1 Genotypic drug susceptibility testing results of selected representative isolates[b]

Patient	Isolate no.	Collection yr	WGS *eis* promoter mutation (% of reads)	WGS *rrs* 1401 mutation (% of reads)	Sanger sequencing *eis* promoter	MTBDR*sl* result *eis* promoter	MTBDR*sl eis* result interpretation	MTBDR*sl* result *rrs*	MTBDR*sl rrs* result interpretation	WGS vs MTBDR*sl eis* promoter	Lineage
P3	WGS-3	2009	−6 G > A (17)	WT	−6 G > K	All WT bands present, no MUT band	WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Discrepant (not detectable by *sl*)	2.2
P14	WGS-22	2007	−32 C > T (91)	WT	−32 C > Y	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	4.1.1.3
	WGS-22	2007	NA	NA	No *eis* promoter mutation detected (additional Sanger sequencing of *rrs* locus found *rrs* 1401A > G)	All WT bands present, no MUT band	WT	WT1 missing, MUT1 present	*rrs* 1401 A > G	NA	NA
P17	WGS-26	2008	−10 G > A (63) and −15 C > G (36)	WT	−10 G > R and −15 C > S	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (detectable by *sl*)	2.2
P19	WGS-30	2009	−14 C > T (17)	*rrs* 1401 A > G (21)	Failed	Failed	NA	Failed	NA	NA	2.2
P27	WGS-48	2014	−14 C > T (67)	WT	−14 C > T	WT2 missing, MUT1 present	*eis* promoter mutation −14	WT1 and WT2 present, no MUT band	WT	Concordant	4.1.1.3
P29	WGS-50	2010	−37 G > T (22)	*rrs* 1401 A > G (35)	−37 G > K	All WT bands present, no MUT band	WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Discrepant (detectable by *sl*)	2.2
P31	WGS-54	2010	−12 C > T (18) and −14 C > T (9)[a] and −37 G > T (60)	WT	−12 C > Y and −14 C > Y[a] and −37 G > K	WT3 present; WT1 and WT2 weakly present, MUT present	Heteroresistance, *eis* promoter mutations −12 or −10, −14, −37 and WT mixed (not interpretable without additional information)	WT1 and WT2 present, no MUT band	WT	Concordant	4.9
P34	WGS-58	2012	−14 C > T (11) and −37 G > T (11)	*rrs* 1401 A > G (53)	−14 C > Y[a] and −37 G > K[a]	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P40	WGS-71	2012	−14 C > T (45)	*rrs* 1401 A > G (8)	−14 C > Y	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P40	WGS-72	2012	−14 C > T (39) and −10 G > A (12)	*rrs* 1401 A > G (41)	Failed	All WT bands present and MUT band present	Heteroresistance, *eis* promoter mutation −14 and WT	WT1 (weak) and WT2 present, MUT1 present	Heteroresistance, *rrs* 1401 A > G and WT	Concordant	2.2
P47	WGS-79	2015	−8 C > A (93)	WT	Failed	All WT bands present and MUT band present	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	2.2
P65	WGS-97	2015	−104 G > A (62)	WT	−104 G > R	All WT bands present, no MUT band	WT	WT1 and WT2 present, no MUT band	WT	Discrepant (not detectable by *sl*)	4.9
Summary				7/12 WT, 5/12 *rrs* 1401 A > G and WT	9/12 confirmed WGS, 3/12 failed	6/12 WT, 1/12 *eis* promoter −14 MUT, 3/12 *eis* promoter −14 MUT and WT, 1/12 combination of *eis* promoter mutations, 1/12 NA		5/12 WT, 5/12 *rrs* 1401 and WT, 1/12 1× WT, 1× *rrs* 1401 (P14), 1/12 NA		5/12 concordant, 4/12 discrepant (not detectable by *sl*), 2/12 discrepant (detectable by *sl*), 1/12 no result	8/12 lineage 2.2, 2/12 lineage 4.9, 2/12 lineage 4.1.1.3

[a]Difficult to distinguish from background noise.

[b]Changes in *rrs* or *eis* promoter are indicated as nucleotide changes using the IUPAC nucleotide code. WGS, whole-genome sequencing; *sl*, MTBDR*sl* assay; WT, wild type; MUT, mutant; NA, not applicable.

TABLE 2 Phenotypic drug susceptibility testing results of selected representative isolates[b]

Patient	Isolate No.	Collection yr	Amikacin pDST result (routine diagnostics)	gDST vs pDST (routine diagnostics) of AMK resistance	Amikacin pDST result (repeat by SU)	Kanamycin pDST result (SU)	Kanamycin MIC (μg/ml)
P3	WGS-3	2009	S	Discrepant	ND	Failed to regrow	NA
P14	WGS-22	2007	R	Discrepant	R	S	1.25 and intermediate growth at all measured concentrations
	WGS-22	2007	R[a]	Concordant[a]	ND	R	>10
P17	WGS-26	2008	R	Discrepant	S	R	>10
P19	WGS-30	2009	R	Concordant	R	R	>10
P27	WGS-48	2014	S	Concordant	ND	Failed to regrow	NA
P29	WGS-50	2010	R	Concordant	ND	R	>10
P31	WGS-54	2010	R	Discrepant	ND	R	10
P34	WGS-58	2012	R	Concordant	R	R	>10
P40	WGS-71	2012	R	Concordant	ND	R	>10
P40	WGS-72	2012	S	Concordant	ND	R	>10
P47	WGS-79	2015	R	Discrepant	ND	R	>10
P65	WGS-97	2015	S	Concordant	S	S	2.5
Summary			8/12 R, 4/12 S	7/12 concordant, 5/12 discrepant	7/12 ND, 4/12 confirmed DST at diagnosis, 1/12 discrepant to diagnosis	8/12 R, 2/12 S, 2/12 failed	7/12 > 10, 1/12 10, 1/12 2.5, 1/12 1.25 and intermediate growth, 2/12 failed

[a]Result from the original diagnostic isolate.

[b]Changes in *rrs* or *eis* promoter are indicated as nucleotide changes using the IUPAC nucleotide code. SU, Stellenbosch University; WGS, whole-genome sequencing; gDST, genotypic drug susceptibility testing; pDST, phenotypic drug susceptibility testing; AMK, amikacin; R, resistant; S, susceptible; ND, not done; NA, not applicable.

TABLE 3 *eis* promoter mutations detected in sample set 1 by the MTBDR*sl* assay as part of routine diagnostics[a]

Patient	Isolate	MTBDR*sl* result *eis* promoter (banding pattern)	*eis* promoter mutation	AMK pDST result
Pa-1	NHLS-1	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-2	NHLS-2	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-3	NHLS-3	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-4	NHLS-4	WT2 and MUT1 missing	−10 G > A or −12 C > T	S
Pa-5	NHLS-5	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-6	NHLS-6	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-7	NHLS-7	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-8	NHLS-8	WT1-3 and MUT1 present	WT and −14 C > T mixed	S
Pa-9	NHLS-9	WT1-3 and MUT1 present	WT and −14 C > T mixed	S

[a]WT, wild type; pDST, phenotypic drug susceptibility testing; AMK, amikacin; S, susceptible; MUT, mutation.

subpopulation. However, the *eis* promoter mutant subpopulation was found to be the dominant subpopulation by both WGS (*eis* promoter mutation, −32 C > T in 91% of reads) and Sanger sequencing, indicating that the −32 C > T mutation may not be the reason for the intermediate growth under KAN pressure. For this isolate, additional pDST under KAN pressure was conducted, and subsequent MTBDR*sl* and Sanger sequencing revealed the *rrs* 1401 mutation but not the −32 C > T *eis* promoter mutation as being present in this subpopulation. Phenotypic DST for this isolate showed high KAN resistance (MIC > 10 μg/ml). This subpopulation had been present in a concentration below the detection limit of the pDST (1%) in the original culture but is clinically relevant, as treatment with KAN could have failed due to high-level KAN resistance (20).

In addition to the *eis* promoter mutations, the presence of the *rrs* 1401 mutation was investigated (Tables 1 and 2). Phenotypic DST revealed AMK resistance in 8/12 isolates at diagnosis, but for only 4/8, the genotypic marker *rrs* 1401 was detected by MTBDR*sl* and/or WGS. For two isolates with no *rrs* 1401 mutation, pDST was repeated, confirming

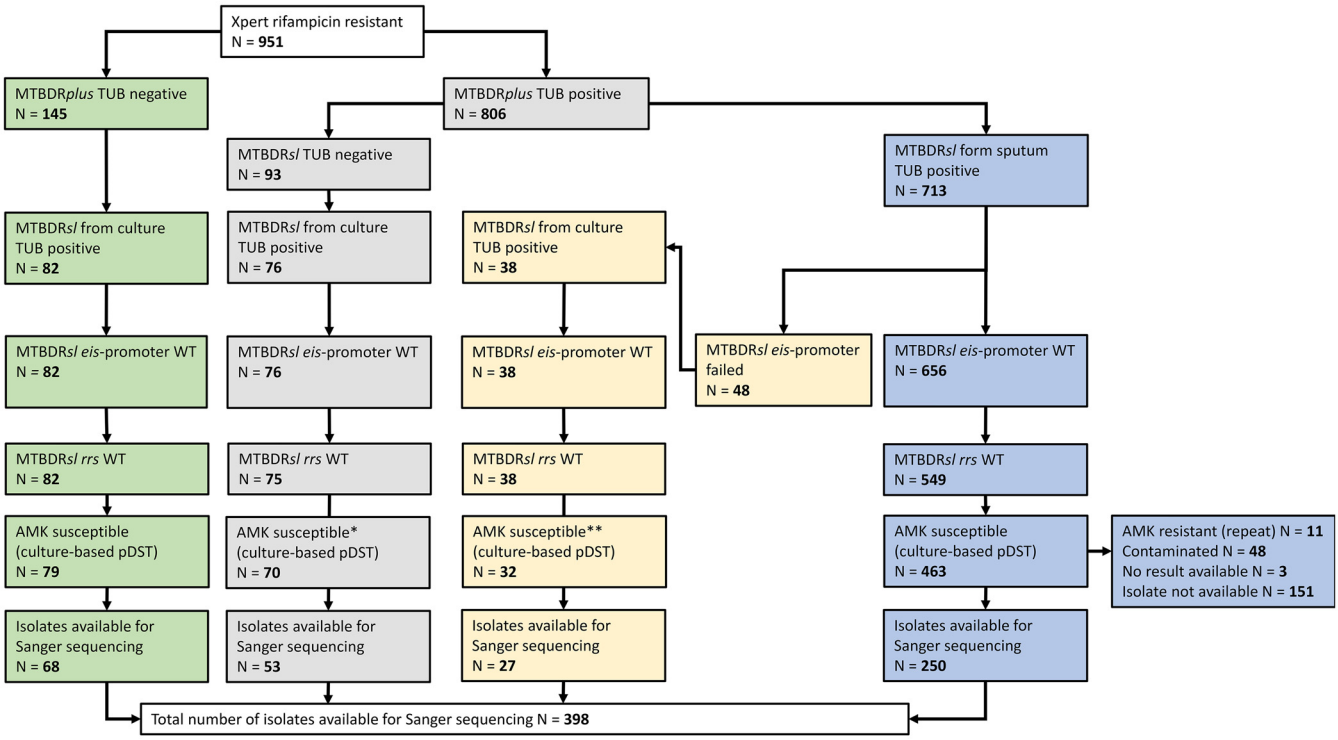

FIG 2 Flowchart describing the sample selection for sample set 1. WT, wild type; TUB, tuberculosis control band of the assay; AMK, amikacin; pDST, phenotypic drug susceptibility testing. *, the remaining 5 cultures were contaminated and pDST could therefore not be performed; **, the remaining 6 cultures were contaminated, and pDST could therefore not be performed.

TABLE 4 *eis* promoter mutations and kanamycin MICs of isolates diagnosed as *eis* promoter wild type by the MTBDR*sl* assay[b]

Patient	Isolate	*eis* promoter mutation	Detectable by MTBDR*sl*[a]	AMK pDST result	KAN pDST result	KAN MIC (ug/ml)
Pa-10	NHLS-10	−10 G > C	No	S	S	2.5
Pa-11	NHLS-11	−12 C > T	Yes	S	S	2.5
Pa-12	NHLS-12	−100 C > T	No	S	S	2.5
Pa-13	NHLS-13	−10 G > C	No	S	S	2.5
Pa-14	NHLS-14	−10 G > C	No	S	S	2.5
Pa-15	NHLS-15	−50 T > C	No	S	S	2.5
Pa-16	NHLS-16	−100 C > T	No	S	S	2.5
Pa-17	NHLS-17	−12 C > T	Yes	S	R	5
Pa-18	NHLS-18	−10 G > C	No	S	Failed regrowth	Failed regrowth
Pa-19	NHLS-19	−10 G > A	Yes	S	R	10
Pa-20	NHLS-20	−100 C > Y	No	S	Failed regrowth	Failed regrowth

[a]"Detectable by MTBDR*sl*" refers to those mutations for which the MTBDR*sl* provides specific banding patterns (see text).
[b]MICs are reported as the lowest concentration tested at which no growth was observed; however, the MIC can be lower than the reported number. S, susceptible; R, resistant; AMK, amikacin; KAN, kanamycin.

the phenotypic resistance for one isolate, whereas the other was phenotypically susceptible, matching the genotypic results. Genotypic and phenotypic results correlated for 3/4 isolates that were typed AMK susceptible at diagnosis, but for 1, MTBDR*sl* detected heteroresistance (i.e., WT and *rrs* 1401 present). WGS, however, did not detect the *rrs* 1401 mutation, suggesting a false-positive MTBDR*sl* result (Tables 1 and 2).

This study used comprehensive data sets that nevertheless bare limitations (for a more comprehensive discussion of the limitations, see Supplemental File 1). Not all isolates of set 1 were available for Sanger sequencing; the proportion of missed *eis* promoter mutations could therefore be higher. Despite analyzing data collected over 25 years, no conclusions about the prevalence of *eis* promoter mutations across that period can be drawn, as sample set 2 was a convenience sample from several studies. All WGS isolates were screened for *eis* promoter mutations, but only representatives were further analyzed. However, in combination, our data provide insights on the type and frequency of *eis* promoter mutations present in South Africa and reflect the complexity of antibiotic resistance in *M. tuberculosis*. Our results indicate the most reliable option for comprehensive individual DST to be a combination of genotypic methods, including (targeted) WGS and the phenotypic analysis of consecutively collected isolates of a patient. This reduces the limitations of current diagnostic algorithms and allows adaptation to newly emerging resistance markers (5, 21) but remains an unaffordable option for low- and middle-income countries where most tuberculosis (TB) cases occur. With more and less expensive WGS-based tools becoming available, targeted use of this strategy for severe cases could nevertheless be implemented (22).

The prevalence of *eis* promoter mutations detected in routine surveillance data and the proportion of missed low-level KAN resistance were low in this setting but nevertheless represent a potential cause of treatment failure. WHO released new tuberculosis treatment guidelines in 2019, no longer recommending the use of KAN (23). However, some *eis* promoter mutations (e.g., −14 C > T) also cause low-level resistance to AMK, which remains part of the WHO-recommended treatment guidelines. More importantly, though, many countries may not be able to timely implement the new treatment recommendations and will continue using AMK or KAN (23, 24). It therefore remains important to continue monitoring the prevalence of *eis* promoter mutations in circulating *M. tuberculosis* to preserve as many treatment options as possible.

Ethics. This study was designed and carried out in accordance with relevant guidelines and regulations. It was reviewed and approved by the Health Research Ethics Committee of Stellenbosch University (HREC) and the Western Cape Province Department of Health.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, DOCX file, 0.06 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.01 MB.

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Hain Lifesciences donated consumables used to generate some of the data analyzed; however, Hain Lifesciences had no say in the results or interpretation.

We declare no conflict of interest.

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