Mycobacterium tuberculosis Genetic Drug Susceptibility Testing Using Whole Genome Sequencing

TROPICAL DISEASES RESEARCH CENTRE

Presenter:
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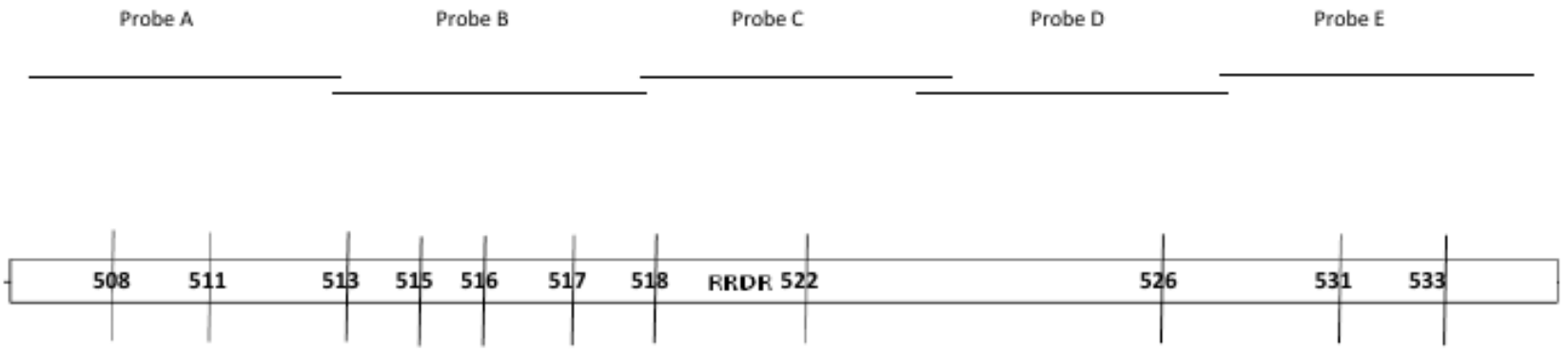
Scientific Officer

Introduction

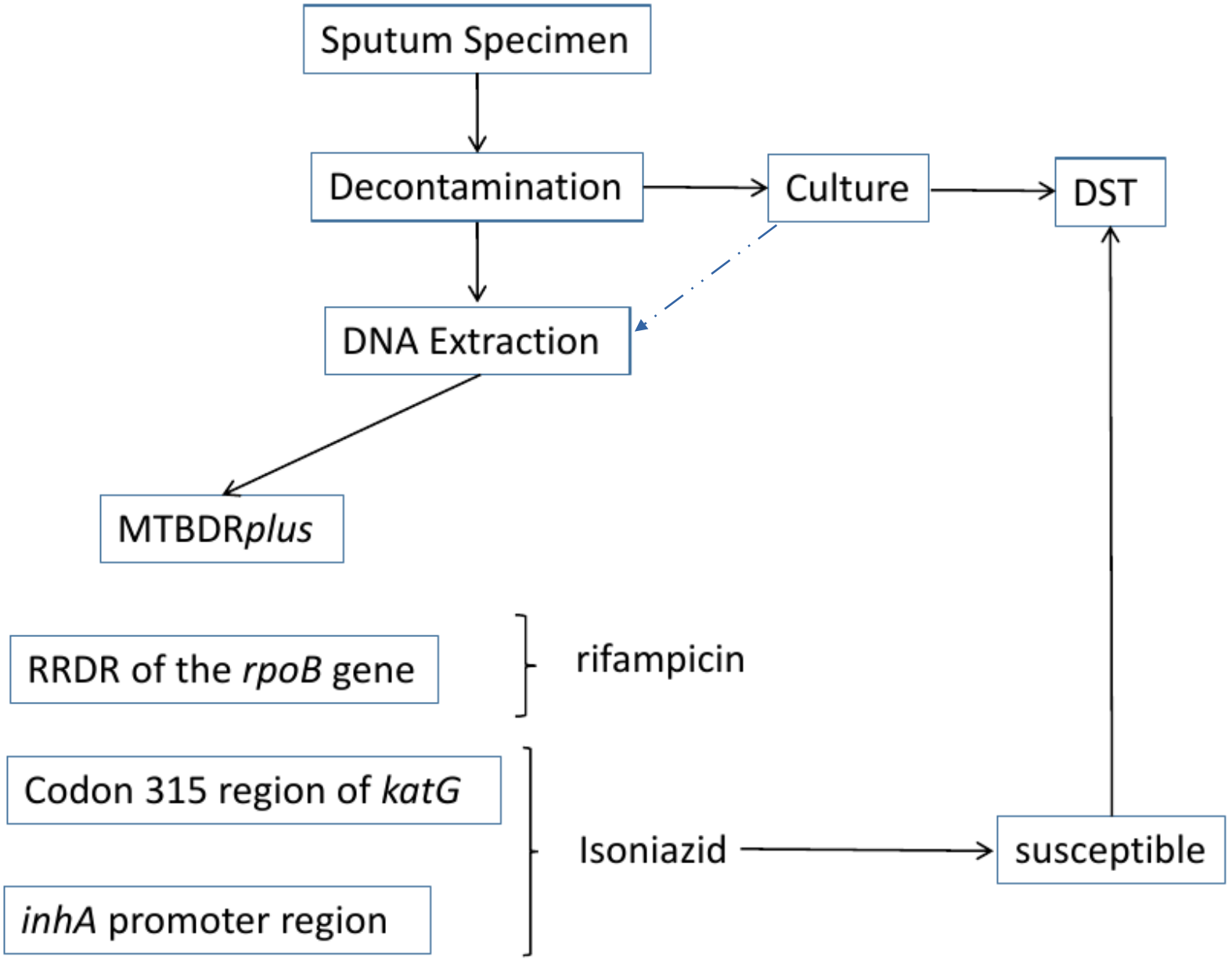
- GeneXpert instruments utilised diagnose TB and rifampicin resistant
- TDRC refereral lab for Rif Resistant isolates
- DST done to confirm INH and RIF resistance on Line Probe Assay and MGIT (Phenotypic DST)
- Molecular DST for 2 line drugs used to treat drug resistant TB limited to fluoroquinolines

- **New and repurposed drugs not covered!!!**

What does an Xpert Rif resistant result mean?



Routine isoniazid drug susceptibility testing

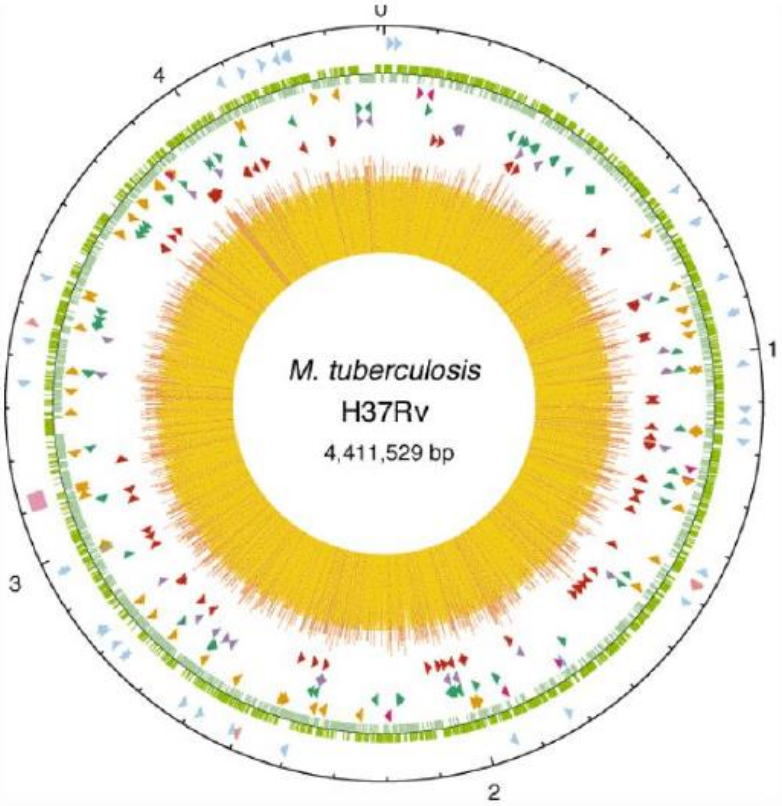


Disputed *rpoB* mutations

	rpoB codon	*rpoB* base change	No of isolates	RIF MIC range	RBT MIC range	Xpert
1	WT	WT	27	<1 - <100	<0.125	NONE
2	508	6bp deletion	2	<1	<0.125	Probe A
3	511	T>C	14	<1 - >1	<0.125	Probe A
4	513	6bp deletion	2	>100		
5	513	C>A	3	>20 - >100	>2	Probe B
6	513	A>C	2	>50 - >100	0.5 – 1.0	Probe B
7	513	A>T	2	>50 - >100	>2	Probe B
8	515	6bp deletion	2	>100	>2	Probe B
9	516	3bp deletion	4	>1 - <100	0.25 – 0.5	Probe B
10	516	G>T	15	<1 - <100	<0.125 – 1.0	Probe B
11	516	GA>TG	1	<1	<0.125	Probe B
12	516	GA>TT	1	>1 <5	<0.125	Probe B
13	516	A>C	2	<1	<0.125	MISSED??
14	516	A>T	81	>1 - <100	<0.125 - >2	Probe B
15	517	3bp deletion	3	<1 - <10	<0.125	Mixed B + C
16	518	3bp deletion	1	>1 <5	<0.125	Probe B
17	522	C>T	2	>100	0.125	Probe C
18	526	C>A	3	<1	<0.125	Probe D
19	526	C>G	19	>50 - >100	>2	Probe D
20	526	C>T	13	>50 - >100	>2	Probe D
21	526	CA>TG	1	>1 <5	<0.125	Probe D
22	526	A>G	3	>20 - >100	>2	Probe D
23	526	A>T	4	<1 - <10	<0.125 - <2	Probe D
24	531	C>G	4	>100		
25	531	C>T	146	<1 - <100	0.125 - >2	Probe E
26	531	CG>AC	1	>100		
27	531	CG>TT	2	>100	>2	Probe E
28	533	T>C	10	<1 - <50	<0.125 – 1.0	Probe E
29	511 + 516	T>G + G>T	1	>5 <10	>1<2	Probe A only
30	516 + 529	G>A + G>T	1	>100		
31	513 + 516	C>G + A>T	1	>100	>2	Probe B only
32	512 + 516	C>G + A>G	1	>1 <5	<0.125	Probe B only
33	511 + 526	T>C + C>T	1	>1 <5	<0.125	Probe A only

The Road Map

4,411,529 base pairs
4,000 genes



The outer circle shows the scale in Mb, with 0 representing the origin of replication. The first ring from the exterior denotes the positions of stable RNA genes (tRNAs are blue, others are pink) and the direct repeat region (pink cube); the second ring inwards shows the coding sequence by strand (clockwise, dark green; anticlockwise, light green); the third ring depicts repetitive DNA (insertion sequences, orange; 13E12 REP family, dark pink; prophage, blue); the fourth ring shows the positions of the PPE family members (green); the fifth ring shows the PE family members (purple, excluding PGRS); and the sixth ring shows the positions of the PGRSsequences (dark red). The histogram (centre) represents G + C content, with <65% G + C in yellow, and >65% G + C in red. The figure was generated with software from DNASTAR.

Two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.

Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence
Nature **393**, 537-544 (11 June 1998)

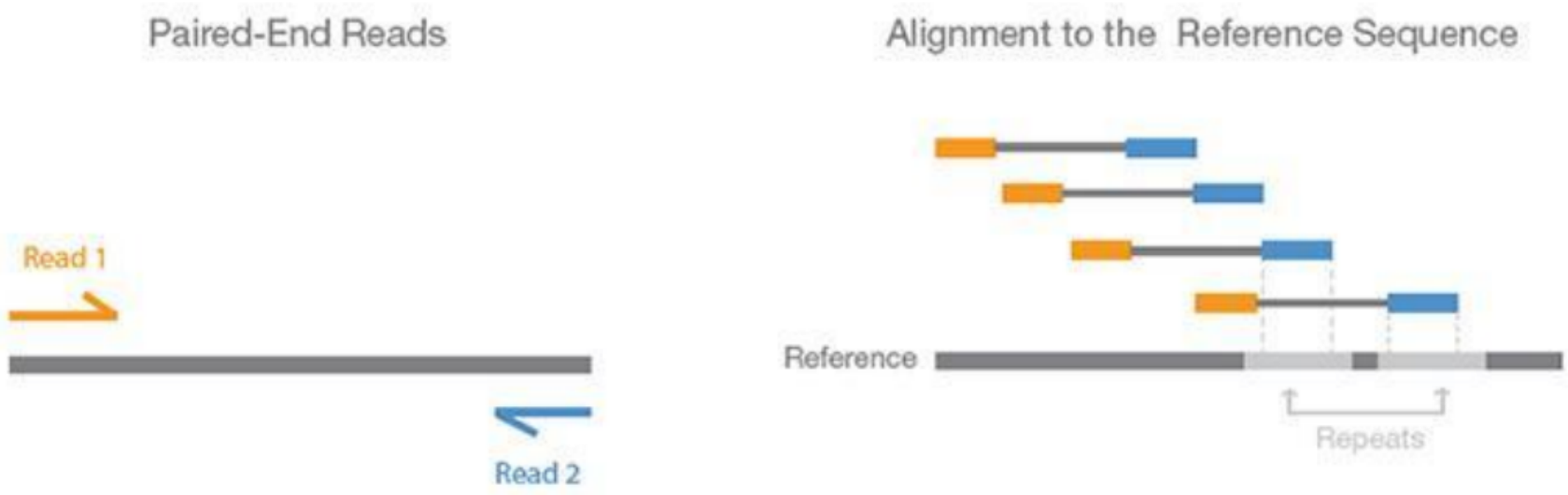
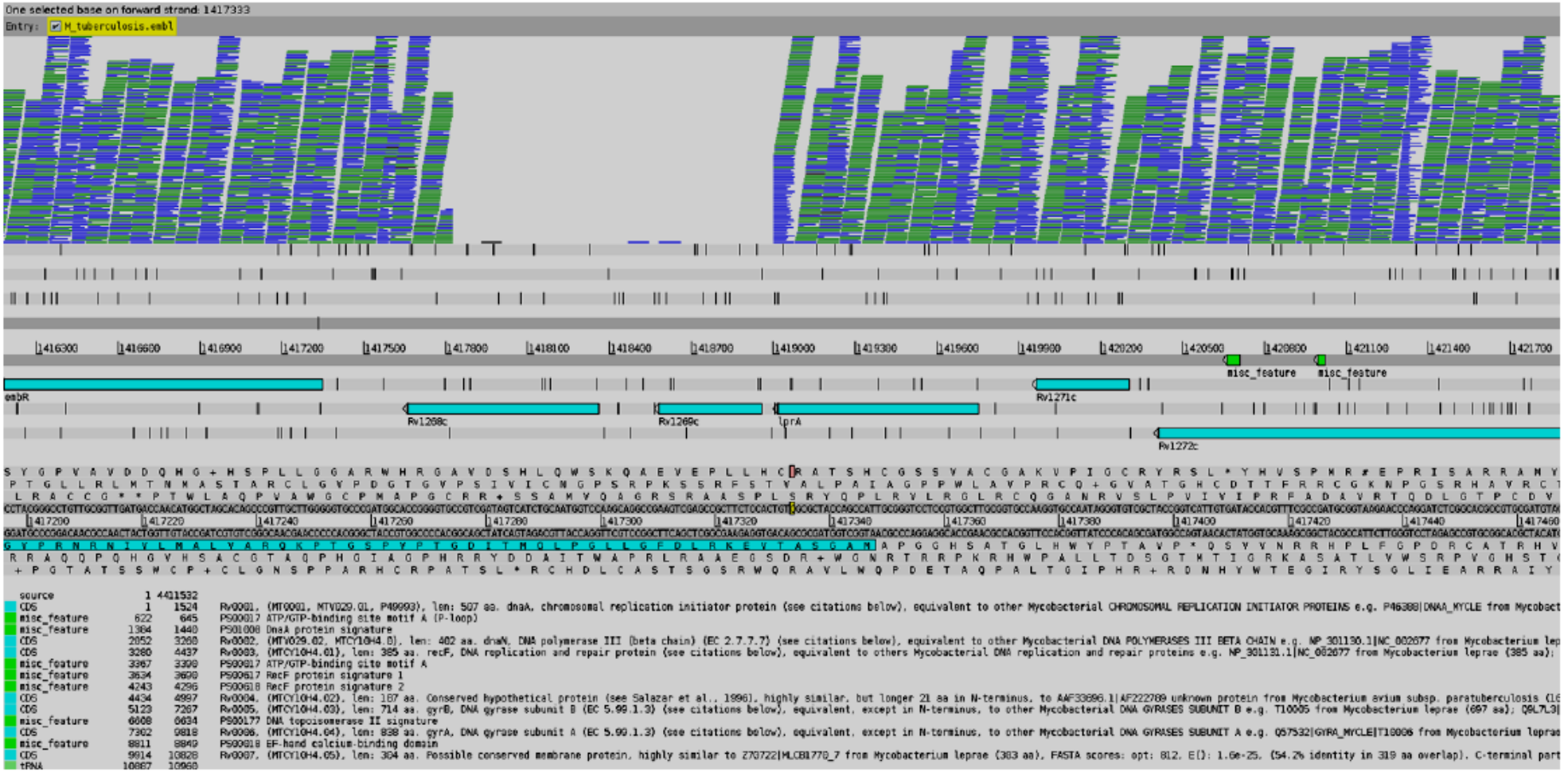
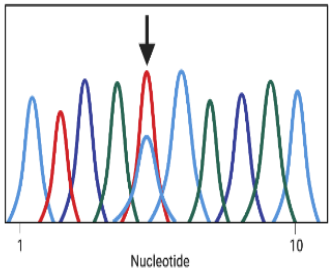


Figure 2.2: Paired-End Sequencing and Alignment: Paired-end sequencing enables both ends of the DNA fragment to be sequenced. Because the distance between each paired read is known, alignment algorithms can use this information to map reads over repetitive regions more precisely. This results in much better alignment of reads, especially across difficult to sequence, repetitive regions of genome (http://www.illumina.com/technology/next-generation-sequencing/paired-end-sequencing_assay.html).


One selected base on forward strand: 1417333
Entry: M_tuberculosis.embl
misc_feature
misc_feature
embR
Rv1271c
Rv1268c
Rv1269c
lprA
Rv1272c
source 1 4411532
CDS 1 1524 Rv0001, (MTV029.01, P49993), len: 507 aa. dnaA, chromosomal replication initiator protein (see citations below), equivalent to other Mycobacterial CHROMOSOMAL REPLICATION INITIATOR PROTEINS e.g. P46388|DNAA_MYCLE from Mycobact
misc_feature 622 645 PS00017 ATP/GTP-binding site motif A (P-loop)
misc_feature 1384 1440 PS01008 DnaA protein signature
CDS 2052 3268 Rv0002, (MTV029.02, MTCY10H4.01), len: 402 aa. dnaN, DNA polymerase III (beta chain) (EC 2.7.7.7) (see citations below), equivalent to other Mycobacterial DNA POLYMERASES III BETA CHAIN e.g. NP_301130.1|NC_002677 from Mycobacterium lep
CDS 3280 4437 Rv0003, (MTCY10H4.01), len: 385 aa. recF, DNA replication and repair protein (see citations below), equivalent to others Mycobacterial DNA replication and repair proteins e.g. NP_301131.1|NC_002677 from Mycobacterium leprae (385 aa);
misc_feature 3367 3390 PS00017 ATP/GTP-binding site motif A
misc_feature 3634 3690 PS00617 RecF protein signature 1
misc_feature 4243 4296 PS00618 RecF protein signature 2
CDS 4434 4997 Rv0004, (MTCY10H4.02), len: 187 aa. Conserved hypothetical protein (see Salazar et al., 1996), highly similar, but longer 21 aa in N-terminus, to AAF33896.1|AF222789 unknown protein from Mycobacterium avium subsp. paratuberculosis (16
CDS 5123 7267 Rv0005, (MTCY10H4.03), len: 714 aa. gyrB, DNA gyrase subunit B (EC 5.99.1.3) (see citations below), equivalent, except in N-terminus, to other Mycobacterial DNA GYRASES SUBUNIT B e.g. T10005 from Mycobacterium leprae (697 aa); Q6L7L3|
misc_feature 6008 6034 PS00177 DNA topoisomerase II signature
CDS 7302 9818 Rv0006, (MTCY10H4.04), len: 838 aa. gyrA, DNA gyrase subunit A (EC 5.99.1.3) (see citations below), equivalent, except in N-terminus, to other Mycobacterial DNA GYRASES SUBUNIT A e.g. Q57532|GYRA_MYCLE|T10006 from Mycobacterium leprae
misc_feature 8811 8849 PS00018 EF-hand calcium-binding domain
CDS 9914 10828 Rv0007, (MTCY10H4.05), len: 304 aa. Possible conserved membrane protein, highly similar to Z70722|MLCB1770_7 from Mycobacterium leprae (303 aa), FASTA scores: opt: 812, E(): 1.6e-25, (54.2% identity in 319 aa overlap). C-terminal part
tRNA 10887 10960

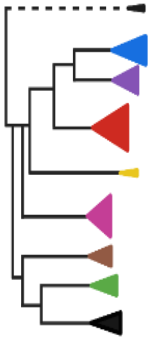
Drug resistance

Heteroresistance

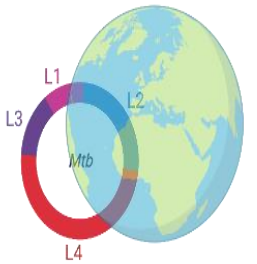


Outbreak detection

Epidemiology



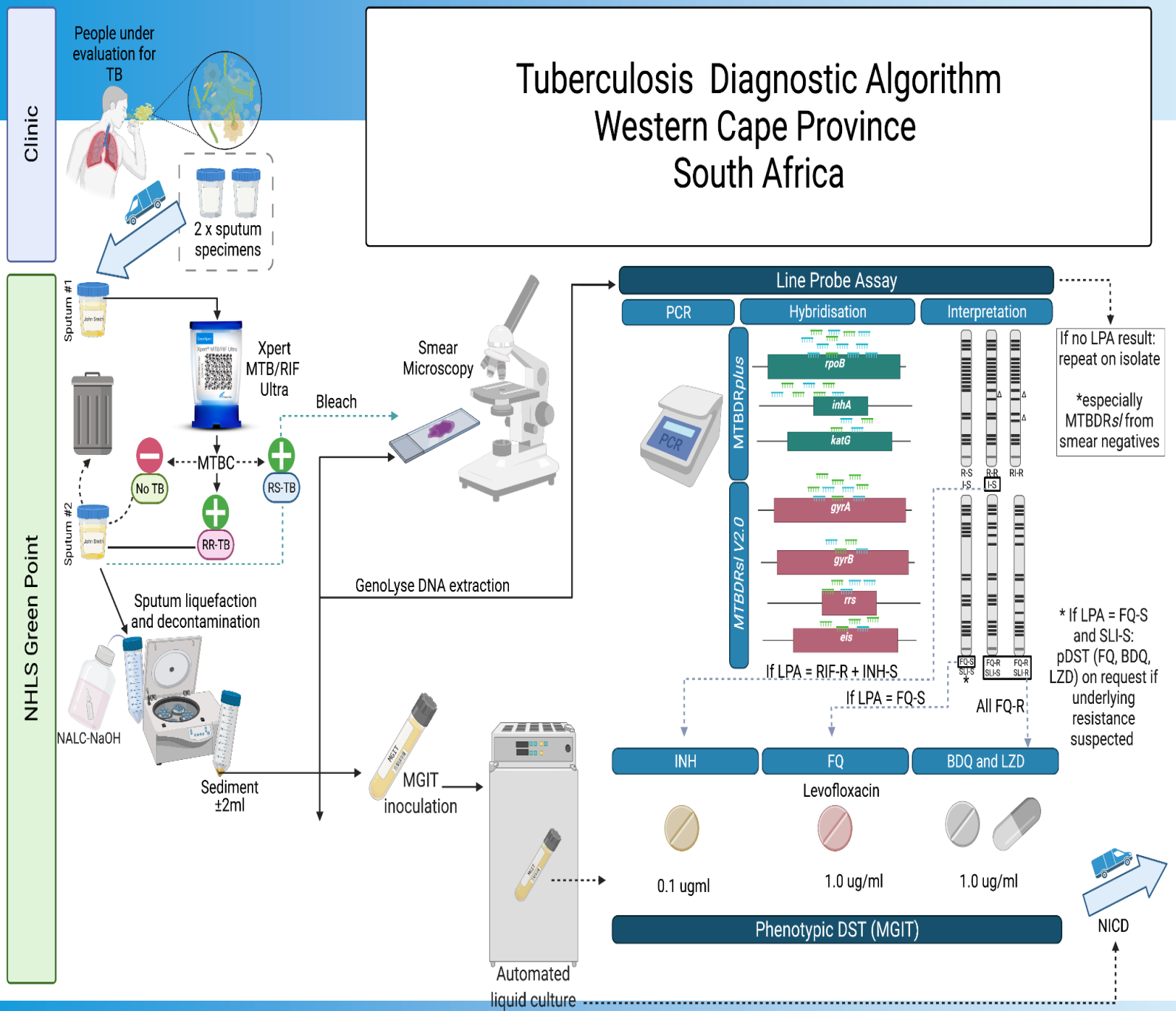
Surveillance



Mycobacterium tuberculosis

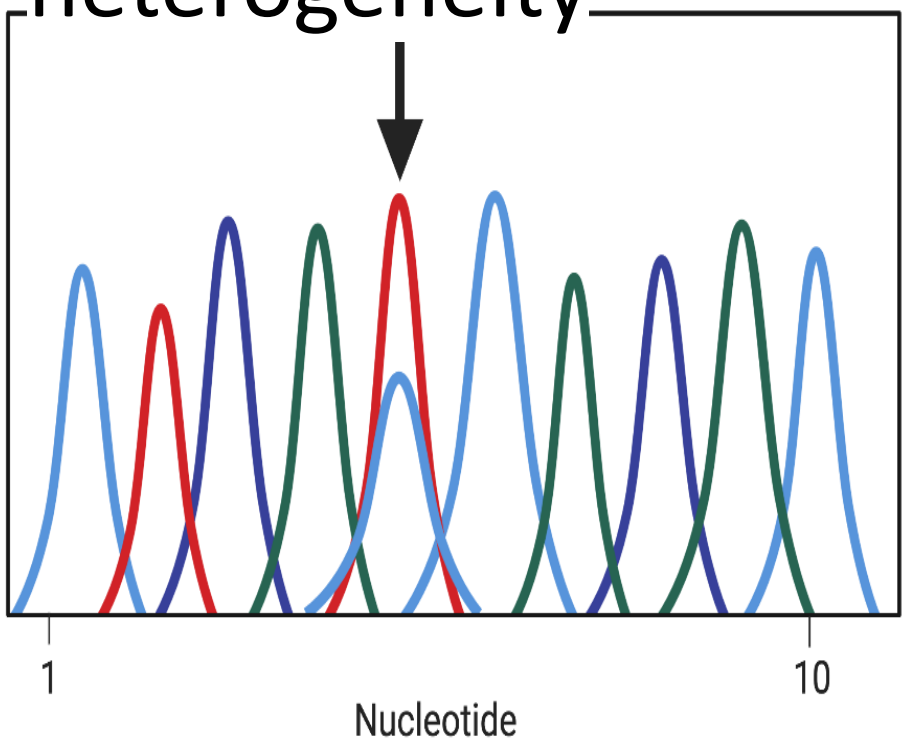
Drug resistance

Determining the drug susceptibility profiles of disease causing organisms based on known genotype-phenotype associations



- PZA not tested
- Ethambutol not tested
- New drugs - not always tested

Population heterogeneity



the quality or state of being diverse in character or content

A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*

Paolo Miotto, Belay Tessema, Elisa Tagliani, Leonid Chindelevitch, Angela M. Starks, Claudia Emerson, Debra Hanna, Peter S. Kim, Richard Liwski, Matteo Zignol, Christopher Gilpin, Stefan Niemann, Claudia M. Denkinger, Joy Fleming, Robin M. Warren, Derrick Crook, James Posey, Sebastien Gagneux, Sven Hoffner, Camilla Rodrigues, Iñaki Comas, David M. Engelthaler, Megan Murray, David Alland, Leen Rigouts, Christoph Lange, Keertan Dheda, Rumina Hasan, Uma Devi K. Ranganathan, Ruth McNerney, Matthew Ezewudo, Daniela M. Cirillo, Marco Schito, Claudio U. Köser, Timothy C. Rodwell

European Respiratory Journal 2017 50: 1701354; **DOI:** 10.1183/13993003.01354-2017

TABLE 2

Overview of proposed confidence levels for grading mutations associated with phenotypic resistance

	Symbol	LR⁺ and OR	
		p-value	value
High (Hi) confidence for association with resistance Strong association of the mutation with phenotypic drug resistance; sufficient evidence that the mutation confers or is strongly associated with drug resistance	#-	<0.05	>10
Moderate (Mo) confidence for association with resistance Moderate association of the mutation with phenotypic drug resistance; additional data desirable for improved evidence that the mutation confers or is strongly associated with drug resistance	#-	<0.05	5< ... ≤10
Minimal (Mi) confidence for association with resistance Weak association of the mutation with phenotypic drug resistance; inconclusive evidence that the mutation confers or is strongly associated with drug resistance. Substantial additional data required	#-	<0.05	1< ... ≤5
No association with resistance No evidence of association between the mutation and drug resistance	#-	<0.05	≤1
Indeterminate No statistically significant threshold reached; additional data required	Indeter	≥0.05	

The table shows the thresholds applied to likelihood ratios (LR) and odds ratios (OR) to grade the association of mutations with phenotypic drug resistance. LR⁺: positive likelihood ratio. "Additional data" is defined as a requirement for 1) more phenotypically drug resistant and susceptible isolates tested with the mutation in question; and/or 2) better understanding of the mechanism of drug resistance (*e.g.* to investigate epistasis, or the interactions between drug-resistance conferring mutations, lineage-specific genetic factors and compensatory mutations [23, 24] or synergistic factors when more than one mutation is required to confer resistance [25]).

Phelan *et al. Genome Medicine* (2019) 11:41
https://doi.org/10.1186/s13073-019-0650-x

Genome Medicine

SOFTWARE Open Access

Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs

Jody E. Phelan[1†], Denise M. O'Sullivan[2†], Diana Machado[3], Jorge Ramos[3], Yaa E. A. Oppong[1], Susana Campino[1], Justin O'Grady[4], Ruth McNerney[5], Martin L. Hibberd[1], Miguel Viveiros[3], Jim F. Huggett[2,6†] and Taane G. Clark[1,3,7*†]

TB-Profiler

- High computing power and storage needed
- 22 drugs can be reported on
- Not all mutations causing resistance have been identified

Genetic variants and their association with phenotypic resistance to bedaquiline in *Mycobacterium tuberculosis*: a systematic review and individual isolate data analysis

Nabila Ismail, Emmanuel Rivière*, Jason Limberis, Stella Huo, John Z Metcalfe, Rob M Warren, Annelies Van Rie*

Summary

Background Bedaquiline is a crucial drug for control of rifampicin-resistant tuberculosis. Molecular drug resistance assays could facilitate effective use of bedaquiline and surveillance of drug resistance emergence. To facilitate molecular assay development, we aimed to identify genomic markers of bedaquiline resistance.

Lancet Microbe 2021; 2: e604–16

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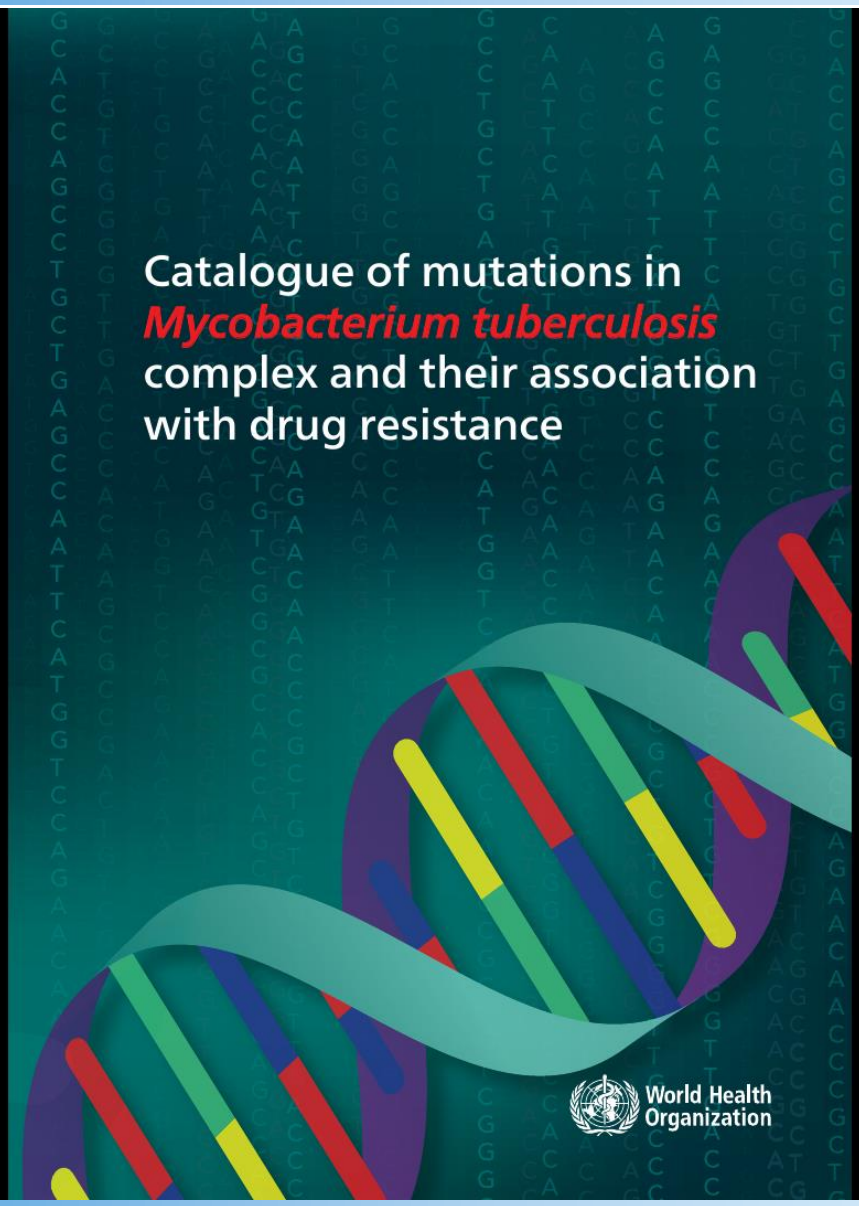
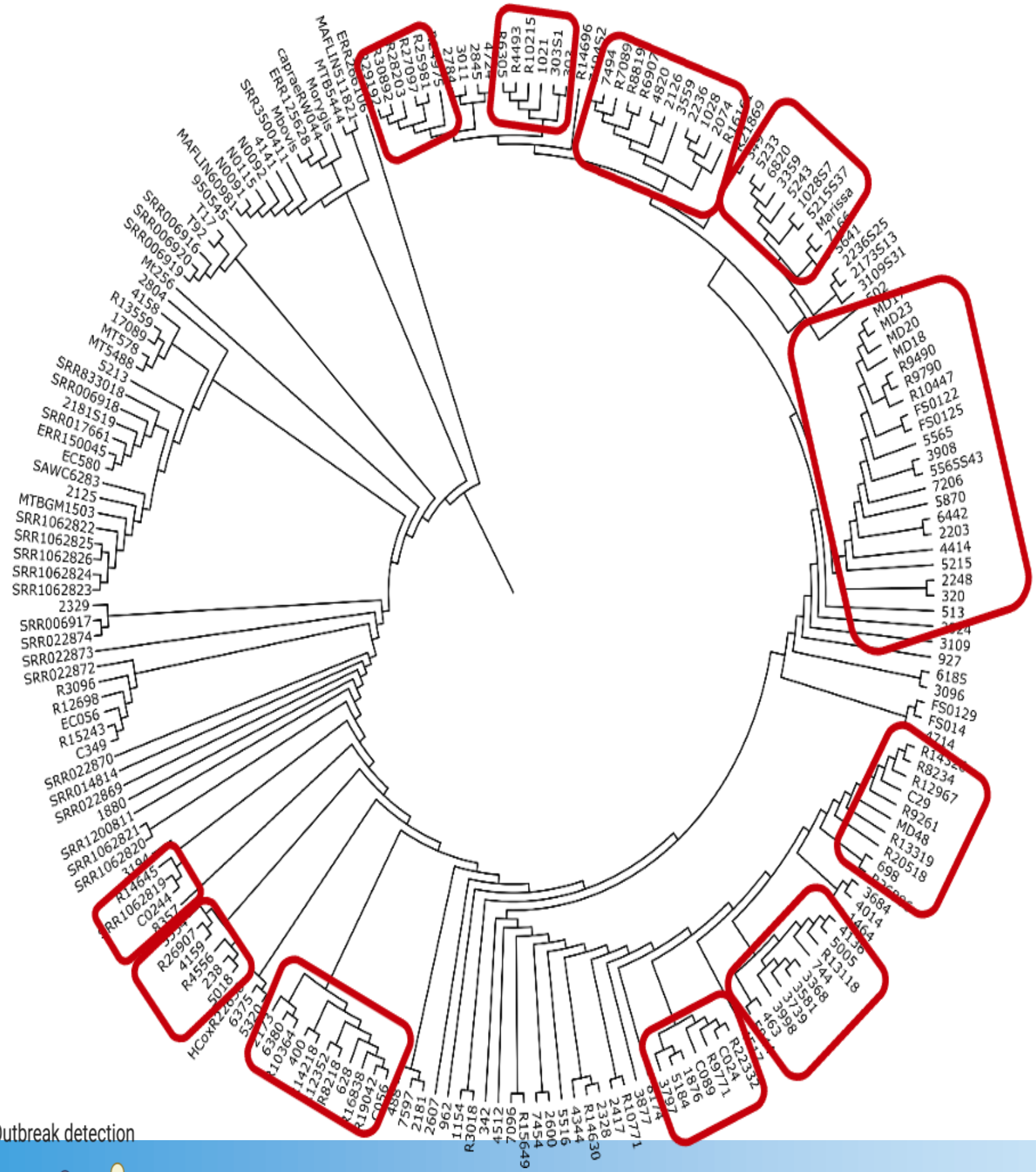

Catalogue of mutations in
Mycobacterium tuberculosis
complex and their association
with drug resistance
World Health
Organization

Linezolid

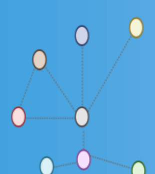
Only *rplC* C154R was found to be a marker for resistance (group 1), resulting in a sensitivity of 38.2% (95% CI, 29.6–47.4%). This may be an underestimate, as the PPV of phenotypic DST is unlikely to be high, with a prevalence of resistance of only 1.1% in this dataset (95% CI, 0.9–1.3%). This finding is consistent with earlier findings that this is the dominant LZD resistance mutation *in vitro* and in clinical isolates *(7)*. In fact, the PPV of this mutation (73%; 95% CI, 61–84%) was comparable to the PPVs of *rrl* g2270t and g2814t, two other well-documented LZD resistance mutations in MTBC and other bacteria (i.e. 70%; 95% CI, 35–94%; and 75%; 95% CI, 48–93%, respectively *(7)*). As resistance mutations in *rrl* were rarer and more diverse, however, the PPV|SOLOs for these *rrl* mutations did not meet the criteria used in this analysis.

Drug	Variant (common name)	MUT present_pheno S	MUT absent_pheno S	MUT present_pheno R	MUT absent_pheno R	SENSITIVITY	SPECIFICITY	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	OR SOLO	INITIAL CONFIDENCE GRADING	SUPPORTING DATASET	ADDITIONAL GRADING CRITERIA	FINAL CONFIDENCE GRADING
LZD	rplC_C154R	17	10878	47	76	38.2%	99.8%	73.4%	71.2%	57.9%	82.2%	375.720	Assoc w R	ALL+WHO		1) Assoc w R
LZD	rrs_c-187t	1122	0	1	0	100.0%	0.0%	0.1%	0.1%	0.0%	0.5%	NA	Not assoc w R	WHO		5) Not assoc w R
LZD	rrl_c344t	57	1065	0	9	0.0%	94.9%	0.0%	0.0%	0.0%	6.3%	0.000	Not assoc w R	WHO		5) Not assoc w R

Phylogeny



Outbreak detection



Mycobacterium tuberculosi

Molecular Epidemiology

WAY FORWARD

- 2 Line Phenotypic DST needs to be implemented as this is GOLD std
- All rifampicin resistant strains to be sequenced
- Strengthen BIOINFORMATICS