MEDGENOME

mocra
Infectious Disease Genetics

MYCOBACTERIUM TUBERCULOSIS SEQUENCING REPORT

Patient Name / Ref No:	Sample ID/Order ID:
Date of Birth / Age:	Sample Type:
Gender:	Date of Sample Collection:
Referring Clinician:	Date of Order Booking:
Hospital:	Date of Report:

Test Requested:

TEST RESULTS

The sample was positive for Mycobacterium tuberculosis.
Mutations associated with resistance of high confidence were detected towards first line drugs Rifampicin, Isoniazid, Ethambutol and Pyrazinamide along with second line drugs Ethionamide, Streptomycin and Fluoroquinolone.
However the allele frequency for the mutations detected is between 10% and 90% which shows hetero-resistance.

SMEAR /XPERT TEST RESULTS

Smear Results : NA
Xpert MTB-RIF : MTB and RIF resistance detected (Done at MedGenome)

BRIEF TEST DETAILS

Mycobacterium tuberculosis (Mtb) is a slow-growing bacterium, taking around 6-8 weeks for culture growth, thereby delaying tuberculosis (TB) diagnosis. Currently available molecular tests like CBNAAT- Gene Xpert MTB/RIF (Xpert) and Line Probe Assay (LPA) provide faster diagnosis and screen limited hot-spot drug resistance mutations [1,2]. Whole genome sequencing (WGS) offers the opportunity to screen not only the loci included in rapid molecular tests, but also other known resistance-associated loci thereby enabling identification of new drug resistance-associated mutations that are not explained by currently available diagnostics.

The test uses probe- based enrichment capture to sequence the whole genome (4.4Mb) of Mtb. The sequencing is performed on the Illumina HiSeq platform. Raw sequence reads are adapter-trimmed and bases with Phred quality > 20 are aligned to Mtb reference strain H37Rv (GenBank NC_018143.2). Variant calling is done using LoFreq or Genome Analysis Toolkit (GATK) followed by variant annotation with Variant Effect Predictor (VEP) pipeline [3]. All the mutation results is considered based on Mtb specific numbering system. Resistance mutations are provided for the first and the second line tuberculosis drugs loci covered by MTBDRplus LPA assay[2], the mutations as per published papers [4,5,6] and the latest ReSeq database[7]. Any additional drug resistance mutations of interest can be shared separately. SNPs based lineage prediction is performed using SNP-IT tool [8]. This methodology has 100% sensitivity to resistance variants profiled by LPA and 97.7% accuracy with the phenotypic drug susceptibility tests for six anti-tuberculosis drugs[9].

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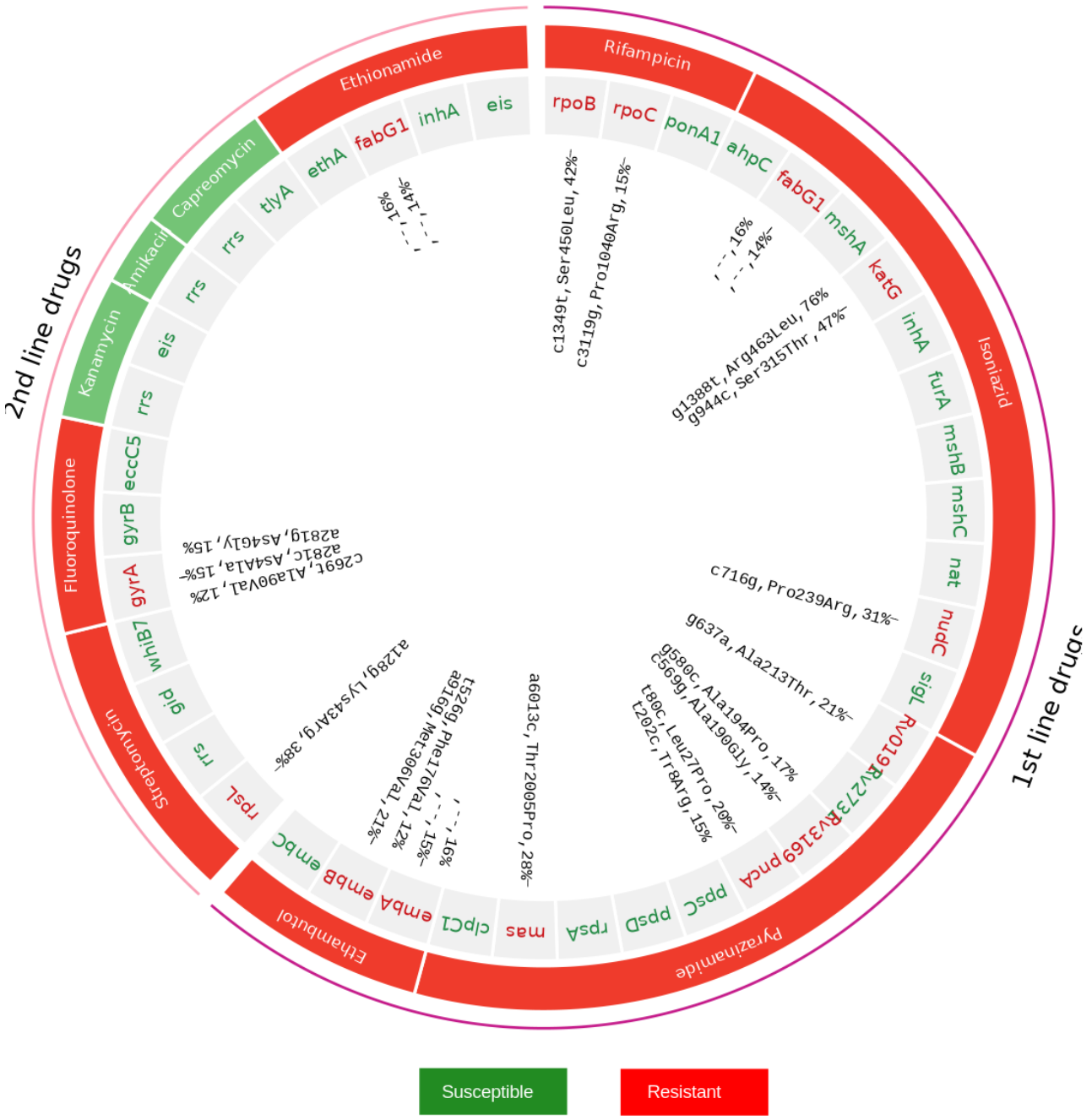
Genes screened for Mtb identification

Locus of Interest	Read-Depth (X)	Gene Coverage (%)
rpoB	1610.8	100
mpt64	2000.3	100
hsp65	1659.5	100

Mutations associated with drug resistance



Name/Sample ID: Rajesh Kumar / 7669883
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Details of genes/variants associated with 1st and 2nd line drugs identified in the specimen

Drug	Gene	Mutation, Allele %	Read Depth (X)	Gene Coverage (%)
Rifampicin	rpoB	c1349t,Ser450Leu,42%	1610.8	100
	rpoC	c3119g,Pro1040Arg,15%	1624.9	100
	ponA1		1810.1	100
Isoniazid	ahpC		2162.6	100
	fabG1-inhA	,,14% ; ,,16%	1672.5	100
	mshA		1392.8	100
	katG	g1388t,Arg463Leu,76% ; g944c,Ser315Thr,47%	1657.9	100
	inhA		1511.7	100
	furA		1628.1	100
	mshB		1620.4	100
	mshC		1566.9	100
	nat		1719.9	100
	nudC	c716g,Pro239Arg,31%	1783.8	100
	sigL		1508.3	100
Pyrazinamide	Rv0191	g637a,Ala213Thr,21%	1709.0	100
	Rv2731		1409.4	100
	Rv3169	c569g,Ala190Gly,14% ; g580c,Ala194Pro,17%	1677.1	100
	pncA	t80c,Leu27Pro,20% ; t202c,Trp68Arg,15%	1535.0	100
	ppsC		1604.3	100
	ppsD		1560.9	100
	rpsA		1898.0	100
	mas	a6013c,Thr2005Pro,28%	1480.4	100

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Drug	Gene	Mutation, Allele %	Read Depth (X)	Gene Coverage (%)
Pyrazinamide	clpC1		1640.3	100
Ethambutol	embA	,,16% ; ,,15%	1652.7	100
	embB	t526g,Phe176Val,12% ; a916g,Met306Val,21%	1476.5	100
	embC		1604.0	100
Streptomycin	rpsL	a128g,Lys43Arg,38%	1777.4	100
	rrs		1505.0	100
	gid		1329.6	100
	whiB7		1804.6	100
Fluoroquinolone	gyrA	c269t,Ala90Val,12% ; a281c,Asp94Ala,15% ; a281g,Asp94Gly,15%	1659.5	100
	gyrB		2101.7	100
	eccC5		1801.0	100
Kanamycin	rrs		1505.0	100
	eis		1511.6	100
Amikacin	rrs		1505.0	100
Capreomycin	rrs		1505.0	100
	tlyA		1567.2	100
Ethionamide	ethA		1834.8	100
	fabG1-inhA	,,14% ; ,,16%	1672.5	100
	inhA		1511.7	100
	eis		1511.6	100
Cycloserine	alr		1695.4	100

* - Not Screened by LPA

Hetero-resistance is defined in this report as a proportion of reads for a given allele between 10% and 90%.

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The variant confidence is reported based on likelihood ratio (LR) value associated with mutation to resistance in the ReSeqTB database, Miotto et al and LPA as follows:

- High – LR ≥; 10, high confidence that the mutation confers or is associated with resistance
- Moderate – LR ≥; 5 and <10, additional data requires to improve or verify the evidence that the mutation confers or is associated with resistance
- Minimal – LR ≥; 1 and < 5, less confidence or inconclusive evidence that the mutation confers or is associated with resistance. Additional data required.

Drug	Susceptible	Resistance
Gene	Normal	Mutated

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Details of genes/variants associated with newer drugs identified in the specimen

Drug	Gene	Mutation, Allele %	Read Depth (X)	Gene Coverage (%)
Bedaquline	Rv0678		1668.6	100
	atpE		2055.5	100
	pepQ		1764.2	100
	ddn		1822.4	100
Clofazime	Rv0678		1668.6	100
	Rv0191	g637a,Ala213Thr,21%	1709.0	100
Delamanid	fbiA		1428.0	100
	fbiB		1561.1	100
	fbiC		1753.1	100
	fgd1		1704.3	100
	ddn		1822.4	100
Linezolid	rrl		1818.0	100
	rplC	c493t,Arg165Trp,14%	1721.2	100
Pretomanid	fbiC		1753.1	100
	ddn		1822.4	100

* - Not Screened by LPA

Drug	Insufficient evidence
Gene	Insufficient evidence

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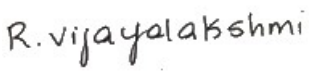
LINEAGE

Lineage 2 (Beijing)

DISCLAIMER

- The test was developed, and its performance characteristics were determined by MedGenome Labs.
- The test results are dependent on factors like time and quality of the specimen collected and the antibiotics administered.
- The results are provided based on the current knowledge and understanding of genotype-phenotype relationships.
- The hetero-resistance with low frequency value below the limit of detection may affect the lineage typing results.
- Correlate with clinical findings.

---------- END OF REPORT ----------



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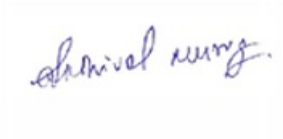

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REFERENCES

1. WHO Global TB Programme, (2013) Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children.
2. HainLifesciences (2012) GenoType MTBDRplus VER 2.0.
3. McLaren W et al (2016) The Ensembl Variant Effect Predictor. Genome Biol, 17:122.
4. Miotto P et al (2017) A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis. European Respiratory Journal, 50: 1701354.
5. Jeong et al (2018) Delamanid, Bedaquiline, and Linezolid Minimum Inhibitory Concentration Distributionsand Resistancerelated Gene Mutations in Multidrug-resistant and Extensively Drug-resistantTuberculosis in Korea. Ann Lab Med, 38:563-568.
6. Wasserman S et al (2019) Linezolid resistance in patients with drug-resistant TB and treatment failure in South Africa. J Antimicrob Chemother, 74: 2377-2384.
7. Ezewudo M, Borens A, Chiner-Oms A et al (2018) Integrating standardized whole genome sequence analysis with a global Mycobacterium tuberculosis antibiotic resistance knowledgebase. Sci Rep, 8:15382.
8. Lipworth S et al (2019) SNP-IT Tool for Identifying Subspecies and Associated Lineages of Mycobacterium tuberculosis Complex. Emerg Infect Dis, 25:482-488.
9. Soundararajan L et al (2020) Whole genome enrichment approach for rapid detection of Mycobacterium tuberculosis and drug resistance-associated mutations from direct sputum sequencing. Tuberculosis, 121:101915.

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