Clinical Utility of Target-based Next-generation Sequencing for Drug Resistant TB: a pilot from Mumbai, India

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Conflict of Interest:
The author has declared no conflict of interest.

Background

- India is estimated to account for a **quarter of the MDR-TB patients** (~1,24,000) globally.

- Mumbai, a densely populated city in Maharashtra, is a hot spot for DR-TB with **24% of MDR-TB in treatment naïve and 41% in previously treated cases with a high frequency of fluoroquinolone(FQ) resistance** among these MDR-TB cases.

- Targeted Next Generation Sequencing (tNGS) is a promising technology that can provide rapid information on well-known resistance conferring mutations across currently used anti-TB drugs

Objectives

- To **assess the clinical application and utility of tNGS** for DR-TB in a high-burden setting within an Indian Public Health Reference laboratory in order to support rapid clinical decision making for individualized regimen design.

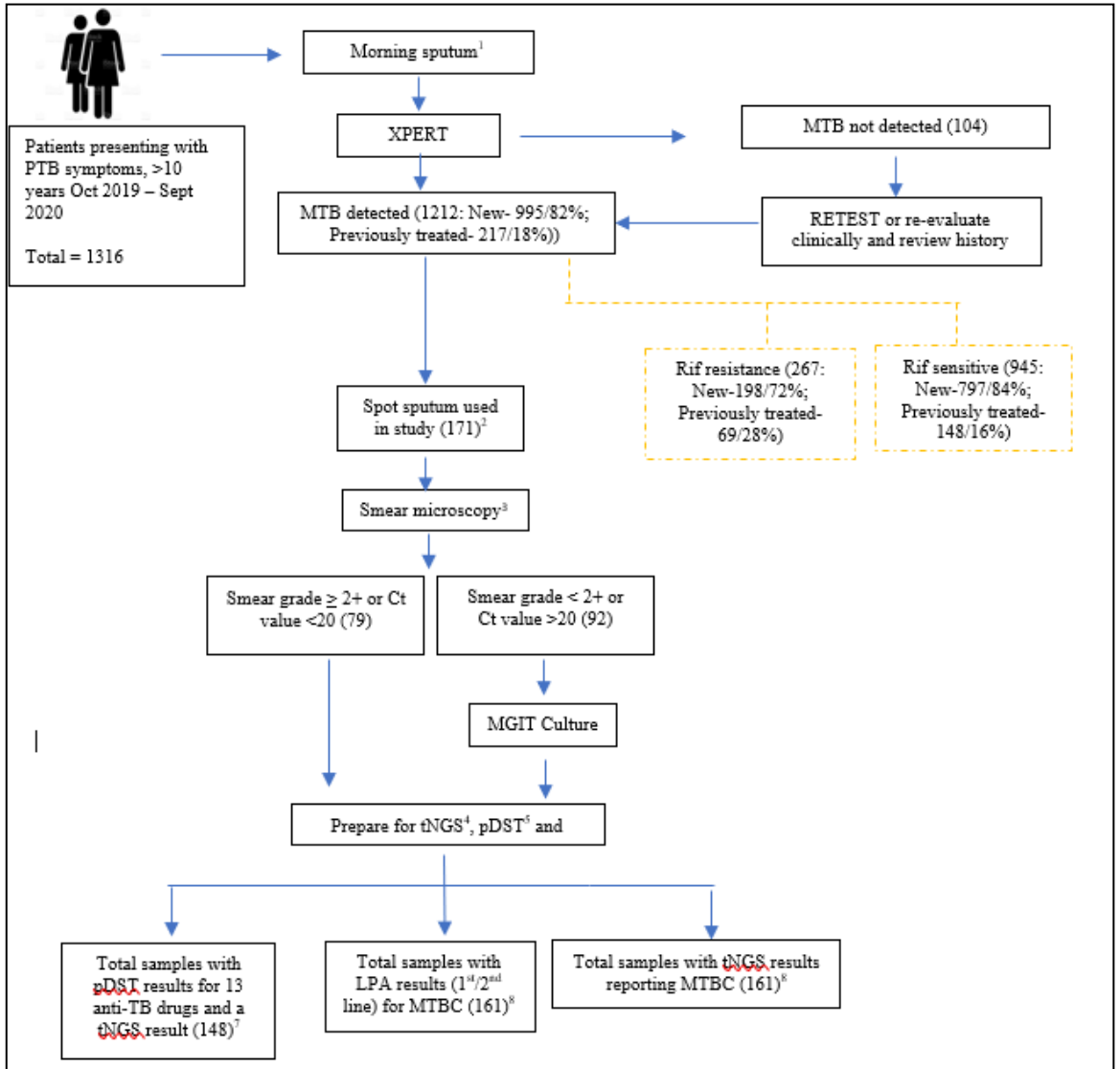
- To **define the opportunities and challenges for tNGS** as a diagnostic tool for DR-TB

Methods

- **Study site:** JJ hospital TB Lab with samples collection from M-east ward, Mumbai
- **Study samples:** Sputum samples tested MTB positive by Xpert MTB/Rif were sent for tNGS and conventional testing (pDST, LPA, MGIT). NGS samples were processed using Deeplex MycTB-kit (GenoScreen, France) and sequenced on a MiSeq platform (Illumina, USA).
- **Study process:**
    a. Resistance profiles were compared between tNGS, LPA and pDST results.
    b. Concordance, sensitivity and specificity, and resistance frequency were compared for all drugs.
    c. Data representing samples with phenotypic resistance/sensitive samples and the correlate genotypic resistance information from the tNGS were analysed.
- **Ethics approval:** This study was approved by the ethics committee of the Grant Medical College & Sir J J Group of Hospitals, Mumbai, India, and was approval by the Medical Director of MSF, Operational Centre Brussels.

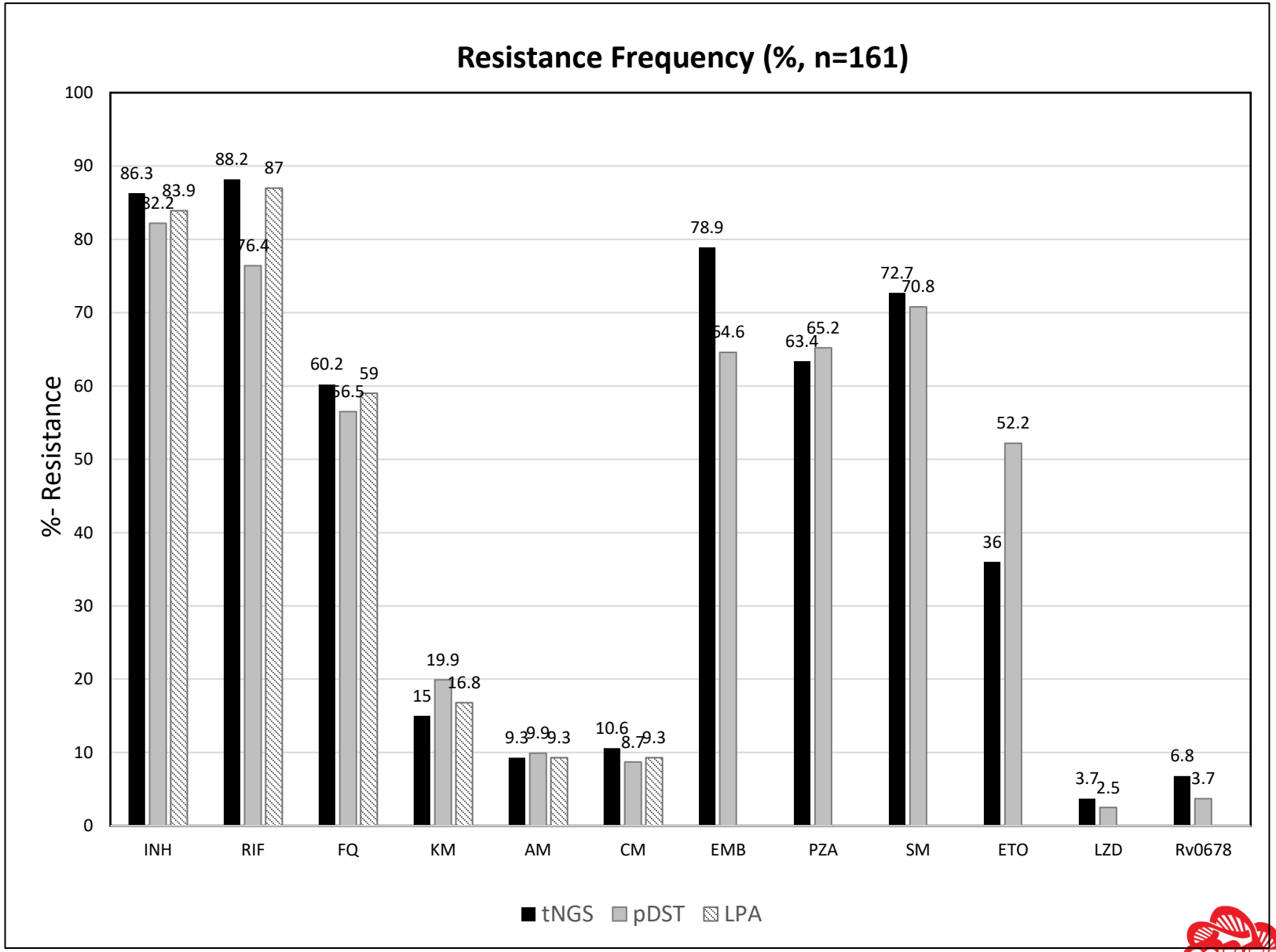
Demographics & Workflow

Characteristics	Number	Percentage
Total	**161**	**100**
Age group (in years)		
12-19	47	29
20-29	50	31
30-39	31	19
40-49	11	7
50 and above	22	14
Median (IQR)	24 (20-40)	
Sex		
Male	69	43
Female	92	57
Healthcare institution		
Patients from public sector	108	67
Patients from private practitioners	53	33
Culture at baseline		
Positive	148	92
Negative	13	8
Resistance profile		
Sensitive	15	9.3
Hr-TB	1	0.6
Qr-TB	2	1.2
Other	1	0.6
RR/MDR-TB	142	88.2
WHO 2020 definitions		
PreXDR-TB (Q or injectable)	74	52.1
XDR-TB (Q and injectable)	24	16.9
WHO 2021 definitions		
PreXDR-TB (Q)	83	58.5
XDR-TB (Q + Lzd or Bdq*)	13	9.2
Previous TB		
Yes	40	24.8
No	113	70.2
Unknown	8	4.9



RESULTS cont…

Methods (MGIT pDST, LPA, and tNGS) were compared by assessing the frequency of resistance for each drug across the patient cohort.



Results continue...

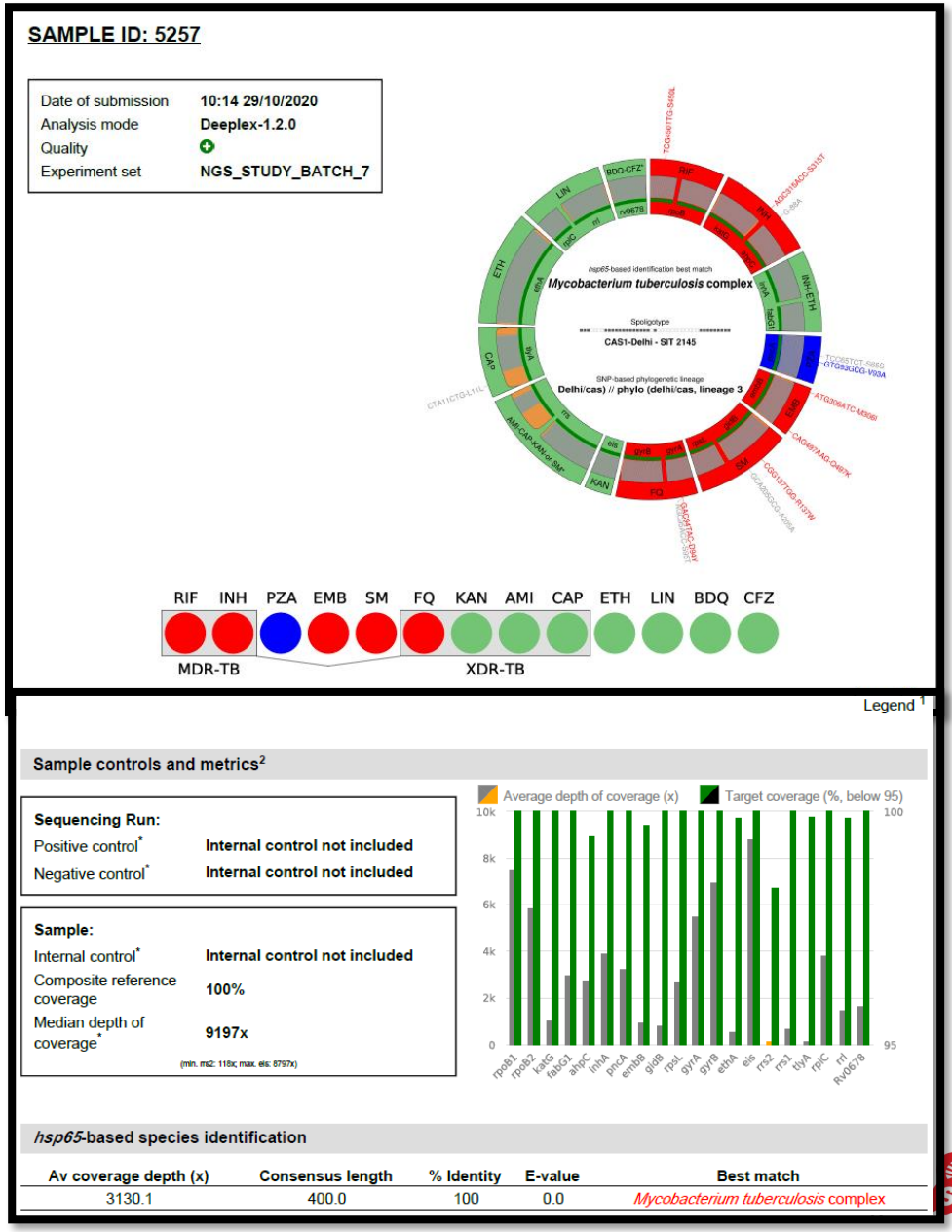
- **Sensitivities** were consistent with reported literature, except for LZD and CFZ/BDQ (rv0678)
- **Specificity** was high (>90%) for most drugs; except for SM/EMB/RIF
- 1% (2/161) of cases were found to have **mixed infections**
- 10 % (16/161) harboured **hetero resistance**.
- 8% (13/161) processed for tNGS were **culture negative** with 8/13 resistant to all 1st -line as well as various 2nd-line drugs.
- **Lineage 2 (Beijing)** was the dominant (55%) strain with the highest degree or DR-TB followed by Lineage 3 (20%) and Lineage 1 (11%).

	Phenotypic Resistant Genotype					Pheontypic Sensitive Genotype					All inclusive		Excl. Unchar		Unchar
Drug	R	Rh	S	U	Tot	R	Rh	S	I	Tot	Sensitivity	Specificity	Sensitivity	Specificity	
INH	130		1	1	132	1		15	0	16	99.2	93.2	99.2	93.2	
											[95.8, 99.9]	[71.7, 98.9]	[95.8, 99.9]	[71.7, 98.9]	0.7%
RIF*	123	2	0	0	125	11	3	14	0	28	100.0	56.6	100.0	56.6	
											97.0, 100]	[37.0, 73.3]	97.0, 100]	[37.0, 73.3]	0.0%
EMB*§	89	4	0	15	108	19		25	0	44	100.0	56.8	100.0	56.8	
											[96.4, 100]	[42.4, 70.3]	[95.9, 100]	[42.4, 70.3]	9.9%
PZA§	90		12	3	105	2		40	0	42	88.6	95.2	88.2	95.2	
											[81.1, 93.3]	[84.2, 98.7]	[80.5, 93.1]	[84.2, 98.7]	2.0%
SM	110		2	2	114	3		31	0	34	98.2	91.2	98.2	91.2	
											[93.8, 99.5]	[77.0, 97.0]	[93.7, 99.5]	[77.0, 97.0]	1.4%
FQ	88	6	4	0	98	1		55	0	56	95.7	98.2	95.7	98.2	
											[89.4, 98.3]	[90.6, 99.7]	[89.4, 98.3]	[90.6, 99.7]	0.0%
KM	19	1	11	2	33	3		109	4	116	65.6	97.3	63.3	97.3	
											[48.3, 79.6]	[92.4, 99.1]	[45.5, 78.1]	[92.4, 99.1]	4.0%
AM	14		2	0	16	0		129	3	132	87.5	100.0	87.5	100.0	
											[64.0, 96.5]	[97.1, 100]	[64.0, 96.5]	[97.1, 100]	2.0%
CM	8		5	1	14	8		119	7	134	64.3	93.7	61.5	93.7	
											38.8, 83.7]	[88.1, 96.8]	[35.5, 82.3]	[88.1, 96.8]	5.4%
ETO§	49		16	14	79	3		64	0	67	79.7	95.5	75.4	95.5	
											[69.7, 87,1]	[87.6, 98.5]	[63.7, 84.2]	[87.6, 98.5]	9.6%
LZD†	2		2	0	4	1		142	1	144	50.0	99.3	50.0	99.3	
											[15.0. 85.0]	[96.2, 99.9]	[15.0. 85.0]	[96.2, 99.9]	0.7%
CFZ/BDQ†#	2		4	0	6	6	1	134	0	141	33.3	96.8	33.3	96.8	
											[9.7, 70]	[90.1, 98.0]	[9.7, 70]	[90.1, 98.0]	0.0%

Clinical Utility of tNGS

- Our study demonstrated sensitivities consistent with those reported literature with a high specificity for most of the drugs recommended under new WHO guidelines. Hence**, tNGS has utility in supporting or even replacing pDST for regimen design**.

- Though resistance-conferring mutations to newer/repurposed drugs (LZD, CFZ, and BDQ) are still evolving, tNGS predictions demonstrated higher detection rates for these drugs than pDST. Hence, **tNGS can help identify newly evolving resistance** which will improve case management and care.



Clinical Utility of tNGS – cont…

- Low level mutations (borderline), particularly for Rif, were observed. Hence, **early detection may help mitigate resistance amplification especially to FQ in PDR-TB treatment**.

- The **existence of mixed infection and or hetero-resistance pose serious treatment challenges with present standard of care testing**. Hence, tNGS has a key advantage.

- Viable but non-culturable bacilli (VBNC) in liquid medium can pose serious implications to patient care with an under diagnosis of resistance on pDST (i.e. “no growth) leading to either no treatment or empirical treatment. Hence, **tNGS direct sputum testing has an edge in diagnosing these cases**.

- **tNGS can monitor trends in strain prevalence** and provide a better understanding of the disease in circulation within the community. Hence, this information can guide program policy and best practices for TB control.

Drug resistance associated variants[3]

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Confidence	PMID
embB	4247429	ATG306GTG	100	254.50	M306V	EMB	High	Campbell PJ et al., 2011
gidB	4408061	CAT48AAT	99.6	394.25	H48N	SM	n/a	n/a
gyrA	7581	GAC94TAC	99.5	806.00	D94Y	FQ	High // High	ReSeqTb
inhA	1674481	TCG94GCG	99.7	1882.50	S94A	INH, ETH	High	Nebenzahl-Guimaraes H et al., 2014
katG	2155168	AGC315ACC	99.9	377.00	S315T	INH	High	ReSeqTb
rpoB	761139	CAC445GAC	99.5	3613.50	H445D	RIF	High	ReSeqTb
pncA	2288949	inserG	88.5		frameshift	PZA		

Uncharacterized variants[3]

Uncharacterized variants designate sequence variants of as yet unknown association with drug sensitivity or resistance.

No uncharacterized variants detected in any gene target.

Spoligotype

Octal code/ Binary code	Av. coverage depth (x)	SIT	SITVIT occurence	Clade
703777740003771	2685.2	26	896	CAS1-Delhi
11100001111111111111110000000000000111111111				

SNP-based phylogenetic lineage

Delhi/cas) // phylo (delhi/cas, lineage 3

Potential mixed infection

Mixed infection is signaled by a phylogenetic variant detected at less than 95%, indicating the simultaneous presence of 1 strain harboring this variant present at this percentage and another strain sharing the same sequence as the reference at this position, present at approx. 100% minus this percentage.

Not detected.

Opportunity:

•Direct sputum-based testing for 13 drugs provides a quicker turnaround of results for individual case management

•tNGS can be used for molecular epidemiological investigation

Challenges:

- Paucibacillary cases (Ped-TB, EPTB, and PLHIV) still require culture pDST.
- MycTB requires an upgrade of drug-targets for newer drugs (DLM, PTM, IMP) and added targets for CFZ (rv1979c) and BDQ (atpE, pepQ) with clinical significance
- More user-friendly data reporting is required for accurate and consistent results interpretation.

Study limitations:

- Unavailability of pDST for BDQ.
- Limitation of cross-resistance for CFZ/BDQ for primary resistance prediction is inaccurate.
- DST EQA panel was available only for drugs currently recommended by the national guideline. This limited the potential reliability of pDST conduct for the additional drugs in the study.

Conclusion

- Overall data supports the use of tNGS for resistance-prediction profiling across currently used drugs.

- The use of tNGS may lead to early diagnosis by virtue of sputum based testing in comparison to culture based methods. Allowing for early initiate on a more appropriate treatment regimen.

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