

The Next Frontier in Tuberculosis Investigation: Automated Whole Genome Sequencing for *Mycobacterium tuberculosis* Analysis

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INTRODUCTION

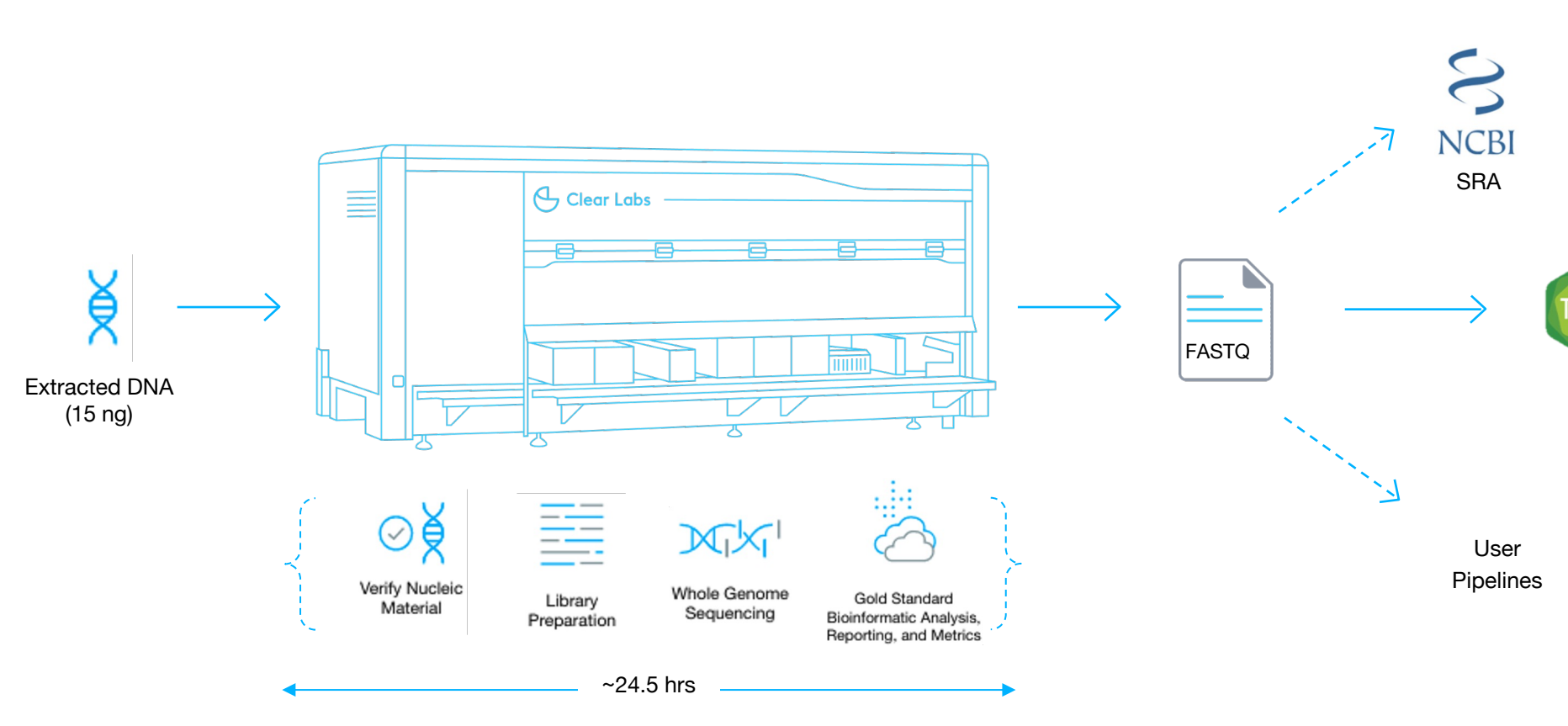
- Mycobacterium tuberculosis* (MTB) is a preventable and curable disease, but there are still tens of millions of new cases of tuberculosis (TB) resulting in more than one (1) million deaths reported every year worldwide¹.
- Multidrug-resistant (MDR) tuberculosis is a major health problem and seriously threatens worldwide TB control and prevention initiatives¹.
- Failure to detect novel antimicrobial resistance (AMR) genotypes and mutations within the MTB genome can lead to inaccurate prescription of patient treatment regimes, posing a risk of treatment failure².
- Whole genome sequencing (WGS) is an agnostic approach to screen the entire pathogen genome for the detection of specific single nucleotide polymorphisms (SNPs) to discriminate known MTB complex (MTBC) (sub)lineages and has significant potential to replace traditional culture-based phenotypic diagnostic tests, which can typically take weeks to months³.
- Integrating WGS with automation will enable laboratorians to perform complex workflows with minimal training and has been proven successful for application in the food safety and infectious diseases space^{4,5}.

The Clear Dx™ Automated Platform



METHODS

Overview of the Microbial Surveillance WGS Workflow



¹ Tuberculosis. Available online: <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>
² Dohali, M. et al. Advancing tuberculosis management: the role of predictive, preventive, and personalized medicine. *Front. Microbiol.* 2023, 14, 1225438.
³ Drug Resistant Tuberculosis: The Next Global Health Crisis? CDC Congressional Testimony. Available online: <https://www.cdc.gov/od/oc/media/pressroom/2015/02/0151208.htm>
⁴ Abdullah, K. et al. Applications of Clear Dx whole genome sequencing system in SARS-CoV-2 diagnostics. *J. Infect. Public Health.* 2022, 15(6), 894-895.
⁵ Holland, I. & Davies, J.A. Automation in the Life Science Research Laboratory. *Front. Bioeng. Biotechnol.* 2020, 8, 571777.

RESULTS

Sequencing Results of MTBs & NTMs Using WGS

Sample ID	Predicted Taxon	Sub-Lineage	Assembly Length (bp)	Number of Contigs	Estimated Depth of Coverage
NSPHL_Strain_8	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,355,471	127	119.63
NSPHL_Strain_9	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,372,144	122	60.37
NSPHL_Strain_10	<i>Mycobacterium tuberculosis</i>	4.1.2.1	4,316,704	142	33.78
NSPHL_Strain_11	<i>Mycobacterium tuberculosis</i>	4.1.1.1	4,344,545	118	100.73
NSPHL_Strain_12	<i>Mycobacterium tuberculosis</i>	4.1.1.3	4,335,708	124	58.77
NSPHL_Strain_13	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,356,191	139	62.57
NSPHL_Strain_14	<i>Mycobacterium tuberculosis</i>	3.1.2	4,343,589	125	114.74
NSPHL_Strain_15	<i>Mycobacterium tuberculosis</i>	4.8	4,335,730	115	52.41
NSPHL_Strain_16	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,326,334	239	20.66
NSPHL_Strain_17	<i>Mycobacterium tuberculosis</i>	4.1.2.1	4,326,395	159	34.55
36359772	<i>Mycobacterium tuberculosis</i>	2.2.1	4,288,293	189	45.65
36360342	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,312,674	222	33.05
36360344	<i>Mycobacterium tuberculosis</i>	2.2.1	4,257,211	278	33.06
36360347	<i>Mycobacterium tuberculosis</i>	4.6.2.2	4,258,952	267	32.12
36360353	<i>Mycobacterium tuberculosis</i>	4.1.2	4,322,326	233	34.83
36360355	<i>Mycobacterium tuberculosis</i>	4.1.2	4,203,142	593	21.84
36360361	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,306,182	247	34.21
36360364	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,314,878	195	45.81
36360369	<i>Mycobacterium tuberculosis</i>	4.1.2	4,292,556	272	36.49
36360376	<i>Mycobacterium tuberculosis</i>	4.1.2	4,300,124	293	31.64
36360377	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,360,550	150	100.91
36360388	<i>Mycobacterium tuberculosis</i>	4.6.2.2	4,314,959	121	98.35
Z008267	<i>Mycobacterium tuberculosis</i>	2.2.1	4,280,868	298	25.63
Z008268	<i>Mycobacterium tuberculosis</i>	2.2.1	4,322,819	159	43.21
Z008270	<i>Mycobacterium tuberculosis</i>	4.6.2.2	4,309,779	118	78.37
Z008271	<i>Mycobacterium tuberculosis</i>	1.2.1.2.1	4,355,604	136	58.3
Z008272	<i>Mycobacterium tuberculosis</i>	2.2.1	4,314,821	181	36.07
Z008273	<i>Mycobacterium tuberculosis</i>	1.2.1.2	4,341,999	153	50.4
Z008274	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,332,833	166	37.56
Z008275	<i>Mycobacterium tuberculosis</i>	2.2.1	4,319,340	196	35.28
Z008276	<i>Mycobacterium tuberculosis</i>	2.2.1.1	4,305,996	191	29.81
Z008277	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,348,679	120	92.59
Z008278	<i>Mycobacterium tuberculosis</i>	1.1.1.1	4,347,657	114	156.87
Z008279	<i>Mycobacterium tuberculosis</i>	4.6.2.2	4,321,629	139	38.43
ATCC_35734	<i>Mycobacterium tuberculosis</i>	La1.2.BCG	4,229,722	138	46.66
ATCC_35822D-2	<i>Mycobacterium tuberculosis</i>	4.9	4,285,606	122	60.58
NR-122	<i>Mycobacterium tuberculosis</i>	4.9	4,348,364	124	54.23
NR-59207	<i>Mycobacterium tuberculosis</i>	La1.8.1	4,242,260	183	24.48
NR-44263	<i>Mycobacteroides abscessus</i>	-	5,133,647	21	47.31
NR-44274	<i>Mycobacteroides abscessus</i>	-	5,188,746	30	37.27
NR-49658	<i>Mycobacterium canettii</i>	-	4,341,653	223	23.38
ATCC_6841	<i>Mycocyclotherium fortuitum</i>	-	6,279,033	47	48.01
ATCC_14470	<i>Mycobacterium goodii</i>	-	7,368,456	278	27.74
NR-49070	<i>Mycobacterium palustre</i>	-	5,768,069	204	42.5

Whole genome sequence information were successfully generated for all MTB and NTM isolates tested

Sample ID	Sample Size	Assembly Length (bp) Mean ± S.D.	Number of Contigs Mean ± S.D.	Mean Q Scores Mean ± S.D.	Mean Read Length (bp) Mean ± S.D.
NSPHL_Strain_8 (MTB)	4	4,335,605 ± 2,228	172.67 ± 8.74	36.12 ± 0.06	147.43 ± 0.78
NSPHL_Strain_12 (MTB)	3	4,305,569 ± 5,272	201.00 ± 8.28	36.19 ± 0.01	147.46 ± 0.34
NSPHL_Strain_17 (MTB)	3	4,324,000 ± 5,569	166.67 ± 2.42	36.08 ± 0.04	147.49 ± 0.81
ATCC_35734 (MTB)	3	4,224,952 ± 4,199	141.67 ± 5.51	35.56 ± 0.05	145.82 ± 0.95
ATCC_35822D-2 (MTB)	5	4,282,387 ± 3,431	129.00 ± 4.95	35.49 ± 0.05	144.77 ± 1.93
NR-122 (MTB)	16	4,336,706 ± 7,704	136.47 ± 10.11	35.49 ± 0.34	147.21 ± 1.33
NR-59207 (MTB)	3	4,238,013 ± 6,183	192.67 ± 8.50	34.71 ± 0.00	145.99 ± 0.57
ATCC_6841 (NTM)	3	6,274,061 ± 4,522	56.67 ± 9.07	35.73 ± 0.09	146.60 ± 1.18
NR-44263 (NTM)	4	5,130,504 ± 3,015	29.25 ± 8.88	35.73 ± 0.08	144.11 ± 1.68
NR-44274 (NTM)	4	5,189,594 ± 3,121	31.25 ± 7.09	35.84 ± 0.09	147.98 ± 0.11
NR-49070 (NTM)	2	5,768,681 ± 865	205.00 ± 1.41	36.03 ± 0.61	144.26 ± 3.20

Clear Dx™ Microbial Surveillance WGS workflow is highly robust

Data and results presented in poster has been published: Ng, J.H.J. et al. The Next Frontier in Tuberculosis Investigation: Automated Whole Genome Sequencing for *Mycobacterium tuberculosis* Analysis. *Int. J. Mol. Sci.* 2024, 25, 7909. <https://doi.org/10.3390/ijms25147909>

MTB AMR and Drug Class Characterization Using WGS

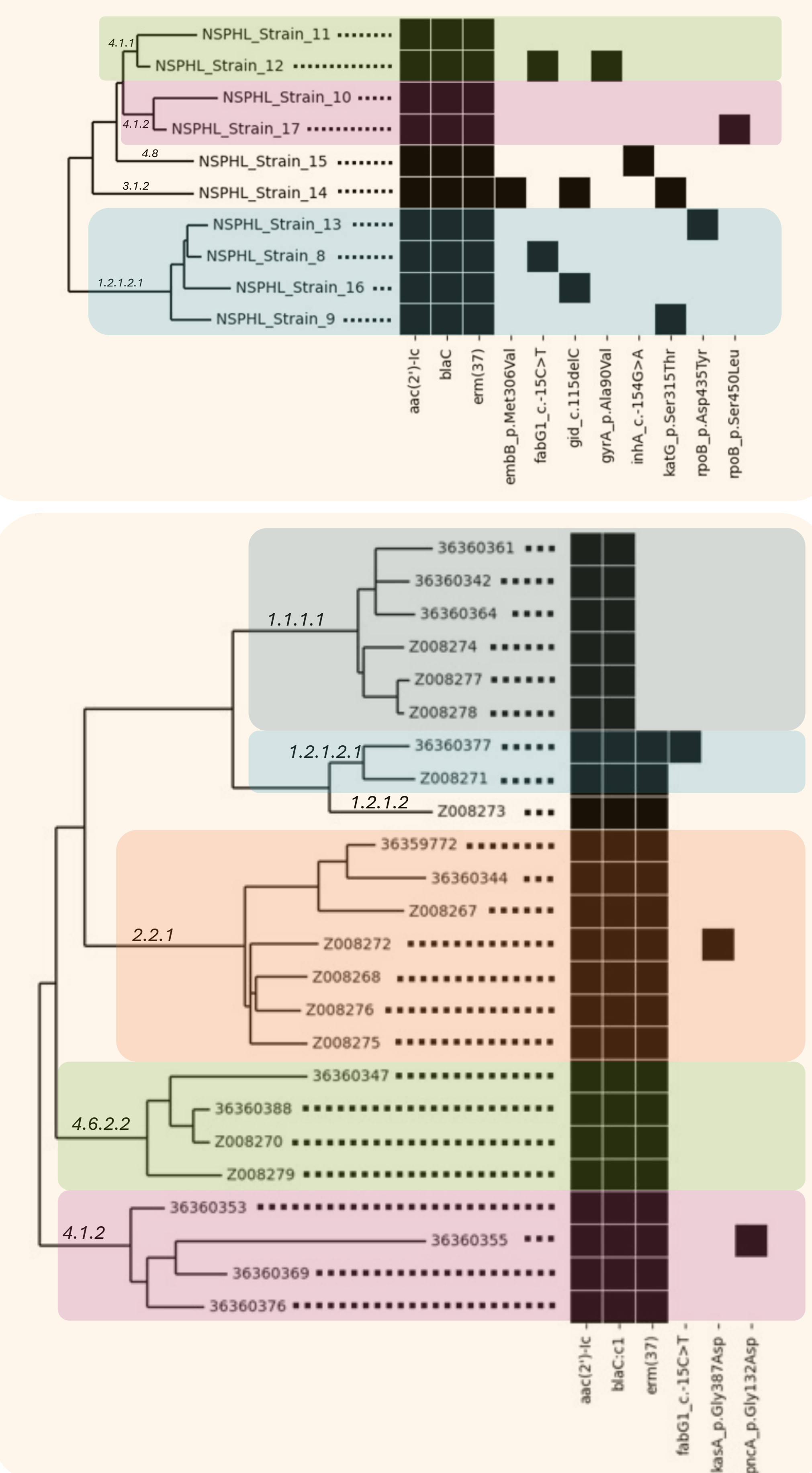
Sample ID	Sub-Lineage	Predicted AMR Drug Classes	Identified AMR Genes / Mutations	TB DR Type*
NSPHL_Strain_8	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethionamide	aac(2)-Ic, blaC, erm(37), fabG1_c-15C>T	HR-TB
NSPHL_Strain_9	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid	aac(2)-Ic, blaC, erm(37), katG_p.Ser415Thr	HR-TB
NSPHL_Strain_10	4.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
NSPHL_Strain_11	4.1.1.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
NSPHL_Strain_12	4.1.1.3	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Fluoroquinolones, Ethionamide	aac(2)-Ic, blaC, erm(37), fabG1_c-15C>T, gyrA_p.Ala90Val	HR-TB
NSPHL_Strain_13	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Rifampicin	aac(2)-Ic, blaC, erm(37), rpoB_p.Asp435Yr	RR-TB
NSPHL_Strain_14	3.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethambutol, Streptomycin	aac(2)-Ic, blaC, erm(37), embB_p.Met306Val, gid_c.115delC, katG_p.Ser415Thr	HR-TB
NSPHL_Strain_15	4.8	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethambutol	aac(2)-Ic, blaC, erm(37), inhA_c-154G>A	HR-TB
NSPHL_Strain_16	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Streptomycin	aac(2)-Ic, blaC, erm(37), gid_c.115delC	Other
NSPHL_Strain_17	4.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Rifampicin	aac(2)-Ic, blaC, erm(37), rpoB_p.Ser450Leu	RR-TB
36359772	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360342	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
36360344	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360347	4.6.2.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360353	4.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360355	4.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Pyrazinamide	aac(2)-Ic, blaC, erm(37), pncA_p.Gly132Asp	Other
36360361	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
36360364	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
36360369	4.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360376	4.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
36360377	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid	aac(2)-Ic, blaC, erm(37), fabG1_c-15C>T	HR-TB
36360388	4.6.2.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008267	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008268	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008270	4.6.2.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008271	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008272	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Pyrazinamide	aac(2)-Ic, blaC, erm(37), ksaA_p.Gly387Asp	HR-TB
Z008273	1.2.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008274	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
Z008275	2.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008276	2.2.1.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
Z008277	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
Z008278	1.1.1.1	Aminoglycoside, Beta-Lactam	aac(2)-Ic, blaC	Sensitive
Z008279	4.6.2.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37), rhpC_c-59C>T	Sensitive
ATCC_35734	La1.2.BCG	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Pyrazinamide	aac(2)-Ic, blaC, erm(37), pncA_p.His57Asp	Other
ATCC_35822D-2	4.9	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid	aac(2)-Ic, blaC, erm(37), rhpC_c-54C>T, katG_c-11139_36437del	HR-TB
NR-122	4.9	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	aac(2)-Ic, blaC, erm(37)	Sensitive
NR-59207	La1.8.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Pyrazinamide	aac(2)-Ic, blaC, erm(37), pncA_p.His57Asp	Other

Detailed AMR and drug class characterization using WGS

Sample ID	Sub-Lineage	Predicted AMR Drug Classes	Antimicrobial Susceptibility Test (AST)				
			Ethambutol 5.0 ug/mL	Isoniazid 0.1 ug/mL	Isoniazid 0.4 ug/mL	Rifampin 1.0 ug/mL	Pyrazinamide 100 ug/mL
NSPHL_Strain_8	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethionamide	Sensitive	Resistant	Resistant	Sensitive	Sensitive
NSPHL_Strain_9	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid	Sensitive	Resistant	Resistant	Sensitive	Sensitive
NSPHL_Strain_10	4.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide			Not Available		
NSPHL_Strain_11	4.1.1.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
NSPHL_Strain_12	4.1.1.3	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Fluoroquinolones, Ethionamide	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
NSPHL_Strain_13	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Rifampicin	Sensitive	Sensitive	Sensitive	Resistant	Sensitive
NSPHL_Strain_14	3.1.2	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethambutol, Streptomycin	Sensitive	Resistant	Resistant	Sensitive	Sensitive
NSPHL_Strain_15	4.8	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Isoniazid, Ethambutol	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
NSPHL_Strain_16	1.2.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Streptomycin			Not Available		
NSPHL_Strain_17	4.1.2.1	Aminoglycoside, Beta-Lactam, Lincosamide/Macrolide, Rifampicin			Not Available		

Genotypic AMR highly concordant with phenotypic AST results

Phylogenetic Analysis of Clinical Isolates Using WGS



WGS provides higher phylogenetic resolution and AMR profiles

CONCLUSION

- Clear Dx™ Microbial Surveillance WGS is a fully automated, end-to-end solution for TB characterization.
- Robust, field-tested and easy-to-use application.
- High quality and reproducible data.
- System accepts dsDNA and amplicons as input material.
- Empower public health laboratories to effectively characterize, monitor and survey for MTBs.