Tuberculosis Drug Resistance Profiling by Targeted Next-Generation Sequencing in Sub-Saharan Africa

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BACKGROUND

- Drug susceptibility testing (DST) is essential for diagnosing and treating DR-TB effectively, but culture-based methods are time and resource-intensive.
- Culture-free targeted next-generation sequencing (tNGS) can comprehensively detect drug resistance mutations but its practicality in routine settings requires further exploration.
- We implemented Oxford Nanopore Technologies' tNGS assay for TB (Nanopore tNGS) in accredited clinical laboratories in Johannesburg, South Africa and Lusaka, Zambia.

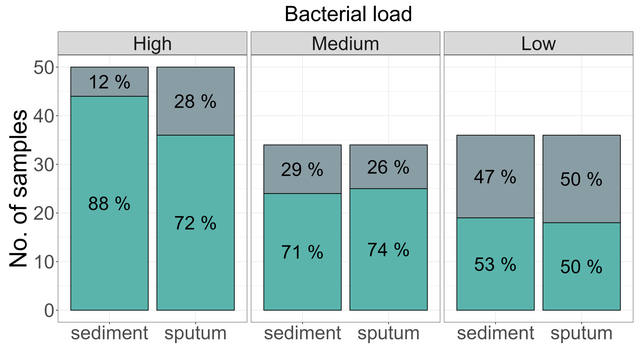


Figure 1: Sequencing results of sputum (native sputum) and sediment (processed sputum) samples, grouped by semi-quantitative GeneXpert MTB/RIF Ultra categories of bacterial load.

METHODS

- Sputum samples were collected from patients recruited as part of an ongoing prospective observational cohort study.
- On-site lab procedures included automated DNA extraction, amplification of 25 gene regions linked to drug resistance across 18 TB drugs, and real-time Nanopore sequencing and analysis with compact MinION sequencers.
- We sequenced native sputa and decontaminated sediments (processed sputa) of 120 TB patients with a GeneXpert MTB/RIF Ultra semi-quantitative result of "low" to "high".
- Sequencing results were compared to local routine DST; phenotypic MGIT DST for first-line drugs and in South Africa GeneXpert MTB/XDR.

Nanopore tNGS can rapidly provide comprehensive TB drug resistance profiles directly from sputum samples but challenges remain.

RESULTS

- Sequencing results and predicted DR calls were available within 24 hours after DNA extraction.
- We obtained sequencing results and detected MTB in 78/120 (65%) native sputum samples and 86/120 (72%) decontaminated sediments (p=0.24, **Figure 1**).
- Complete sequencing results, covering all 25 gene targets, were obtained for 37/64 (58%) sputum and 55/64 (86%) sediment samples (p<0.001, **Figure 2**). For incomplete samples, the median number of targets missed in sputum was lower than in sediments (8 vs.11).
- Sequencing success rates differed at the two sites with 22/49 (45%) and 57/71 (80%) successful sequencing results from sputum in Zambia and South Africa, respectively (p<0.001).
- tNGS from sediments had a specificity of 95% or higher across all drugs compared to local DST. Sensitivity for rifampicin was high. For isoniazid and pyrazinamide, sensitivity was 56% and 17%, respectively compared to local MGIT DST (**Figure 3**).

Reference Test (drug)	TP	FP	FN	TN	Sens (95% CI)	Spec (95% CI)
GeneXpert MTB/RIF (rifampicin)	15	3	1	60	0.94 (0.72–0.99)	0.95 (0.87–0.98)
phenotypic MGIT DST	21	4	13	235	0.62 (0.45–0.76)	0.98 (0.95–0.99)
(rifampicin)	11	3	1	52	0.92 (0.65–0.99)	0.95 (0.86–0.98)
(isoniazid)	6	1	5	52	0.55 (0.28–0.79)	0.98 (0.90–1.00)
(ethambutol)	2	0	1	64	0.67 (0.21–0.94)	1.00 (0.94–1.00)
(pyrazinamide)	2	0	6	57	0.25 (0.07–0.59)	1.00 (0.94–1.00)
GeneXpert MTB/XDR *	7	3	4	276	0.64 (0.36–0.85)	0.99 (0.97–1.00)
(isoniazid)	6	1	3	48	0.67 (0.36–0.88)	0.98 (0.89–1.00)
(ethionamide)	1	2	1	54	0.50 (0.09–0.91)	0.96 (0.87–0.99)

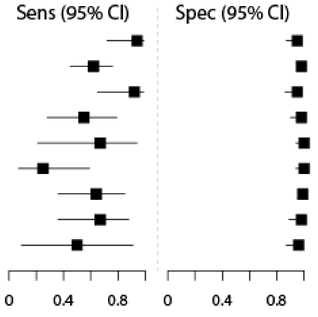


Figure 2: Sediment Nanopore tNGS sensitivity (Sens) and specificity (Spec) compared to locally available drug susceptibility testing (DST). Drugs for which no resistance was detected are not shown individually.
* (isoniazid, ethionamide, amikacin, kanamycin, capreomycin, fluoroquinolones)

CONCLUSIONS & OUTLOOK

- Nanopore tNGS was implemented successfully at both sites and detected TB and DR-associated mutations.
- Sequencing from sputum and sediment samples was comparable.
- The Nanopore bioinformatic pipeline has been updated and sequencing results will be reanalysed to improve DR detection. Further validation using whole-genome sequencing and phenotypic DST to second-line TB drugs is planned.
- Factors affecting sequencing performance such as operator differences or batch-to-batch variability of kits need to be investigated.
- In conclusion, Nanopore tNGS promises a feasible and rapid sequencing approach for comprehensive genotypic DR profiling in TB high-burden countries but technical and logistic challenges complicate its routine implementation.

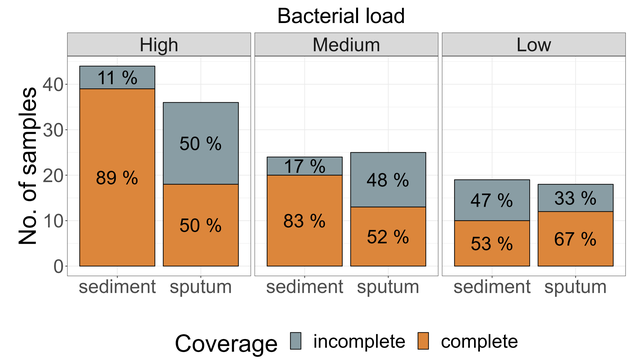


Figure 3: Proportions of complete (covering all gene targets) and incomplete sequencing results in MTB-positive sputum (native sputum) and sediment (processed sputum) samples, grouped by semi-quantitative GeneXpert MTB/RIF Ultra categories of bacterial load.

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