SOUTHERN AFRICAN CENTRE FOR INFECTIOUS DISEASE SURVEILLANCE

# Genome profiling of multidrug resistance tuberculosis among patients in Tanzania

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# Background

- Multidrug resistance TB (MDR-TB) defined as resistance to at least Rifampin (RIF) and Isoniazid (INH), is a threat to global eradication of TB
- Drug resistant TB is due to accumulation of mutations in genes
  - **INH** - *katG, inhA, ahpC, kasA;* **RIF** – *rpoB*, Ethambutol-*embB*
  - **Pyrazinamide** (PZA)– *pncA;* ***S*treptomycin** (STR) - *rpsL* and *rrs*
- Prevalence of MDR
  - Globally, 4.1% of new TB cases, 19% treated cases (WHO, 2017)
  - Tanzania, 60,575 new cases (1.1%) ; 2,576 treated cases (3.9%) (NTLP, 2015)
- Most studies on resistance to TB drugs have been done using DST, few by GeneXpert MTB/RIF (Cepheid) that do not allow the detection of new mutations

# Background

- Whole genome sequencing (WGS) detect new mutations (SNPs) in important proteins, regulatory enzymes and promoter genes &

- The availability of Whole genome sequencing (WGS) has improved the understanding anti-TB drug resistance

- Hypothesis
  - The evolution of drug resistance TB is primarily driven by drug therapy or de novo micro-evolution of new drug resistance strains
- Research Question
  - What are the genomic diversity and drivers of evolution of drug resistance TB strains that have evolved special mechanisms that facilitate drug resistance acquisition?

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# Materials & Methods

- Study area: Kibong'oto Infectious Diseases Hospital

- Study design: Unmatched case control study
  - 31 subjects – MDR-TB
  - 10 subjects – non MDR-TB
- Ethical consideration - Research and Publications Committee of MUHAS, Tanzania

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# Diagnostic Procedures

1st line drugs - RHEP
2nd line - Kanamycin/ Ofloxacin

TB patients Cases + Control

Sputum samples

Drug Susceptibility Testing

Microscopy Culture

DNA extraction

PCR amplification

DNA sequencing (Illumina Miseq®)

Statistical Analysis

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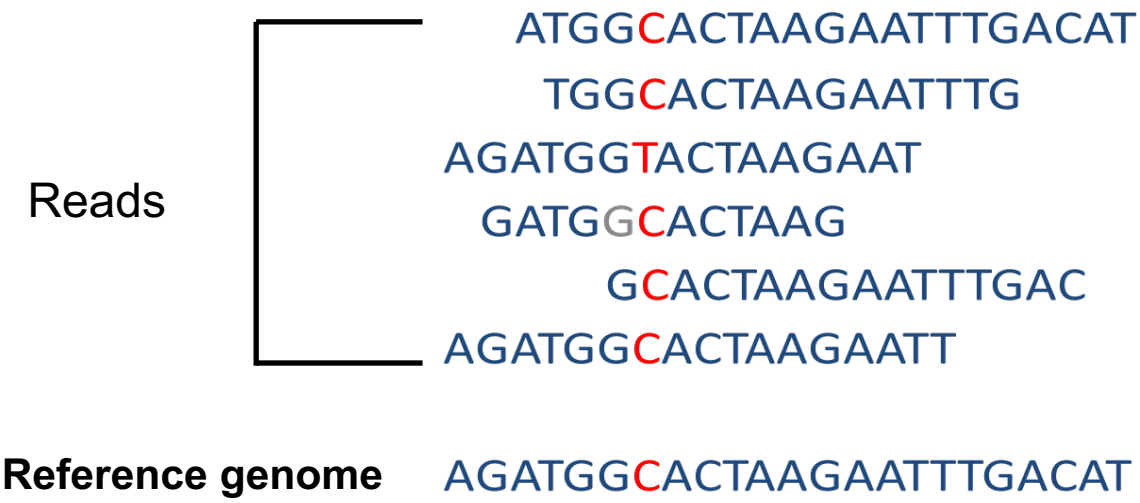
# Data Analysis

1. Alignment of reads to a reference genome




2. Construction of phylogeny

Using all SNPs, a maximum likelihood phylogeny was constructed using RaXML

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# Results & Discussion

| Drug | Gene | SNP Mutation (% in resistance isolates) |
|---|---|---|
| Isoniazid | *katG* | S315T (77.4%), S315R (3.2%), W328L (3.2%), G316S (3.2%) |
| | *inhA* | I21T (3.2%) |
| | *Rv1482c-fabG1* | T8C (6.5%), C15T (3.2%) |
| | *oxyR'-ahpC* | C52T (3.2%) |
| Rifampin | *rpoB* | S450L (41.9%), H445Y (9.7%), H445R (6.7%), Q432E (12.9%), H445L (3.2%), D435V (6.5%), H445D (3.2%), H445D (3.2%), K446Q (3.2%), S431T; L430P (3.3%), L452 P(3.2%), L464M (3.3%) |
| Streptomycin | *rpsL* | K88R (16.1%), K88M (12.9%), K43R (3.2%) |
| | *rrs* | C905G (3.2%), C517T (3.2%) |

| Drug | Target gene | SNP Mutation ((% in resistance isolates) |
|---|---|---|
| Ethambutol | *embB* | Q497R(23.3%),M306V(16.7%),G406S(10%),D1024N(10%),M306T(6.7%), |
| | *embC* | Y334H(10%), D354A(3.2%), M306I(3.2%) C16T(10%) |
| Pyrazinamide | *pncA* | V128G (20%), D49G (3.2%), D63A (3.2%), A193AT (3.2%), P69L (6.5%), A46V (3.2%), |
| Ethionamide | *Rv1482cfabG1* | I21T (3.2%) |
| | *InhA* | |
| Amikacin | *rrs* | C517T (3.2%) |
| Flouroquinolones | *gyrB* | R446C (3.2%) |

A range of mutations that drive resistance to anti-TB drugs, suggesting diversity in the drug resistance of *M. tuberculosis* isolates in Tanzania

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# Results & Discussion

- Resistances to INH and RIF and ETH were predominantly in the *katG*, *rpoB* and *embB* genes respectively, reflecting ongoing transmission of these strains in our local settings

- The S315T and S450L amino acid substitutions in the *katG* and *rpoB* genes, respectively, were the most prevalent mutations found in MDR-TB isolates, and concurs with other studies (Unissa et al. 2015: Silva et al. 2003; Höfling et al. 2005).

- More mutations in *katG* & *rpoB* gene, is a reliable biomarker or hot spot region for determining of MDR-TB in our local settings

- The detected mutations mediating resistance to first line drugs correlated with phenotypic DST results

# Discussion & Conclusion

- Majority of MDR TB isolates were from lineage 4 (LAM) 41.9% (13/31), which is one of the predominant lineage in Tanzania (Mbugi *et al*. 2015)

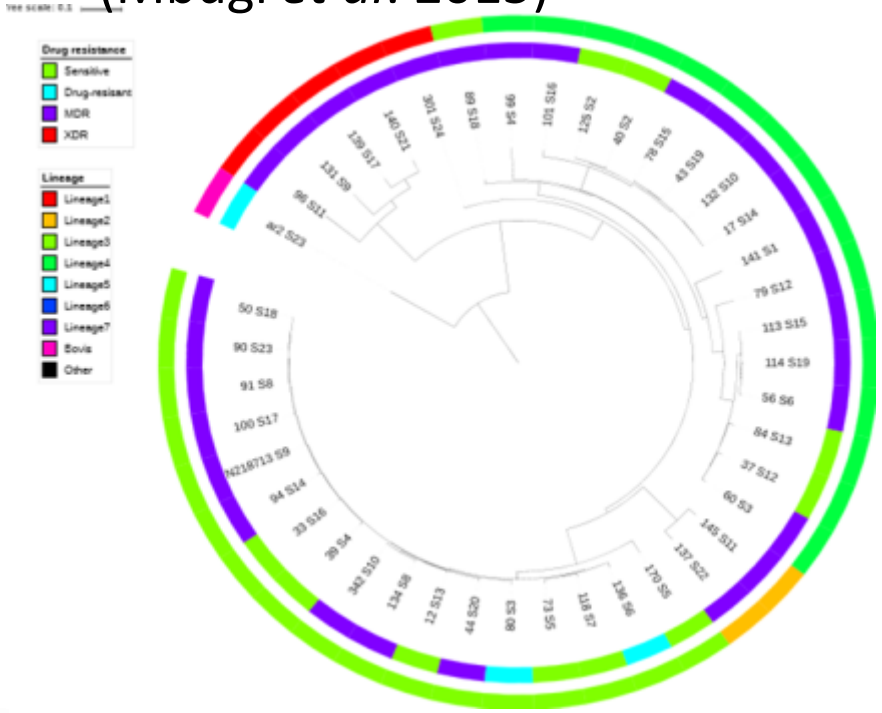


Fig: Phylogenetic tree showing the variations between the patterns of drug resistance (DR) and genotypic family of *M. tuberculosis* strains

- In conclusion, a diversity of mutations that could assist with developing rapid diagnostics for clinical patient management
- Inspite of challenges associated with WGS in resource limited countries, WGS is cost effective and provides actionable results with reference to infection control
- Ongoing: WGS of >isolates to increase sample size

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