Epidemiology of multi-drug resistant *Mycobacterium tuberculosis* in South Africa: A molecular analysis of historical samples

Nontuthuko E Maningi[1, 2,] Luke T Daum[3], Sue A Worthy[3], John D Rodriguez[3], Remco PH Peters[1,4], Matsie Mphahlele[2], Gerald W Fischer[3], James P Chambers[5], P Bernard Fourie[1]

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

[1]University of Pretoria, Department of Medical Microbiology, Pretoria, South Africa
[2] Tuberculosis Laboratory, South African Medical Research Council, Pretoria, South Africa
[3]Longhorn Vaccines & Diagnostics, San Antonio and Bethesda, USA
[4]Anova Health Institute, Johannesburg, South Africa
[5] The University of Texas, San Antonio TX, USA

Introduction

- Multidrug-resistant (MDR) tuberculosis (TB) has emerged as a global epidemic, with at least 450,000 new cases estimated to occur in 2012 worldwide (1)
- Extensively drug-resistant tuberculosis (XDR-TB) strains was first defined in 2006; by 2012 a total of 90 countries have reported at least one case of XDR-TB (2)
- In 2009 South Africa accounted for more than 2.4% of the global MDR-TB burden – 10,000 cases (3)
- XDR strains are resistant to first line drugs, any of the fluoroquinolones and one of the second-line injectable drugs (amikacin, kanamycin or capreomycin)
- The study aim is to confirm phenotypic MDR-TB resistance profile and to perform whole-genome MDR-TB sequencing to characterize known mutations and deduce novel polymorphisms

Results

- Three of the 240 MDR cultures submitted to LPA had mutations on *gyr*A and *rrs* genes (*gyr*A: S91P and A90X, T95S; *rrs*: G1484T and A1401R)
- Mutations in the *rpo*B gene were most frequent at codon 531, 526 and 516 (43.14%, 25.49% and 7.84% respectively)
- Five novel mutations on the *rpo*B gene were detected (WC: D516V, T480A, Q253R, V249M and V251Y; GP: V251F
- Most *kat*G gene mutations were at codon 315 (42.2%)
- Six novel stop codons were discovered in the *kat*G gene (GP: W477STOP, Q88STOP, W198STOP, W477STOP, W412STOP; WC: W198STOP)
- In the *inh*A gene, two novel mutations were detected from Gauteng province isolates (S100A and I200T)

Figure 1: Methods flow

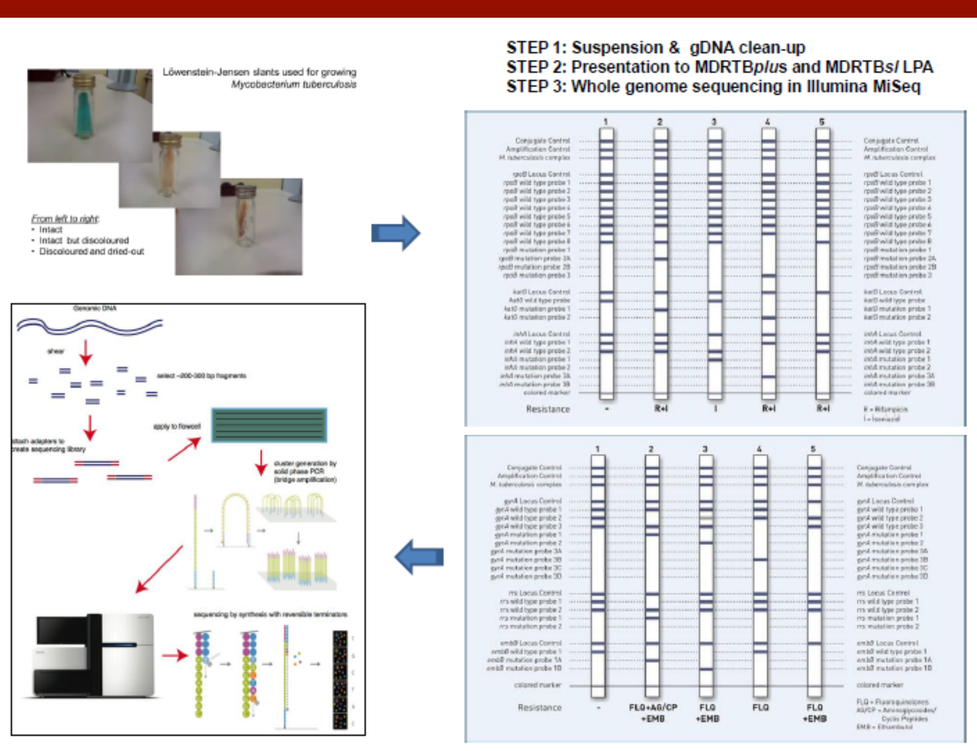
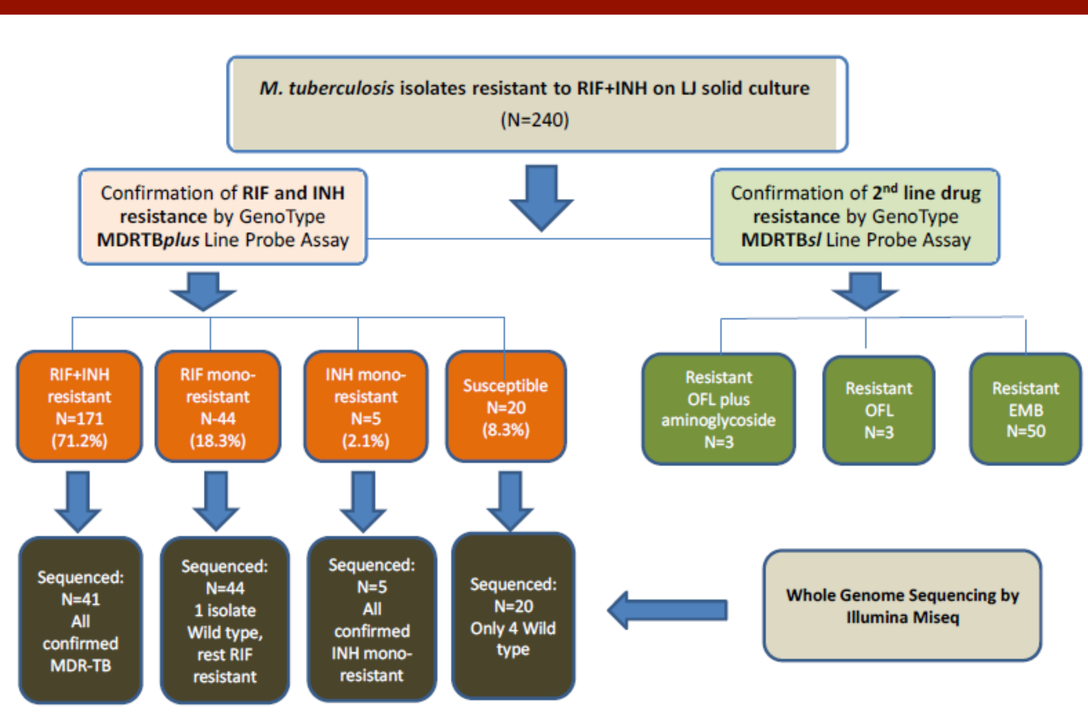


Figure 2. Confirmation of resistance profile



Material & methods

- *Mycobacterium tuberculosis* isolates collected between 1993 and 1995 from the Western Cape (WC) and Gauteng Province (GP) in South Africa and phenotypically determined to be resistant to at least rifampicin and isoniazid (N=240) were DNA extracted and the MDR-TB status of the samples confirmed using a commercial line probe assay (GenoType MDRTB*plus* and GenoType MDRTB*sl*, Hain Lifescience) (**Fig. 1**)
- Mutations in genes known to confer resistance to anti-TB drugs were characterized and discrepant results between line probe assay and conventional DST culture methods were resolved by Illumina MiSeq next-generation sequencing by methods described elsewhere (4) (**Fig. 2**)

Discussion & conclusion

- This study revealed novel mutations and confirmed old and newly emerging mutations
- One isolate had mutations by whole gene sequencing to *rpo*B, *kat*G, *gyr*A, *pnc*A, *rrs* and *rps*L
- The novel stop codons on the *kat*G gene indicate an evolution in *kat*G resistance
- Indications are that, as per the current definition, XDR-TB already existed in South Africa in the early 1990s
- Next generation whole gene/genome sequencing offers a definitive platform for rapid detection and characterisaion of tuberculosis cases with drug resistance

References / Notes

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