# Tracking cross-border transmission of the Rwanda MDR-TB R3 Clone

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## R3clone in Rwanda

374 rifampicin resistant (RR) *M. tuberculosis* (*Mtb*) strains collected from 1991-2021

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Genome sequencing, mapping and transmission phylodynamics

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Single clone responsible for 70% of RR-TB
Termed the R3clone (Rifampicin Resistant Rwanda clone)

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Clone is resistant to all 1st line drugs and has an *rpoC* compensatory mutation

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R3clone has been spread since rifampicin was introduced (late 1980's)

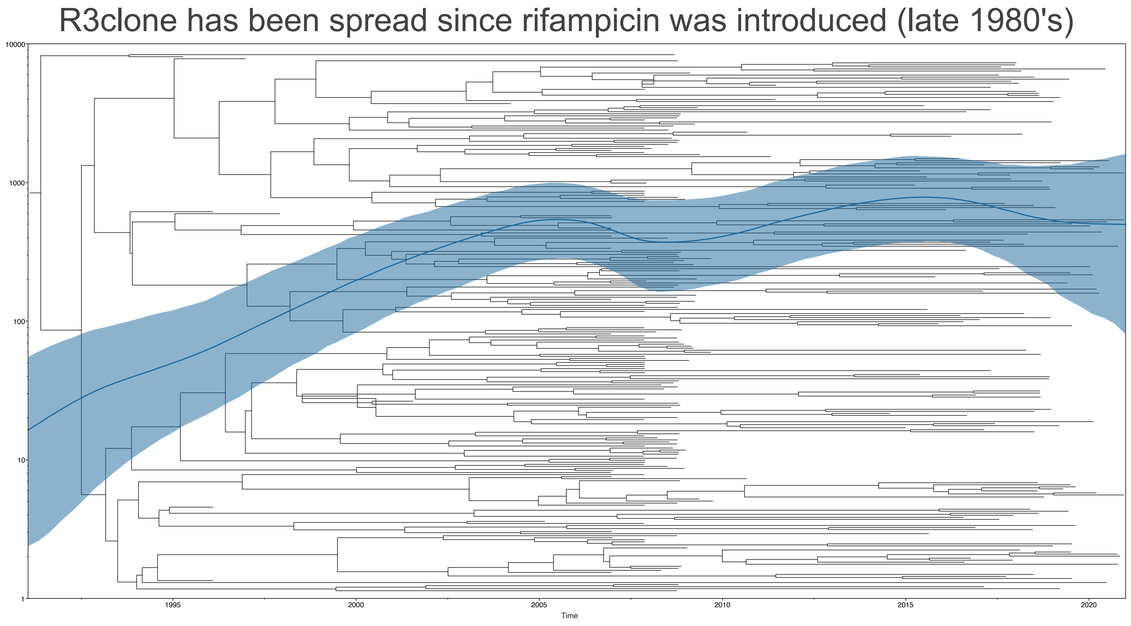



**Figure 1. Phylodynamics of the R3clone in Rwanda.** BEAST was used to estimate the transmission dynamics including time (branch length on X-axis) and bacterial population size (black line with blue HPD on Y-axis).

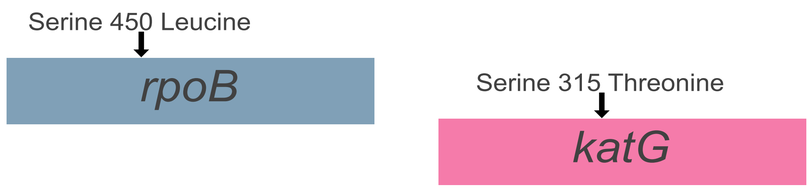
### Is the R3clone found outside Rwanda?

## Creating a diagnostic signature

### Spoligotype

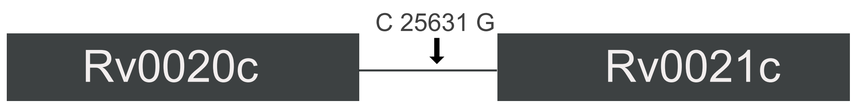
- 1111111111111111111111111111111100001110111

### Drug resistance mutations




### Clone-specific SNP

1. Find a SNP common to all R3clone strains but absent from all other Rwanda strains
2. Search for this SNP in all *M. tuberculosis* strains in the SRA
   - Confirmed SNP is specific for R3Clone strains




### Can we use these signatures to quickly find R3clone isolates?

## Detecting the R3clone

### Low cost screening of isolates

- 143 RR-TB isolates from Burundi (2011-2013)
  - No genome data
- Spoligotype and *rpoB* sequencing performed
  - 37 with patterns matching R3clone
- Further subjected to R3clone SNPqPCR
- 25 sample (17%) detected as R3clone
  - Confirmed using WGS

### Automated screening of SRA

- SRA data searched using TB-profiler website
- Extract all with the same sub-lineage as R3clone (L4.6.1.2)
- Read mapping and transmission clustering
- 47 samples from outside Rwanda confirmed R3clone
  - 7 from neighbouring countries (6 DRC and 1 Uganda)
  - 4 from Europe (Belgium and UK)
  - Remaining 37 without country information

### Screening via Deeplex Myc-TB

- Retrospective convenience sampling in Bukavu, DRC
- 3 R3clone strains detected via Spoligtype/DR mutation combination

### Clone is present in neighbouring countries and beyond

## Phylogeography

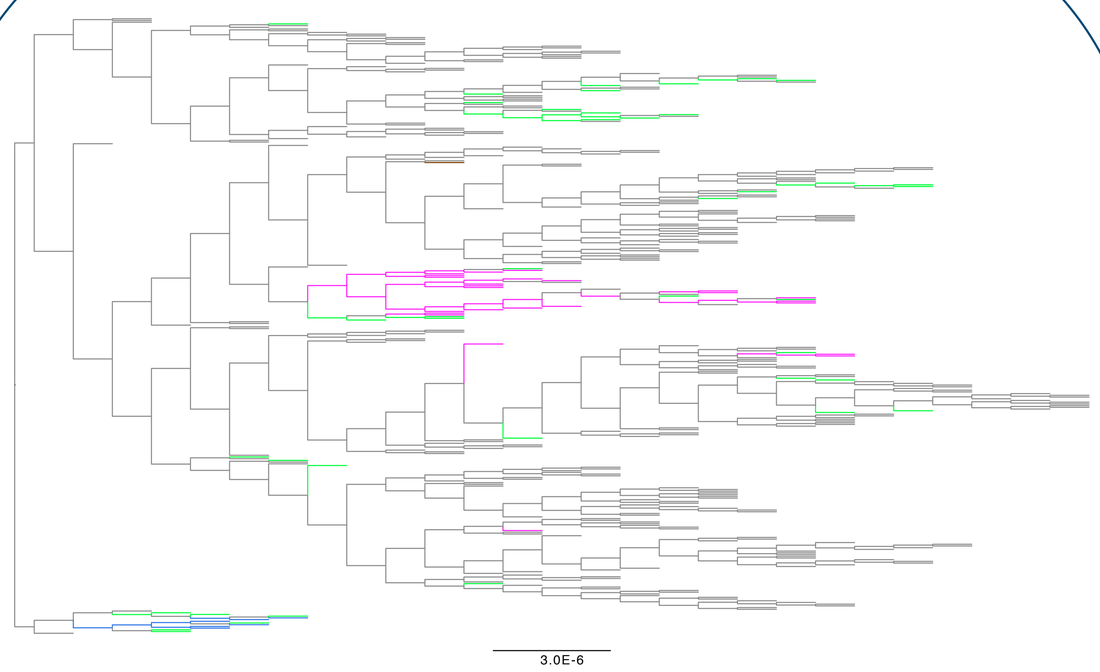



**Figure 2. Phylogeny of R3clone coloured by country.** RAxML-NG was used to build a maximum likelihood tree of all R3clone strains. Branches in coloured in the following way: Grey-Rwanda; Pink-Burundi; Blue-DRC; Brown-Uganda; Green-UK/Belgium/Unknown. Internal branches are coloured based on the origin of majority of descendant strains

## Conclusions

1. The R3clone is still prevalent in Rwanda
   - 18/32 (56%) of sampled RR-TB cases in 2021
2. Detecting the clone can be done via multiple methods
   - Spoligotype/DR patterns allows Deeplex Myc-TB detection
   - SNP qPCR also possible
3. R3clone present in neighbouring countries
   - Likely multiple introductions from Rwanda
   - More sampling of region needed to determine full cross border picture
   - Transmission studies/interventions need to look beyond national borders

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