Next-generation sequencing for drug-resistant TB
Madagascar experience with MinION

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Plan

1. TB in Madagascar
2. Drug resistance surveillance and previous insights on transmission
3. DNA-Sequencing implementation process, platform structure and workflow
4. Sample results
5. Early case examples of DNA-Sequencing public health information
6. Challenges to come
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## TB in Madagascar

<table>
<thead>
<tr>
<th>Estimates of TB burden*, 2017</th>
<th>Number (thousands)</th>
<th>Rate (per 100 000 population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality (excludes HIV+TB)</td>
<td>13 (7.8–20)</td>
<td>52 (31–79)</td>
</tr>
<tr>
<td>Mortality (HIV+TB only)</td>
<td>0.65 (0.29–1.2)</td>
<td>2.6 (1.1–4.5)</td>
</tr>
<tr>
<td>Incidence (includes HIV+TB)</td>
<td>61 (39–87)</td>
<td>238 (154–340)</td>
</tr>
<tr>
<td>Incidence (HIV+TB only)</td>
<td>1.5 (0.69–2.7)</td>
<td>6 (2.7–11)</td>
</tr>
<tr>
<td>Incidence (MDR/RR-TB)**</td>
<td>0.44 (0.087–1.1)</td>
<td>1.7 (0.34–4.2)</td>
</tr>
</tbody>
</table>

### Incidence and Funding Trends

#### Incidence Trend (2000–2016)

- **Incidence**
- **Notified (new and relapse)**
- **Incidence (HIV+TB only)**

#### Funding by Source (US$ millions, 2006–2018)

- **Grants (excluding Global Fund)**
- **Global Fund**
- **Domestic**

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**Sources**

- WHO – Madagascar tuberculosis country profile (2018)
- Ministry of Health of Madagascar, Programme national de lute à la tuberculose (2018)
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Drug resistance surveillance

Grandjean Lapierre S., Knoblauch A. et al. [Unpublished – copyrights Institut Pasteur de Madagascar]
Insights on transmission

![Maps illustrating spatial clustering of TB cases and potential transmission areas.](image)

**Fig. 4** Spatial signatures of TB identified by the Kulldorff spatial scan method. 

- **A**: Spatial clustering of TB cases and patients with genotypic clustered isolate.
- **B**: Distribution of isolates families in each spatial clustering of patients with genotypic clustered isolates.

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Implementation process

**Illumina & nanopore sequencing of MDR and controls**

- September 2017
- February 2018
- March 2018
- April 2018
- May 2018
- September 2018
- March 2019

- Retrospective Sample selection
- Research assistant onboarding
- Training & summit

**Prospective nanopore sequencing**
Platform structure and workflow

1. Review of the current methods available for the sequencing of *Mycobacterium tuberculosis* complex

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  1.2.3 Next-generation sequencing: Platforms and considerations ................... 5
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WHO – FIND, The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex: technical guide, 2018
Platform structure and workflow

Figure 7
The Madagascar project TB sequencing and analysis workflow.

Institut Pasteur de Madagascar
Hosts Madagascar’s NTP reference laboratory
Mycobacteria Unit – “Level 3” laboratory

Pre-PCR dedicated space
Sequencing lab dedicated space
Biosafety level 3 culture capacity

One full time equivalent post-doctoral researcher
Three part time laboratory technicians

“Level 3” laboratory
Platform structure

“Level 2” laboratory

“Level 1” laboratory
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Sample results

- Madagascar TB DNA-Sequencing program
  - Over 100 retrospective isolates
  - Over 50 prospective isolates
  - Turn around times of 3-4 working days post culture
  - All MDR/RR isolates in the country
  - All phenotype – GeneXpert – HAIN discordant isolates in MDR-TB high risk patients
  - Other research oriented projects
Sample results

The EPI2ME *M. tuberculosis* AMR pipeline on the EPI2ME platform provides real-time identification of first-second-line drug resistance of TB samples.
Sample results

Quality Control
1. *Porechop* was run to trim adapter sequences from reads and discard reads with adapters found in the middle. More detailed information can be found in *porechop.log*. For quality control plots of the reads after this step, see *plot.prs*.
2. Reads were aligned to the TB reference NC_000962.3 using *Minimap2*.
3. All reads which did not map to NC_000962.3.3a were removed. Prior to filtering, there were 345130 reads. After filtering there remained 20392. This means 98.68% of reads mapped to NC_000962.3.3a. For more stats on the pre-filtered reads see *stats.pre* and for post-filtered reads see *stats.post*. For quality control plots of the reads after this step (and read percent identity to NC_000962.3.3a) see *plot.post*. Stats were produced with *bamtools* and plots with *Pleido*.

Mykrobe Analysis
A summary of the susceptibility information from *Mykrobe* is shown here. For the full report, see *mykrobe*. If resistance is identified for a drug then the predicted resistant variant(s) is/are given, along with supporting information.

- Rifampicin  
  Prediction: Resistant
  Called by rmpA, rmpA2, rmpA3
  Reference median depth: 0
  Alternate median depth: 2

- Amikacin  
  Prediction: Susceptible

- Streptomycin  
  Prediction: Susceptible

- Ethambutol  
  Prediction: Susceptible

- Pyrazinamide  
  Prediction: Resistant
  Called by pzaA, pzaA2, pzaA3
  Reference median depth: 0
  Alternate median depth: 2

- Kanamycin  
  Prediction: Susceptible

- Gatifloxacin  
  Prediction: Susceptible

- Quinolones  
  Prediction: Susceptible

- Isoniazid  
  Prediction: Resistant
  Called by rpsD_3917, rpsD_0317
  Reference median depth: 0
  Alternate median depth: 2

mykrobe
  BC03_predictARN
  plot.post
  BC03_post_filering.pdf
  plot.prs
  BC03_post_filering.pdf
  porechop_log
  porechop.log
  stats.post
  BC03_post_filering.bt
  stats.prs
  BC03_post_filering.txt

Author: Michael Hall | michael.hall@bels.ac.uk | 2019-04-18
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Public health information

• **Is there MDR person to person transmission in Madagascar?**
  Rare geospatial and molecular clustering of confirmed MDR-TB cases (2012-2017)

![Map and tree diagram showing clustering of MDR-TB cases](image)

• **How should we re-design Madagascar’s drug resistance surveillance laboratory testing algorithm?**
  National resistome-guided review of analytical performance of rapid molecular assays like GeneXpert and HAIN

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Individual patient information

- Investigation of atypical phenotypic DST profiles
  Recognition of Non Tuberculosis Mycobacteria
  Suspicion of mixed infections

- WGS is not yet used for systematic prospective DST
Early successes

- Early integration with NTP
- Locally recruited and trained human resources
- International partnership and mentoring
- Systematic data sharing in CRyPTIC database
- Research & routine diagnostics shared platform
Challenges to come

- Training & advocacy for clinicians and NTP managers
- Integration of diagnostics with therapeutic guidelines
- Improvement of turn around times
- Delocalization towards “level 2” and “level 1” laboratories
- Strengthening of the procurement system and general business case for NGS in LMICs
- Sustained funding
Thanks to a team of teams