NGS for diagnosis and management of drug resistant tuberculosis

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The roadmap to new TB diagnostics to achieve End TB and Global Plan targets

**Improve TB case detection**

1. Triage test (high NPV)
   Or **ideally**
2. Highly sensitive stand-alone detection test

**Universal access to DST**

1. TB confirmation with rapid integrated DST for critical drugs
2. Test for cure
3. Comprehensive DST to cover the extended portfolio of drugs
4. DR surveillance
5. Control transmission

**Support TB elimination**

1. LTBI: Test to identify high risk of progression to active disease
2. Incipient TB test: to identify early subclinical TB

New Diagnostics Working Group
Whole genome sequencing

- Species and lineage ID
  Evolution studies
- Resistance to first-, second-line and new drugs
- Epidemiological and transmission analysis

Public health: anti-TB Drug Resistance Surveys and Surveillance

Development and evaluation of user-friendly tools

KNOWLEDGE INCREASE

NGS have *measurable* parameters that can be used for QC

✓ Diagnosis of DR TB
✓ Transmission analysis
✓ Drug resistance survey and routine surveillance
✓ Approaching new MDR TB drugs
Prevalence of resistance estimated through sequencing compared with phenotypic testing

EVIDENCE

Large overlap between resistance determined by genetic sequencing, after adjustment for sensitivity, and the true prevalence of drug resistance

✓ Accuracy of genetic sequencing is very good at predicting phenotypic resistance to rifampicin, isoniazid, the fluoroquinolones, and (among rifampicin-resistant cases) injectable drugs
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A cluster of multidrug-resistant Mycobacterium tuberculosis among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study

Case Definition Proposal

- Resistance phenotype as on table 3; i.e. INH high-level R, RIF R, CAP R, PZA R, quinolones S, amikacin S.
- Specific set of resistance mutations: see table 3
- MIRU-VNTR: 2-2-4-2-4-3-3-2-4-2-2-5-1-4-3-3-4-4-2 and/or WGS core genome MLST with <4 SNP (to be discussed)

MTBseq: a comprehensive pipeline for whole genome sequence analysis of Mycobacterium tuberculosis complex isolates

Elisa Tagliani, Daniela Maria Crillo, Cusca Kódmón, Marijke J van der Werf and the EUSeqMyTB Consortium

Published: April, 2018 - DOI: https://doi.org/10.1016/S1473-369X(18)30132-4
Whole genome sequencing

- Species and lineage ID
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- Public health: anti-TB Drug Resistance Surveys and Surveillance
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  KNOWLEDGE INCREASE
WHO-recommended rapid diagnostics for DR-TB

Xpert ULTRA
- Integrated/automated qPCR
- Fully nested amplification
- Results in 1h17m
- Technical expertise: none
- TB detection by targeting two different multi-copy genes (IS6110 & IS1081)
- High-resolution melt curve analysis for rpoB detection
- Additional PCR modifications to improve sensitivity and specificity

MTBDRplus
- Reverse hybridization, colorimetric reaction
- Results in 6-7 h
- Technical expertise: some
- Biosafety lev 2
- Detect resistance in TB to Rifampicine (RpoB gene) and Isoniazide (InhA, KatG genes),

MTBDRsl
- Reverse hybridization, colorimetric reaction
- Results in 6-7 h
- Technical expertise: some
- Biosafety lev 2
- Detect resistance in TB to the fluoroquinolones (FQs; ofloxacin, moxifloxacin and levofloxacin) and the second-line injectable drugs (SLIDs; amikacin, kanamycin and capreomycin),

Xpert ULTRA
MTBDRplus
MTBDRsl
Suboptimal performance of rapid tests

- inhA t-8a and katG S315T; pncA G132A
- Lineage LAM

- inhA I21T and fabG1 L203L and katG A106V; pncA WT
- Lineage Beijing

- katG S315T; pncA H51D
- Lineage S-type

I491F not targeted by Xpert MTB/RIF
Not detected
Not treated=TRANSMITTED

LEGEND
Pink circle: strains harbouring rpoB I491F
Light blue circle: strains harbouring rpoB mutations other than I491F
Purple shadow: clustered strains (≤5 alleles)
Fast advancement in knowledge and standardization
<table>
<thead>
<tr>
<th>Drug (phenotypic testing)</th>
<th>Gene</th>
<th>High confidence mutations</th>
<th>Moderate confidence mutations</th>
<th>Minimal confidence mutations</th>
<th>No association with resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>mkhA-mabA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>katG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-line (group B)</td>
<td>AMK</td>
<td>rrs</td>
<td>a1401g, g148t</td>
<td>c-12t, g-37t</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KAN</td>
<td>eis</td>
<td>c-14t, g-10a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAP</td>
<td>rrs+eis</td>
<td>a1401g, a514c, c1402t, g148t</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>thylA</td>
<td>N236K, Pooled frameshifs and premature Stop codons</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rrs</td>
<td>a1401g, a514c, c514t, c462t, c513t, e517t</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-line (group C)</td>
<td>ETH/PTH</td>
<td>mkhA</td>
<td>c-15t+I194T, c-15t+I549A</td>
<td>c-15t</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>etkA</td>
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</tbody>
</table>
Building the framework for standardized clinical laboratory reporting of next generation sequencing data for resistance-associated mutations in *Mycobacterium tuberculosis* complex

Jeffrey A. Tornheim,1,‡ Angela M. Starks,2,‡ Timothy C. Rodwell,3,‡ Jennifer L. Gardy,5,6 Timothy M. Walker,7 Daniela Maria Cirillo,8 Lakshmi Jayashankar,9 Paolo Miotto,8 Matteo Zignol,10 Marco Schito11

2a. One-Page Report

2b. Long Report Resistance Predictor

2c. Long Report NGS Statistics
Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC) to speed up diagnosis of tuberculosis

ECOFF definition of individual drugs

Rancoita P et al AAC 2018
Fowler et 2019 in preparation
Some \textit{rpoB} mutants are associated to false-sensitive DST for rifampicin

“Disputed mutations” cause DISCREPANT genotypic/phenotypic results

\textit{in silico} analysis of the effect of disputed mutations on the structural interaction between the RpoB protein and rifampin

The binding affinity towards rifampin is affected

➢ Strains should be considered RESISTANT to rifampin

INH resistance is a heterogeneous phenotype

- Possibility to use different isoniazid doses depending on the type of mutation detected
  - If \textit{inhA} mutations only are detected INH could be used; high doses are likely to be effective.
  - If \textit{katG} mutations only are detected, use of high doses is an option.
    - Most \textit{katG} mutations (other than 315 codon) confer moderate resistance that might be treated with higher doses of INH; even the most common S315T variant leads to a variable range of resistance.
    - In the absence of additional mutations affecting the \textit{inhA} gene (and \textit{ethA} gene, so far uniquely detectable by sequencing approaches), ethionamide can be considered an option for the intensive phase of the shorter regimen.
  - If \textit{inhA} + \textit{katG} mutations are concurrently detected, INH drug use should be avoided, since these patterns are linked to high resistance levels.
  - \textbf{Additional} mutations in \textit{katG} and \textit{inhA} not included in LPA increase MIC
WGS to predict sensitivity

Analysis of 10,209 MTB isolates
16 Countries, 6 continents
All major lineages represented

- Resistance to H, R, E, Z was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity,
- Susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity.
- 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted.
- On the 4037 phenotypic profiles predicted to be pan-susceptible, 3952 (97.9%) were correctly predicted.
Simulated Negative Predictive Values for Individual Drugs and Complete Drug Profiles

Negative predictive values are shown for individual drugs and complete drug profiles, according to the simulated prevalence of resistance to each drug, or within each drug profile (any resistance).

➢ WGS can predict profiles of susceptibility to first-line anti-TB drugs with a degree of accuracy sufficient for clinical use.
Bedaquiline resistance emerges under treatment
Drugs MICs distribution and correlation with mutations from Cryptic-Italy collection

**Delamanid**
- 1 MDR isolate with \( ddn\_Pro5fs \)
- 1 MDR isolate with \( fbiB\_Arg88Ser \)
- 1 isolate with \( ddn\_Trp20^* \)
- 1 isolate with \( ddn\_Leu90fs \)

**Linezolid**
- 3 isolates with \( rrl\_g2814t \)
- 1 isolate with \( rrl\_c344t \)
- 1 isolate with the \( rplC\_Val16\_Asp18dup \)
- 7 isolates with \( rrl\_c344t \)

**Bedaquiline**
- 1 MDR isolate with \( Rv0678\_Arg38fs \)
- 1 MDR isolate with \( Rv0678\_Ile67fs \)
- 1 INH-mono R isolate with \( Rv0678\_Leu136Pro \)
- 1 MDR isolate with \( Rv0678\_Gly121Arg \)

**Genes analyzed for DLM**
- \( ddn\): \( Rv3547\): -100 bp (3986744-3987299)
- \( fgd1\): \( Rv0407\): -100 bp (490683-491793)
- \( fbiA\): \( Rv3261\): -100 bp (3640443-3641538)
- \( fbiB\): \( Rv3262\): (3641535-3642881)
- \( fbiC\): \( Rv1173\): -100 bp (1302831-1305501)

**Genes analyzed for LZD**
- \( rplC\): \( Rv0701\) (800809-801462)
- \( rrl\): \( RvnR02\) (1473658-1476795)

**Genes analyzed for BDQ**
- \( Rv0678\): -100 bp (778890-779487)
- \( atpE\): \( Rv1305\) (1461045-1461290)
- \( pepQ\): \( Rv2535c\): +100 bp (2859300-2860518)
Deeplex-MycTB (Genoscreen): hi-plex amplification of main TB drug resistance targets (1st and 2nd line plus LZD and BDQ/CFZ) plus mycobacterial species identification (hsp65) and MTBC genotyping targets (spoligotyping + phylo SNPs)

Targeted Next Generation Sequencing on ONT device

MinION (Oxford Nanopore Technologies) is a portable and real-time device for DNA and RNA sequencing
Training and technology transfer

We provide training to strengthen tuberculosis national laboratory networks and to support operational research

Background

We provide training to strengthen tuberculosis national laboratory networks and to support operational research

Since 2013

- Trained >300 TB HCW in 20 different countries
- > 200 TB specialists over 26 countries in America, Africa, Asia and Europe

Mid/long-term goals

- to support the implementation of genomic platforms to provide diagnostic of DR TB in hubs in Africa and Asia
- To provide tech transfer
- to explore the possibility to be involved in AMR in LIC
Precision global health in the management of TB
Merging precise individual care and large-scale programmatic functions

Consider:
- DST, preference for oral route, DR prevalence, h/o previous treatment, tolerability, drug-drug interactions, severe forms

STANDARDIZED SHORTER MDR-TB REGIMEN
4-6 Amk-M-Pto-Cfz-Z-Hhd/E / 5 M-Cfz-Z-E

TB POSITIVE
Rif (PRE-XDR-TB or XDR-TB)

TREATMENT FOR DRUG SUSCEPTIBLE TB
(2HRZE/4HR)

FIRST-LINE RAPID DIAGNOSTIC
XPERT MTB/RIF FOLLOWED BY CULTURE

TB-POSITIVE Rif-R

LPA-SL

PATIENT DATA FOR DOSAGE PRECISION

TARGETED NEXT GENERATION SEQUENCING AND MIC

CLINICAL DECISION SUPPORT SYSTEM
ARTIFICIAL INTELLIGENCE

CENTRAL DATABASE FEED-BACK LEARNING LOOP FOR ADJUSTMENT OF DX, RX, APPROACHES

SOCIAL SUPPORT & COUNSELLING

DRUG PROCUREMENT SYSTEM PRECISION SUPPLY

PHARMACOVIGILANCE SYSTEM BIOMARKERS TO PERSONALISE DURATION

Adapted from Mario C. Raviglione Global Health
Thank you!