Sequencing for the surveillance of drug–resistant TB

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Surveillance of drug-resistant TB: Current situation
Global project on anti-TB drug resistance surveillance

Collects representative data from the TB patient population to
- Estimate the magnitude of drug resistance
- Understand trends in drug resistance over time
- Guide planning, resource allocation and policy development

From 1994

Until now

Data from 160 countries or 97% of the world’s TB cases

Oldest and largest global surveillance project for anti-microbial resistance (AMR)
Global status in 2017
Rifampicin-resistant (RR) TB among new patients

3.5% of new patients have RR-TB (including MDR-TB)
Global status in 2017
Rifampicin-resistant (RR) TB among previously treated patients

18% of previously treated patients have RR-TB (including MDR-TB)
Surveillance of drug-resistant TB: Challenges
Coverage of data in 2017
Periodic surveys versus continuous surveillance systems

Challenge: moving from periodic surveys to continuous surveillance systems

Rifampicin testing result for $\geq 80\%$ bacteriologically confirmed new cases
Nationally representative sample of patients

Source of data
- Surveillance
- Survey
- No data
- Not applicable
Challenge: more recent data needed from many countries

Coverage of data in 2017
Rifampicin-resistant TB among new and previously treated patients
Coverage of data in 2017
Second-line resistance among MDR-TB patients

Challenge: data needed for other drugs beyond rifampicin
Surveillance of drug-resistant TB: Incorporating sequencing
Role of sequencing

• Overcomes challenges of phenotypic-based surveillance by allowing testing
  • of a greater range of drugs
  • in less time
  • for more countries

• Additional information beyond prevalence of resistance
  • Other genotypic information, e.g. transmission
  • Future analyses to explore the potential of new diagnostics and new drugs
Proof of concept
A multi-country population-based study

Background

• Multi-partner project coordinated by WHO NTPs, Supranational TB Reference Laboratories (SRLs), BMGF, USAID, TB Alliance
• Representative surveys of TB patients already conducted in seven countries
  Azerbaijan, Bangladesh, Belarus, Pakistan, Philippines, South Africa, Ukraine

Methodology

• Sequencing of culture isolates from 7,094 patients SRLs by either
  whole genome sequencing (Illumina or Ion Torrent)
  targeted gene sequencing (Sanger)
• Role of mutations classified according to established framework
  see webinar by Paolo Miotto on 12 Feb
• Accuracy of genetic sequencing compared to phenotypic testing
• Bayesian approach used to account for sensitivity and specificity of sequencing in estimates of prevalence of resistance among TB patients

Proof of concept
A multi-country population-based study

Findings

• Sensitivity of sequencing varies between drugs and patient groups, for example

<table>
<thead>
<tr>
<th>Drug</th>
<th>Locus</th>
<th>RIF-susceptible cases</th>
<th>RIF-resistant cases</th>
<th>All cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no. of isolates</td>
<td>sensitivity % (95%CI)</td>
<td>no. of isolates</td>
</tr>
<tr>
<td>RIF</td>
<td>rpoB</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>INH</td>
<td>katG &amp; inhA</td>
<td>6,065</td>
<td>81 (66-90)</td>
<td>953</td>
</tr>
</tbody>
</table>

Detailed results by drug, patient group and geographical site given in reference below.

• Overall, sequencing produces consistent results
• Comparative advantages of sequencing
  greater range of drugs, less time, more countries
• Practicalities of implementation in countries?
  see webinar by Andrea Cabibbe on 19 Feb

Prevalence of resistance
Isoniazid phenotypic testing versus sequencing of katG and inhA

Consistent results – large overlap between sequencing and phenotypic testing

Incorporating sequencing into surveys

12,000 samples from 13 countries

Azerbaijan
Bangladesh
Belarus
Djibouti
Pakistan
Philippines
South Africa
Ukraine
DR Congo
Eritrea
Eswatini
Ethiopia
Indonesia
Examples from the field

Whole genome sequencing of culture isolates from national survey in Eswatini

**Eswatini**
(sample size 1,008 new patients)

- Presumptive TB patient
  - Xpert MTB/RIF
    - Negative for *M. tuberculosis* complex
    - Positive for *M. tuberculosis* complex
      - Culture in MGIT
        - Negative for *M. tuberculosis* complex
        - Positive for *M. tuberculosis* complex
          - Phenotypic drug susceptibility testing
          - Whole genome sequencing

National TB Reference Laboratory

Partners: National TB Control Programme, WHO, US CDC, SRL Kampala, SRL Milan (Ospedale San Raffaele)
Examples from the field

Targeted deep sequencing on sputum from national survey in DR Congo

DR Congo
(sample size 1,500 new patients)

Presumptive TB patient

Sputum smear microscopy

Positive sputum smear microscopy

Negative sputum smear microscopy

Xpert MTB/RIF

Positive for M. tuberculosis complex

Negative for M. tuberculosis complex

Sputum preservation in ethanol

Targeted deep sequencing

Health centre enrolling patients in survey

Partners: National TB Programme, WHO, SRL Antwerp (ITM), UZ Leuven, UC Louvain
Global data platform at WHO
For surveillance of drug-resistant TB by sequencing

- Cloud-based software at WHO (from March 2019)
- Adapted from multi-partner ReSeqTB (relational sequencing platform), hosted by Critical Path Institute (see webinar by Tim Rodwell on 5 Feb)
- To support countries with
  - Analysis through standardized pipeline
  - Interpretation of mutations by expert knowledgebase (see webinar by Paolo Miotto on 12 Feb)
  - Linkages with patient data and phenotypic results
  - Safeguarding of data
- A “living” platform – interpretation updated as new information become available
- Can inform future policy on sequencing and other diagnostics
Tagliani, Hassan, Waberi, de Filippo, Falzon et al (2015). Culture and Next-generation sequencing-based drug susceptibility testing unveil high levels of drug-resistant-TB in Djibouti: results from the first national survey. Scientific Reports. 7(1):17672


Thank you