Surveillance of resistance to fluoroquinolones and pyrazinamide

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20 years of surveillance of anti-TB drug resistance
20 years of surveillance of anti-TB drug resistance: lab methods unchanged

- Phenotypic drug susceptibility testing

- First-line DST on new and previously treated TB cases:
  - rifampicin
  - isoniazid
  - ethambutol
  - streptomycin

- Second-line DST on MDR-TB cases only:
  - fluoroquinolones (ofloxacin, moxifloxacin, levofloxacin): the most commonly used in the country is tested – usually ofloxacin
  - injectable agents (kanamycin, amikacin, capreomycin) the most commonly used in the country is tested – usually kanamycin
Challenges in anti-TB drug resistance surveys

- Organization/logistics:
  - Dedicated human resources at NRL and NTP
  - Duration of 1-2 years
  - Specimen transport within the country (from clinics to NRL)
  - Specimen transport outside the country (from NRL to SRL)

- Laboratory:
  - Capacity for culture and DST
  - Workload at NRL
Limitation of current anti-TB drug resistance surveillance

- DST limited to rifampicin and isoniazid plus fluoroquinolones and injectable agents on MDR-strains → not useful to investigate feasibility of introduction of new drugs and regimens

- Surveys are difficult to repeat at regular intervals → limited understanding of time trends worldwide
Drug resistance surveillance: vision for the future

1. Expand the range of drugs to be tested to include also:
   - pyrazinamide, fluoroquinolones, injectable agents and new drugs

2. Use of high throughput sequencing technologies

3. Increased frequency to be able to monitor time trends
Surveillance of resistance to fluoroquinolones and pyrazinamide
Objectives of the FQLs and PZA surveillance project

- **Primary objective:**
  - to assess the prevalence of resistance to FQLs (OFX, MFX) and PZA in new and previously treated, RIF-susceptible and RIF-resistant TB cases.

- **Secondary objectives:**
  - to assess proportions of cross-resistance between OFX, MFX, GFX, and LFX;
  - to assess the correlation between phenotypic DST of OFX and MFX (gold standard) and sequencing of gyrA and gyrB;
  - to assess the correlation between phenotypic DST of PZA and sequencing of pncA;
  - to evaluate the feasibility of using sequencing technologies for surveillance of drug resistance in TB.
## Project sites

<table>
<thead>
<tr>
<th>Country</th>
<th>Survey site</th>
<th>Survey status</th>
<th>No. of patients (new - retreatment)</th>
<th>SRL</th>
<th>Responsible laboratory</th>
<th>Sequencing pncA, gyrA, gyrB (method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azerbaijan</td>
<td>nationwide</td>
<td>survey completed in 2013</td>
<td>789 (549 - 240)</td>
<td>Borstel, Germany</td>
<td>Borstel</td>
<td>Borstel (Sanger)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>nationwide</td>
<td>survey completed in 2011</td>
<td>1,344 (1,050 - 291)</td>
<td>Antwerp, Belgium Milan, Italy</td>
<td>Antwerp</td>
<td>Milan (Illumina)</td>
</tr>
<tr>
<td>Belarus</td>
<td>Minsk city</td>
<td>survey completed in 2011</td>
<td>224 (156 - 68)</td>
<td>Stockholm, Sweden</td>
<td>Stockholm</td>
<td>Stockholm (Sanger)</td>
</tr>
<tr>
<td>Pakistan</td>
<td>nationwide</td>
<td>survey completed in 2013</td>
<td>1,593 (1,379 - 212)</td>
<td>Karachi, Pakistan Milan, Italy</td>
<td>Karachi, Antwerp</td>
<td>Karachi (Sanger)</td>
</tr>
<tr>
<td>South Africa</td>
<td>Gauteng &amp; Kwazulu-Natal provinces</td>
<td>enrolment ongoing</td>
<td>2,000 * (1,500 - 500) *</td>
<td>Johannesburg, South Africa</td>
<td>Johannesburg</td>
<td>Johannesburg (Illumina - whole genome)</td>
</tr>
</tbody>
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* Expected number as enrolment of patients is still ongoing
Laboratory work
6 SRLs: Antwerp, Borstel, Johannesburg, Karachi, Milan, Stockholm

- **FQLs:**
  - all strains tested for resistance to OFX (2.0 μg/ml) and MFX (0.5 μg/ml) (MGIT);
  - all strains resistant to OFX &/or MFX tested for resistance to GFX (2.0 μg/ml), MFX (2.0 μg/ml), LFX (1.5 μg/ml) and OFX (2.0 μg/ml);
  - all strains resistant to OFX &/or MFX and 10% of susceptible undergo gyrA & gyrB sequencing (Azerbaijan, Belarus, Bangladesh, Pakistan); all strains undergo gyrA & gyrB sequencing (South Africa).

- **PZA:**
  - all strains undergo sequencing of pncA;
  - all strains with pncA mutation and 10% of wild type strains tested for resistance to PZA (MGIT) (Bangladesh, Pakistan); all strains tested for resistance to PZA (MGIT) (Azerbaijan, Belarus, South Africa);
  - strains with discordant phenotypic and sequencing results tested with modified Wayne method.
Preliminary findings on PZA resistance

- PZA resistance is low in the general population with exception of EEU
- PZA resistance is lower than RIF resistance
- PZA resistance significantly higher in RIF-resistant strains (30%-80%)
- PZA resistance higher previously treated TB cases

When comparing sequencing with phenotypic DST:
  - Sensitivity: 80%-90%
  - Specificity: 99%
Preliminary findings on fluoroquinolones resistance

- OFX 2.0µg/ml resistance is generally lower than RIF resistance
- MOX 2.0µg/ml resistance very low
- OFX/MXF 2.0µg/ml resistance higher in RIF-resistant strains and previously treated TB cases
- Suboptimal correlation between testing of OFX 2.0µg/ml vs. MXF 0.5 µg/ml
- Limited cross resistance between OFX 2.0µg/ml and MXF2.0µg/ml / GFX2.0µg/ml
Next steps of current project

- Updated analyses in June and September
- Dissemination of part of the results in October-November
- Inclusion of additional countries (EEU, Asia)
- Extend sequencing to other genes ($rpoB$, $inhA$, $katG$)
- Collect treatment outcome data
Acknowledgments

Implementing group:
- NRLs and NTPs of Azerbaijan, Bangladesh, Belarus, Pakistan, South Africa
- SRLs of Antwerp, Borstel, Johannesburg, Karachi, Milan, Stockholm
- WHO (CO, RO, HQ)

Donor Agencies:
- BMGF
- USAID
- TB Alliance

Technical partners:
- CDC
- NIH
Coming up

- Drug resistance surveillance data published yearly on global TB reports: 2014 supplement on DR surveillance and response

- New guidance being developed to include rapid molecular technologies (Xpert MTB/RIF and genome sequencing) in surveillance of drug resistance in tuberculosis