DST for detection of DR TB - roll out of Xpert in South Africa and overview of other technologies: what are the gaps?

Mark Nicol
Division of Medical Microbiology and Institute for Infectious Diseases and Molecular Medicine, University of Cape Town and National Health Laboratory Service

IOM/IMCAS Workshop on DR TB, Beijing 16-18 Jan 2013
Disclosure

The University of Cape Town (PI Mark Nicol) has received funding from FIND to support evaluation and demonstration studies for GeneXpert.

No other conflicts of interest.
Limitations of conventional diagnostics for drug-resistant TB

Jan 08 to June 09
- 73 patients diagnosed with DR-TB did not start treatment
- 53% died whilst waiting for their results
- median time of death was 25 days from sputum sampling.

Khayelitsha Annual Activity Report 2008-2009 MSF, Western Cape Department of Health, City of Cape Town, University of Cape Town CIDER
Genetic mutation
Gives rise to resistance
GENOTYPIC RESISTANCE

MTB fails to grow or survives
in presence of antibiotic
PHENOTYPIC RESISTANCE

Patient fails to respond to
therapy with the drug
CLINICAL RESISTANCE
Genetic mutation
Gives rise to resistance
GENOTYPIC RESISTANCE

MTB fails to grow or survives
in presence of antibiotic
PHENOTYPIC RESISTANCE

Patient fails to respond to
therapy with the drug
CLINICAL RESISTANCE

Detected by molecular testing
e.g. Xpert, LPA, sequencing

Detected by culture-based testing
e.g. MGIT, MODS

Usually undetected but may result in treatment failure or relapse
Genetic mutation
Gives rise to resistance
GENOTYPIC RESISTANCE

MTB fails to grow or survives in presence of antibiotic
PHENOTYPIC RESISTANCE

Patient fails to respond to therapy with the drug
CLINICAL RESISTANCE

Detected by molecular testing e.g. Xpert, LPA, sequencing

Detected by culture-based testing e.g. MGIT, MODS

Usually undetected but may result in treatment failure or relapse

Problems:
- Genotype-phenotype relationship unclear
- Strain background may affect penetrance
- Unknown mutations
- Geographic variability
- Heteroresistance and mixed infections

Problems:
- Slow
- Complex for some drugs (MICs close to critical concentration)
- Multiple methods, standardization
- Single concentration
- Biosafety

Problems:
- Too late!
Culture-based DST

• Principle: critical concentration of a drug at which
  • susceptible strains don’t grow
  • resistant strains grow

• 3 broad approaches
  1. Absolute concentration
  2. Resistance ratio
  3. Proportion

• Several different applications of these approaches
  • e.g., liquid culture (MGIT, MODS, colorimetric assays), solid culture (LJ, Middlebrook, TLA, nitrate reduction), phage-based assays

• Agar proportion method still considered the reference standard for most drugs
Principle of culture-based DST

Genotypic DST, e.g., rifampicin resistance

RIF binds to β-subunit of DNA-dependent RNA polymerase (rpoB) prevents initiation of transcription

81 bp RRDR – 95% RIF\textsuperscript{R} mutations
Line probe assays: GenoType MTBDRplus

1) DNA isolation (culture/sputum) → 2) PCR amplification → 3) Reverse hybridisation and signal detection

Pictures: www.hain-lifescience.de/en/
# Accuracy of GenoType MTBDRplus

<table>
<thead>
<tr>
<th></th>
<th>Rifampicin</th>
<th>Isoniazid</th>
<th>Multidrug-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity,</strong> %</td>
<td>98.9% (94.3 – 100.0)</td>
<td>94.2% (88.4 – 97.6)</td>
<td>98.8% (93.7 – 100.0)</td>
</tr>
<tr>
<td>95% CI</td>
<td>99.4% (98.0 – 99.9)</td>
<td>99.7% (98.3 – 100.0)</td>
<td>100% (99.0 – 100.0)*</td>
</tr>
<tr>
<td><strong>Specificity,</strong> %</td>
<td>99.3% (98.1 – 99.9)</td>
<td>98.2% (96.5 – 99.2)</td>
<td>99.8% (98.8 – 100.0)</td>
</tr>
<tr>
<td>95% CI</td>
<td>99.3% (98.1 – 99.9)</td>
<td>98.2% (96.5 – 99.2)</td>
<td>99.8% (98.8 – 100.0)</td>
</tr>
<tr>
<td><strong>Overall accuracy,</strong> %</td>
<td>99.3% (98.1 – 99.9)</td>
<td>98.2% (96.5 – 99.2)</td>
<td>99.8% (98.8 – 100.0)</td>
</tr>
<tr>
<td>95% CI</td>
<td>99.3% (98.1 – 99.9)</td>
<td>98.2% (96.5 – 99.2)</td>
<td>99.8% (98.8 – 100.0)</td>
</tr>
<tr>
<td><strong>PPV,</strong> % (95% CI)</td>
<td>97.9% (92.7 – 99.7)</td>
<td>99.1% (95.3 – 100.0)</td>
<td>100% (95.8 – 100.0)*</td>
</tr>
<tr>
<td><strong>NPV,</strong> % (95% CI)</td>
<td>99.7% (98.5 – 100.0)</td>
<td>97.9% (95.8 – 99.2)</td>
<td>99.7% (98.5 – 100.0)</td>
</tr>
</tbody>
</table>
Genotype MTBDR<em>plus</em> test impact?

Khayelitsha MDR cases diagnosed between September 2007 and June 2008

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Median Days to Treatment Initiation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear Negative, DST on positive culture</td>
<td>88</td>
<td>19</td>
</tr>
<tr>
<td>Smear Negative, PCR on positive culture</td>
<td>69</td>
<td>25</td>
</tr>
<tr>
<td>Smear Positive, DST on positive culture</td>
<td>59</td>
<td>16</td>
</tr>
<tr>
<td>Smear Positive, PCR on sputum</td>
<td>37</td>
<td>17</td>
</tr>
</tbody>
</table>

Slide courtesy Helen Cox, MSF
Genotype MTBDRplus test impact?

Khayelitsha MDR cases diagnosed between September 2007 and June 2008

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Median Days to Treatment Initiation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear Negative, DST on positive culture</td>
<td>88</td>
<td>19</td>
</tr>
<tr>
<td>Smear Negative, PCR on positive culture</td>
<td>69</td>
<td>25</td>
</tr>
<tr>
<td>Smear Positive, DST on positive culture</td>
<td>59</td>
<td>16</td>
</tr>
<tr>
<td>Smear Positive, PCR on sputum</td>
<td>37</td>
<td>17</td>
</tr>
</tbody>
</table>

Only 30% of cases are smear positive

Slide courtesy Helen Cox, MSF
Xpert MTB/RIF for the diagnosis of TB

Figure 1. The *rpoB* gene core region target sequence and molecular beacon technology. (A) The *rpoB* gene, the nucleotide sequence of the core region and the localization of the complementary overlapping molecular beacon probes that span the complete core region. (B) The stem-and-loop structure of a molecular beacon and onset of fluorescence following binding to a complementary DNA strand. The loop structure of the molecular beacon contains the complementary oligonucleotide probe sequence, and the fluorophore and quencher molecules are attached to the ends of the stem structure. Following hybridization, conformational change in the probe leads to separation of the fluorophore and quencher molecules and onset of fluorescence.

Lawn, Nicol. Future Microbiol 2001; 6(9): 1067-82
Sputum liquefaction and inactivation with 2:1 SR

End of hands-on work

Time-to-result: 1 h 45 min

Concentrates bacilli and removes inhibitors

Sample is automatically filtered and washed

Ultrasonic lysis of filter captured organisms to release DNA

DNA molecules are mixed with dry PCR reagents

Semi-nested real-time amplification and detection in integrated reaction tube

Printable test result

Lawn, Nicoll. Future Microbiol 2001; 6(9): 1067-82
Xpert MTB/RIF: TB detected, rifampicin sensitive
Xpert MTB/RIF: TB detected, rifampicin resistant
Accuracy of Xpert MTB/RIF for detection of rifampicin resistance

• Evaluation study
  • Comparison with phenotypic DST:
    • sensitivity 98% specificity 98%
  • After sequencing discordant isolates
    • sensitivity 99% specificity 100%

• Early demonstration study
  • Cases identified in Khayelitsha and Paarl
    • Rif R on Xpert, Rif S on LPA (confirmed WT on sequencing)
    • Sensitivity 99 (96-100) % specificity 96 (95-97) %
      205/208 (679/706)
  • Revised ADF: sensitivity 94%, specificity 98%

• Version 4 cartridge, improved specificity

Boehme CC et al. NEJM 2010; 11:1005-15
Boehme CC et al. Lancet 2011; 377:1495-505
Xpert: time to detection of RR TB
Initial default: Xpert vs. Routine

Time to treatment of all microbiologically confirmed cases

Percent treated

Time (days)

- Smear or culture positive
- Xpert or culture positive

P<0.001
TB SUSPECTS

TB and DR-TB contacts, non-contact symptomatic individuals, re-treatment after relapse, failure and default

Collect one sputum specimen at the health facility under supervision

- GXP positive
  - GXP positive
    - Rifampicin sensitive
      - Treat as TB
        - Start on Regimen 1
        - Send one specimen for microscopy
      - Follow up with microscopy
      - Collect one specimen for culture and DST for Rifampicin, Isoniazid, Fluoroquinolone and Aminoglycoside
      - Follow up with microscopy and culture
    - Rifampicin resistant
      - Treat as MDR-TB
        - Refer to MDR-TB Unit
      - Collect one specimen for microscopy and LPA
    - Rifampicin unsuccessful
      - Treat as TB
        - Start on Regimen 1
        - Collect one specimen for microscopy and LPA
      - Collect one specimen for culture and LPA or culture and DST (for R and H)
        - Treat with antibiotics and review after 5 days
        - Do chest x-ray
        - Poor response to antibiotics
          - Clinically TB
          - TB on chest x-ray
          - Treat as TB
            - Start on Regimen 1
            - Refer to MDR-TB Unit
        - LPA/DST results
          - Resistant to R and H
          - R only
            - Treat as TB
              - Start on Regimen 1
              - Review culture results
            - Poor response
              - Consider other diagnosis
              - Refer for further investigation
      - HIV positive
      - HIV negative
        - Collect one sputum specimen for a repeat GXP
        - Treat with antibiotics
          - Good response
            - No further follow up
            - Advise to return when symptoms recur
          - Poor response
            - Consider other diagnosis
            - Refer for further investigation

- GXP negative
  - GXP unsuccessful
    - Collect one sputum specimen for a repeat GXP
Cumulative number of GeneXpert instrument modules and Xpert MTB/RIF cartridges procured under concessional pricing

- SA Public sector: >720,000 assays since Q2 2011
- Remains 50% of total cartridge HBDC purchase
- Excludes private sector

Data provided by FIND


Slide courtesy Wendy Stevens, NHLS
Xpert testing sites in South Africa (Oct 2012)

100 testing centres
143 analysers
*20 clinic placements
Gx4: 51
Gx16: 90
GX48:2

Slide courtesy Wendy Stevens, NHLS
RIF resistance detected by Xpert in SA

- 7,799 RR cases detected Mar 2011-Oct 2012
  - 7.1% of all Xpert positive cases
  - Regional variation (4.9-8.8%)
  - Stable over time period

- PPV for RR = 88% with LPA / 89% with phenotypic DST
  - Implies very high specificity (99.1%)

Data source: Wendy Stevens, NHLS
False positive Xpert RIF-resistant results

1000 TB suspects

150 Xpert pos

10 RIF resistant

1 false RIF resistant
RIF resistance detected by Xpert in SA

- 7,799 RR cases detected Mar 2011-Oct 2012
  - 7.1% of all Xpert positive cases
  - Regional variation (4.9-8.8%)
  - Stable over time period

- PPV for RR = 88% with LPA / 89% with phenotypic DST
  - Implies very high specificity (99.1%)

- Poor adherence to testing algorithm
  - 46% of patients with RR on Xpert received confirmatory culture
    - Exception Western Cape (84%)
    - Culture for 2nd line DST not available/delayed

- High apparent rates of RIF mono-R
  - 20% with LPA, 12% with MGIT DST

Data source: Wendy Stevens, NHLS
Has Xpert made a difference?

Delay to treatment initiation (from diagnostic sputum sample)

Programme and LPA implementation

Xpert implementation

Slide courtesy Helen Cox, MSF
What are the gaps in an Xpert-driven algorithm and how do other technologies fill these?

- No routine INH susceptibility testing
  - Does this matter?

- Complexity of algorithms
  - Need for multiple samples for RR cases

- Cost
  - Low-cost culture-based methods (MODS, TLA, colorimetric)

- Delay for 2\textsuperscript{nd} line DST
  - MTBDRs/
  - Xpert MTB/XDR
  - Sequencing
  - Microarray (TB-Biochip and others)
  - Phage-based assays
  - Trek sensititre MYCOTB
MTBDRs/ – rapid detection of XDR

- Fluoroquinolones: sensitivity 55-92% (lower in Vietnam and Russia)
- SLI: sensitivity varies markedly by drug and by region; mutation patterns complex
- EMB: sensitivity poor (≈60%)
- Specificity high for FQ and SLI, poorer for EMB (phenotypic testing problematic)
- Good ‘rule in’ for XDR but not ‘rule out’
- Utility will vary geographically

Picture: www.hain-lifescience.de/en/

Sequencing

• Targeted sequencing e.g. pyrosequencing
  • Relatively high throughput (XDR rather than MDR detection)
    • 1 day: 100 strains x 1 drug OR 12 strains x 7 drugs

• Whole genome sequencing

• Advantages of sequencing
  • Broad identification of specific mutations
  • Can distinguish missense from silent mutations
  • May detect mixed allelic variants

• However
  • Well trained staff
  • Costly equipment
  • Bioinformatics challenges
  • Centralized model

Engstrom JCM 2012; 50: 2026 and Kontsevaya JCM 2011; 49: 2832
Tension between centralized/decentralized model

2\textsuperscript{nd} line testing

- Sophisticated technology
- Broader testing
- Quality assurance
- Cost-effective

First-line (RIF) screening

- Reduced TAT
- Reduced default
- Improved communication
- Less reliance on logistics

Centralized testing

Decentralized testing
Acknowledgements

• FIND
  • Catharina Boehme
  • Pamela Nabetan
  • Christen Gray

• University of Cape Town
  • Neisha Mohess
  • Silindile Mbhele
  • Widaad Zemanay
  • Steve Lawn

• NHLS
  • Wendy Stevens
  • Andrew Whitelaw
  • Gerrit Coetzee
  • John Simpson
  • Marlein Bosman

• MSF
  • Helen Cox
  • Cheryl McDermid

• City of Cape Town
  • Virginia De Azevedo

• Provincial Government of the Western Cape

• Patients

• Funders:
  • The Wellcome Trust
  • EDCTP