Investigation of discrepancies between phenotypic and molecular methods for detection of rifampicin susceptibilities from a tertiary diagnostic laboratory in Pretoria: towards a consensus in \textit{rpoB} mutations

Kudzai B Nyazema	extsuperscript{1}, John F Antiabong	extsuperscript{2}, Omowunwii O Onwuegbuna	extsuperscript{3}, Lesibana A Malinga	extsuperscript{4}, Nontombi M Mbelle	extsuperscript{1,3}

1. Department of Medical Microbiology, University of Pretoria, South Africa 2. Centre for Infectious Diseases Research and Experimental Therapeutics, Baylor Institute, Texas 3. National Health Laboratory Service, Pretoria, South Africa 4. Tuberculosis Platform, South African Medical Research Council, Pretoria, South Africa
TB epidemiology

- TB remains a major health concern worldwide
  - high incidence and mortality rate
- Globally, estimated 10.4 million incident cases in 2016\(^1\)
  - Highest burden in the WHO African Region, WHO South-East Asia Region & the WHO Western Pacific Region
  - South Africa accounted for 4% of the global total
MDR – TB Burden

- Emergence and prevalence of drug resistant *Mycobacterium tuberculosis* hinders progress in TB control
  - Rifampicin resistant TB (RR-TB)
  - Multi drug resistant TB (MDR-TB)
  - Extensively drug resistant TB (XDR-TB)
- ~4.1% of new cases and 19% of previously treated cases are MDR/RR – TB
MDR-TB burden

Lengthy\textsuperscript{1,2}:
- Standard regimen (18 – 24 months)
- Short regimen (9 – 12 months)

Complex\textsuperscript{1,2}:
Multiple drugs of different classes

Costly\textsuperscript{1,2}:
US$2000 – 20 000 per patient
First line Drug Susceptibility Testing (DST): Phenotypic

- **Agar Proportion**
  - Gold standard
  - Solid media-based test based on CFU count comparison
  - Approximately 21 days

- **Sensititre MycoTB MIC Plate**
  - 96-well microtiter plate testing 1 isolate against 12 first and second line drugs
  - Determines MIC values

- **BACTEC MGIT 960 SIRE Kit**
  - Liquid-based test based on mycobacterial growth in the presence of known drugs’ critical concentration
  - 4 – 13 days
First Line Drug Susceptibility Testing (DST): Genotypic

- Xpert MTB/Rif$^{5,6,7}$
  - Hemi-nested real-time PCR based assay
  - MTBC and RIF resistance

- GenoTypeMTBDRplus$^{5,6,7}$
  - PCR amplification and reverse hybridization assay
  - MTBC, RIF and INH resistance

- Sequencing$^6$
  - Targeted gene sequencing
  - Whole genome sequencing
Diagnostic Dilemma

- Discrepancies observed for RIF
- Mutations occur outside targeted hotspot\(^5\)
- Phenotypic DST may not detect low level resistance for RIF\(^7\)
  - “Disputed mutations”
  - Below critical concentration
- Existence of heteroresistant populations\(^8\)
  - $\geq 1\%$ resistant strains detected by conventional DST (proportion method)
- Other resistance mechanisms
  - Compensatory mutations; efflux pumps
- Variability of H37Rv?
Problem Statement

- DST diagnostic methods have short-comings
  - Affects patient management
- Correct characterisation of different $rpoB$ mutations causing different RIF susceptibilities is of importance
- Will the utility of clinical strains as a reference provide a true reflection of RIF resistance in comparison to H37Rv?
Aim

To investigate discrepancies between GenoTypeMDRTB\textit{plus} and phenotypic DST for RIF and design an \textit{rpoB} consensus sequence
Objectives

• To compare DST results from LPA and conventional DST
• To sequence and analyse PCR amplified *rpoB* gene
• To develop an *in silico* consensus sequence from susceptible isolates to be used as reference and compared to H37Rv results
Materials and Methods

- Culture
- Microscopy
- Purity Check
- MTBC ID
- Phenotypic DST
- Genotypic DST
- PCR and Gel electrophoresis
- Sequencing
Data Analysis

• Kappa values and agreement were calculated using STATA 13
  • $\kappa < 0.4$, $0.4 - 0.7$ and $0.71 - 1.0$ indicating low agreement, substantial agreement and “perfect” agreement

• Sequencing data was edited using Chromaspro version 1.45 & multiple alignment done using Bioedit ClustalW

• Consensus sequence was also developed using the same software

• A phylogenetic tree was constructed applying the PHYLIP – Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm
Results

Table 1: Comparison between phenotypic and genotypic DST results with regards to RIF

<table>
<thead>
<tr>
<th></th>
<th>Phenotypic DST</th>
<th>Total</th>
<th>Agreement (%)</th>
<th>kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GenoTypeMTBDRplus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>11</td>
<td>3</td>
<td>14</td>
<td>90%</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>19</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>Sanger Sequencing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>100%</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>19</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>
## Results

Table 2: *rpoB* mutations as detected by sequencing

<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Frequency</th>
<th>Mutation detected by GenoTypeMTBDR<em>plus</em> assay</th>
<th>Phenotype by MGIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S531L</td>
<td>5/10 = 0.5</td>
<td>MUT3</td>
<td>Resistant</td>
</tr>
<tr>
<td>H526S</td>
<td>1/10 = 0.1</td>
<td>-</td>
<td>Susceptible</td>
</tr>
<tr>
<td>H526D</td>
<td>1/10 = 0.1</td>
<td>MUT2B</td>
<td>Resistant</td>
</tr>
<tr>
<td>D516V</td>
<td>2/10 = 0.2</td>
<td>MUT1</td>
<td>Resistant</td>
</tr>
<tr>
<td>+514F</td>
<td>1/10 = 0.1</td>
<td>-</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
Figure 1: UPGMA Phylogenetic tree constructed with rpoB sequences. S = Susceptible; R = Resistant
Discussion

• Rapid and accurate diagnosis of TB is important
• Results on agreement between phenotypic and genotypic DST in support of literature
  • Kappa = 1.0: relatively higher than previous studies
• Mutations detected noted in literature but at relatively lower frequencies
Discussion

• Discrepancies occur because
  • Molecular tests detect resistance at genetic level and not ultimate phenotypic expression
  • Conventional DST unable to detect low level resistance due to presence of disputed mutations such as H526D detected in our study
• Targeted gene sequencing allows for detection of “disputed” rpoB mutations
Conclusion

• Sequencing could help minimise discrepancies observed between DST methods
• Similar results between H37Rv and the developed consensus sequence highlights better performance of Sanger sequencing for DST in comparison to GenoTypeMTBDRplus
Current & Future Work

• Whole genome sequencing
  • Possible IS6110 disruptions involved in RIF resistance?
• Development of a consensus sequence at genome level to observe the differences if any, of DST results obtained by H37Rv compared to the clinical isolates
References

Thank you