Application of Whole Genome Sequencing (WGS) to Diagnosis of Drug Resistance in Tuberculosis

Global Consortium for Drug-resistant TB Diagnostics (GCDD)

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Molecular Diagnostics for Drug Resistant TB (DRTB)

Rapid Molecular Tests:
- Hain
- GeneXpert
- Pyrosequencing

Features:
- Inexpensive
- Fast
- Highly localized
  - Usually evaluate the presence of point mutations
Considerations for Use of Knowledge Gained from Molecular Testing

1. Phenotypic prediction based on discovered single nucleotide polymorphisms (SNPs)

Table 4: Prevalence of rrs A1401G mutation among GCDD “injectable” resistant isolates

<table>
<thead>
<tr>
<th>Region</th>
<th>AMKR</th>
<th>CAPR</th>
<th>KANR</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>85%</td>
<td>89%</td>
<td>81%</td>
</tr>
<tr>
<td>Moldova</td>
<td>65%</td>
<td>63%</td>
<td>26%</td>
</tr>
<tr>
<td>Philippines</td>
<td>100%</td>
<td>100%</td>
<td>81%</td>
</tr>
<tr>
<td>South Africa</td>
<td>91%</td>
<td>92%</td>
<td>88%</td>
</tr>
</tbody>
</table>

Table 5: Prevalence of eis promoter C-12T mutation among GCDD “injectable” resistant isolates

<table>
<thead>
<tr>
<th>Region</th>
<th>AMKR</th>
<th>CAPR</th>
<th>KANR</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Moldova</td>
<td>10%</td>
<td>25%</td>
<td>54%</td>
</tr>
<tr>
<td>Philippines</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>South Africa</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

2. Regional Differences in Prevalence

Table 3: Prevalence of gyrA mutation among GCDD Fluoroquinolone resistant isolates

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Isolates</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 A→G</td>
<td>121</td>
<td>41.44%</td>
</tr>
<tr>
<td>94 G→A</td>
<td>41</td>
<td>14.04%</td>
</tr>
<tr>
<td>90 C→T</td>
<td>84</td>
<td>28.77%</td>
</tr>
<tr>
<td>91 T→C</td>
<td>6</td>
<td>2.05%</td>
</tr>
<tr>
<td>88 G→T</td>
<td>4</td>
<td>1.37%</td>
</tr>
<tr>
<td>74 G→C &amp; 75 C→G</td>
<td>1</td>
<td>0.34%</td>
</tr>
<tr>
<td>105 T→G &amp; 112 G→C</td>
<td>2</td>
<td>0.68%</td>
</tr>
<tr>
<td>No* gyrA mutation</td>
<td>33</td>
<td>11.30%</td>
</tr>
</tbody>
</table>

* No mutation in QRDR except for Codon 95
Considerations for Use of Knowledge Gained from Molecular Testing

1. Phenotypic prediction based on discovered single nucleotide polymorphisms (SNPs)
Considerations for Use of Knowledge Gained from Molecular Testing

1. Phenotypic prediction based on discovered single nucleotide polymorphisms (SNPs)

   1. Consideration: Variable MIC for individual point mutations

       1. Likely causes:
          1. Highly localized consideration of genetic variation.
          2. Not all resistance conferring mutations are known
          3. Multiple functional pathways could cause resistance

       2. Partial solution: Combination of point mutations can help
Considerations for Use of Knowledge Gained from Molecular Testing

2. Important information that a broader look at the genome could provide:

2. Treatment Failure:
   2. exogenous secondary infection?
   3. Endogenous evolution?
   4. Heteroresistance?

3. Population Genetics:
   2. Metagenomic analysis
   3. Population dynamics

4. Contact Tracing:
   2. Identification of point of original and any secondary infection(s)
   3. Early identification of an outbreak
Whole Genome Sequencing (WGS) as a Tool for a Broader Genomic Perspective

**Process:**

1. Sample Culture
2. DNA Extraction
3. Library Preparation
   1. Often includes (or is followed by) Amplification
4. WGS
   1. Illumina: Genome Analyzer, HiSeq, MiSeq, etc.
   2. Roche: 454
   3. Life Technologies: Ion Torent
   4. Pacific Biosciences: RS
   5. Etc.
5. Bioinformatics
WGS *Mycobacterium tuberculosis* (MtB)

What to consider:

1. MtB has a circular genome
   1. 4.4 million bps
   2. Unbalanced genome (62.5% GC content)
      1. Will suffer from GC bias in the amplification step
2. WGS usually means mass processing
   1. Illumina Hiseq: needs 96 isolates
   2. Slow growth rate can become a big problem
GCDD’s Approach to WGS

Process:

1. **Platform:** Pacific Biosciences RS system
   1. Very long reads
   1. Easy de novo assembly
   2. Easy mapping to a reference genome
2. No base quality score drop at the end of the read
   1. Needs much lower sequencing depth
3. **No systematic bias**
   1. No GC Bias
4. Can sequence one isolate at a time.
   1. Important for its utility in diagnostics
Illumina’s GC Bias Affecting Coverage Depth

Illumina GC Bias Plot

- Normalized Coverage
- Windows at GC%
PacBio Coverage Depth

![Pacbio GC Bias Plot]

- Normalized Coverage
- Windows at GC%

GC% of 100 base windows vs. Fraction of normalized coverage
GCDD’s Approach to WGS

What has been done:

1. Developed an in-house bioinformatic pipeline (PacDAP) for base and variant calling.
2. Reliable base calling: PacDAP registers an uniform error rate of 50 in PHRED scale in base calling.
3. Higher than all other sequencing platforms including Sanger, and
4. Significantly higher than industry standard (score of 30)

2. Can identify SNPs on a genomic scale

3. Have identified 28,963 unstable loci in the genome
GCDD’s Approach to WGS

What has been done:

4. Rapidly detects all major mutations associated with resistance to seven drugs:
   1. First line: Rifampicin, Isoniazid
   2. Three aminoglycosides: Amikacin, Kanamycin, and Capreomycin
   3. Two fluoroquinolones: Moxifloxacin and ofloxacin

2. 366 isolates from four countries have been sequenced:
   1. India, Moldova, the Philippines, South Africa
   2. Have identified 28,963 unstable loci in the genome
Results of PacDAP analysis:

1. Novel Markers of Resistance:

Table 7. Number of the unannotated SNPs associated with drug resistance

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Novel SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH&lt;sup&gt;R&lt;/sup&gt;</td>
<td>37</td>
</tr>
<tr>
<td>RIF&lt;sup&gt;R&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>MOX&lt;sup&gt;R&lt;/sup&gt;/OFX&lt;sup&gt;R&lt;/sup&gt;</td>
<td>42</td>
</tr>
<tr>
<td>AMK&lt;sup&gt;R&lt;/sup&gt;</td>
<td>35</td>
</tr>
<tr>
<td>CAP&lt;sup&gt;R&lt;/sup&gt;</td>
<td>31</td>
</tr>
<tr>
<td>KAN&lt;sup&gt;R&lt;/sup&gt;</td>
<td>29</td>
</tr>
</tbody>
</table>
Results of PacDAP analysis:

2. **Lineage:** Have identified mutations on a genomic scale for determining lineage at a much finer scale than MIRU/Spoligo Typing

3. **Outbreak boundaries:** Identified specific markers for two outbreaks in India

4. **Compensatory Markers**

5. **Precursor Markers**

6. **Host Specific Markers**

7. **Evolutionary Markers**

8. **Molecular Clock loci**

<table>
<thead>
<tr>
<th>SNP Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain Specific</td>
<td>24</td>
</tr>
<tr>
<td>Outbreak Specific</td>
<td>8</td>
</tr>
<tr>
<td>Host Specific</td>
<td>78</td>
</tr>
<tr>
<td>Evolutionary Path Specific</td>
<td>3</td>
</tr>
<tr>
<td>Evolutionary State Specific</td>
<td>11</td>
</tr>
<tr>
<td>Compensatory</td>
<td>3</td>
</tr>
<tr>
<td>Precursor</td>
<td>2</td>
</tr>
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</table>
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  - The Philipinnes
  - South Africa

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