New Frontiers: Innovation and Access

The Future of DST
Next Generation Technologies

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TB Diagnostics Advisor
MSF-Access Campaign

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Paradigm shift

**Culture Based Testing** (Phenotypic)
- cDST or pDST

Measured bacterial growth in the presence at a critical concentration of an anti-TB drug which correlates with a poor clinical outcome

**Molecular Based Testing** (Genotypic)
- mDST or gDST

Mutations linked to resistant phenotypes.
Most knowledgeable: INH, RIF, PZA, FQ, and SLIs

**Research**

**New Tools**
- LPA, CBNAAT, DNA Chip, and sequencing-based technologies

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**Solid/Liquid (MGIT) cDST and MIC plate-based technologies**
### Features: phenotypic vs. genotypic

<table>
<thead>
<tr>
<th>pDST</th>
<th>gDST</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Requires establishment of Critical Concentrations</td>
<td>• Requires knowledge of mechanisms related to DR</td>
</tr>
<tr>
<td>• Requires BSL3 facilities</td>
<td>• Requires BSL2 facilities</td>
</tr>
<tr>
<td>• Requires culture</td>
<td>• Potentially culture free</td>
</tr>
<tr>
<td>• Time to results: weeks to months</td>
<td>• Time to results: hours to days</td>
</tr>
</tbody>
</table>

Current WHO approved molecular technologies have **limited capacity**, covering only specific gene targets or regions of DNA.
Pipeline molecular technologies

- Expanded use and access
- Diversified placement
- Multi-disease testing
- Remain limited in capacity

Centralized high-throughput platforms
WHO data review July 2019

Hain FluoroType
Abbott m2000
BD Max
Roche Cobas

Cepheid XDR
Moblio Truenat MTB/RIF

<table>
<thead>
<tr>
<th>Gene</th>
<th>Encoded drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>katG</td>
<td>Isoniazid (may also include Ethionamide)</td>
</tr>
<tr>
<td>inhA</td>
<td></td>
</tr>
<tr>
<td>Atpc-OxyR</td>
<td></td>
</tr>
<tr>
<td>fabG1</td>
<td></td>
</tr>
<tr>
<td>gyrA</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>gyrB</td>
<td></td>
</tr>
<tr>
<td>rrs</td>
<td>Amikacin, Kanamycin, Capreomycin</td>
</tr>
<tr>
<td>eis promoter</td>
<td></td>
</tr>
</tbody>
</table>

Currently Available Chip based Real Time PCR Tests

- Truenat™ MTB
- Truenat™ HBV
- Truenat™ H1N1
- Truenat™ Dengue
- Truenat™ Chikungunya
- Truenat™ Dengue/Chikungunya
- Truenat™ HCV
- Truenat™ HIV-1
- Truenat™ Rabies
- Truenat™ CT
- Truenat™ NG
- Truenat™ CT/NG
- Truenat™ Salmonella
- Truenat™ Trich
- Truenat™ Malaria Py/Pf
- Truenat™ Malaria PF
- Truenat™ HPV-16
- Truenat™ HPV-18
- Truenat™ HPV-31
- Truenat™ HPV-45
- Truenat™ HPV-52
- Truenat™ HPV-58
- Truenat™ HPV-66
- Truenat™ HPV-68
- Truenat™ HPV-16
- Truenat™ HPV-18
- Truenat™ HPV-31
- Truenat™ HPV-45
- Truenat™ HPV-52
- Truenat™ HPV-58
- Truenat™ HPV-66
- Truenat™ HPV-68
- Truenat™ Malaria PF
- Truenat™ Malaria Py/Pf
- Truenat™ MTB Plus
- Truenat™ MTB
- Truenat™ MTB-RIF Dx
- Truenat™ HBV
- Truenat™ H1N1
- Truenat™ Dengue
- Truenat™ Chikungunya
- Truenat™ Dengue/Chikungunya
- Truenat™ HCV
- Truenat™ HIV-1
- Truenat™ Rabies
- Truenat™ CT
- Truenat™ NG
- Truenat™ CT/NG
- Truenat™ Salmonella
- Truenat™ Trich
- Truenat™ Malaria Py/Pf
- Truenat™ Malaria PF
- Truenat™ HPV-16
- Truenat™ HPV-18
- Truenat™ HPV-31
- Truenat™ HPV-45
- Truenat™ HPV-52
- Truenat™ HPV-58
- Truenat™ HPV-66
- Truenat™ HPV-68
- Truenat™ Malaria PF
- Truenat™ Malaria Py/Pf
- Truenat™ MTB Plus

Trials start this year, WHO review 2020
Trials underway, WHO review 2020
Rifampicin resistance studies

Rifampicin (RIF)

Bactericidal antibiotic that inhibits the bacterial DNA-dependent RNA polymerase.

Target: β-subunit of the RNA polymerase (encoded by rpoB), blocking elongation of the RNA chain.

513, 526, 531 > 90% RR cases

Others outside of rpoB (81bp): V146F and I572F
Silent Mutations: F506, T508, Q510, L511, Q513, F514, T525, A532, L533, P535

Mutations in a “hot-spot” region of 81 bp of rpoB gene (Rifampicin resistance-determining region) → RIF resistance (> 95%)

Slide courtesy of P. Miotto (2016)
Fluoroquinolone resistance studies

81% FQ mutations in QRDRs

**gyrA**
- Main Loci: 90, 91, 94
- Non FQ-R: E21Q, S95T, G668D, G247S, A384V
- Silent: I614I, A830A

**gyrB**
- Main Loci: 461, 494, 499
- Silent: T221T, V265V, A334A
# Markers of resistance to anti-TB drugs

<table>
<thead>
<tr>
<th>Anti-TB Drug</th>
<th>Gene Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>$katG$, $inhA$, $ndh$, $aphC$, $oxyR$, $mshA$, $furA$</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>$rpoB$</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>$embB$, $aftA$, $embA$, $embC$, $ubiA$</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>$pncA$, $rpsa$, $panD$</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>$rpsl$, $rrs$, $gidB$</td>
</tr>
<tr>
<td>Amikacin/Kanamycin</td>
<td>$rrs$, $eis$, $whibB$</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>$rrs$, $tlyA$, $eis$, $whibB$</td>
</tr>
<tr>
<td>Fluroquinolones</td>
<td>$gyrA$, $gyrB$, $mfpA$, $pstB$, $lfrA$, $corD$</td>
</tr>
<tr>
<td>Eth/Prothionamide</td>
<td>$ethA$, $ethR$, $inhA$, $ndh$, $mshA$, $furA$</td>
</tr>
<tr>
<td>p-aminosalycyclic acid</td>
<td>$thyA$, $dfiA$, $folC$, $ribD$</td>
</tr>
<tr>
<td>Cycloserine/Terizidone</td>
<td>$alr$, $ald$, $ddl$, $cycA$</td>
</tr>
<tr>
<td>Linezolid</td>
<td>$rrl$, $rplC$</td>
</tr>
<tr>
<td>Clofazamine</td>
<td>$mmpR$ (Rv0678)</td>
</tr>
<tr>
<td>Bedaquiline</td>
<td>$mmpR$ (Rv0678), $atpE$, $mmpL5$, $mmpS5$, $pepQ$</td>
</tr>
<tr>
<td>Delamanid</td>
<td>$ddn$, $fdg1$, $fbiA$, $fbiB$, $fbiC$</td>
</tr>
</tbody>
</table>

- Know where to identify resistance conferring mutations
- Defined highest frequency gene targets
New WHO guidance 2018

- **Systematic review** of pheno/genotypic data
- Defined new critical concentrations
- **Identify resistance associated mutations**
- Critical concentrations for reclassified drugs
- Outlined drug preparations and protocols per media
- Reliability of pDST per drug
- Recommended testing

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https://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/

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**Group** | **Medicine** | **Abbreviation** | **Critical concentrations (µg/ml) for DST by medium** |
--- | --- | --- | --- |
**Group A** | Levofloxacin (CC) | LEF<sup>2,3</sup> | L<sub>Löwenstein Jensen</sub> 2.0, M<sub>Middlebrook 7H10</sub> 1.0, M<sub>Middlebrook 7H11</sub> 1.0 |
| Moxifloxacin (CC) | MXF<sup>2,3</sup> | - | - |
| Moxifloxacin (CB) | MXF<sup>4</sup> | 2.0 | 1.0 |
| Bedaquiline<sup>5</sup> | BDQ | - | - |
| Linezolid<sup>6</sup> | LZD | 1.0 | 1.0 |
| **Group B** | Clarithromycin | CLZ | - | - |
| Cycloserine | CS | - | - |
| Terizidone/Terizidone | TZD | - | - |
| **Group C** | Ethambutol<sup>7</sup> | E | 2.0, M<sub>Middlebrook 7H10</sub> 5.0, M<sub>Middlebrook 7H11</sub> 7.5 |
| Delamanid<sup>8</sup> | DLM | - | 0.016, 0.06 |
| Pyrazinamide<sup>9</sup> | PZA | - | 100.0 |
| Imipenem-cilastatin | IMP/CLN | - | - |
| Meropenem | MPM | - | - |
| Amikacin<sup>10</sup> | AMK | 30.0, 2.0 | 2.0, 2.0 |
| (Or Streptomycin) | [S] | 4.0, 2.0 | 2.0 |
| Ethionamide | ETO | 40.0 | 5.0 |
| Protionamide | PTO | 40.0 | 10.0 |
| Paraaminosalicyclic acid | PAS | - | 2.5 |

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**Notes:**
- LEF<sup>2,3</sup>, MXF<sup>2,3</sup>, MXF<sup>4</sup>, BDQ, LZD, CLZ, CS, TZD, E, DLM, PZA, IMP/CLN, MPM, AMK, [S], ETO, PTO, PAS

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New Frontiers: Innovation And Access
8th TB Symposium – Ministry of Health of the Republic of Uzbekistan and Médecins Sans Frontières
<table>
<thead>
<tr>
<th>Drug</th>
<th>Genes</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INH</strong></td>
<td>katG, inhA, mabA (fabG1) ahpC-oxyR</td>
<td>Se: 84% / Sp: 98%</td>
</tr>
<tr>
<td><strong>rif</strong></td>
<td>rpoB</td>
<td>Se: 96% / Sp: 99%</td>
</tr>
<tr>
<td><strong>FQ</strong></td>
<td>gyrA, gyrB</td>
<td>Se: 89% / Sp: 100%</td>
</tr>
<tr>
<td><strong>AMK</strong></td>
<td>els, rrs</td>
<td>Se: 79% / Sp: 100%</td>
</tr>
</tbody>
</table>

Still accumulating data for LZD, CFZ, BDQ, DLM, etc

**Prediction accuracy when including all mutations, even low confidence**

**Grading:**
- **High**
- **Moderate**
- **Minimal**
- **None**
Increasing knowledge to grade & interpret variants

- 100,000 isolates: MDR, pre/XDR (6600 currently)
- Perform WGS / MICs
- Link data: pDST, MIC, genetic variants for grading and interpretation

CRyPTIC
Comprehensive Research on Tuberculosis:

- Bedaquiline
- Delamanid
- Clofazimine
- Linezolid
- Ethionamide
- PAS
- Levofloxacin
- Moxifloxacin
- Kanamycin
- Amikacin
- Capreomycin
- Pyrazinamide
- Ethambutol
- Rifabutin
- Rifampicin
- Isoniazid

http://www.crypticproject.org

NGS Illumina

customized microtitre plates

New Frontiers: Innovation And Access
8th TB Symposium – Ministry of Health of the Republic of Uzbekistan and Médecins Sans Frontières
Can we move to sequencing for DR-TB?
Next generation sequencing (NGS)

Approaches for DR-TB:
- **Whole genome**: complete DNA sequence, requires isolate (Surveillance Tool)
- **Target-based**: select genes, enriched from direct specimens (Clinical Tool)

**Strengths**
- High accuracy for known resistance
- High throughput (200 strains/batch)
- Rapid runtime 3-4 days/batch
- Cheaper than phenotypic testing
- Systems open and adaptable as new knowledge is acquired

**Limitations**
- Knowledge on mutations for newer medicines is incomplete
- Contribution of hetero-resistance is not well understood
- The importance of efflux pumps and compensatory mechanisms remains unclear
Target-based sequencing: Genoscreen Deeplex MycTB

- Target amplification from direct sputum sediment = **FAST**
- A 24-plex amplicon preparation before sequencing = **FOCUSED**
- Identifies species, genotype, and drug resistance profiling for 18 genes targets = **FUNDAMENTAL**

### Drug & Gene target

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<tr>
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<th>Gene target</th>
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<td>Rifampicin</td>
<td>rpoB</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>inhA, fabG1, katG, ahpC</td>
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<tr>
<td>Clofazimine</td>
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</table>

### Deeplex®-MycTB, an all-in-one NGS-based diagnostic test for *M. tuberculosis*

- 24 gene targets amplified
- Only 24 genes sequenced
- Analysis/ Report

**Clinical sample**
- DNA extraction
- Multiplex PCR
- Library preparation
- Deep sequencing
- Secured cloud-based analysis

**Turnaround time**: 36-48h, **Hands-on time**: 4h
The overall pooled sensitivity for predicting resistance by WGS:

- 91% (87–94) for \( rpoB \) (rifampicin)
- 86% (74–93) for \( katG, \) \( inhA, \) and \( fabG \) promoter combined (isoniazid)
- 54% (39–68) for \( pncA \) (pyrazinamide)
- 85% (77–91) for \( gyrA/gyrB \) combined (ofloxacin/levofloxacin)
- 88% (81–92) for \( gyrA/gyrB \) combined (moxifloxacin)

For nearly all drugs and most settings, there was a correlation in the estimated prevalence of drug resistance by sequencing and the estimated prevalence by phenotypic testing.
New technical guidance

- Reviews current NGS methods for sequencing *Mycobacterium tuberculosis* complex species
- Describes the utility and workflows of whole genome and target-based sequencing
- Defines the principles behind using resistance-conferring mutations to detect DR-TB
- Illustrates the accuracy of sequencing in a multi-country, population-based study for determining DR-TB
- Provides considerations for implementation in LMICs

https://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/
Future programmatic response

**CONSIDERATIONS:**
- pDST
- preference for oral route
- DR prevalence
- previous treatment
- tolerability
- drug-drug interactions
- severe forms

**STANDARDIZED SHORTER MDR-TB REGIMEN**
4-6 Amk-M-Pto-Cfz-Z-Hhd-E / 5 M-Cfz-Z-E

**TREATMENT FOR DRUG SUSCEPTIBLE TB**
(2HRZE/4HR)

**Rapid Diagnostic**
(Xpert MTB/RIF)

**TB-POSITIVE RIf-R**

**LPA-SL**

**TP-POSITIVE RIf-S**

**TARGETTED SEQUENCING ACCESSIBLE**

**CONSIDERATIONS:**
- (PRE-XDR-TB or XDR-TB)
- Precision treatment (following WHO standards)

**LONGER MDR-TB REGIMEN**

**SOCIALLY SUPPORT & COUNSELLING**

**DRTB-SURVEILLANCE**

**PHARMACOVIGILANCE**

**DRUG PROCUREMENT**

**CENTRAL DATABASE**

**FEED-BACK LEARNING LOOP FOR ADJUSTMENT OF DX, RX, APPROACHES**

**Adapted from Mario C. Raviglione Global Health**
Summary

- **Rapid molecular technologies remain useful**
  - Triage at near point of care (RR/FQR)
  - High-throughput testing (reference level)
  - But remain limited in capacity (targets)

- **NGS will play a significant role in the future**
  **Target-based sequencing** *(Clinical diagnosis)*
  - Rapid and accurate diagnosis using graded-mutation encyclopedia
  - Complete resistance profile in one test
  - Eliminates pDST for many drugs (INH, RIF, FQ, AMK/S)
  - pDST is unreliable for EMB, ETO/PTO, CS, PAS – rely on mutational correlates of resistance
  - Building evidence for mutation correlates of resistance for LZD, CFZ, BDQ, DLM

  **Whole genome sequencing** *(Surveillance/Transmission)*
  - Identify new mutations as strains evolve
  - Study the role of efflux pumps
  - Understand compensatory mechanisms underpinning resistance
  - Evaluate transmission events

- **Future convention will rely on gDST over pDST as more knowledge is gained.**

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