OVERVIEW

1. Available diagnostic tools
2. Culture: the gold standard?
3. Molecular tools in development
4. Molecular diagnostic utilization
5. Diagnostic discrepancies
6. Sequence-based assays
7. Clinical trial applications
ADVANCES IN AVAILABLE DIAGNOSTIC TOOLS

- Tuberculin
- Conventional microscopy
- Solid Cultures
- Conventional, phenotypic DST
- Conventional PCR
- Strip speciation
- Conventional chest X-ray
- IGRAs
- LED/FM microscopy
- Liquid Culture
- Molecular DST (LPAs)
- Xpert MTB/RIF & Ultra
- Portable X-ray
- Urine LAM
- MIC Sensititer plate
- TB LAMP
- PET-CT
DIAGNOSTIC TESTS ACROSS TUBERCULOSIS DISEASE SPECTRUM

- Over 30% of the world's population is infected (2.2 B)
- Only ~5–10% will develop clinical disease (110 M)

Researchers understand little about what distinguishes individuals whose infection progresses to active tuberculosis (TB) from those whose infection remains latent for decades.
CULTURE: GOLD STANDARD

Pathogen-specific challenges

• TB grows slowly (3-4 weeks)
• Contamination issues
• Laboratory delays
  – Poor sample quality
  – Batch runs
  – Adjudication
  – Results back to patient

Phenotypic DST

• Additional 4-6 weeks (1\textsuperscript{st} line)
• Additional 4-6 weeks (2\textsuperscript{nd} line)
  – Limited capacity
Established challenges
• Huge investment in past decade
• Technical capacity and retention
• Infrastructure and biosafety
• Quality issues and contamination rates

Emerging challenges
• Maintenance of infrastructure and capacity
• Appropriate infection control measures and programs for staff screening
• New costs and legislation for international transfer

Need for rapid POC tests
MOLECULAR GENOMIC REVOLUTION

Tech in development
• Phage-based
• Breath detection
• Biomarkers
NEW TOOLS: PROBE-BASED GENEXPERT MTB/RIF ASSAY

• WHO endorsed in 2010 & FDA cleared in 2013
• Provide result from sputum in 2 hours
  – Identify TB and determine resistance to Rifampicin (Rif)
    ▪ Ultra-cartridge as sensitive as culture but lower specificity
    ▪ New “Omni” form factor for point-of-care applications
    ▪ New XDR cartridge will expand the drug menu
LOW UTILIZATION OF NEW TOOLS

Clouse et al. 2017 PLoS One

Cazabon et al. ERJ in press

“Xpert utilization was low even though the majority of sites had access to the test”
Clouse et al. 2017 PLoS One
CONCORDANCE AMONG DIFFERENT DST ASSAYS

DST methods evaluated
- L-J proportion
- MGIT 960
- GeneXpert MTB/RIF
- HAIN MTBDRplus
- MycoTB Sensititre
- Phage lab-based qPCR

Why the discrepancies?
- Phenotypic test issues for some drugs
- Unknown, rare or unique Single Nucleotide Polymorphisms (SNPs)
- Epidemiological cut-off and critical concentrations poorly characterized
- Low-level mixed populations (hetero-resistance)

Banu S et al, 2014. JCM
NEXT GENERATION SEQUENCING

Drug-resistance

Virulence determinants

Genotyping

Evolution

Identification

Phylogenetic

Population structure

All-in-one
NEXT GENERATION SEQUENCING FOR DRUG RESISTANCE

**Whole Genome**
- **Strengths**
  - Full genome
  - Comprehensive
- **Weaknesses**
  - Slow
  - Culture dependent
  - Expensive
  - Bioinformatics

**Targeted Amplicon**
- **Strengths**
  - Sequence direct from sputum
  - Simpler and faster
  - Deeper sequencing
  - Up to several hundred loci
- **Weaknesses**
  - Less information
  - Prior knowledge of targets
  - Optimization

Need for a comprehensive database to provide a priori information regarding *Mtb* drug loci and mutations associated with drug resistance
### Defined Criteria

1. **Statistical**
2. **Homoplasy**
3. **MIC**
4. **Clin outcome**
5. **Functional Genetics**

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<th>Gene</th>
<th>AA Change</th>
<th>NUC Change</th>
<th>Res w/ Mutation</th>
<th>Susc w/ Mutation</th>
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<td>0.075</td>
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- **LR>10** - High confidence that the mutation confers drug or is associated with resistance.
- **LR <10 or >5** - Additional data desirable for improving evidence that the mutation confers or is associated with drug resistance (include data that is available such as numerator, denominator, and lineage).
- **LR<5 or >1** - Inconclusive evidence that the mutation confers or is associated with drug resistance. Substantial additional data required (suggest additional clinical test to follow up).
- **LR<1** - No evidence of association between mutation and drug resistance.
SEQUENCING IN CLINICAL TRIALS

- Transmission
- Relapse vs. re-infection
- Drug resistance
- Hetero-resistance

**Elucidating Emergence and Transmission of Multidrug-Resistant Tuberculosis in Treatment Experienced Patients by W**

**Abstract**

Background: RIFAQUIN was a tuberculosis chemotherapeutic drug. The study aimed to evaluate the emergence and epidemiological data of MDR-TB cases identified in individual patients (2-15 years) from 2011 to 2015.

Methods: DNA samples from 68 paired samples of Mycobacterium tuberculosis were used to identify the MDR strain. The samples were grouped according to the resistance pattern and the results were analyzed.

Results: Out of the 68 samples, 22 were resistant to RIFAQUIN. The resistance pattern was similar across all samples.

Conclusions: The study found that RIFAQUIN resistance is emerging in the studied population. The results highlight the need for alternative treatment options for drug-resistant TB.

Keywords: Whole genome sequencing, Mycobacterium tuberculosis, RIFAQUIN resistance.

**First Evaluation of Tuberculosis Clinical Misdiagnosis Using High Fidelity Amplicon Sequencing**

**Abstract**

Background: Tuberculosis (TB) is a leading cause of death worldwide. Accurate diagnosis is crucial for effective treatment. This study aimed to evaluate the accuracy of a high-fidelity amplicon sequencing method compared to conventional methods.

Methods: Two groups of samples were analyzed: a control group with known TB status and a test group with suspected cases. The sequencing method was compared to standard culture methods.

Results: The sequencing method correctly identified all the samples with known TB status, while the culture methods had a 10% false negative rate.

Conclusions: The high-fidelity amplicon sequencing method is a promising tool for rapid and accurate diagnosis of TB.

Keywords: High-fidelity amplicon sequencing, Tuberculosis, diagnostic accuracy.
HETERO-RESISTANCE

Hetero-resistance (low-level mixed populations)

• The presence of a small number of organisms that are resistant to an antimicrobial drug within a population that are susceptible to the drug

• May explain why failure to eradicate an infection occurs in some patients treated with seemingly appropriate antibiotics

Sensitivity of detecting hetero-resistance

Sanger: 30-50%

WGS: 5-10%

Culture: 1-5%

Targeted: 0.01%
**Predicting Drug Resistance**

- Targeted amplicon sequencing such as the Single Molecule Overlapping Read (SMOR) assay can reduce the sequencing error rate from 1% to 0.01%
- Potential to identify populations of resistant bacteria with sensitivity that exceeds current gold standard methods

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Resistance</th>
<th>AMK DST</th>
<th>SMOR</th>
<th>rrs SNP (% R Allele)</th>
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<td>MDR</td>
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<td>S</td>
<td>none</td>
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<td>1/19/2010</td>
<td>MDR</td>
<td>S</td>
<td>S</td>
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<td>7/20/2011</td>
<td>MDR</td>
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<td>S</td>
<td>1401G (0.94%)</td>
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<td>pre-XDR</td>
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<td>4/30/2012</td>
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<td>S</td>
<td>1401G (3.4%)</td>
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<td>12/21/2012</td>
<td>XDR</td>
<td>R</td>
<td>R</td>
<td>1401G (29.6%)</td>
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Metcalfe et al. 2017. Am J Respir Crit Care Med
CAN NGS BE USED TO ASSESS HOST PHARMACOGENOMICS?

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**Pharmacogenetic Study of Drug-Metabolising Enzyme Polymorphisms on the Risk of Anti-Tuberculosis Drug-Induced Liver Injury: A Meta-Analysis**

Yu Cai, JiaYong Yi, ChaoHui Zhou, XiZhong Shen

1 Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai, People’s Republic of China. 2 Departments of Orthopedics, Zhongshan Hospital, Fudan University, Shanghai, People’s Republic of China.

**Abstract**

**Background:** There are first-line antituberculosis drugs, isoniazid, rifampicin, and pyrazinamide, may induce liver injury, especially isoniazid. This antituberculosis drug-induced liver injury (ATLI) ranges from a mild to severe form, and the associated mortality cases are not rare. In the past decade, many investigations have focused the association between drug-metabolising enzyme (DME) gene polymorphisms and risk for ATLI; however, these studies have yielded contradictory results.

**Methods:** PubMed, EMBASE, ISI web of science and the Chinese National Knowledge Infrastructure database were systematically searched to identify relevant studies. A meta-analysis was performed to examine the association between polymorphisms from four DME genes (CYP2E1, GSTM1 and GSTT1) and susceptibility to ATLI. Odds ratios (ORs) and 95% confidence intervals (Cis) were calculated. Heterogeneity among articles and their publication bias were also tested.

**Results:** 38 studies involving 2225 patients and 4906 controls were included. Overall, significantly increased ATLI risk was associated with slow NAT2 genotype and GSTM1 null genotype when all studies were pooled into the meta-analysis. Significantly increased risk was also found for CYP2E1*1A in East Asians when stratified by ethnicity. However, no significant results were observed for GSTT1.

**Conclusions:** Our results demonstrated that slow NAT2 genotype, CYP2E1*1A and GSTM1 null have a modest effect on genetic susceptibility to ATLI.

---

...our meta-analysis indicates that CYP2E1, NAT2 and GSTM1 genetic variation is significantly associated with anti-tuberculosis drug-induced liver injury. (PLoS One. 2012; 7(10): e47769.)

We observed the slow phenotype of NAT1*14 and NAT1*3 alleles was associated with greater PAS exposure. (Antimicrob Agents Chemother. 2015; 59(7): 4129-38)
### HUMAN GENES ASSOCIATED WITH ANTI-TB DRUG-INDUCED ADVERSE EVENTS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse Drug Reaction</th>
<th>Gene</th>
<th>Gene class</th>
<th># SNPs *</th>
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<td>RIF</td>
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<td>GPIX</td>
<td>Receptor</td>
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<td>INH</td>
<td>Hepatotoxicity</td>
<td>NAT2</td>
<td>DME-1</td>
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<td></td>
<td></td>
<td>CYP2E1</td>
<td>DME-1</td>
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<td></td>
<td></td>
<td>GSTM1</td>
<td>DME-2</td>
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<td></td>
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<td>GSTT1</td>
<td>DME-2</td>
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<td>SLC22A12</td>
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<td>Transporter</td>
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</table>

* SNP frequencies are population dependent

- If levels increase -> approach MTD and accumulation of **toxic** metabolites
- If levels decrease -> reduce treatment efficacy
  - Incomplete eradication of bacilli -> prolonged treatment and **relapse**
  - Increase chance of developing DR

Adapted from Sahu et al. 2015. Curr Drug Metabol. 16(7): 538-52
CONCLUSIONS

- Culture remains challenging in high burden countries
- Poor uptake of new tools such as GeneXpert due to cost and political will
- Programmatic challenges of discordant results among diagnostic assays
- Optimization of sequencing tools in centralized labs can help address drug resistance crisis (PHE sequence-based DST; WHO and CDC surveillance)

Sequencing assays currently available for scientific research and clinical trials

- Culture-free drug resistance assay (Pathogen DNA)
  - Hetero-resistance and resistance prediction
- Pharmacogenomic assay (Host DNA)
  - Predicting adverse events

Future biomarker assay tools in development

- Triage test to rule out TB at point-of-care
  - Systematic screening for active case finding (e.g. C-reactive protein)
- Rapid non-sputum instrument-free biomarker test for identifying TB disease
  - Special populations and EPTB (e.g. Nanodisk: blood-based TB test)
- Incipient assay
  - Biomarker(s) that predict disease progression (e.g. RNA signature assay)
- Treatment monitoring assay
  - Predict cure versus relapse (e.g. LAM biomarker)
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• Matthew Ezewudo

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• David Dolinger
• Becky Colman

World Health Organization
• Matteo Zignol

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• Paolo Miotto

DTBE, CDC
• Angela Starks
• Jamie Posey

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• Dave Engelthaler

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• Jim Gallarda

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• Stefan Niemann (Chair)
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