

# Molecular Methods in the Diagnosis of Drug Resistant Tuberculosis

Dr Sahajal Dhooria

What is drug resistant TB?

# Definitions

- MDR TB-- defined as resistance to isoniazid and rifampicin, with or without resistance to other anti-TB drugs
- XDR-TB-- defined as resistance to at least Isoniazid and Rifampicin (i.e. MDR-TB) plus resistance to any of the fluoroquinolones and any one of the second-line injectable drugs (amikacin, kanamycin, or capreomycin)

# The Global Situation-WHO

# How it began?

- Early 1990s – MDR TB outbreaks, in New York and Florida as well as in countries in Europe and South America (often associated with HIV in hospital populations)
- 1994-WHO joined forces in with the International Union against Tuberculosis and Lung Disease and other partners to develop a set of guidelines [21] for the surveillance of resistance to 4 of the 5 first line anti-tuberculosis drugs: HRES

# SRL

- Supranational Reference Laboratory (SRL) Network
- SRLs provide technical assistance to NRL during the preparation, implementation, and evaluation of survey results
- Currently 29 SRLs worldwide
- Coordinated by the Laboratory of Mycobacteriology of the Tropical Institute in Antwerp, Belgium

M Zignol, et al. *Bull World Health Organ* 2012;90:111–119D

# Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance



Abigail Wright, Matteo Zignol, Armand Van Deun, Dennis Falzon, Sabine Ruesch Gerdes, Knut Feldman, Sven Hoffner, Francis Drobniewski, Lucia Barrera, Dick van Soolingen, Fadila Boulabhal, C N Paramasivan, Kai Man Kam, Satoshi Mitarai, Paul Nunn, Mario Raviglione, for the Global Project on Anti-Tuberculosis Drug Resistance Surveillance\*

## Summary

**Background** The Global Project on Anti-Tuberculosis Drug Resistance has been gathering data since 1994. This study provides the latest data on the extent of drug resistance worldwide.

**Methods** Data for drug susceptibility were gathered from 90 726 patients in 83 countries and territories between 2002 and 2007. Standardised collection of results enabled comparison both between and within countries. Where possible, data for HIV status and resistance to second-line drugs were also obtained. Laboratory data were quality assured by the Supranational Tuberculosis Reference Laboratory Network.

**Findings** The median prevalence of resistance to any drug in new cases of tuberculosis was 11·1% (IQR 7·0–22·3). The prevalence of multidrug resistance in new tuberculosis cases ranged from 0% in eight countries to 7% in two provinces

**Lancet 2009; 373: 1861–73**

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See [Comment](#) page 1822

\*For members see webappendix

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Stop TB Department, WHO,  
Geneva, Switzerland

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Wright A. Lancet 2009; 373: 1861–73

- Median prevalence of resistance to any drug in new cases of tuberculosis --11.1% (IQR 7.0–22.3)
- Median prevalence of MDR tuberculosis in new tuberculosis cases -- 1.6% (IQR 0.6–3.9)
- Median prevalence of resistance to any drug in previously treated cases -- 25.1% (IQR 6.0–46.3).

Wright A. Lancet 2009; 373: 1861–73



# Latest Report

## Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007–2010

Matteo Zignol,<sup>a</sup> Wayne van Gemert,<sup>a</sup> Dennis Falzon,<sup>a</sup> Charalambos Sismanidis,<sup>a</sup> Philippe Glaziou,<sup>a</sup>  
Katherine Floyd<sup>a</sup> & Mario Raviglione<sup>a</sup>

**Objective** To present a global update of drug-resistant tuberculosis (TB) and explore trends in 1994–2010.

**Methods** Data on drug resistance among new and previously treated TB patients, as reported by countries to the World Health Organization, were analysed. Such data are collected through surveys of a representative sample of TB patients or surveillance systems based on routine drug susceptibility testing. Associations between multidrug-resistant TB (MDR-TB) and human immunodeficiency virus (HIV) infection and sex were explored through logistic regression.

**Findings** In 2007–2010, 80 countries and 8 territories reported surveillance data. MDR-TB among new and previously treated cases was highest in the Russian Federation (Murmansk oblast, 28.9%) and the Republic of Moldova (65.1%), respectively. In three former Soviet Union countries and South Africa, more than 10% of the cases of MDR-TB were extensively drug-resistant. Globally, in 1994 to 2010 multidrug resistance was observed in 3.4% (95% confidence interval, CI: 1.9–5.0) of all new TB cases and in 19.8% (95% CI: 14.4–25.1) of previously treated TB cases. No overall associations between MDR-TB and HIV infection (odds ratio, OR: 1.4; 95% CI: 0.7–3.0) or sex (OR: 1.1; 95% CI: 0.8–1.4) were found. Between 1994 and 2010, MDR-TB rates in the general population increased in Botswana, Peru, the Republic of Korea and declined in Estonia, Latvia and the United States of America.

**Conclusion** The highest global rates of MDR-TB ever reported were documented in 2009 and 2010. Trends in MDR-TB are still unclear in most settings. Better surveillance or survey data are required, especially from Africa and India.

Abstracts in **عربي**, **中文**, **Français**, **Русский** and **Español** at the end of each article.

Bull World Health Organ 2012;90:111–119D

# What the WHO says?

- In 2010, only 16% of the TB patients estimated to have MDR-TB were diagnosed and given appropriate treatment
- 2007-2010-- proportion of new TB cases reported as showing MDR -- 0% to 28.9%
- Proportions exceeding 12%
  - Belarus (25.7%)
  - Estonia (18.3%)
  - Several oblasts of the Russian Federation (with Murmansk having the highest level, 28.9%)
  - Tajikistan (Dushanbe city and Rudaki district, 16.5%)

*Bull World Health Organ 2012;90:111–119D*

- Proportion of previously treated cases having MDR-TB -- 0% to 65.1%
- Countries or subnational areas with proportions exceeding 50%
  - Belarus (60.2%)
  - Lithuania (51.5%)
  - Republic of Moldova(65.1%)
  - Five oblasts of the Russian Federation,
  - Tajikistan (Dushanbe city and Rudaki district, 61.6%)

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- Largest country that conducted a nationwide survey in the reporting period was China, where proportion of MDR
  - New -- 5.7%
  - Previously treated cases -- 25.6%

- XDR-TB identified in 77 countries globally
- Combined data from 57 countries and 3 territories proportion of MDR-TB cases with XDR was 9.4% (95% CI: 7.4–11.6) (1994-2010 data)
- Proportion of MDR-TB cases that were XDR exceeded 10% in
  - Estonia (19.7%)
  - Latvia (15.1%)
  - South Africa (10.5%)
  - Tajikistan (Dushanbe city and Rudaki district, 21.0%)

- Estonia, Latvia and the United States, surveillance data suggest that both TB and MDR-TB rates have been falling for more than a decade

- Only 34 countries and settings have a system in place to routinely test all patients with MDR-TB for secondline anti-TB drug resistance
- Generally countries with established or emerging economies

*Bull World Health Organ 2012;90:111–119D*

# What does it say about India?

- “Whereas China has been able to conduct a nationwide survey, India and the Russian Federation -- the other two large countries that, with China, contribute to more than 50% of the estimated global burden of MDR-TB – have only produced reliable subnational level data to date.”



Southeast Asian region								
Burma (Myanmar), 2003	SVY	733	73 (10.0%, 7.8–12.5)	48 (6.5%, 4.8–8.7)	34 (4.6%, 3.2–6.5)	0 (0.0%, 0.0–0.4)	29 (4.0%, 2.6–5.7)	4 (0.5%, 0.1–1.4)
India								
Ernakulam District, Kerala State, 2004	SVY	305	85 (27.9%, 22.9–33.3)	27 (8.9%, 5.9–12.6)	11 (3.6%, 1.8–6.4)	3 (1.0%, 0.2–2.8)	6 (2.0%, 0.7–4.2)	3 (1.0%, 0.2–2.8)
Gujarat State, 2006	SVY	1571	335 (21.3%, 19.1–23.7)	173 (11.0%, 9.4–12.8)	40 (2.5%, 1.8–3.5)	3 (0.2%, 0.0–0.6)	37 (2.4%, 1.7–3.2)	13 (0.8%, 0.4–1.4)
Mayhurbhanj District, Orissa State, 2001†	SVY	282	15 (5.3%, 3.0–8.6)	7 (2.5%, 1.0–5.0)	2 (0.7%, 0.1–2.5)	0 (0.0%, 0.0–1.1)	2 (0.7%, 0.1–2.5)	1 (0.4%, 0.0–2.0)
Hoogli district, West Bengal State, 2001†	SVY	263	44 (16.7%, 12.4–21.8)	27 (10.3%, 6.9–14.6)	8 (3.0%, 1.3–5.9)	0 (0.0%, 0.0–1.1)	8 (3.0%, 1.3–5.9)	3 (1.1%, 0.2–3.3)
Indonesia, Mimika District, Papua Province, 2004	SVY	101	14 (13.9%, 7.8–22.2)	13 (12.9%, 7.0–21.0)	2 (2.0%, 0.2–7.0)	0 (0.0%, 0.0–2.9)	2 (2.0%, 0.2–7.0)	0 (0.0%, 0.0–2.9)

Wright A. Lancet 2009; 373: 1861–73

# Indian Data

- Data from studies conducted by TRC and NTI, have found MDR-TB levels of less than 1% to 3% in new cases and around 12% in re-treatment cases <sup>1,2</sup>
- Overall emergence of resistance to rifampicin in only 2% of patients<sup>3</sup>
- High level (18%) of initial resistance to isoniazid

<sup>1</sup>Mahadev B. *Indian J Tuberc* 2005; 52(1): 5-10

<sup>2</sup>CN Paramasivan. *Int J Tuberc Lung Dis* 2002; 6 (6): 479-484

<sup>3</sup>TRC, ICMR, Chennai, India. *Int J Tuberc Lung Dis* 2001; 5(1); 40-45

- Two community based state level drug resistance surveillance (DRS) studies by RNTCP in Gujarat and Maharashtra
- Prevalence of MDR-TB to be about 3% in new cases and 12-17% in re-treatment cases<sup>1</sup>

<sup>1</sup>Ramachandran R. *Int J Tuberc Lung Dis* 2009; 13(9): 1154-1160.

# Cause of Emergence of Drug Resistance

**Table 1.1 Causes of inadequate treatment <sup>1</sup>**

<b>Providers/Programmes: Inadequate regimens</b>	<b>Drugs: Inadequate supply/quality</b>	<b>Patients: Inadequate drug intake</b>
<ul style="list-style-type: none"> <li>-Absence of guidelines or inappropriate guidelines</li> <li>-Non-compliance with guidelines</li> <li>-Inadequate training of health staff</li> <li>-No monitoring of treatment</li> <li>-Poorly organized or funded TB control programmes</li> </ul>	<ul style="list-style-type: none"> <li>-Non-availability of certain drugs (stock-outs or delivery disruptions)</li> <li>-Poor quality</li> <li>-Poor storage conditions</li> <li>-Wrong dosages or combination</li> </ul>	<ul style="list-style-type: none"> <li>-Poor adherence (or poor DOT)</li> <li>-Lack of information</li> <li>-Non-availability of free drugs</li> <li>-Adverse drug reactions</li> <li>-Social and economic barriers</li> <li>-Malabsorption</li> <li>-Substance abuse disorders</li> </ul>

**Revised National Tuberculosis Control Programme. DOTS-Plus Guidelines January 2010**  
 Central TB Division, Directorate General of Health Services, Ministry of Health & Family Welfare

# Mechanisms of Resistance for Anti-TB Drugs

# Initial Molecular Assays for Rifampicin Resistance

The Beginnings

[Lancet.](#) 1993 Mar 13;341(8846):647-50.

**Detection of rifampicin-resistance mutations in  
*Mycobacterium tuberculosis*.**

[Telenti A](#), [Imboden P](#), [Marchesi F](#), [Lowrie D](#), [Cole S](#), [Colston MJ](#), [Matter L](#), [Schopfer K](#), [Bodmer T](#).

# The First Step

- RNA polymerase subunit beta (rpoB) gene cloned
- 122 isolates of M tuberculosis
- Direct amplification and sequencing of a 411 bp rpoB fragment
- Mutations involving 8 conserved aminoacids identified in 64 of 66 rifampicin-resistant isolates, but in none of 56 sensitive isolates
- All mutations clustered within a region of 23 aminoacids (69bp)



- Strategy developed: PCR-SSCP (polymerase chain reaction-single-strand conformation polymorphism)
- Allowed efficient detection of all known rifampicin-resistant mutants.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1993, p. 2054–2058  
0066-4804/93/102054-05\$02.00/0  
Copyright © 1993, American Society for Microbiology

Vol. 37, 1

# Direct, Automated Detection of Rifampin-Resistant *Mycobacterium tuberculosis* by Polymerase Chain Reaction and Single-Strand Conformation Polymorphism Analysis

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- SSCP is based on the fact that separated strands of DNA adopt a folded conformation as a result of self complementarity and intramolecular interactions
- A single nucleotide mutation usually leads to an altered conformation that can be identified as a change in DNA strand mobility by nondenaturing gel electrophoresis
- To this PCR adds the possibility of precisely selecting and amplifying mutation loci for analysis

Telenti A. . Antimicrob Agents Chemother 1993; 37: 2054-2058.

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1994, p. 1095–1098  
0095-1137/94/\$04.00+0  
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Vol. 32, No. 4

## Characterization by Automated DNA Sequencing of Mutations in the Gene (*rpoB*) Encoding the RNA Polymerase $\beta$ Subunit in Rifampin-Resistant *Mycobacterium tuberculosis* Strains from New York City and Texas

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Received 18 October 1993/Returned for modification 7 December 1993/Accepted 3 January 1994

- Automated DNA sequencing used to characterize mutations associated with rifampin resistance
- 69-bp region of the rpoB gene
- Greater than 90% of rifampin-resistant strains have sequence alterations in this region
- Most were missense mutations

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1994, p. 2380–2386  
0066-4804/94/\$04.00+0  
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Vol. 38, No. 1

## Characterization of Rifampin Resistance in Pathogenic Mycobacteria

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Tokyo, Japan<sup>7</sup>; and Armauer-Hansen Institute, Wurzburg, Germany<sup>8</sup>*

Received 11 March 1994/Returned for modification 1 June 1994/Accepted 11 August 1994

# PCR-HDF

- PCR-heteroduplex formation assay (PCR-HDF)
- 305-bp PCR product of each test strain mixed with 305-bp PCR product from rifampin-susceptible *M. tuberculosis* H37Rv.
- Samples heated in a thermal cycler then cooled slowly
- Resulting DNA duplexes analyzed by electrophoresis on a mutation detection enhancement gel

Williams D L. Antimicrob Agents Chemother 1994; 38: 2380-2386

- These bands represent the 305-bp homoduplex plus various species of heteroduplexes migrating in the gels distinct from the band representing the homoduplex
- Single band representing the homoduplex at 305 bp detected in rifampin-susceptible isolates



## Tubercle and Lung Disease

### Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay

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**S U M M A R Y.** *Setting:* Multidrug resistant *Mycobacterium tuberculosis* strains are threatening TB control in the world. Rapid diagnosis of resistance is essential for adequate treatment and optimal control of the disease.

**Objective:** Evaluation of a new technique (Line Probe Assay, LiPA) for easy and rapid detection of Rifampicin resistance (RMP<sup>R</sup>) of *M. tuberculosis*.

**Design:** After amplification of the region of the RNA polymerase, involved in RMP<sup>R</sup>, the amplified product is hybridized with a set of 10 oligonucleotides immobilized onto a membrane strip. From the pattern obtained the presence or absence of RMP<sup>R</sup> *M. tuberculosis* can be assessed. 67 clinical samples positive in culture for *M. tuberculosis* were analyzed with LiPA and results were compared with classical susceptibility testing.

**Results:** In vitro drug sensitivity testing identified 46 rifampicin sensitive and 21 resistant strains. In 65 of the 67 specimens LiPA results matched classical testing. In two RMP<sup>R</sup> cases LiPA showed a sensitive pattern.

**Conclusion:** In contrast to culture and sensitivity testing, where results take on average 6 weeks, LiPA testing is an easy and rapid (< 48 h) method of detecting RMP<sup>R</sup> *M. tuberculosis* in clinical samples. Results correlated in 97% of the samples. In the two RMP<sup>R</sup> samples with a sensitive LiPA pattern another mechanism of resistance is suspected.

# Line Probe Assay

- Based on the reverse hybridization principle
- Oligonucleotide probes immobilized as parallel lines at known locations on a nitrocellulose strip

- Extracting DNA from cultures or directly from clinical samples
- Amplifying the RIF resistance determining region of the *rpoB* gene using PCR
- Biotinylated PCR products then hybridized with the immobilized probes
- Results determined by colorimetric development
- The *M. tuberculosis* isolate considered RIF susceptible if all of the wild-type S probes give a positive signal and all of the R probes react negatively
- RIF resistance indicated by absence of one or more wildtype S probes
- a positive reaction obtained with one of the four R probes -- RIF resistance is due to one of the four most frequently observed mutations

# Innogenetics

# Innogenetics NV

- An international in vitro diagnostics company(founded in 1985) with headquarters in Ghent, Belgium
- Develops and markets diagnostic assays in the fields of infectious diseases, oncology, neurodegeneration, transplantation and genetic testing, with a special focus on molecular diagnostics and multiparameter testing
- Company acquired in September 2010 by the Japanese Fujirebio Inc. Group

## Company

- [Mission and Vision](#)
- [Innogenetics Group](#)
- [Distribution Network](#)
- [Partnerships & Alliances](#)



## Company Description

Innogenetics NV is an international diagnostic company that develops and markets diagnostic products to improve therapy management and patient health.

Innogenetics develops and markets a range of diagnostic assays with a focus on molecular diagnostics and multiparameter testing. Its products are sold in over 120 countries through its 6 subsidiaries and a large number of distributors.



## Have a question?

Look at our FAQ, go to our general contact page or contact us by:

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Fax.: +32/9 329 19 11  
E-mail: [info@innogenetics.com](mailto:info@innogenetics.com)

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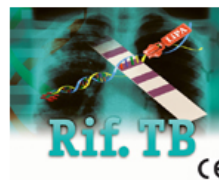
### INNO-LiPA Rif.TB

#### One strip - one test MTB complex and rapid rifampicin susceptibility testing.

INNO-LiPA Rif.TB is a **line probe assay** for the detection of *Mycobacterium tuberculosis* complex and its resistance to rifampicin.

An amplification method starting directly from clinical sample preparation is available for research use only, not for use in diagnostic procedures: INNO-LiPA Rif. TB outer. Contact Customer Support for details.

Article number: 80663 (20T - CE), 80664 (20T - CE)



#### Features & Benefits

- Detects *M. tuberculosis* complex and sensitivity/resistance to rifampicin (RMP) simultaneously on one strip
- Detects mixed populations, allowing early detection of emerging resistant strains
- Targets the *rpoB* gene where mutations associated with RMP resistance are located<sup>1</sup>
- Quick visual interpretation<sup>2</sup>
- Fast resistance profile obtained before standard susceptibility testing
- Automation is possible using **Auto-LiPA 48**

#### Have a question?

Look at our FAQ, go to our general contact page or contact us by:

Ph.: +32/9 329 13 29  
Fax.: +32/9 329 19 11  
E-mail: [info@innogenetics.com](mailto:info@innogenetics.com)

#### Customer support:

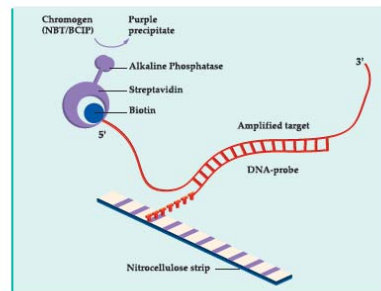
- (Material) Safety Data Sheets
- IFUs / Package Inserts
- Other support requests

#### Customer satisfaction surveys:



## INNO-LiPA Rif.TB

Fast, easy, and highly specific DNA hybridization test

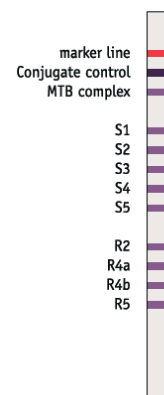


Test principle based on reverse hybridization:

INNO-LiPA major steps and total incubation time

1 Hybridization	30 min
2 Stringent wash	10 min
3 Colorimetric detection	60 min
Total	< 2 hrs

### Easy interpretation



INNO-LiPA Rif.TB simultaneously detects the *Mycobacterium tuberculosis* complex and the presence of mutations in the *rpoB* gene associated with resistance to rifampicin (RMP), which is considered a marker for multi-drug resistant strains.<sup>1</sup>

The strip contains 5 probes for detection of sensitive genotypes (S1-S5) and 4 probes for detection of resistant genotypes (R2, R4a, R4b, R5). RMP resistance is indicated by the absence of one or more "sensitive" probes, possibly accompanied by the appearance of one or more "mutant" probes.



## Evaluation of a Line Probe Assay Kit for Characterization of *rpoB* Mutations in Rifampin-Resistant *Mycobacterium tuberculosis* Isolates from New York City

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Received 14 November 1996/Returned for modification 16 January 1997/Accepted 6 February 1997

**A commercial line probe assay kit (Inno-LiPA Rif.TB) for rapid identification of mutations in the *rpoB* gene associated with rifampin resistance in *Mycobacterium tuberculosis* was evaluated with a collection of 51 rifampin-resistant strains. Nine distinct *rpoB* mutations were identified. Concordances with automated sequence results for five wild-type kit probes and four probes for specific mutations were 94.1 and 100%, respectively. Overall concordance of the line probe assay kit with phenotypic rifampin susceptibility testing results was 90.2%.**

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- DNA sequencing studies have shown that greater than 95% of the RIF-resistant strains have mutations within an 81 base pair hot-spot region (codons 507–533) of the *rpoB* gene<sup>1</sup>
- *Though more* than 50 mutations within this region have been characterized by automated DNA sequencing, the majority involve point mutations at codons 516, 526, or 531<sub>2</sub>
- More than 90% of RIF-resistant TB is also resistant to INH, making RIF-resistance a good surrogate marker for MDR-TB<sup>1,3</sup>

<sup>1</sup>Cavusoglu C. J Clin Microbiol 2002, 40:4435-4438

<sup>2</sup>Ahmad S. Diagn Microbiol Infect Dis 2002, 44:245-252

<sup>3</sup>Drobniewski FA. J Med Microbiol 1998, 47:189-196

Research article

Open Access

## **A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis**

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\* Corresponding author    †Equal contributors

- Included studies that met the following pre-determined criteria:
  - Comparison of INNO-LiPA with a reference standard (including proportion method, radiometric BACTEC 460 method, and minimum inhibitory concentration method)
  - Evaluation of a minimum of ten RIF-sensitive and ten RIF-resistant samples

Morgan M, et al. *BMC Infectious Diseases* 2005, **5:62**

- Fifteen articles using the commercial INNO-LiPA Rif. TB kit included in this review
  - Exclusively on culture isolates--11
  - Directly on clinical specimens--1
  - On both isolates and clinical specimens—3
- Evaluated a total of 1738 specimens (mean 91; range 20 to 411), 1164 (67%) of which were RIF-resistant

# Subgroup Analysis

- 4 studies that tested LiPA directly on clinical specimens
  - Sensitivity -- 80% to 100%
  - Specificity for all four studies -- 100%
- 4 studies determined the number of RIF-resistant samples that were also INH-resistant, hence MDR-TB.
- On average, 91% of RIF-resistant samples were also INH-resistant

- LiPA yielded high overall sensitivity and specificity with a maximum joint sensitivity and specificity of 97% , a positive predictive value of 83%, while a negative LiPA
- In high prevalence area with 5% RIF-resistant strains
- PPV—84%
- NPV--99%
- Clinically meaningful increase in the probability of RIF-resistance from 5% to 83% if a test is positive, while a negative test would virtually rule out RIF-resistance

- If the baseline prevalence of rifampicin resistance is 1%
  - PPV -- 66%, i.e. one false positive test for every two true positives
- Prevalence of RIF-resistance in the study—67%
- Differs significantly from the prevalence of MDRTB seen in routine clinical practice settings, even in high prevalence regions



- The cost of the commercial LiPA kit is \$45 per sample tested. When additional costs for import and transport are taken into account, the actual cost per sample is as high as \$116 [27]
- Might be cost effective when weighed against the costs of undetected drug resistant TB

# Genotype MTB DR and MTB DR Plus

- GenoType® MTBDR detects the common mutations in the rpoB and katG genes responsible for resistance to rifampicin and isoniazid, respectively
- Involves DNA extraction PCR, solid phase reverse hybridization and detection of the resistance mutations
- Genotype® MTBDRplus assay detects additional mutations in the rpoB gene and also in the inhA gene promoter region, giving a higher sensitivity in resistance detection

Research article

Open Access

## Direct susceptibility testing for multi drug resistant tuberculosis: A meta-analysis

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# Aim

- Compared the sensitivity, specificity and time to results of four direct drug susceptibility testing tests with the conventional indirect testing for detection of resistance to rifampicin and isoniazid in *M. tuberculosis*:
  - Nitrate Reductase Assay (NRA)
  - Microscopic Observation Drug Susceptibility (MODS)
  - Genotype<sup>®</sup> MTBDR (*Hain Life Sciences, Nehren, Germany*)
  - Genotype<sup>®</sup> MTBDRplus (*Hain Life Sciences, Nehren, Germany*)

# Results

S No	Test	Rifampicin		Isoniazid	
		Pooled sensitivity	Pooled specificity	Pooled sensitivity	Pooled specificity
1	NRA	99	100	94	100
2	MODS	99	98	92	96
3	Genotype MTBDR	99	98	71	100
4	Genotype MTBDR plus	99	99	96	100

- Genotype<sup>®</sup> MTBDR test -- detect the most common mutations for INH resistance in the *katG* gene (account for 50–80% of INH resistance in *M tuberculosis*) [17]
- Genotype<sup>®</sup> MTBDRplus detect additional mutations in the *katG* gene and also in the *inhA* promoter region for isoniazid resistance [18]

## ***Time to results (TTR)***

- MODS and NRA tests--average TTR < 23 days
- Conventional indirect testing—2 months
- MODS 7–14
- Contamination and indeterminate results in phenotypic methods may prolong the time to the final
- Genotypic assays— 1-2 days

# 2012-PloS One

- The performance of the commercially available Genotype MTBDRplus molecular assay was compared to conventional methods including AFB smear, culture and drug susceptibility testing (DST) using both an absolute concentration method on LJ media and broth-based method using the MGIT 960 system Among 500 AFB smear-positive sputum specimens



- Most common genetic mutations conferring INH resistance were located in the *katG* gene at codon 531 with *inhA* mutations much less likely in this study
- MTBDRplus assay detected 97% of all RIF resistant cases with the most common genetic mutations occurring in the 530–533 base pair region of *rpoB* gene

- Lower sensitivity of the MTBDRplus test for INH compared to conventional methods is likely due to genetic mutations conferring INH resistance that are located outside the katG and inhA genes
- 94% all missed cases of INH resistance with genotypic testing were in INH mono-resistant cases, thus mitigating clinical consequences may be mitigated, as initial treatment regimens for INH mono-resistance incorporate standard first line therapy and outcomes of INH mono-resistance TB have been found to be similar to drug susceptible TB

# Xpert Mtb/RIF (GeneXpert System, Cepheid)

- Xpert MTB/RIF is a TB-specific automated, cartridge-based NAAT based on the GeneXpert multi-disease platform
- Developed by Cepheid, Inc. (Sunnyvale, USA) in partnership with the Foundation for Innovative New Diagnostics (FIND) and the University of Medicine and Dentistry of New Jersey (Newark, USA) with support from the US National Institutes of Health and the Bill & Melinda Gates Foundation

# Xpert MTB/RIF

- Automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF)
- Uses real-time polymerase-chain reaction (PCR) assay to amplify an MTB-specific sequence of the *rpoB* gene
- Probed with molecular beacons for mutations within the rifampin-resistance determining region

Boehme CC. N Engl J Med. 2010 September 9; 363(11): 1005–1015

- MTB/RIF test platform (GeneXpert, Cepheid) integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification and amplicon detection
- Only manual step -- addition of a bactericidal buffer to sputum before transferring a defined volume to the cartridge
- MTB/RIF cartridge is then inserted into the GeneXpert device, which provides results within 2 hours



# NIH Public Access

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## Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

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### Abstract

**Background**—Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

- 1730 patients with suspected drug-sensitive or MDR pulmonary TB
- Peru, Azerbaijan, South Africa, and India
- Three sputum specimens each
  - 2 specimens processed with *N-acetyl-L-cysteine and sodium hydroxide* before microscopy, solid and liquid culture, and the MTB/RIF test
  - 1 specimen used for direct testing with microscopy and the MTB/RIF test
- Shubhada Shenai, Ph.D., Camilla Rodrigues, M.D. [P.D. Hinduja National Hospital and Medical Research Centre (Hinduja), Mumbai, India]



# Results

- Sensitivity in smear positive, culture-positive patients – 98.2% (551 of 561)
- Smear-negative tuberculosis -- 72.5% (124 of 171)
- Specificity – 99.2% (604 of 609 patients)
- Smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 percentage points and a third by 5.1 percentage points, to a total of 90.2%.

- As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria (sensitivity) and
- 504 of 514 (98.1%) with rifampin-sensitive bacteria (specificity)
- Sequencing resolved all but two cases in favor of the MTB/RIF assay

# **Evaluation of the Xpert MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in a High HIV Prevalence Setting**

Grant Theron<sup>1\*</sup>, Jonny Peter<sup>1,\*</sup>, Richard van Zyl-Smit<sup>1</sup>, Hridayesh Mishra<sup>2</sup>, Elizabeth Streicher<sup>3</sup>, Samuel Murray<sup>1</sup>, Rodney Dawson<sup>1</sup>, Andrew Whitelaw<sup>4</sup>, Michael Hoelscher<sup>5</sup>, Surendra Sharma<sup>2</sup>, Madhukar Pai<sup>6</sup>, Robin Warren<sup>3</sup>, and Keertan Dheda<sup>1,7,8</sup>

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**Am J Respir Crit Care Med Vol 184. pp 132–140, 2011**

- Single archived spotsputum samples from 496 South African patients with suspected TB.
- *Mycobacterium tuberculosis* culture positivity and phenotypic resistance to rifampicin served as reference standards

- Xpert MTB/RIF detected 95% (95% confidence interval [CI], 88–98%; 89 of 94) of smearpositive culture-positive cases and the specificity was 94% (91– 96%; 320 of 339)
- The sensitivity in smear-negative cases was 55% (35–73%; 12 of 22) when the analysis was restricted to 1 ml of unprocessed sputum and culture time-to-positivity of less than or equal to 28 days

- Compared with smear microscopy (n = 94), Xpert MTB/ RIF detected an additional 17 cases (n = 111) representing an 18% (11–27%; 111 vs. 94) relative increase in the rapid TB case detection rate
- Compared with smear microscopy, the inclusion of Xpert MTB/RIF-positive culture-negative TB cases (ruled-in by an alternative diagnostic method) resulted in the detection of a further 16 cases (n = 127), thus significantly increasing the rapid TB case detection rate to 35% (95% CI, 26–45%; 94 to 111 vs. 94 to 127; P , 0.01), the overall specificity to 99.1% (97–100%; 320 of 323; P , 0.001), and sensitivity in smear-negative TB to 60% (P = 0.12).
- Performance strongly correlated with smear status and culture time-to-positivity.

- In patients infected with HIV compared with patients uninfected with HIV Xpert MTB/RIF showed a trend to reduced sensitivity ( $P = 0.09$ ) and significantly reduced negative predictive value ( $P = 0.01$ )
- The negative predictive value for rifampicin resistance was 99.4%

# WHO and Xpert

- The WHO has endorsed Xpert MTB/RIF in 2010

WHO. STAG-TB Report of the Tenth Meeting. Publication No. WHO/ HTM/2010.18.  
Geneva, Switzerland: World Health Organization; 2010.



- FIND (Foundation for Innovative New Diagnostics) is an NGO
- Invested in the development of Xpert MTB/RIF
- Negotiated a price reduction agreement with Cepheid for 145 high burden and developing countries including India)
- Would provide GeneXpert 4-module with desktop for \$17,000
- Each test cost would be around \$10

ORIGINAL ARTICLE

**Rapid detection of rifampicin, isoniazid and streptomycin resistance in *Mycobacterium tuberculosis* clinical isolates by high-resolution melting curve analysis**

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# PGI Data

- Evaluate high-resolution melting (HRM) curve analysis assay for detection of mutations in three drug resistance– associated genes of *Mycobacterium tuberculosis*

- Clinical isolates of *Myco. tuberculosis* phenotypically resistant to rifampicin (n = 29), isoniazid (n = 35) and streptomycin (n = 34) were analysed for mutations in *rpoB*, *katG* and *rpsL* genes, respectively, by HRM curve analysis and DNA sequencing
- HRM curve assay resulted in 11 clearly distinguishable melt curves denoting eight types of mutations responsible for drug resistance. For the three drugs, respectively, the sensitivity of HRM curve assay was found to be 93.1%, 80% and 61.8% compared to the phenotypic resistance patterns, and 93.1, 93.3 and 100% in comparison with the DNA sequencing

- The sensitivity and specificity of HRM curve assay was found to be comparable to DNA sequencing
- The assay offers the advantage of high throughput, single step, rapid work flow and cost effectiveness and can be utilized as a rapid screening method for detection of drug-resistant tuberculosis

# PGI Data

- Study of 36 strains of M tuberculosis analysed by InnoLipa Rif TB
- Specificity was 100%
- Most frequently observed mutation was His-526-Tyr
- Correlation between results of LiPA and conventional DST was 100%

# Unpublished Data

- Analyze the frequency of gene mutations associated with resistance to Rifampicin, Isoniazid and Streptomycin among *Mycobacterium tuberculosis* isolates from North India.
- 102 (59.6%) were untreated newly diagnosed cases and 69 (40.3%) were re-treated cases.
- Drug susceptibility testing was performed for Rifampicin, Isoniazid, Streptomycin and ethambutol by LJ - proportion method
- Drug resistant isolates were then characterized using molecular methods i.e PCR amplification, Restriction Fragment Length Polymorphism and sequence analysis was performed for screening of mutations in the drug target genes that encode to *rpoB*, *katG*, *inhA* and *rpsL*.

- Among 171 consecutive *M. tuberculosis* isolates, 28 (16.47%) were MDR-TB
- 6(5.9%) of these were from newly diagnosed untreated cases, 23 (33.3%) were from re-treated cases
- Among the drug resistant isolates, *rpoB* mutations were found in 100% (31/31) of RIF-resistant isolates and the most common mutation was S531L in 74.2% (23/31)
- In Isoniazid resistant isolates mutations were found in 79.6% (35/44) of INH-resistant isolates at *katG*315



# Unpublished Data

- *rpsL* mutations were screened found in 48.9% (24/25), k43 mutation were found in 42.5%(21/49), of SM-resistant isolates. In this study new restriction enzyme, NlaIV, was used for screening of k88 (AAG-AGG) mutation in *rpsL* gene

- . *rpsL* mutations were screened found in 48.9% (24/25), k43 mutation were found in 42.5%(21/49), of SM-resistant isolates. In this study new restriction enzyme, NlaIV, was used for screening of k88 (AAG-AGG) mutation in *rpsL* gene

# Limitations

- None of the molecular tests established targets all possible genes or mechanisms (some are not identified yet) involved in resistance, and thus, a variable proportion of resistant strains will not be detected
- The second inherent limitation is the detection limit of > 10% mutant DNA in a mixture of wild-type and mutant DNA
- If the proportion of resistant cells in an isolate is less than that amount, it can hardly be detected by molecular methods, whereas classical susceptibility testing might give a more sensitive test result in these cases

# Microarray

- Back in the 90s when the possibility to spot multiple probes on a solid format opened the possibility to measure the expression levels of many genes in a single reaction, a technology known as microarray or DNA biochip, there was great expectation on the application of this technology for the rapid diagnosis of tuberculosis and the detection of drug-resistant bacilli (Gingeras et al., 1998; Troesch et al., 1999)
- However, after a decade of several attempts it has not yet become a reality for common use in the tuberculosis diagnostic laboratory (Sougakoff et al., 2004; Vernet et al., 2004)

# Summary

- A number of new methods and assays have been developed without detection of drug resistance in *M. tuberculosis*
- However, rapid methods are not a replacement for culture, most are not reliable when used with smear-negative specimens; conventional AST is still needed to confirm cases of XDR-TB; to test for resistance to drugs other than isoniazid and rifampin
- None of the methods detect all resistant strains; and in many parts of the world, the existing infrastructure is inadequate for these assays to be used on a widespread basis
- Moreover, it is not yet clear that use of these assays, without other changes in overall diagnosis and treatment programs, will have the effect on TB control that is needed in many areas