結核病的分子藥物敏感性試驗
Molecular Drug Susceptibility for Tuberculosis

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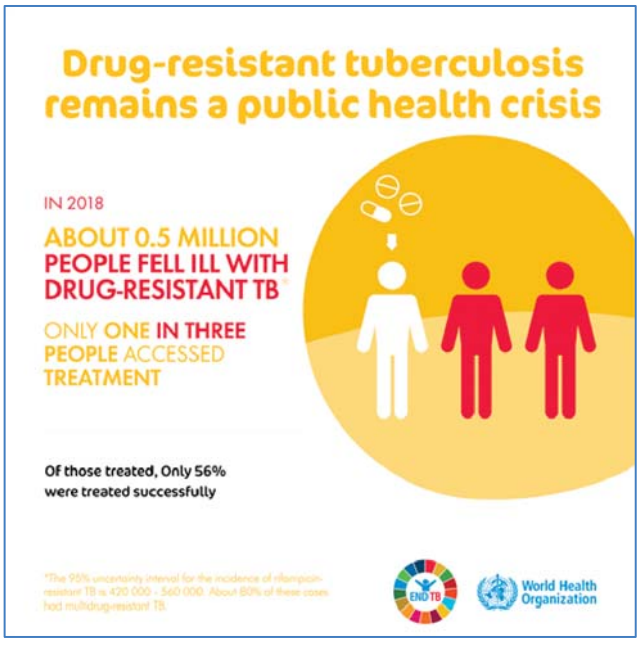
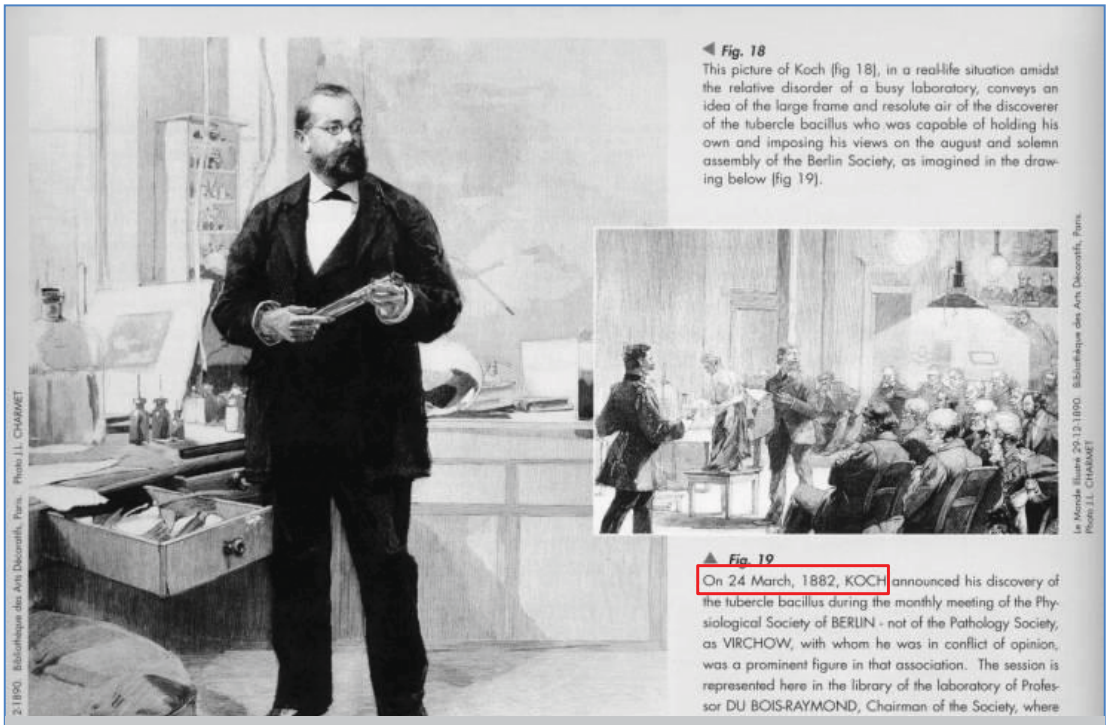
大綱

- 抗藥性結核簡介
- 分子藥物敏感性試驗
- 台灣分子藥物敏感性試驗的現況
- 第二線藥物的分子藥物敏感性試驗
- 未來發展
- 結論





◀ *Fig. 18*
This picture of Koch (fig 18), in a real-life situation amidst the relative disorder of a busy laboratory, conveys an idea of the large frame and resolute air of the discoverer of the tubercle bacillus who was capable of holding his own and imposing his views on the august and solemn assembly of the Berlin Society, as imagined in the drawing below (fig 19).

▲ *Fig. 19*
On 24 March, 1882, KOCH announced his discovery of the tubercle bacillus during the monthly meeting of the Physiological Society of BERLIN - not of the Pathology Society, as VIRCHOW, with whom he was in conflict of opinion, was a prominent figure in that association. The session is represented here in the library of the laboratory of Professor DU BOIS-RAYMOND, Chairman of the Society, where

人類未來與這致命的疾病奮戰時，面臨的
不再是未知的敵人，而是真實的細菌。 Robert Koch

Tuberculosis Therapy

Table 1.–Landmarks in tuberculosis (TB) therapy

Date	Landmark
1944	SM and PAS
1948	Randomised trial, SM *versus* PAS *versus* SM/PAS
1952	Triple therapy, isoniazid/SM/PAS, 24 months
1960s	EMB replaces PAS, 18 months
1970s	RIF added to INH/EMB/SM, 9 months
1980s	PZA added to INH/RIF, 6 months

SM: streptomycin; PAS: para-amino salt of salicylic acid; RIF: rifampicin; EMB: ethambutol; INH: isonicotinic acid hydrazide; PZA: pyrazinamide.

Eur Respir J 2002; 20: Suppl. 36, 87s–94s

Streptomycin-Resistant TB

BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 30 1948

STREPTOMYCIN TREATMENT OF PULMONARY TUBERCULOSIS

A MEDICAL RESEARCH COUNCIL INVESTIGATION

The following gives the short-term results of a controlled investigation into the effects of streptomycin on one type of pulmonary tuberculosis. The inquiry was planned and directed by the Streptomycin in Tuberculosis Trials Committee, composed of the following members: Dr. Geoffrey Marshall (chairman), Professor J. W. S. Blacklock, Professor C. Cameron, Professor N. B. Capon, Dr. R. Cruickshank, Professor J. H. Gaddum, Dr. F. R. G. Heaf, Professor A. Bradford Hill, Dr. L. E. Houghton, Dr. J. Clifford Hoyle, Professor H. Raistrick, Dr. J. G. Scadding, Professor W. H. Tytler, Professor G. S. Wilson, and Dr. P. D'Arcy Hart (secretary). The centres at which the work was carried out and the specialists in charge of patients and pathological work were as follows:

Brompton Hospital, London.—Clinician: Dr. J. W. Crofton, Streptomycin Registrar (working under the direction of the honorary staff of Brompton Hospital); Pathologists: Dr. J. W. Clegg, Dr. D. A. Mitchison.

Colindale Hospital (L.C.C.), London.—Clinicians: Dr. J. V. Hurford, Dr. B. J. Douglas Smith, Dr. W. E. Snell; Pathologists (Central Public Health Laboratory): Dr. G. B. Forbes, Dr. H. D. Holt.

Harefield Hospital (M.C.C.), Harefield, Middlesex.—Clinicians: Dr. R. H. Brent, Dr. L. E. Houghton; Pathologist: Dr. E. Nassau.

Bangour Hospital, Bangour, West Lothian.—Clinician: Dr. I. D. Ross; Pathologist: Dr. Isabella Purdie.

Killingbeck Hospital and Sanatorium, Leeds.—Clinicians: Dr. W. Santon Gilmour, Dr. A. M. Reevie; Pathologist: Professor J. W. McLeod.

Northern Hospital (L.C.C.), Winchmore Hill, London.—Clinicians: Dr. F. A. Nash, Dr. R. Shoulman; Pathologists: Dr. J. M. Alston, Dr. A. Mohun.

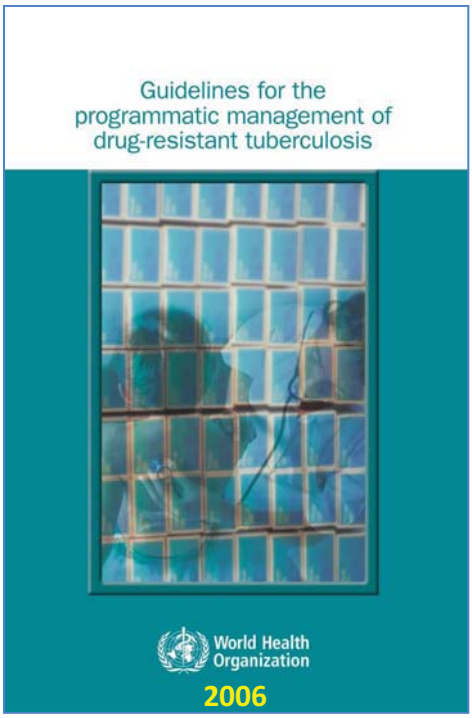
Sully Hospital, Sully, Glam.—Clinicians: Dr. D. M. E. Thomas, Dr. L. R. West; Pathologist: Professor W. H. Tytler.

The clinicians of the centres met periodically as a working subcommittee under the chairmanship of Dr. Geoffrey Marshall; so also did the pathologists under the chairmanship of Dr. R. Cruickshank. Dr. Marc Daniels, of the Council's scientific staff, was responsible for the clinical co-ordination of the trials, and he also prepared the report for the Committee, with assistance from Dr. D. A. Mitchison on the analysis of laboratory results. For the purpose of final analysis the radiological findings were assessed by a panel composed of Dr. L. G. Blair, Dr. Peter Kerley, and Dr. Geoffrey S. Todd.

Drug-resistant TB: a Man-made Phenomenon

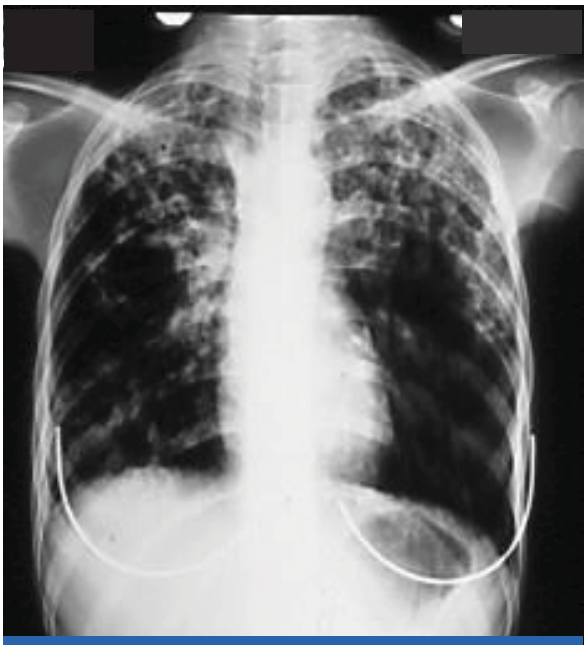
TABLE 1.1 **Causes of inadequate antituberculosis treatment** (1)

HEALTH-CARE PROVIDERS: INADEQUATE REGIMENS	DRUGS: INADEQUATE SUPPLY/QUALITY	PATIENTS: INADEQUATE DRUG INTAKE
Inappropriate guidelines	Poor quality	Poor adherence (or poor DOT)
Noncompliance with guidelines	Unavailability of certain drugs (stock-outs or delivery disruptions)	Lack of information
Absence of guidelines	Poor storage conditions	Lack of money (no treatment available free of charge)
Poor training	Wrong dose or combination	Lack of transportation
No monitoring of treatment		Adverse effects
Poorly organized or funded TB control programmes		Social barriers
		Malabsorption
		Substance dependency disorders



- Short-course chemotherapy for patients infected with drug-resistant strains may create even more resistance to the drugs in use
  - The "amplifier effect" of short-course chemotherapy

正確診斷肺結核，就代表診斷正確？



MDR-TB

培養室(非培養箱)

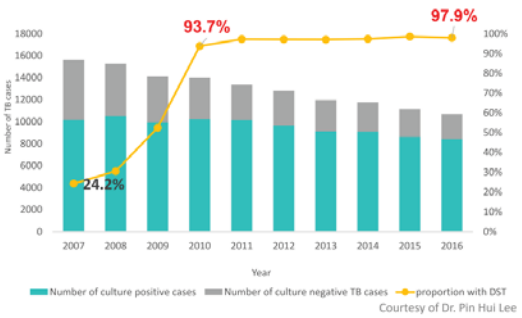
台灣省防癆局(青島西路10號)

藥物敏感性試驗：全面實施

- 所有病人第一次培養陽性的結核分枝桿菌株
- 病人接受治療第五個月及以後培養仍呈陽性
- 陰轉後再度培養陽性的菌株

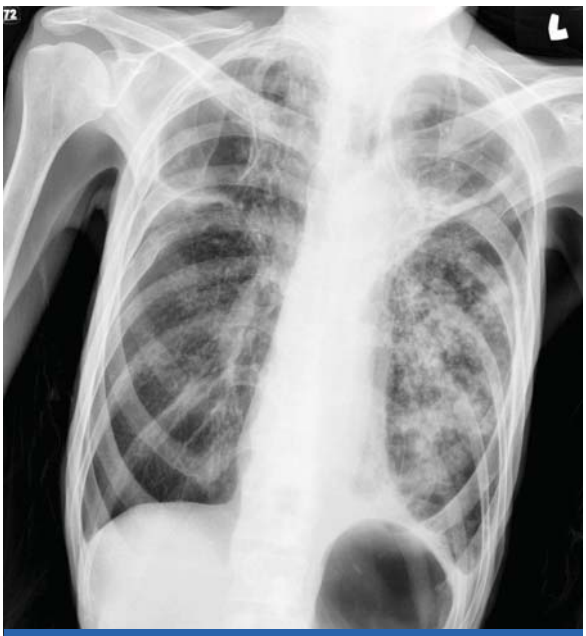
台灣結核病診治指引第6版

Number and Proportion of Culture Positive TB Cases with Baseline Drug Susceptibility Testing Results



Courtesy of Dr. Pin Hui Lee

PLoS ONE 2019;14(4):e0214792



59 y/o, Male, INH-Resistant TB

藥物敏感性試驗：品質提升

INT J TUBERC LUNG DIS 17(1):113–119
©2013 The Union
http://dx.doi.org/10.5588/ijtld.12.0521

Proficiency of drug susceptibility testing for *Mycobacterium tuberculosis* in Taiwan, 2007–2011

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*Reference Laboratory of Mycobacteriology, Research and Diagnostic Center, Centers for Disease Control, Department of Health, Taiwan; †International Union Against Tuberculosis and Lung Disease, Paris, France; ‡Division of Pulmonary Medicine, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, §Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, ¶Department of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan

SUMMARY

SETTING: Authorised clinical mycobacteriology laboratories in Taiwan.
OBJECTIVE: To evaluate the impact of external quality assessment (EQA) on the quality of drug susceptibility testing (DST) in 2007–2011.
DESIGN: Panels consisting of 20–30 *Mycobacterium tuberculosis* strains were used. Efficiency of 95% in detecting resistance to both isoniazid (INH) and rifampicin (RMP), and of 90% to ethambutol (EMB) and streptomycin (SM) was used to define a competent laboratory.
RESULTS: The proportion of laboratories that fulfilled the competency criteria for all first-line drugs was 16.7% in 2007, increasing to 85.7% in 2008, 86.1% in 2009, 82.4% in 2010, and to 96.8% in 2011 ($P < 0.01$). The mean efficiency in detecting resistance to INH and RMP reached >99% during 2008–2011 ($P = 0.90$ for INH and $P = 0.82$ for RMP), and for EMB it increased from 82.0% in 2007 to 92.2% in 2008 and 99.5% in 2011 ($P < 0.01$), while that for resistance to SM increased from 82.0% in 2007 to 98.1% in 2008 and 99.5% in 2011 ($P < 0.01$). Preparations of inoculum for DST and detection of EMB resistance were the main reasons for non-competence.
CONCLUSION: The EQA programme was effective in improving the competency of clinical laboratories in performing DST for tuberculosis.
KEY WORDS: proficiency; drug susceptibility testing; *Mycobacterium tuberculosis*

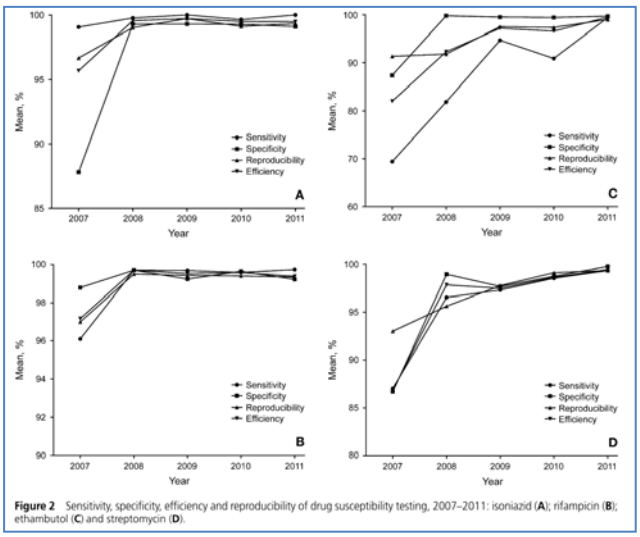
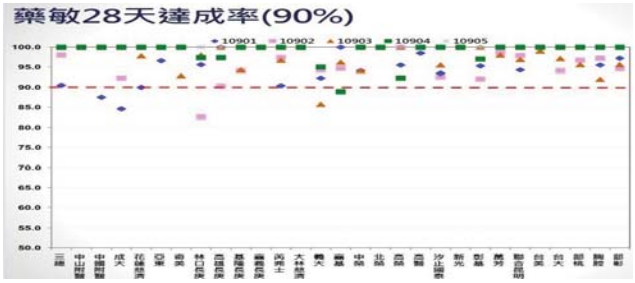


Figure 2 Sensitivity, specificity, efficiency and reproducibility of drug susceptibility testing, 2007–2011: isoniazid (**A**); rifampicin (**B**); ethambutol (**C**) and streptomycin (**D**).

- 所有檢驗必須在有品質保證的實驗室內進行
- 臨床醫師判讀時，必須考慮實驗室的檢驗品質

台灣結核病診治指引第6版

藥物敏感性試驗：速度變快



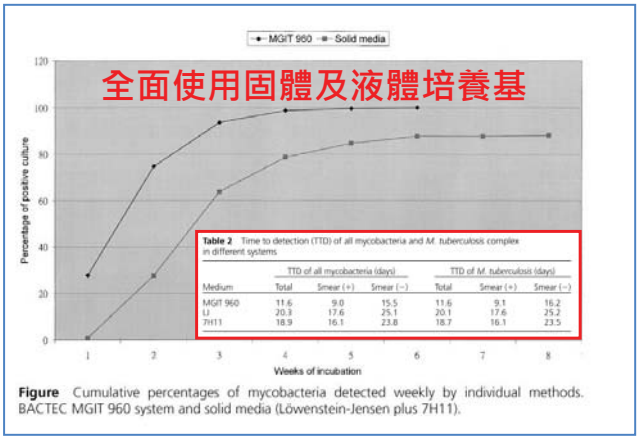
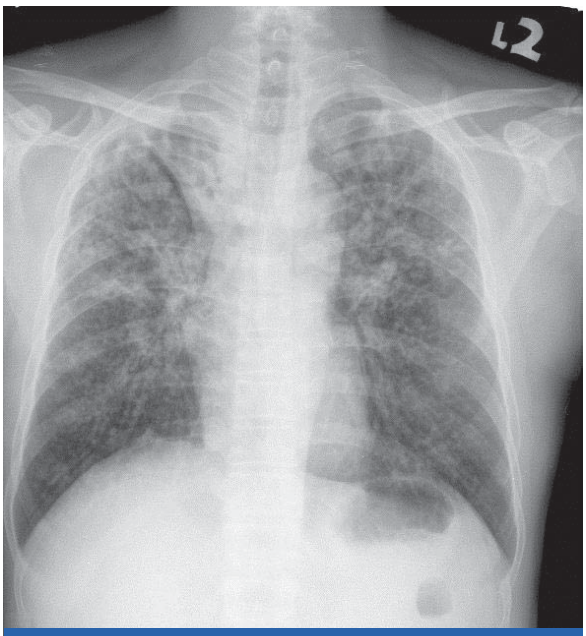
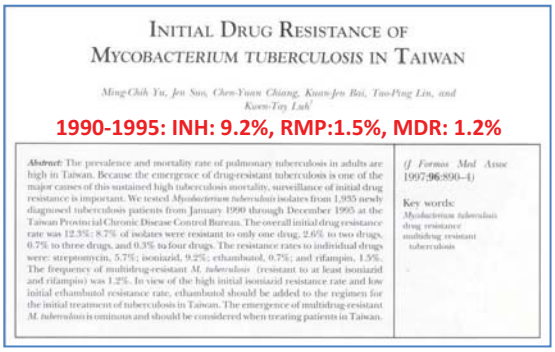


Figure Cumulative percentages of mycobacteria detected weekly by individual methods. BACTEC MGIT 960 system and solid media (Löwenstein-Jensen plus 7H11).



Int J Tuberc Lung Dis 2003;7:569-74

挑戰 1



INITIAL DRUG RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS IN TAIWAN

Ming-Chih Yu, Jen Suo, Chen-Yuan Chiang, Kuan-Jen Bai, Tuo-Ping Liu, and Kuen-Tay Luh

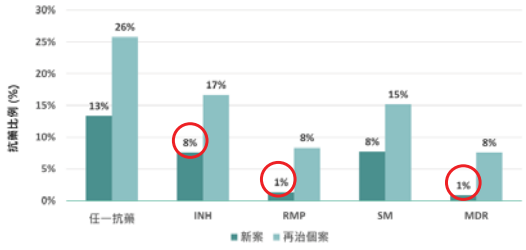
1990-1995: INH: 9.2%, RMP:1.5%, MDR: 1.2%

Abstract: The prevalence and mortality rate of pulmonary tuberculosis in adults are high in Taiwan. Because the emergence of drug-resistant tuberculosis is one of the major causes of this sustained high tuberculosis mortality, surveillance of initial drug resistance is important. We tested *Mycobacterium tuberculosis* isolates from 1,935 newly diagnosed tuberculosis patients from January 1990 through December 1995 at the Taiwan Provincial Chronic Disease Control Bureau. The overall initial drug resistance rate was 12.3%: 8.7% of isolates were resistant to only one drug; 2.6% to two drugs, 0.7% to three drugs, and 0.3% to four drugs. The resistance rates to individual drugs were: streptomycin, 5.7%; isoniazid, 9.2%; ethambutol, 0.7%; and rifampin, 1.5%. The frequency of multidrug-resistant *M. tuberculosis* (resistant to at least isoniazid and rifampin) was 1.2%. In view of the high initial isoniazid resistance rate and low initial ethambutol resistance rate, ethambutol should be added to the regimen for the initial treatment of tuberculosis in Taiwan. The emergence of multidrug-resistant *M. tuberculosis* is ominous and should be considered when treating patients in Taiwan.

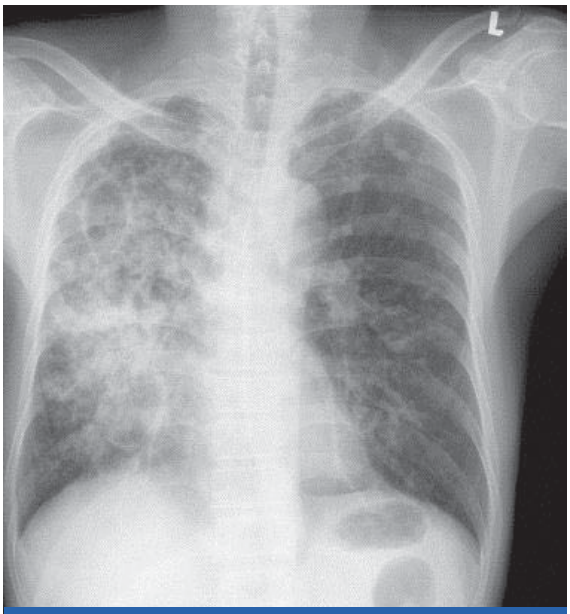
J Formos Med Assoc 1997;96:890-4)

Key words:
Mycobacterium tuberculosis
drug resistance
multidrug resistant tuberculosis

結核病抗藥性監測



Taiwan CDC 2020.5.21

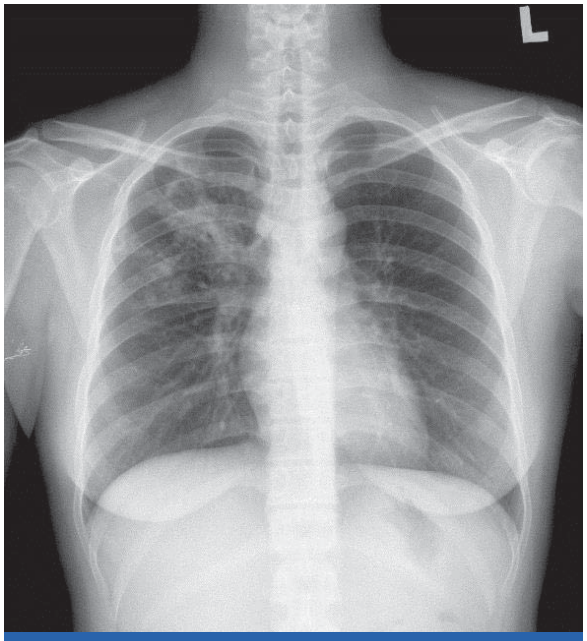


Far-advanced TB, Effective?

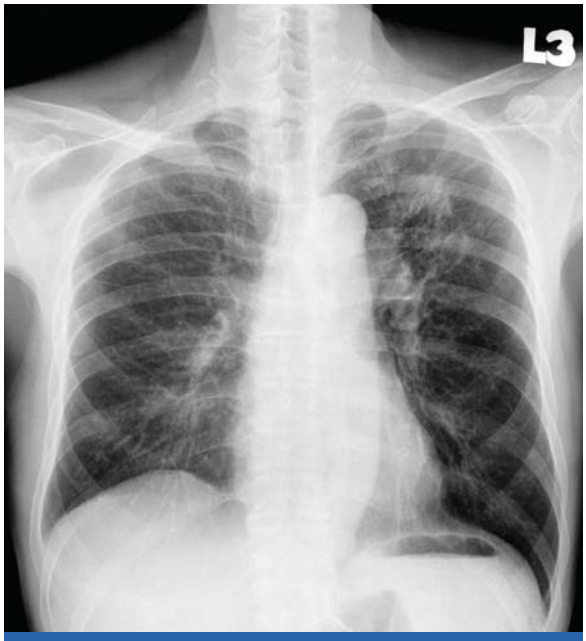
- Standard short-course chemotherapy, based on first-line drugs, is an inadequate treatment for some patients with drug-resistant TB

JAMA 2000;283:2537-45

挑戰 2

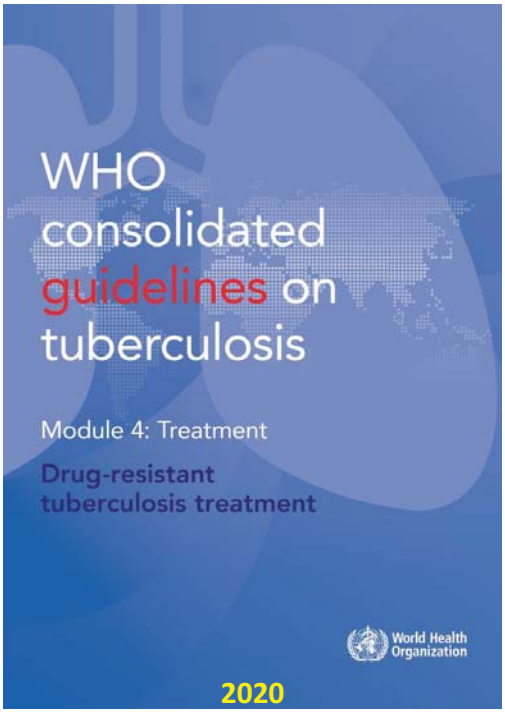


Gout attack----DC Pyrazinamide?



Multidrug-resistant TB---Isolation!

Drug-resistant Tuberculosis



- 1. Regimen for rifampicin-susceptible, isoniazid-resistant tuberculosis
- 2. Shorter all-oral bedaquiline-containing regimen for multidrug- or rifampicin-resistant tuberculosis
- 3. Longer regimens for multidrug- or rifampicin-resistant tuberculosis
- 4. The bedaquiline, pretomanid and linezolid (BPaL) regimen for multidrug-resistant tuberculosis with additional fluoroquinolone resistance
- 5. Monitoring patient response to MDR-TB treatment using culture
- 6. Starting antiretroviral therapy in patients on second-line antituberculosis regimens
- 7. Surgery for patients on MDR-TB treatment
- 8. Care and support for patients with MDR/RR-TB

Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis

Medea Gegia, Nicholas Winters, Andrea Benedetti, Dick van Soolingen, Dick Menzies

Summary

Background The results of some reports have suggested that treatment of isoniazid-resistant tuberculosis with the recommended regimens of first-line drugs might be suboptimal. We updated a previous systematic review of treatment outcomes associated with use of first-line drugs in patients with tuberculosis resistant to isoniazid but not rifampicin.

Methods In this systematic review, we updated the results of a previous review to include randomised trials and cohort studies published in English, French, or Spanish to March 31, 2015, containing results of standardised treatment of patients with bacteriologically confirmed isoniazid-resistant tuberculosis (but not multidrug-resistant tuberculosis—ie, not resistant to rifampicin) in whom failure and relapse were bacteriologically confirmed. Results in patients with drug-sensitive tuberculosis included in the same studies were also analysed. We pooled treatment outcomes with random-effects meta-analysis.

Findings We identified 19 cohort studies and 33 trials with 3744 patients with isoniazid-resistant tuberculosis and 19 012 patients with drug-sensitive disease. The pooled rates of failure or relapse, or both, and acquired drug resistance with all drug regimens were 15% (95% CI 12–18) and 3·6% (2–5), respectively, in patients with isoniazid-resistant tuberculosis and 4% (3–5) and 0·6% (0·3–0·9) in those with drug-sensitive tuberculosis. Of patients with initial isoniazid-resistant tuberculosis with acquired drug resistance, 96% (93–99) had acquired multidrug-resistant disease. Treatment of isoniazid-resistant tuberculosis with the WHO standard regimen for new patients resulted in treatment failure, relapse, and acquired multidrug resistance in 11% (6–17), 10% (5–15) and 8% (3–13), respectively; treatment with the standard WHO regimen for previously treated patients resulted in treatment failure in 6% (2–10), relapse in 5% (2–8), and acquisition of multidrug resistance in 3% (0–6). For patients with drug-sensitive disease treated with the standard retreatment regimen the rates were 1% (0–2), 5% (4–7), and 0·3% (0–0·6).

Interpretation Treatment of isoniazid-resistant tuberculosis with first-line drugs resulted in suboptimal outcomes, supporting the need for better regimens. Standardised empirical treatment of new cases could be contributing substantially to the multidrug-resistant epidemic, particularly in settings where the prevalence of isoniazid resistance is high.

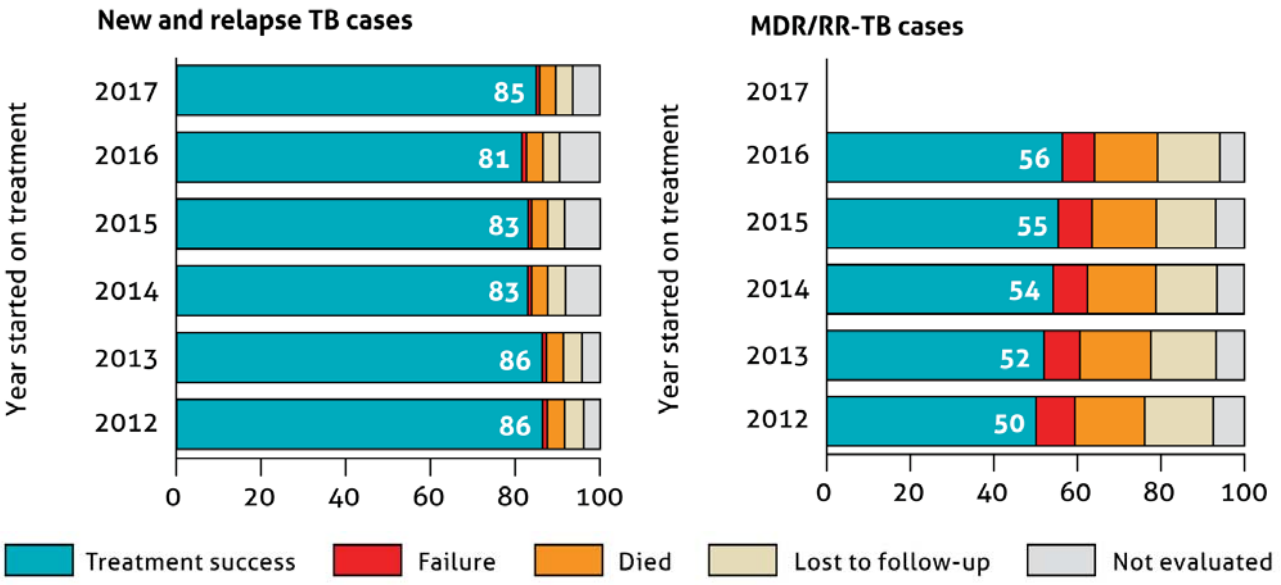
Lancet Infect Dis 2017; 17: 223–34
Published Online November 16, 2016 http://dx.doi.org/10.1016/S1473-3099(16)30407-8
See Comment page 127

Global TB Programme, WHO, Geneva, Switzerland (M Gegia MD); **Montreal Chest Institute, McGill University, Montreal, QC, Canada** (N Winters MSc, A Benedetti PhD, Prof D Menzies MD); **and Mycobacterial Reference Lab, Bilthoven, Netherlands** (Prof D van Soolingen MD)
Correspondence to: Prof Dick Menzies, Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, 2155 Guy Street, Montreal, QC, Canada H3H 2R9
dick.menzies@mcgill.ca

Isoniazid-resistant TB vs. Isoniazid-sensitive TB

- **Higher treatment failure** (11% vs 1%)
- **Relapse** (10% vs 5%)
- **Higher rates of acquired multidrug resistance** (8% vs 0.3%)

Treatment Outcomes for New and Relapse TB Cases , and MDR/RR-TB Cases, 2012–2017 Globally



WHO: 2019 Global TB Report

The NEW ENGLAND JOURNAL of MEDICINE

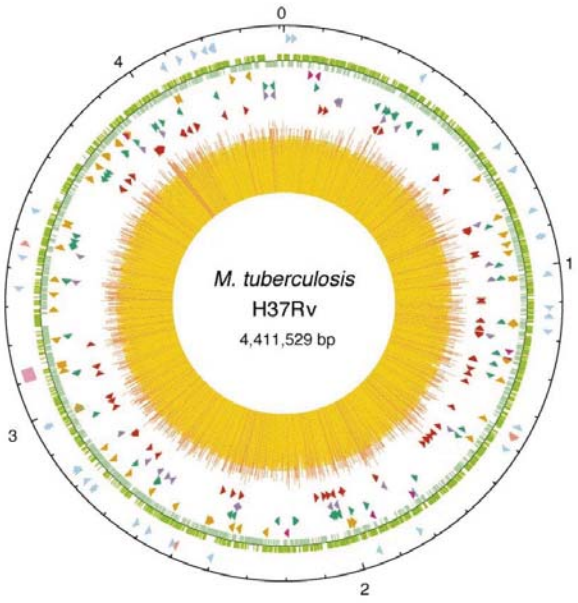
EDITORIALS

Tuberculosis Diagnosis — Time for a Game Change

Peter M. Small, M.D., and Madhukar Pai, M.D., Ph.D.

N Engl J Med 2010;363:1070-1

Drug Resistance-related Genes against Anti-TB Drugs



Drug	Resistance-related genes	Occurrence(%)	Gene function
Rifampicin	*rpoB*	95–99	RNA polymerase subunit B
Isoniazid	*katG*	60–95	Catalase-peroxidase
	inhA	8–43	Promoter region for 2-trans-enoyl-acyl carrier protein reductase
Ethambutol	*embB*	40–68	Arabinosyltransferase
	ubiA	9.5–45.5	5-Phospho-α-d-ribose-1-diphosphate: decaprenyl-phosphate 5-phosphoribosyltransferase
Streptomycin	*rpsL*	70–85	Ribosomal protein S12
	rrs	70–85	16S rRNA
	gidB	N/A	Putative 16S rRNA methyltransferase
Quinolones	*gyrA*	97–98	DNA gyrase subunit A
	gyrB	N/A	DNA gyrase subunit B
Aminoglycosides	*rrs*	86–97	16S rRNA
	eis	N/A	Aminoglycoside acetyltransferase
Pyrazinamide	*pncA*	Up to 99	Amide conversion
	rpsA		S1 ribosomal protein
	panD		Aspartate decarboxylase
	clpC1		Protease

Nature 1998;393:537–44

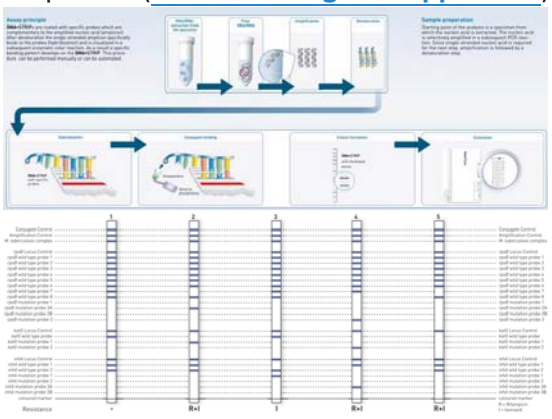
Respirology 2018;23:1098–113

Genotypic Methods

Detection of Rifampicin Resistance

GenoType MTBDRplus Assay

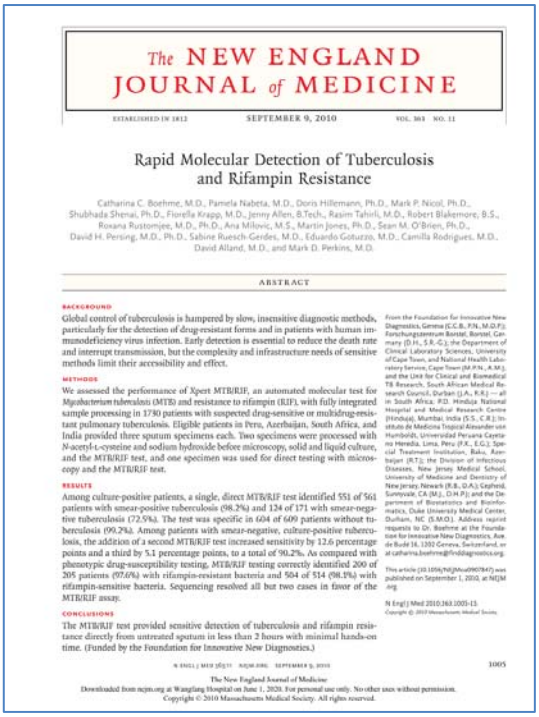
- Technology: PCR and the Strip technology
- Targets: rifampicin (*rpoB* gene) and isoniazid (*katG* gene: high level isoniazid resistance; *inhA* gene: low level resistance)
- Complex to perform and require technical expertise (**Decentralizing: not applicable**)



Xpert MTB/RIF Assay

- Technology: Nested real-time PCR
- Targets: *rpoB* gene - probed with five molecular beacons for mutations within the rifampin-resistance determining region (RRDR)
- Two-hour detection of MTB and rifampin resistance mutations

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance-1



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

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.

METHODS

We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and India provided three sputum specimens each. Two specimens were processed with *N*-acetyl-L-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and one specimen was used for direct testing with microscopy and the MTB/RIF test.

RESULTS

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without tuberculosis (99.2%). Among patients with 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 and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay.

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

From the Foundation for Innovative New Diagnostics, Geneva (C.C.B., P.N., M.D.P.); Forschungszentrum Borstel, Borstel, Germany (D.H., S.R.-G.); the Department of Clinical Laboratory Sciences, University of Cape Town, and National Health Laboratory Service, Cape Town (M.P.N., A.M.), and the Unit for Clinical and Biomedical TB Research, South African Medical Research Council, Durban (J.A., R.R.) — all in South Africa; P.D. Hinduja National Hospital and Medical Research Centre (Hinduja), Mumbai, India (S.S., C.R.); Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru (F.K., E.G.); Special Treatment Institution, Baku, Azerbaijan (R.T.); the Division of Infectious Diseases, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark (R.B., D.A.); Cepheid, Sunnyvale, CA (M.J., D.H.P.); and the Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC (S.M.O.). Address reprint requests to Dr. Boehme at the Foundation for Innovative New Diagnostics, Avenue de Budé 16, 1202 Geneva, Switzerland, or at catharina.boehme@finddiagnostics.org.

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N Engl J Med 2010;363:1005-15.

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1005

The New England Journal of Medicine

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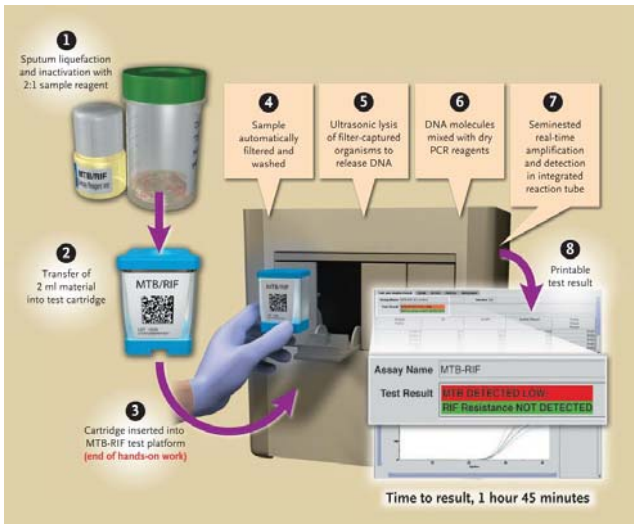


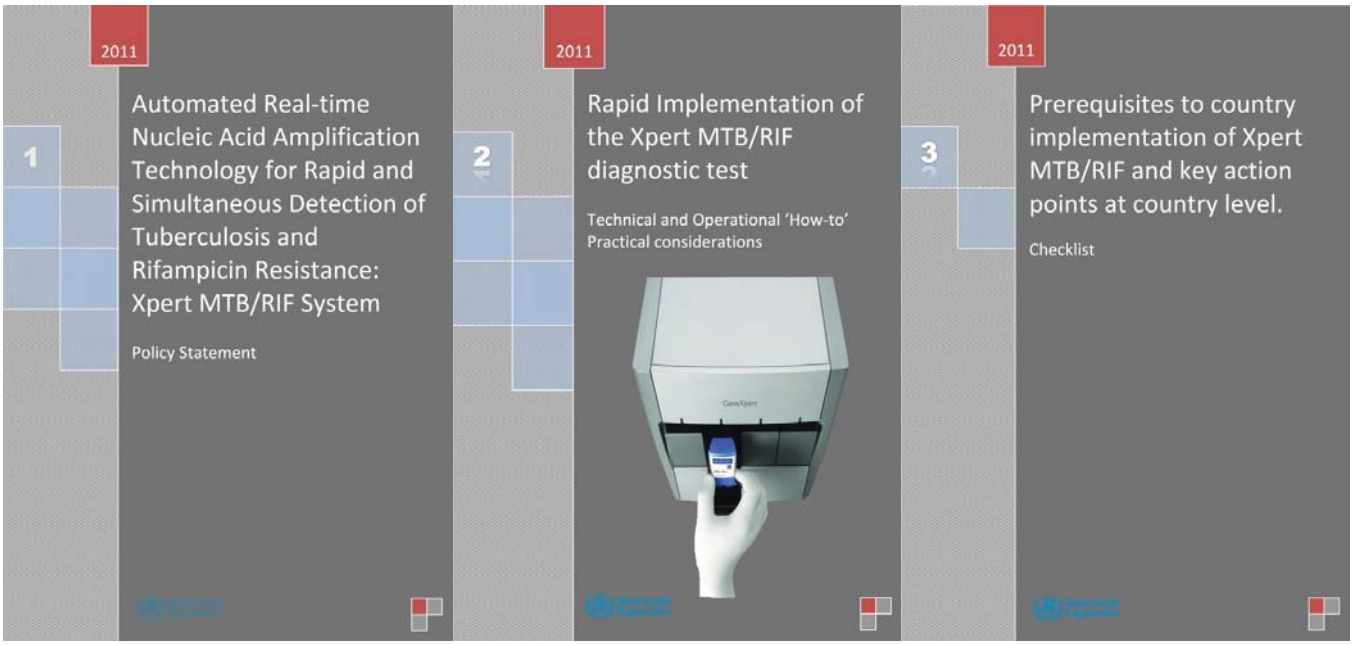
N Engl J Med 2010;363:1005-15

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance-2

Site and Total	Phenotypic Drug-Susceptibility Testing†		Phenotypic Drug-Susceptibility Testing and Discrepant Resolution by Sequencing†	
	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance
Lima, Peru — no./total no. (%)	16/16 (100.0)	190/193 (98.4)	19/19 (100.0)	190/190 (100.0)
Baku, Azerbaijan — no./total no. (%)	47/49 (95.9)	90/94 (95.7)	51/52 (98.1)	90/90 (100.0)
Cape Town, South Africa — no./total no. (%)	15/16 (93.8)	126/126 (100.0)	15/15 (100.0)	126/126 (100.0)
Durban, South Africa — no./total no. (%)	3/3 (100.0)	38/38 (100.0)	3/3 (100.0)	38/38 (100.0)
Mumbai, India — no./total no. (%)	119/121 (98.3)	61/64 (95.3)	121/122 (99.2)	62/62 (100.0)
Total for rifampin resistance				
Correct — no./total no. (%)	200/205 (97.6)	505/515 (98.1)	209/211 (99.1)	506/506 (100.0)
95% CI — %	94.4–99.0	96.5–98.9	96.6–99.7	99.2–100.0
Total for multidrug resistance				
Correct — no. /total no. (%)	195/200 (97.5)		197/199 (99.0)	
95% CI — %	94.3–98.9		96.4–99.7	

N Engl J Med 2010;363:1005-15

WHO Endorsed Xpert MTB/RIF Assay in 2010



Xpert MTB/RIF Assay

疾病管制署/萬芳醫院團隊(越南廣寧)

Feasibility, Diagnostic Accuracy, and Effectiveness of Decentralised Use of the Xpert MTB/RIF Test for Diagnosis of Tuberculosis and Multidrug Resistance: a Multicentre Implementation Study

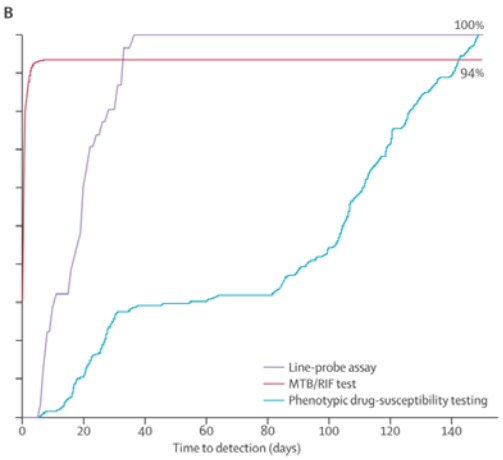
	Sensitivity in rifampicin-resistant cases	Specificity in rifampicin-sensitive cases	Positive predictive value	Negative predictive value
Lima, Peru	22/23 (95·7%, 79·0–99·2)	161/162 (99·4%, 96·6–99·9)	95·6%	99·4%
Baku, Azerbaijan	47/50 (94·0%, 83·8–97·9)	160/161 (99·4%, 96·6–99·9)	98·0%	98·1%
Cape Town, South Africa	9/10 (90·0%, 59·6–98·2)	175/178 (98·3%, 95·2–99·4)	77·1%	99·3%
Kampala, Uganda	1/3 (33·3%, 6·1–79·2)	112/113 (99·1%, 95·2–99·8)	54·2%	97·9%
Vellore, India	8/10 (80·0%, 49·0–94·3)	91/93 (97·8%, 92·5–99·4)	80·5%	97·7%
Manila, Philippines	149/154 (96·8%, 92·6–98·6)	97/103 (94·2%, 87·9–97·3)	95·5%	95·9%
Total	236/250 (94·4%, 90·8–96·6)	796/810 (98·3%, 97·1–99·0)	93·2%	98·6%

Data are number of positive results/number tested (%, 95% CI). The reference standard was phenotypic susceptibility testing in Peru, Azerbaijan, Uganda, and the Philippines and genotypic testing by line-probe assay followed by phenotypic drug-susceptibility testing for resistant cases in South Africa and Uganda. MTB=Mycobacterium tuberculosis. RIF=rifampicin.

Table 6: MTB/RIF test sensitivity and specificity for detection of rifampicin resistance after change to software cutoff

- MTB/RIF test for rifampicin resistance
  - Sensitivity: 94.4% (236 of 250)
  - Specificity: 98.3% (796 of 810)

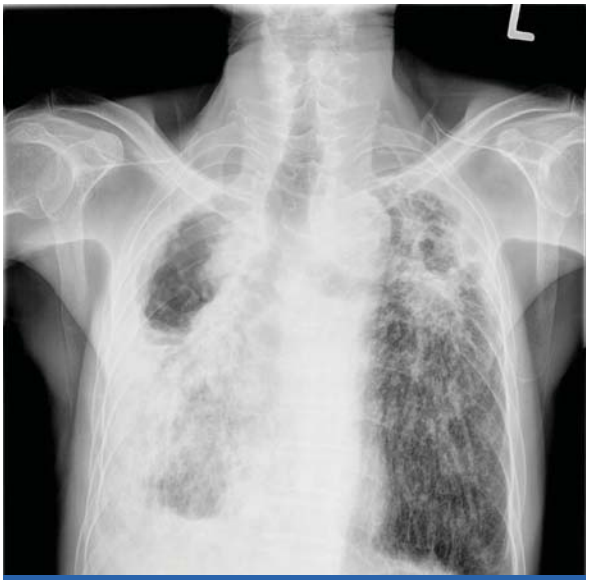
- **Decentralised MTB/RIF test implementation is feasible and could lead to an improvement in tuberculosis care and control**



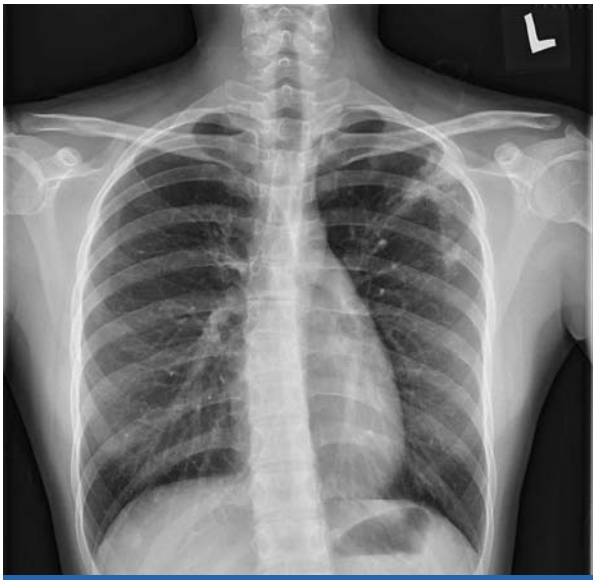
- Median time to detection of rifampicin resistance
  - MTB/RIF test: 1 day (0–1)
  - Phenotypic DST: 106 days (30–124)

Lancet 2011;377:1495–505

Clinical Implications: Rifampin-susceptible

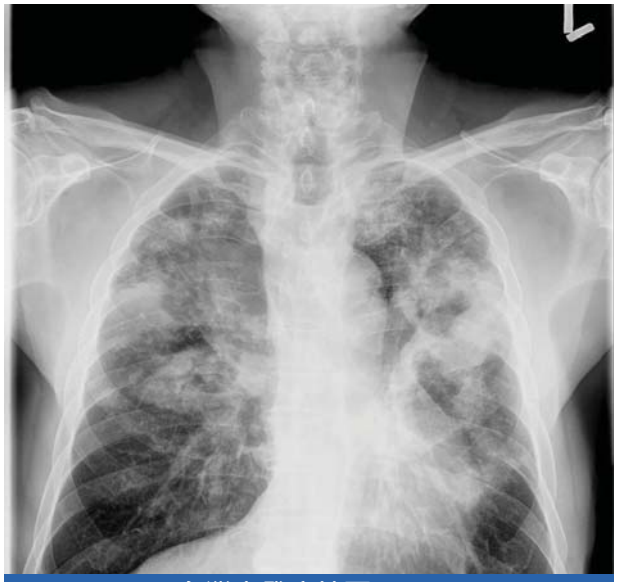


79 y/o, male (Pneumoconiosis)
Sputum AFS (++++)
Xpert: MTB(+), RMP-resistant (-)
Phenotypic DST: all susceptible (1 month)

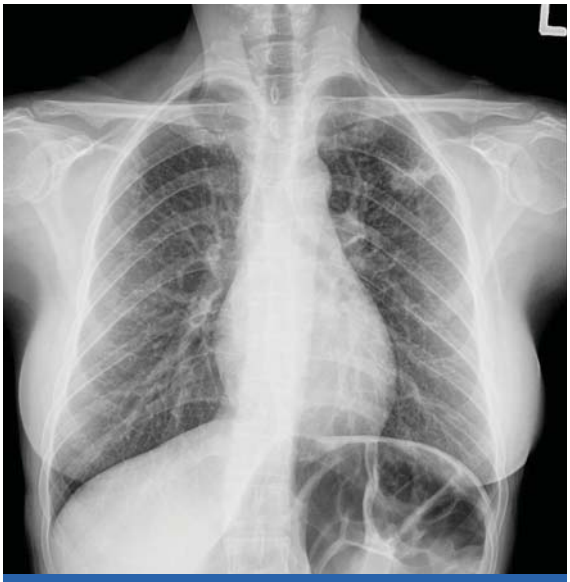


19 y/o, female (外籍學生)
Sputum AFS(-)
Xpert: MTB(+), RMP-resistant (-)
Phenotypic DST: all susceptible (45 days)

Clinical Implications: Rifampin-resistant

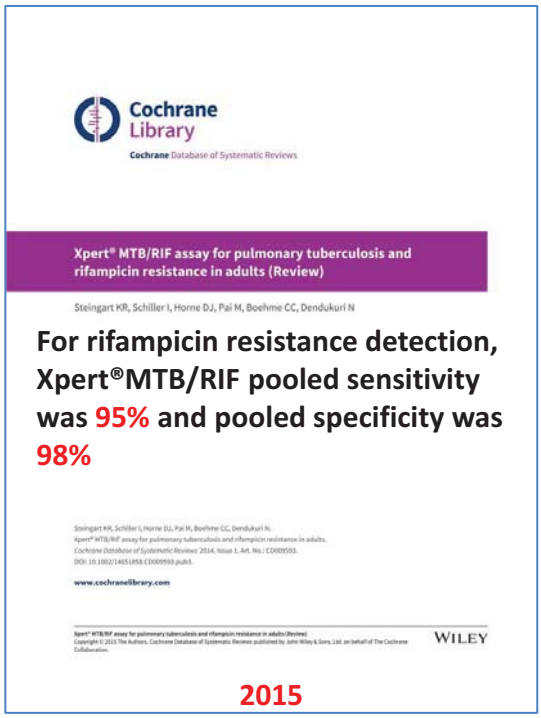


70 y/o, male (台灣高發病地區)
Sputum AFS(++++)
Xpert: MTB(+), RMP-resistant (+)
Phenotypic DST: rifampin-resistant (35 days)



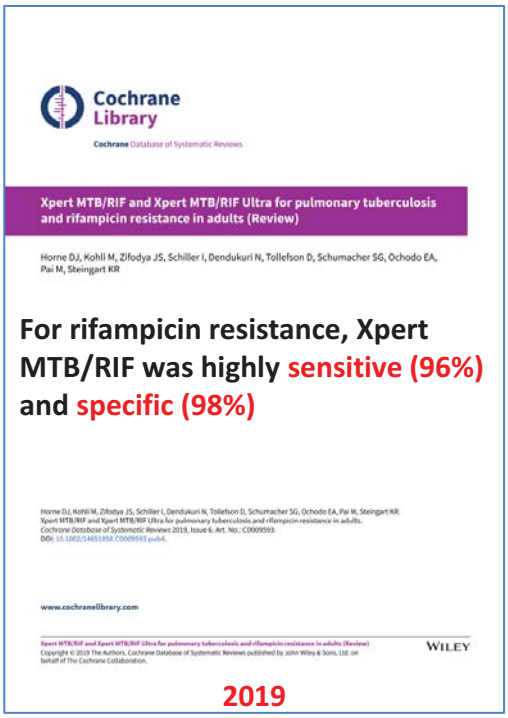
31 y/o, female (Rifampin-resistant TB contact)
Sputum AFS(-)
Xpert: MTB(+), RMP-resistant (+)
Phenotypic DST: rifampin-resistant (41 days)

Cochrane Review



For rifampicin resistance detection, Xpert®MTB/RIF pooled sensitivity was 95% and pooled specificity was 98%

2015



For rifampicin resistance, Xpert MTB/RIF was highly sensitive (96%) and specific (98%)

2019

GenoType MTBDR Assays for the Diagnosis of Multidrug-resistant Tuberculosis: a Meta-analysis

ABSTRACT: The global extensively drug-resistant tuberculosis (TB) response plan calls for implementation of rapid tests to screen patients at risk of drug-resistant TB. Currently, two line probe assays exist, the INNO-LiPA® Rif.TB assay (Innogenetics, Ghent, Belgium) and the GenoType® MTBDR assay (Hain LifeScience GmbH, Nehren, Germany). While LiPA studies have been reviewed, the accuracy of GenoType assays has not been systematically reviewed.

The present authors carried out a systematic review and used meta-analysis methods appropriate for diagnostic accuracy. After the literature searches, 14 comparisons for rifampicin and 15 comparisons for isoniazid were identified in 10 articles that used GenoType MTBDR assays. Accuracy results were summarised in forest plots and pooled using bivariate random-effects regression.

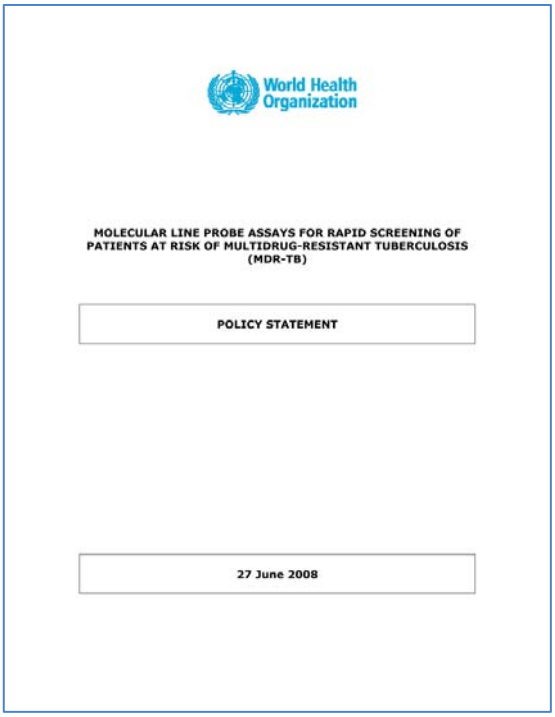
The pooled sensitivity (98.1%, 95% confidence interval (CI) 95.9–99.1) and specificity (98.7%, 95% CI 97.3–99.4) estimates for rifampicin resistance were very high and consistent across all subgroups, assay versions and specimen types. The accuracy for isoniazid was variable, with lower sensitivity (84.3%, 95% CI 76.6–89.8) and more inconsistent than specificity (99.5%, 95% CI 97.5–99.9).

GenoType MDTBR assays demonstrate excellent accuracy for rifampicin resistance, even when used on clinical specimens. While specificity is excellent for isoniazid, sensitivity estimates were modest and variable. Together with data from demonstration projects, the meta-analysis provides evidence for policy making and clinical practice.

KEYWORDS: Diagnostic accuracy, drug resistance, line probe assay, multidrug-resistant tuberculosis, sensitivity and specificity, tuberculosis

Eur Respir J 2008;32:1165–74

WHO Recommended the Use of Line probe Assay



World Health Organization

MOLECULAR LINE PROBE ASSAYS FOR RAPID SCREENING OF PATIENTS AT RISK OF MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB)

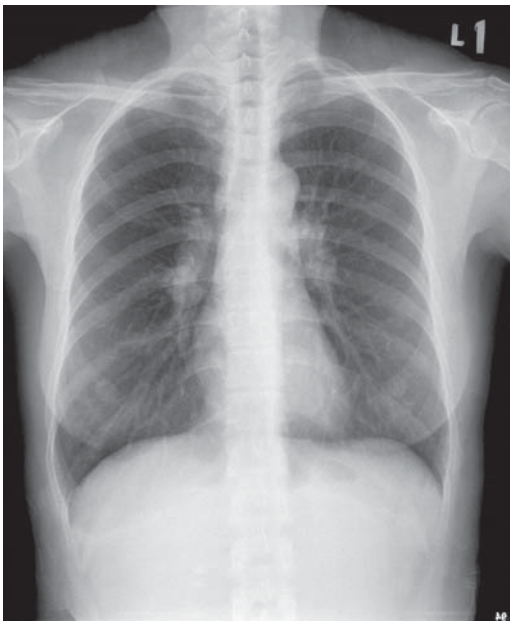
POLICY STATEMENT

27 June 2008

- A specific probe to identify *M. tuberculosis* complex
- Multiple probes to detect the most common mutations in *rpoB* (codons 531,526 and 516)
- Multiple overlapping wild-type (susceptible) probes covering the RRDR region of *rpoB*
- Multiple probes to detect both high-level (*katG* mutations) and low level isoniazid resistance (*inhA* mutations)
- Strip technology, with appropriate assay procedure controls, allowing visual detection of results

Close Contacts of Patients with MDR-TB

MDR-TB



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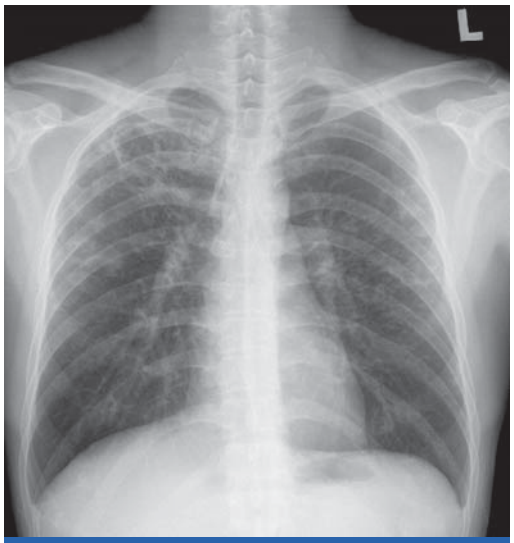


MDR-TB:46天

Performance Assessment of the GenoType MTBDR*plus* Test and DNA Sequencing in Detection of Multidrug-Resistant *Mycobacterium tuberculosis*

TABLE 1. Genotype MTBDR*plus* assay and sequencing results for 242 MDR *Mycobacterium tuberculosis* isolates

Result by the GenoType MTBDR*plus* test	Result by sequencing	No. (%) with the following result by conventional DST:		
		RIF resistant	INH resistant	MDR
Resistant	Resistant	229 (94.6)	198 (81.8)	188 (77.7)
Resistant	Susceptible	2 (0.8)	0 (0)	2 (0.8)
Susceptible	Resistant	8 (3.3)	28 (11.6)	33 (13.6)
Susceptible	Susceptible	3 (1.2)	16 (6.6)	19 (7.9)

- The sensitivity and specificity for RIF-resistant : 95.5% and 100%
- The sensitivity and specificity for INH-resistant: 81.8% and 100%

J Clin Microbiol 2009;47:2520–4

抗藥性結核病檢驗

疾管署參考實驗室

2008年5月始推動MDR-TB需送本署複判

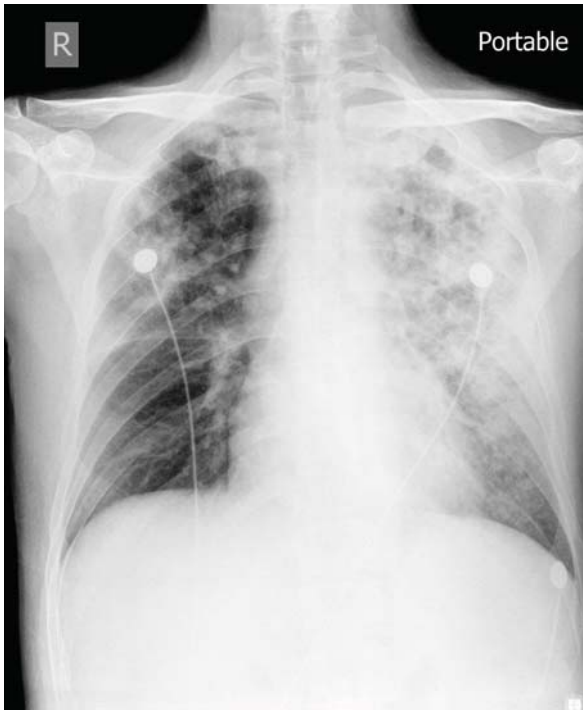
- 複判條件：對INH 且RMP 同時抗藥
- 檢驗方式：
  - 分子鑑定
  - 傳統藥敏 (若分子方法無法判定，再進行傳統藥敏鑑定)
- 經判定為MDR-TB之個案
  - 提供二線藥敏檢測結果

2015年8月1日提供MDR及RMP抗藥個案之二線藥物分子快速檢測

- 抹片陽性痰檢體
  - 檢測FQ、KM、AM及CAP
- 陽性培養菌株
  - 檢測FQ、KM、AM、CAP及PZA

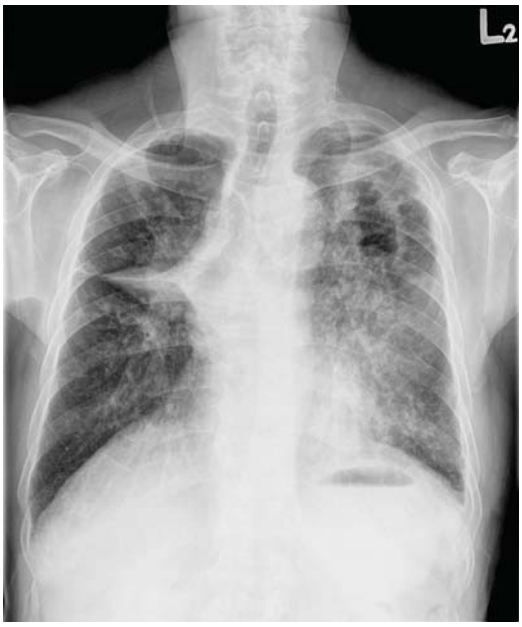
Taiwan CDC 2019.5.8

50 y/o, Male



- Worked in Vietnam
- Sputum AFS (++++)
- GeneXpert test
  - RMP: resistant
- GenoTypeMTBDR*plus* Test
  - INH: resistant
  - RMP: resistant
- Phenotypic DST (40 days later)
  - HERS: resistant

82 y/o, Male



- Underlying disease
  - Pneumoconiosis
- TB History: 15 yrs ago
- GeneXpert test
  - RMP: resistant
- GenoTypeMTBDR*plus* Test
  - INH: susceptible
  - RMP: resistant
- Phenotypic DST
  - INH: resistant
  - RMP: resistant

Line Probe Assays: A Meta-Analysis

GenoType MTBDR*plus* Assay for Rapid Detection of Multidrug Resistance in *Mycobacterium tuberculosis*: A Meta-Analysis

Yuanyuan Bai, Yueling Wang, Chunhong Shao, Yingying Hao, Yan Jin*

Department of Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, PR China

The pooled sensitivity
- **Isoniazid: 0.91 (0.88–0.94)**
- **Rifampicin: 0.96 (0.95–0.97)**

The pooled specificity
- **Isoniazid: 0.99 (0.98–0.99)**
- **Rifampicin: 0.98 (0.97–0.99)**

Methods

We searched PubMed, EMBASE, and Cochrane Library databases to identify studies according to predetermined criteria. A total of 40 studies were included in the meta-analysis. QUADAS-2 was used to assess the quality of included studies with RevMan 5.2. STATA 13.0 software was used to analyze the tests for sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and area under the summary receiver operating characteristic curves. Heterogeneity in accuracy measures was tested with Spearman correlation coefficient and Chi-square.

Results

Patient selection bias was observed in most studies. The pooled sensitivity (95% confidence intervals were 0.91 (0.88–0.94) for isoniazid, 0.96 (0.95–0.97) for rifampicin, and 0.91(0.86–0.94) for multidrug-resistance. The pooled specificity (95% CI) was 0.99 (0.98–0.99) for isoniazid, 0.98 (0.97–0.99) for rifampicin and 0.99 (0.99–1.00) for multidrug-resistance, respectively. The area under the summary receiver operating characteristic curves ranged from 0.99 to 1.00.

Conclusion

This meta-analysis determined that GenoType MTBDR*plus* had good accuracy for rapid detection of drug resistance to isoniazid and/or rifampicin of *M. tuberculosis*. MTBDR*plus*

PLoS ONE 2016;11: e0150321

Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis

RIF resistance: pooled sensitivity and specificity
- **96.7% (95.6–97.5%) and 98.8% (98.2–99.2%)**

INH resistance: pooled sensitivity and specificity
- **90.2% (88.2–91.9%) and 99.2% (98.7–99.5%)**

@ERSpublications
Line probe assays have high accuracy for detection of RIF resistance and INH resistance
http://ow.ly/USX5305tqFV

Cite this article as: Nathavitharana RR, Cudahy PGT, Schumacher SG, *et al.* Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2017; 49: 1601075 [https://doi.org/10.1183/13993003.01075-2016].

ABSTRACT Only 25% of multidrug-resistant tuberculosis (MDR-TB) cases are currently diagnosed. Line probe assays (LPAs) enable rapid drug-susceptibility testing for rifampicin (RIF) and isoniazid (INH) resistance and *Mycobacterium tuberculosis* detection. Genotype MTBDRplusV1 was WHO-endorsed in 2008 but newer LPAs have since been developed.

This systematic review evaluated three LPAs: Hain Genotype MTBDRplusV1, MTBDRplusV2 and Nipro NTM+MDRTB. Study quality was assessed with QUADAS-2. Bivariate random-effects meta-analyses were performed for direct and indirect testing. Results for RIF and INH resistance were compared to phenotypic and composite (incorporating sequencing) reference standards. *M. tuberculosis* detection results were compared to culture.

74 unique studies were included. For RIF resistance (21 225 samples), pooled sensitivity and specificity (with 95% confidence intervals) were 96.7% (95.6–97.5%) and 98.8% (98.2–99.2%). For INH resistance (20 954 samples), pooled sensitivity and specificity were 90.2% (88.2–91.9%) and 99.2% (98.7–99.5%). Results were similar for direct and indirect testing and across LPAs. Using a composite reference standard, specificity increased marginally. For *M. tuberculosis* detection (3451 samples), pooled sensitivity was 94% (89.4–99.4%) for smear-positive specimens and 44% (20.2–71.7%) for smear-negative specimens.

In patients with pulmonary TB, LPAs have high sensitivity and specificity for RIF resistance and high specificity and good sensitivity for INH resistance. This meta-analysis provides evidence for policy and practice.

Eur Respir J 2017; 49: 1601075

Decreased Time to Treatment Initiation for Multidrug-Resistant Tuberculosis Patients after Use of Xpert MTB/RIF Test, Latvia

Few studies have examined whether the **Xpert MTB/RIF test** improves time to treatment initiation for persons with multidrug-resistant tuberculosis (MDR TB). We determined the impact of this test in **Latvia**, where it was introduced in 2010. After descriptive analyses of pulmonary MDR TB patients in Latvia during 2009–2012, time to treatment initiation was calculated, and univariate and multivariable accelerated failure time models were constructed. Univariate results showed strong evidence of an association between having rifampin-resistant TB detected by Xpert MTB/RIF and reduced time to treatment initiation versus the test not being used. A multivariable model stratifying by previous TB showed similar results. Our finding that in Latvia, **time to treatment initiation was decreased for MDR TB cases** that were rifampin-resistant TB by XpertMTB/RIF has implications for the use of this test in other settings with a high burden of MDR TB in which rifampin resistance is highly predictive of MDR TB.

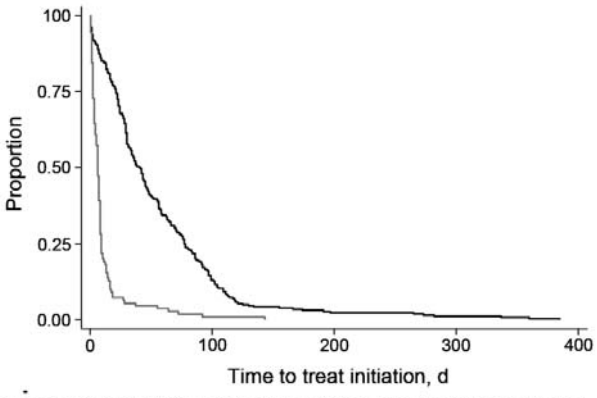


Figure 5. Kaplan-Meier plot of time to treatment initiation by use and results of Xpert MTB/RIF in patients with multidrug-resistant tuberculosis (MDR TB), Latvia, 2009–2012. Shown is time to MDR TB treatment initiation (days) for patients who were not tested by Xpert MTB/RIF (dark gray line) and those who had rifampin-resistant TB by Xpert MTB/RIF (light gray line). MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

Emerg Infect Dis 2016;22:482-90

Impact of GeneXpert MTB/RIF® on Treatment Initiation and Outcomes of RIF-resistant and RIF-susceptible TB Patients in Vladimir TB Dispensary, Russia

- **Background**: The main advantage of GeneXpert MTB/RIF® (Xpert) molecular diagnostic technology is the rapid detection of *M.tuberculosis* DNA and mutations associated with rifampicin (RIF) resistance for timely initiation of appropriate treatment and, consequently, preventing further transmission of the disease. We assessed time to treatment initiation and treatment outcomes of RIF-resistant and RIF-susceptible TB patients diagnosed and treated in Vladimir TB Dispensary, Russia in 2012, before and after implementation of GeneXpert MTB/RIF® diagnostic technology.
- **Methods**: All adult patients suspected of having TB during February–December 2012 underwent a clinical examination, chest x-ray, microscopy, culture, and phenotypic drug susceptibility testing (DST). Starting August 2012 Xpert diagnostic technology became available in the facility. We used logistic regression to compare treatment outcomes in pre-Xpert and post-Xpert periods. Kaplan-Meier curves and log-rank test were used to compare the time to treatment initiation between the groups.
- **Results**: Of 402 patients screened for TB during February–December 2012, 338 were diagnosed with TB (280 RIF-susceptible, 58 RIF-resistant). **RIF-resistant patients in the post-Xpert group started treatment with second-line drugs (SLD) earlier than those in pre-Xpert group (median 11 vs. 37 days, Log-rank p = 0.02).** The hazard ratio for time to SLD treatment initiation was significantly higher in post-Xpert group (HR:2.06; 95%CI:1.09,3.89) compared to pre-Xpert group. Among the 53/58 RIF-resistant TB patients with available **treatment outcome**, 28 (53%) had successful outcomes (cured/completed treatment) including 15/26 (58%) in post-Xpert group versus 13/27 (48%) in pre-Xpert group. The observed difference, however, was **not statistically significant** (OR:0.69; 95%CI:0.23,2.06). Among RIF-susceptible TB cases time to treatment initiation was not significantly different between the groups (2 vs. 3 days, Log-rank p = 0.73). Of 252/280 RIF-susceptible TB cases with treatment outcome, 199 (79%) cases had successful outcome including 94/114 (82%) in post-Xpert group versus 105/138 (76%) in pre-Xpert group (OR:0.68; 95%CI:0.36,1.26).
- **Conclusion**: We observed that **availability of Xpert for initial diagnosis significantly reduced the time to SLD treatment** for **RIF-resistant patients** in the Vladimir TB Dispensary. Although **implementation of rapid diagnostics did not improve treatment outcomes**, early diagnosis of MDR-TB is important for selection of appropriate treatment regimen and prevention of transmission of drug-resistant strains of TB.

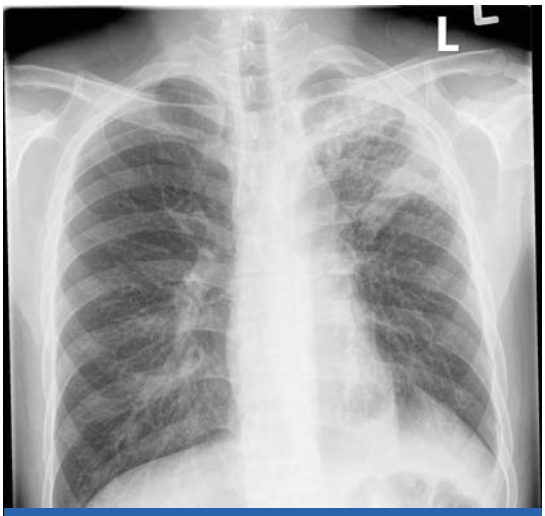
BMC Infect Dis 2020;20:543

Rifampin Drug Resistance Tests for Tuberculosis

Challenging the Gold Standard

J Clin Microbiol 2013;51:2633–40

治療前



- 1st TB history: 2016/7~2017/1 with HRZ(E)
  Phenotypic DST: all susceptible
- 2nd TB history: 2017/9, relapse

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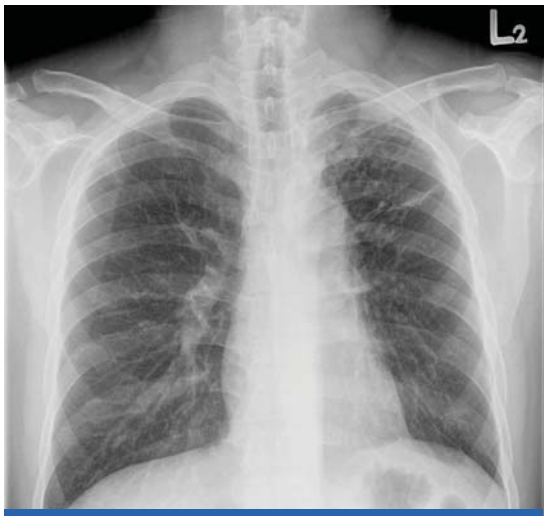

- Phenotypic DST: all susceptible
- Genotypic DST: RMP-R
- Genetic locus: *rpoB* L511P

- Laboratory errors
- Silent mutations
- Mutations outside the 81 base-pair RMP resistance-determining region
- Disputed mutations conferring increased minimal inhibitory concentrations below the critical concentration in some phenotypic drug susceptibility tests
- Heteroresistance

INT J TUBERC LUNG DIS 21(7):721–726

http://dx.doi.org/10.5588/ijtld.17.0140

How should discordance between molecular and growth-based assays for rifampicin resistance be investigated?

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SUMMARY

Molecular tests to detect the presence of *Mycobacterium tuberculosis* and genetic polymorphisms in the *rpo*B gene conferring resistance to rifampicin (RMP) have become integral parts of tuberculosis diagnostics worldwide. These assays are often performed sequentially or in parallel to phenotypic drug susceptibility testing. Discordances between molecular and phenotypic tests invariably occur. Root causes range from pre-, post- and analytic errors to co-existence of non-tuberculous mycobacteria, silent mutations, mutations outside the 81 base-pair RMP resistance-determining region, non-canonical mutations conferring increased minimal inhibitory concentrations below the critical concentration in some phenotypic drug susceptibility tests, and heteroresistance. Resolving discordant results is challenging. This guide aims to assist both clinicians and microbiologists in interpreting discordances by providing a structured approach to manage further investigations. Case scenarios are discussed, including the likelihood of occurrence.

KEY WORDS: RMP; molecular diagnostics; Xpert® MTB/RIF; phenotypic DST; tuberculosis

INT J TUBERC LUNG DIS 16(2):206–208

http://dx.doi.org/10.5588/ijtld.11.0395

TECHNICAL NOTE

False-positive rifampicin resistance on Xpert® MTB/RIF: case report and clinical implications

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SUMMARY

The World Health Organization had endorsed Xpert® MTB/RIF (Xpert) as the initial diagnostic for multidrug-resistant tuberculosis (TB) or TB suspects co-infected with the human immunodeficiency virus. We investigated an unexpected case of rifampicin (RMP) resistance on Xpert using repeat Xpert, smear microscopy, MTBDR*plus* assay, culture, drug susceptibility testing, spoligotyping and *rpo*B gene sequencing. A false-positive result was most likely, given the wild type *rpo*B gene sequence and exclusion of both mixed infection and mixture of drug-susceptible and drug-resistant populations. When decentralising Xpert, test performance characteristics need to be understood by health care workers and methods of confirmation of RMP resistance need to be accessible.

KEY WORDS: tuberculosis; MDR-TB; assay performance; false-positive rifampicin resistance

Isolation of *Mycobacterium tuberculosis* Strains with a Silent Mutation

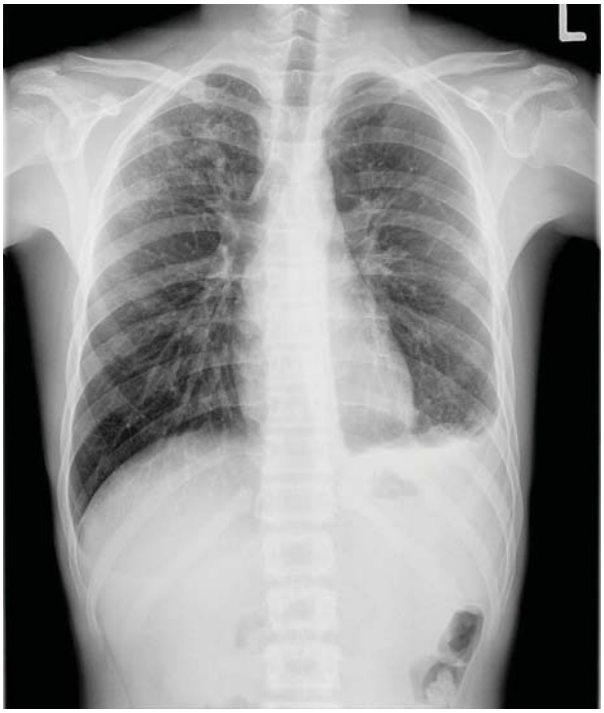
- **Isolation of *Mycobacterium tuberculosis* Strains with a Silent Mutation in *rpoB* Leading to Potential Misassignment of Resistance Category**
  - **Our study provides an alert regarding the transmission of rifampin-susceptible strains of *Mycobacterium tuberculosis* with a silent substitution in codon 514 of *rpoB*. Among 1,450 cases, we identified 12 isolates sharing this mutation and related restriction fragment length polymorphism (RFLP) types. The mutation impaired hybridization with the wild-type probes in three independent commercial assays, which could lead to misassignment of resistance.**

J Clin Microbiol 2011:49:2688-90

- **Silent Mutation in *rpoB* Detected from Clinical Samples with Rifampin-Susceptible *Mycobacterium tuberculosis***
  - **These two isolates (1.4%) had a silent TTC (Phe)-to-TTT (Phe) shift (the same replacement found in the isolates included in the study by Alonso et al).**

J Clin Microbiol 2011;49:3722

Drug-resistant TB: Silent Mutation



- Male, 14 y/o
- Family Hx of TB: uncle (treated as MDR-TB)
- Xpert: RMP-R
- Genotype: INH-S, RMP-R
- Phenotypic DST: all susceptible
- Genetic locus: silent mutation
  - *rpoB* P520P, CCG/CCA

79 y/o, Male

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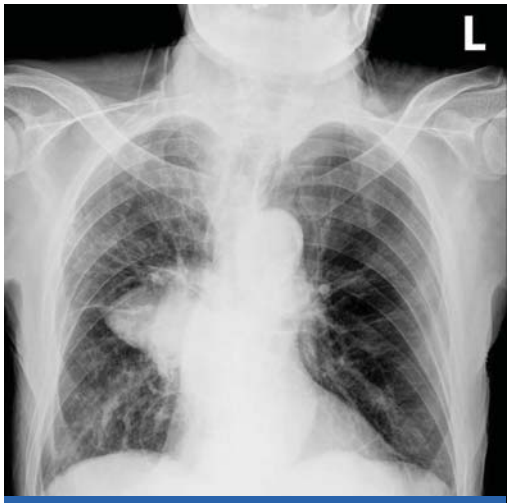

Phenotypic DST: RMP-R, INH-R
Genotypic DST: RMP-S, INH-R

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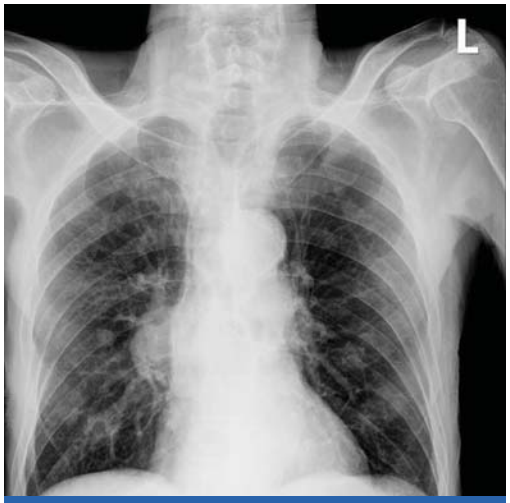

- ***rpoB* gene: no RRDR (RMP resistance determining region) mutation**
- ***katG* S315T mutation**

Clinical Failures Associated with *rpoB* Mutations in Phenotypically Occult Multidrug-resistant *Mycobacterium Tuberculosis*

- **SETTING**: Recently, *Mycobacterium tuberculosis* isolates have been described that test phenotypically susceptible to rifampicin (RMP) yet harbour genotypic *rpo*B mutations.
- **OBJECTIVE**: 1) To investigate the impact of such mutations on clinical outcomes among RMP-susceptible isolates, and 2) to determine the prevalence of *rpo*B mutations among isoniazid (INH) monoresistant isolates at our laboratory and to describe the association between the presence of these mutations and clinical outcomes.
- **METHODS**: *M. tuberculosis* isolates were screened for mutations in the *rpo*B gene using the Cepheid Gene-XpertR MTB/RIF assay. Clinical correlation was made by reviewing patient case notes.
- **RESULTS**: Isolates from 94 patients were found to have **INH-resistant, RMP-susceptible profiles**. Clinical information was available for **52 patients**, including **three whose isolates had *rpo*B mutations**. **All three of these patients had treatment failures**, compared to **two of 49 patients** whose isolates did not have *rpo*B mutations ($P = 0.0005$).
- **DISCUSSION**: We demonstrate a **significant association** between the presence of *rpo*B gene mutations that are not detected at the current RMP critical concentration and treatment failure. We suggest that a review of the current RMP critical concentration is warranted to ensure that RMP is not used inappropriately for the treatment of phenotypically occult multidrug-resistant tuberculosis.

Table Clinical, microbiological and genotypic characteristics of patients with *rpo*B gene mutations and high-level isoniazid resistance

	Clinical characteristics					Microbiological and genotypic characteristics					
						Rifampicin DST			Genotypic mutations		
Patient	Country of birth	Initial site of disease	Previous treatment for TB	Treatment regimen	Treatment failure?	1.0 µg/ml	0.5 µg/ml	0.25 µg/ml	0.125 µg/ml	*rpoB* mutation(s)	*katG* mutation*	Phenotypic resistance to other first-line agents
1	Korea	Pulmonary	Unknown	Unknown	Unknown	Susceptible	Susceptible	Resistant	Resistant	Leu511Pro Met515Ile	Ser315Thr	SZ
2	China	Extra-pulmonary (pleural)	No	2 months RHEZ/ 11 days RE Pth Mfx/ 10 months RE Mfx Cs	Recurrent culture-positive pleural effusion 3 months after commencing treatment	Susceptible	Susceptible	Resistant	Resistant	His526Asn Ala532Val	Ser315Thr	SZ
3	Cambodia	Extrapulmonary (cervical lymph node)	No	2 months RHEZ/ 10 months RE	Progression to culture-positive pulmonary disease 1 year after commencing treatment	Susceptible	Susceptible	Susceptible	Resistant	Asp516Tyr	Ser315Thr	S
4	China	Pulmonary	No	9 days RHEZ/4 months REZ Mfx/4 months RE	Persistent sputum culture positivity 6 months after commencing treatment	Susceptible	Susceptible	Resistant	Resistant	His526Leu	Ser315Thr	S

*Detected by the Genotype MTBDRplus assay.
TB = tuberculosis; DST = drug susceptibility testing; S = streptomycin; Z = pyrazinamide; R = rifampicin; H = isoniazid; E = ethambutol; Pth = prothionamide; Mfx = moxifloxacin; Cs = cycloserine.

Int J Tuber Lung Dis 2011;16:216-20

55 y/o, Female

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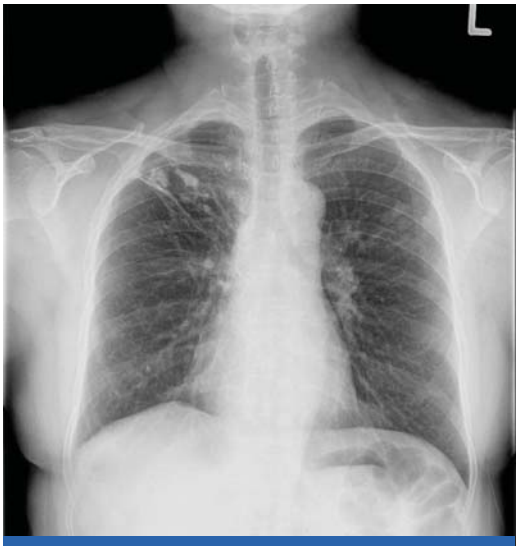

Phenotypic DST: RMP-S
Genotypic DST: RMP-R

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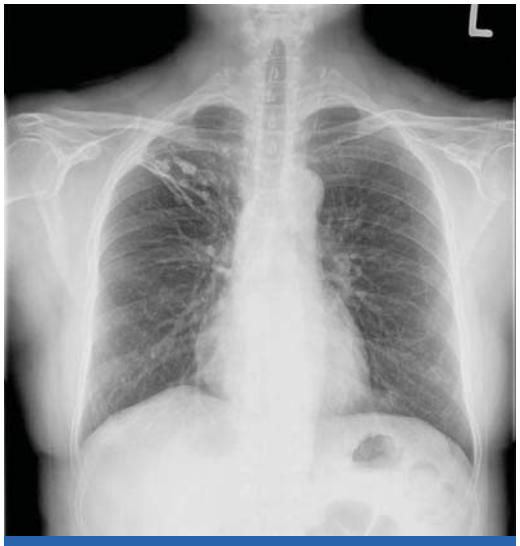


Disputed mutation
***rpoB* L511P**

How Well Do Routine Molecular Diagnostics Detect Rifampin Heteroresistance in *Mycobacterium tuberculosis*?

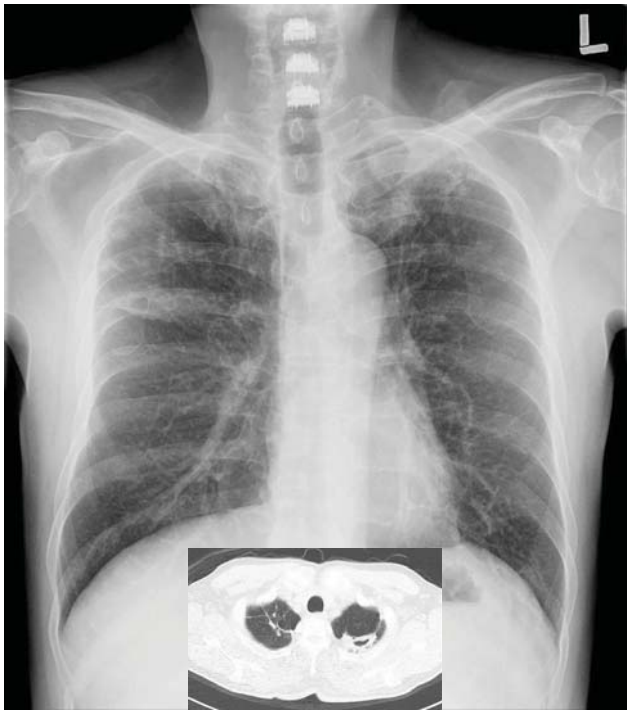
ABSTRACT Rifampin heteroresistance—where rifampin-resistant and -susceptible tuberculosis (TB) bacilli coexist—may result in failed standard TB treatment and potential spread of rifampin-resistant strains. The detection of rifampin heteroresistance in routine rapid diagnostic tests (RDTs) allows for patients to receive prompt and effective multidrug-resistant-TB treatment and may improve rifampin-resistant TB control. The limit of detection (LOD) of rifampin heteroresistance for phenotypic drug susceptibility testing by the proportion method is 1% and, yet, is insufficiently documented for RDTs. We, therefore, aimed to determine, for the four RDTs (XpertMTB/RIF, XpertMTB/RIF Ultra, GenoTypeMTBDR*plus*v2.0, and GenoscholarNTM+MDRTBII), the LOD per probe and mutation, validated by CFU counting and targeted deep sequencing (Deeplex-MycTB). We selected one rifampin-susceptible and four rifampin-resistant strains, with mutations D435V, H445D, H445Y, and S450L, respectively, mixed them in various proportions in triplicate, tested them with each RDT, and determined the LODs per mutation type. Deeplex-MycTB revealed concordant proportions of the minority resistant variants in the mixtures. The Deeplex-MycTB-validated LODs ranged from 20% to 80% for XpertMTB/RIF, 20% to 70% for Xpert Ultra, 5% to 10% for GenoTypeMTBDRplusv2.0, and 1% to 10% for GenoscholarNTM+MDRTBII for the different mutations. Deeplex-MycTB, GenoTypeMTBDR*plus*v2.0, and GenoscholarNTM+MDRTBII provide explicit information on rifampin heteroresistance for the most frequently detected mutations. Classic Xpert and Ultra report rifampin heteroresistance as rifampin resistance, while Ultra may denote rifampin heteroresistance through "mixed patterns" of wild-type and mutant melt probe, melt peak temperatures. Overall, our findings inform end users that the threshold for reporting resistance in the case of rifampin heteroresistance is the highest for Classic Xpert and Ultra to resolve phenotypic and genotypic discordant rifampin-resistant TB results.

KEYWORDS Deeplex-MycTB, GenoscholarNTM+MDRTBII, GenoTypeMTBDR*plus*v2.0, *Mycobacterium tuberculosis*, XpertMTB/RIF, XpertMTB/RIF Ultra, limit of detection, rifampin heteroresistance, rifampin-resistant tuberculosis

- Rifampin **heteroresistance**-where rifampin-resistant and -susceptible tuberculosis (TB) bacilli coexist-may result in failed standard TB treatment and potential spread of rifampin-resistant strains.

J Clin Microbiol 2019;57: e00717-19

57 y/o, Male



- Xpert: RMP-R
- GenoType : INH-R, RMP-R
- Phenotypic DST:
  - High-level INH-S, low-level INH-R
  - RMP-S/RMP-R
  - Prothionamide-R
- Genetic loci
  - *rpoB* L511P: disputed mutation
  - *rpoB* S512T: hot-spot mutation
  - *inhA* C-15T: inhA promoter mutations confer low-level INH resistance, but significantly affect ETH susceptibility

Interpretation of Discordant Rifampicin Susceptibility Test Results Obtained Using GeneXpert vs Phenotypic Drug Susceptibility Testing

- ***Background***. The **3-month difference** in turnaround time between Xpert and conventional phenotypic drug susceptibility testing (pDST) causes patient treatment challenges when pDST rifampin (RIF) susceptibility results and earlier Xpert results disagree, resulting in unnecessary tuberculosis (TB) patient exposure to toxic second-line drugs. Here, the prevalence of **discordant RIF susceptibility test** results, specifically Xpert (resistant) vs pDST (susceptible) results, was determined.
- ***Methods***. Tuberculosis patients enrolled between January 2015 and June 2018 at Beijing Chest Hospital who consecutively tested positive for RIF resistance using Xpert then negative using pDST were studied. DNA sequences and minimal inhibitory concentration (MIC) results provided insights for understanding discordant results.
- ***Results***. Of 26 826 patients with suggestive TB symptoms undergoing Xpert MTB/RIF testing, 728 diagnosed as RIF-resistant were evaluated. Of these, **118 (16.2%) exhibiting Xpert RIF resistance and phenotypic RIF susceptibility** yielded 104 successfully subcultured isolates; of these, 86 (82.7%) harbored *rpoB* gene RIF resistance–determining region mutations and 18 (17.3%) did not. The Leu511Pro (25.0%) and Leu533Pro (17.3%) mutations were most frequently associated with discordant RIF susceptibility test results. Of the 86 isolates with *rpoB* mutations, 42 (48.8%) with MICs ≤1.0 mg/L were assigned to the RIF-susceptible group, with Leu511Pro being the most common mutation observed. Isolates with a very low bacterial load were most frequently misdiagnosed as RIF-resistant by Xpert.
- ***Conclusions***. Approximately **one-sixth** of RIF-resistant TB isolates identified via Xpert yielded discordant pDST results due to questionable interpretation of specific **"disputed" mutations**. Thus, a diagnostic flowchart should be used to correctly interpret Xpert RIF resistance results to best guide patient treatment.

Table 1. Mutations of MTB Isolates Within the RRDR of the *rpoB* Gene by Sanger Sequencing

Mutation Type	No. of Isolates With Different Mutations (n = 104) (%)
Leu511Pro	22 (21.2)
Asp516Val	3 (2.9)
Asp516Tyr	7 (6.7)
Ser522Gln	1 (1.0)
Ser522Leu	1 (1.0)
His526Asn	4 (3.8)
His526Cys	3 (2.9)
His526Gly	1 (1.0)
His526Leu	10 (9.6)
His526Ser	1 (1.0)
Ser531Leu	5 (4.8)
Ser531Cys	1 (1.0)
Leu533Pro	18 (17.3)
Leu511Pro + Met515Ile	2 (1.9)
Leu511Pro + Ser509Arg	1 (1.0)
Leu511Pro + His526Gln	1 (1.0)
Asp516Gly + Ser522Leu	1 (1.0)
Asp516Gly + Asn518Asp	1 (1.0)
His526Asp + Glu541Gly	1 (1.0)
Gln517Gln	1 (1.0)
Heteroresistance	1 (1.0)
Wild-type	18 (17.3)

Abbreviations: MTB, *Mycobacterium tuberculosis*; RRDR, rifampin resistance–determining region.

[a]Heteroresistance was defined as a heterogeneous population of tubercle bacilli harboring wild-type and mutant Asp516Asn according to the sequencing chromatograms.

Open Forum Infect Dis 2020;7:ofaa279

Interpretation of Discordant Rifampicin Susceptibility Test Results Obtained Using GeneXpert vs Phenotypic Drug Susceptibility Testing

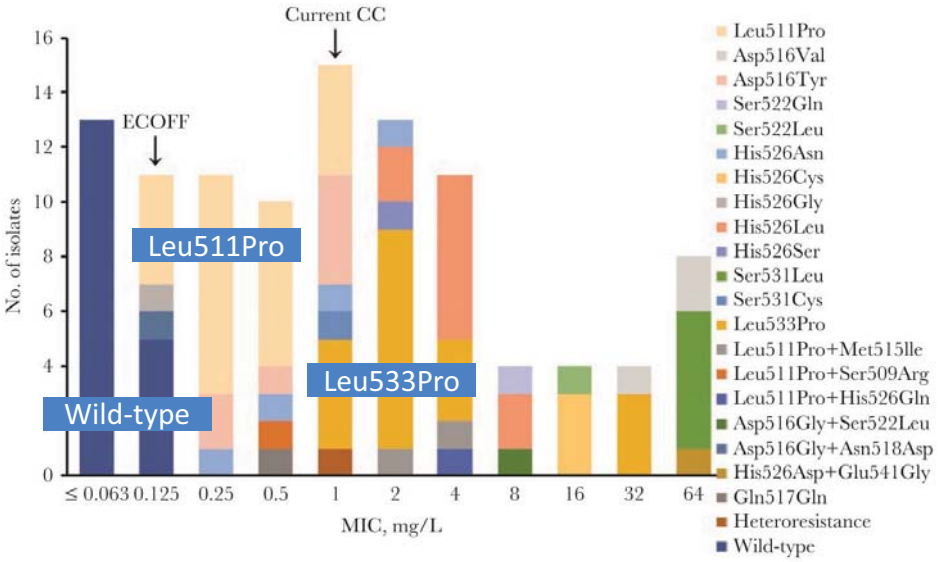
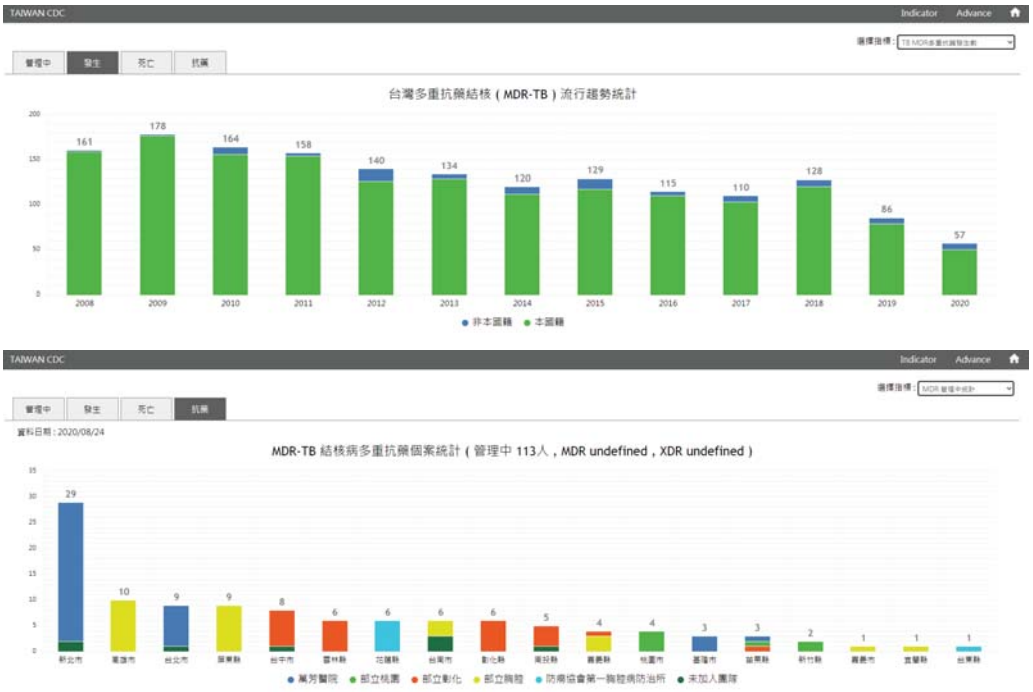


Figure 2. Distribution of *Mycobacterium tuberculosis* isolates with different MICs grouped according to *rpoB* mutation profile. Abbreviations: CC, critical concentration; ECOFF, epidemiological cutoff value; MIC, minimal inhibitory concentration.

Open Forum Infect Dis 2020;7:ofaa279

臺北市立萬芳醫院
-委託財團法人臺北醫學大學財團-

台灣多重抗藥性結核(MDR-TB)



Molecular Screening of Multidrug-resistance Tuberculosis by a Designated Public Health Laboratory in Taiwan

- Taiwan Centers for Disease Control designated a single referral laboratory to provide the GenoType MTBDRplus test for screening high-risk MDR-TB populations nationwide in 2012–2015

GenoType MTBDR*plus*		Corresponding DST results†		Sen.	Spe.	PPV	NPV	Accuracy
		R	S					
RIF resistance	R	93	16	92.1%	97.3%	85.3%	98.6%	96.5%
	S	8	567					
INH resistance	R	109	1	77.9%	99.8%	99.1%	94.6%	95.2%
	S	31	543					
MDR	R	67	2	82.7%	99.7%	97.1%	97.7%	97.7%
	S	14	601					

*710 patients with Genotype TB (+) and culture *M. tuberculosis* isolation (+)

†Only 684 patients with completed DST results

Abbreviations: R, resistance; S, susceptible; Sen., sensitivity; Spe., specificity; PPV, positive predicted value; NPV, negative predicted value; INH, isoniazid, RIF, rifampicin; MDR, multidrug resistance to at least INH and RIF

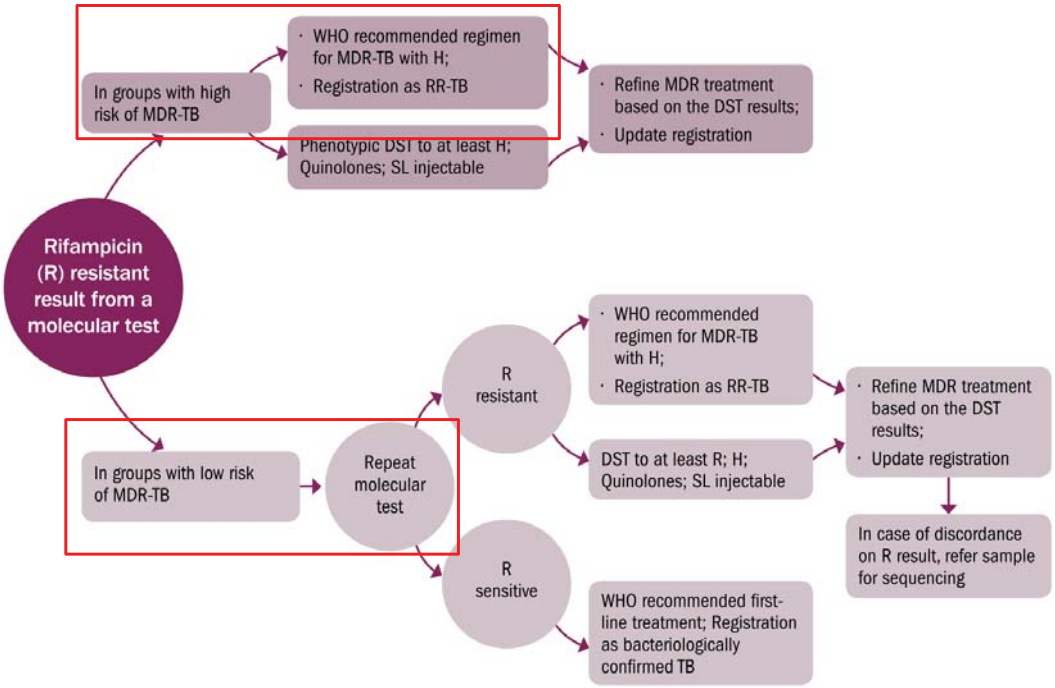
Eur J Clin Microbiol Infect Dis 2017; 36:2431–39

Performance of an Xpert-based Diagnostic Algorithm for the Rapid Detection of Drug-resistant Tuberculosis among High-risk Populations in a Low-incidence Setting

		Conventional DST results, no.			Performance, %			
		Resistant	Susceptible	Total	Sensitivity	Specificity	PPV	NPV
Xpert results, n = 697*								
RIF	Resistant	36	9	45	100	98.6	80.0	100.0
	Susceptible	0	652	652	(90.3–100.0)	(97.4–99.4)	(67.6–88.4)	(99.4–100.0)
	Total	36	661	697				
LPA results, n = 44#								
RIF	Resistant	36	8	44				
	Susceptible	0	0	0				
	Total	36	8	44				
INH	Resistant	26	0	26	96.3	100.0	100.0	94.4
	Susceptible	1	17	18	(81.0–99.9)	(80.5–100.0)	(87.1–100.0)	(71.3–99.2)
	Total	27	17	44				
MDR	Yes	24	2	26	96.0	89.5	92.3	94.4
	No	1	17	18	(79.7–99.9)	(66.9–98.7)	(76.3–97.8)	(71.2–99.2)
	Total	25	19	44				

PLoS ONE 2018;13:e0200755

Algorithm for Interpretation of Results from Molecular Methods



Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. WHO/HTM/TB/2014.11.

Correlation between Genotypic and Phenotypic Testing for Resistance to Rifampin in *M. tuberculosis* Clinical Isolates in Haiti : Investigation of Cases with Discrepant Susceptibility Results

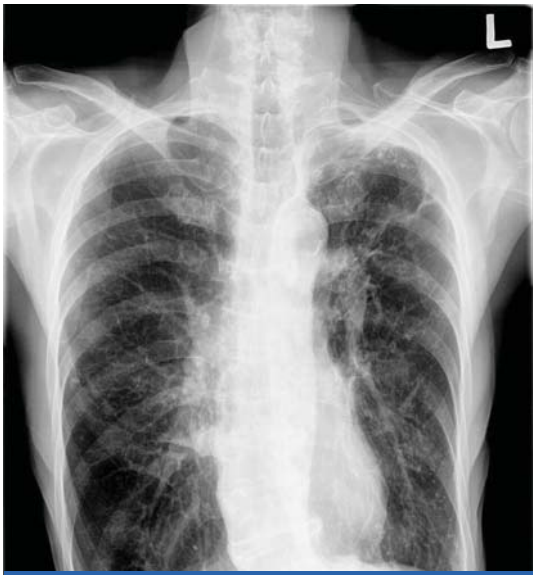
Abstract

The World Health Organization has recommended use of molecular-based tests MTBDRplus and GeneXpert MTB/RIF to diagnose multidrug-resistant tuberculosis in developing and high-burden countries. Both tests are based on detection of mutations in the Rifampin (RIF) Resistance-Determining Region of DNA-dependent RNA Polymerase gene (*rpoB*). Such mutations are found in 95–98% of *Mycobacterium tuberculosis* strains determined to be RIF-resistant by the "gold standard" culture-based drug susceptibility testing (DST). We report the phenotypic and genotypic characterization of 153 consecutive clinical *Mycobacterium tuberculosis* strains diagnosed as RIF-resistant by molecular tests in our laboratory in Port-au-Prince, Haiti. 133 isolates (86.9%) were resistant to both RIF and Isoniazid and 4 isolates (2.6%) were RIF mono-resistant in MGIT SIRE liquid culture-based DST. However the remaining 16 isolates (10.5%) tested RIF-sensitive by the assay. Five strains with discordant genotypic and phenotypic susceptibility results had RIF minimal inhibitory concentration (MIC) close to the cut-off value of 1 µg/ml used in phenotypic susceptibility assays and were confirmed as resistant by DST on solid media. Nine strains had sub-critical RIF MICs ranging from 0.063 to 0.5 µg/ml. Finally two strains were pan-susceptible and harbored a silent *rpoB* mutation. Our data indicate that not only detection of the presence but also identification of the nature of *rpoB* mutation is needed to accurately diagnose resistance to RIF in *Mycobacterium tuberculosis*. Observed clinical significance of low-level resistance to RIF supports the re-evaluation of the present critical concentration of the drug used in culture-based DST assays.

- In 10.5% of TB cases, genotypic resistance to RIF was not confirmed by phenotypic DST
- Our clinical observations suggest that not only detection of the presence but also identification of the nature of *rpoB* mutation is needed for accurate diagnosis of resistance to Rifampin

PLoS ONE 2014;9:e90569

Rifabutin and Rifampin Resistance Levels and Associated *rpoB* Mutations in Clinical Isolates of *Mycobacterium tuberculosis* Complex



Male, 87 y/o
pDST: INH-R, RMP-R, Rifabutin-S
Genetic locus: *rpoB* S522L

Table 1
Observed MIC for RFB and RIF and associated *rpoB* mutations.

Amino acid change (nucleotide changes)	Observed MIC (µg/mL)	
	RIF MIC (Isolates tested)	RFB MIC (Isolates tested)
Group I, RIF-S and RFB-S		
Wildtype	≤0.125 (2), 0.125 (2)	≤0.0625 (37), 0.125 (5)
L511P (CCG)	≤0.25 (2), 0.25 (1)	≤0.0625 (2), 0.0625 (1)
F514F (TTT)	0.125 (2)	≤0.0625 (2)
D516Y (TAC)	0.25 (2), 0.5 (2)	0.0625 (4)
H526N (AAC)	0.125 (2), 0.25 (1)	≤0.0625 (2), 0.125 (1)
H526S (AGC)	0.5 (1), 1 (1)	≤0.0625 (1), 0.125 (1)
H526S (TCC)	0.25 (1)	≤0.0625 (1)
S531C (TGT)	≤0.125 (1)	0.0625 (1)
L533P (CCG)	0.5 (2)	0.125 (1), 0.25 (1)
T508 to S509 deletion	0.5 (1)	≤0.0625 (1)
M515I (ATA) + H526N (AAC)	1 (1)	0.125 (1)
Group II, RIF-R and RFB-S		
D516V (GTC)	8 (3), >8 (15)	0.125 (2), 0.25 (6), 0.5 (10)
D516F (TTC)	2 (1)	0.0625 (1)
S522L (TTG)	2 (1)	0.0625 (1)
H526A (GCC)	2 (1)	0.125 (1)
H526C (TGC)	2 (1), 8 (1)	0.125 (2)
H526G (GGC)	2 (1)	0.125 (1)
H526L (CTC)	2 (2), 4 (1), 8 (1)	0.125 (2), 0.25 (1), 0.5 (1)
N518 deletion	4 (1)	0.125 (1)
S522L (TTG) + K527R (AGG)	8 (1)	0.5 (1)
H526S (TCC) + K527R (CGG)	4 (1)	0.25 (1)
Group III, RIF-R AND RFB-R		
Q513E (GAA)	>8 (2)	1 (2)
Q513K (AAA)	>8 (3)	>8 (3)
Q513L (CTA)	>8 (1)	>8 (1)
Q513P (CCA)	>8 (2)	1 (1), 2 (1)
H526D (GAC)	>8 (3)	8 (2), >8 (1)
G526R (CGC)	>8 (2)[a]	8 (2), >8 (3)
H526Y (TAC)	>8 (1)[a]	8 (2), >8 (2)
S531F (TTC)	>8 (1)	>8 (1)
S531W (TGG)	>8 (3)[a]	4 (1), 8 (2), >8 (1)
S531L (TTG)	>8 (5)[a]	2 (7), 4 (25), 8 (1)
S509R (CGC) + H526Y (TAC)	>8 (1)	8 (1)
S509R (AGG) + H526L (CTC)	>8 (1)	8 (1)
Q510L (CTG) + D516V (GTC)	>8 (1)	1 (1)
L511P (CCG) + S512T (ACC) + D516Y (TAC)	>8 (1)	8 (1)
L511P (CCG + D516Y (TAC)	>8 (2)	2 (2)
Q513L (CTA) + H526N (AAC)	>8 (1)	>8 (1)
F514F (TTT) + S531L (TTG)	>8 (1)	8 (1)
515–521 deletion	>8 (1)	8 (1)
D516E (GAG) + S522L (TTG)	>8 (1)	1 (1)
D516V (GTC) + S531L (TTG)	>8 (1)	4 (1)
D516G (GGC) + L533P (CCG)	>8 (1)	4 (1)
H526Q (CAG) + L533P (CCG)	>8 (1)	1 (1)

[a] These mutations are commonly found in isolates from MDR TB patients and they are known to confer high level resistance to RIF; RIF MIC was performed for limited numbers of those strains. RFB MIC was performed for all strains included in this study.

Diagn Microbiol Infect Dis 2016;85:177–81

Treatment Outcomes of Rifabutin-containing Regimens for Rifabutin-sensitive Multidrug-resistant Pulmonary Tuberculosis

Objectives: The aim of this study was to evaluate whether rifabutin can improve treatment outcomes in patients with rifabutin-sensitive MDR-TB.
Methods: A retrospective cohort study was performed on 76 patients with rifabutin-sensitive MDR-TB who were treated with or without rifabutin between 2006 and 2011.
Results: Overall, 75% (57/76) of patients achieved favorable outcomes, including cure (53/76, 70%) and treatment completion (4/76, 5%). In contrast, 25% (19/76) had unfavorable treatment outcomes, which included treatment failure (6/76, 8%), death (2/76, 3%), loss to follow-up (4/76. 5%), and no evaluation due to transfer to other institutions (7/76, 9%). Rifabutin was given to 52 (68%) of the 76 patients with rifabutin-sensitive MDR-TB. Although favorable treatment outcomes were more frequent in patients who received rifabutin [81% (42/52)] than in those who did not receive rifabutin [63% (15/24)], this difference was not statistically significant ($P = 0.154$). However, in multivariable regression logistic analysis, use of rifabutin was significantly associated with favorable treatment outcomes in patients with rifabutin-sensitive MDR-TB (adjusted odds ratio = 9.80, 95% confidence interval = 1.65–58.37, $P = 0.012$).
Conclusions: These results suggest that the use of rifabutin can improve treatment outcomes in patients with rifabutin-sensitive MDR-TB.

Int J Infect Dis 2017;65:135–141

建議使用Rifabutin:

- **細菌對Rifampicin 抗藥，且藥物感受性試驗證實 Rifabutin對它有效**
- **Cross resistance:台灣的數據為 87%**

台灣結核病診治指引第6版

Molecular Detection of Rifabutin-Susceptible *Mycobacterium tuberculosis*

TABLE 1 Correlations between specific mutations of the *rpoB* genes and patterns of the GenoType MTBDR*plus* assay for identification of RFB-susceptible isolates[a]

Mutation codon no.	Codon	Amino acid change	No. of isolates	No. (%) of RFB-resistant isolates	Pattern by GenoType MTBDR*plus* assay											
					wt1	wt2	wt3	wt4	wt5	wt6	wt7	wt8	mut1	mut2	mut3	mut4
143	CGT/TGT	R→C	1	0 (0)												
146	GTC/TTC	V→F	6	6 (100)												
511	CTG/CCG	L→P	3	0 (0)	■		■	■	■	■	■	■				
513	CAA/AAA	Q→K	10	10 (100)	■			■	■	■	■	■				
	CAA/CTA	Q→L	4	4 (100)	■			■	■	■	■	■				
	CAA/GAA	Q→E	1	1 (100)	■			■	■	■	■	■				
	CAA/CCA	Q→P	7	7 (100)	■			■	■	■	■	■				
516	GAC/TAC	D→Y	14	0 (0)	■	■			■	■	■	■				
	GAC/GTC	D→V	22	0 (0)	■	■			■	■	■	■	■			
	GAC/TTC	D→F	8	0 (0)	■	■			■	■	■	■				
522	TCG/TTG	S→L	5	0 (0)	■	■	■	■			■	■				
526	CAC/CGC	H→R	18	18 (100)	■	■	■	■	■	■		■				
	CAC/TAC	H→Y	53	53 (100)	■	■	■	■	■	■		■		■		
	CAC/GAC	H→D	32	32 (100)	■	■	■	■	■	■		■			■	
	CAC/CAA	H→Q	2	2 (100)	■	■	■	■	■	■		■				
	CAC/CCC	H→P	1	1 (100)	■	■	■	■	■	■		■				
	CAC/TGC	H→C	2	0 (0)	■	■	■	■	■	■		■				
	CAC/CTC	H→L	11	0 (0)	■	■	■	■	■	■		■				
	CAC/ACC	H→T	1	0 (0)	■	■	■	■	■	■		■				
	CAC/AAC	H→N	4	0 (0)	■	■	■	■	■	■		■				
529	CGA/CTA	R→L	1	0 (0)	■	■	■	■	■	■		■				
531	TCG/TTG	S→L	491	491 (100)	■	■	■	■	■	■	■					■
	TCG/TGG	S→W	16	16 (100)	■	■	■	■	■	■	■					
533	CTG/CCG	L→P	27	8 (29.6)	■	■	■	■	■	■	■					

[a] Shading highlights mutations that confer both RIF and RFB resistance.

J Clin Microbiol 2012;50: 2085–2088

Diagnosis of Tuberculosis in Adults and Children

Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines

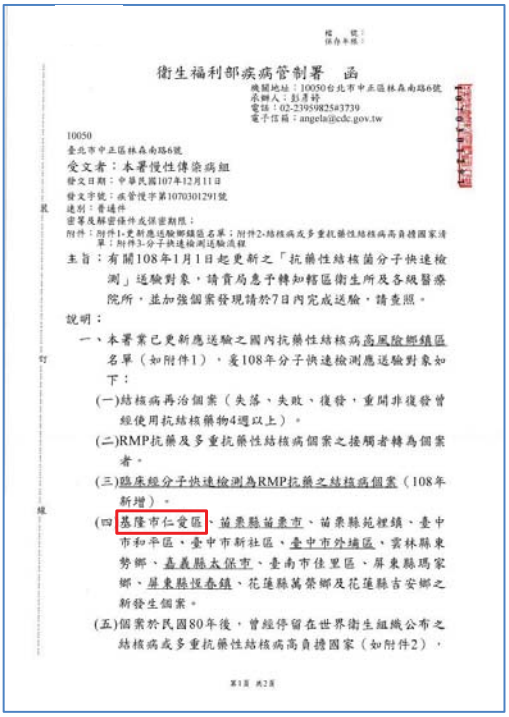
- **The sensitivity and specificity of rapid molecular DST for detecting rifampin resistance are both >97%, indicating that false-positive and false-negative results occur <3% of the time**
- **The sensitivity and specificity of rapid molecular DST for detecting isoniazid resistance are estimated to be 90% and 99%, respectively, indicating that false-positive and false-negative results occur roughly 1% and 10% of the time, respectively**

- **Confirmation of a positive test result for rifampin resistance has been recommended**
  - **To confirm a positive result, genetic loci associated with rifampin resistance (to include *rpoB*), as well as isoniazid resistance (to include *inhA* and *katG*), should be sequenced to assess for MDR-TB**

Clin Infect Dis 2017;64:e1–e33

分子快速檢測送驗對象

- 1. 結核病再治個案(失落、失敗、復發，重開非復發曾經使用抗結核藥物 4 週以上)
- 2. RR-TB 及 MDR-TB 個案之接觸者轉為個案者
- 3. 臨床經分子快速檢測為RMP抗藥之結核病個案
- 4. 國內高風險地區之新發生個案
- 5. 於民國 80 年後，個案過去曾停留在疾病管制署指定應送分子快速篩檢國家，於 1 年內累積達 1 個月以上(即連續任 365 天內，停留時間累積達 30 天以上)



衛生福利部疾病管制署 函

主旨：有關108年1月1日起更新之「抗藥性結核菌分子快速檢測」送驗對象，請貴局惠予轉知轄區衛生所及各級醫療院所，並加強個案發現請於7日內完成送驗，請查照。

說明：

一、本署業已更新應送驗之國內抗藥性結核病高風險鄉鎮區名單（如附件1），及108年分子快速檢測應送驗對象如下：

(一)結核病再治個案（失落、失敗、復發，重開非復發曾經使用抗結核藥物4週以上）。

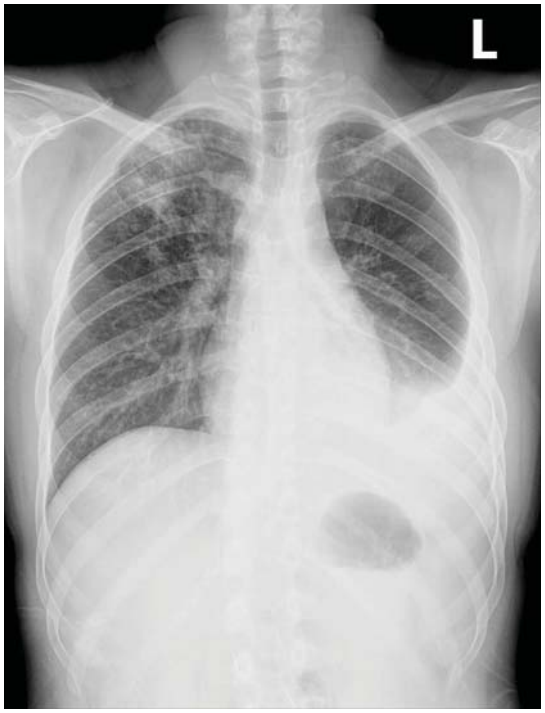
(二)RMP抗藥及多重抗藥性結核病個案之接觸者轉為個案者。

(三)臨床經分子快速檢測為RMP抗藥之結核病個案（108年新增）。

(四)基隆市仁愛區、苗栗縣苗栗市、苗栗縣苑裡鎮、臺中市和平區、臺中市新社區、臺中市外埔區、雲林縣東勢鄉、嘉義縣太保市、臺南市佳里區、屏東縣瑪家鄉、屏東縣恆春鎮、花蓮縣萬榮鄉及花蓮縣吉安鄉之新發生個案。

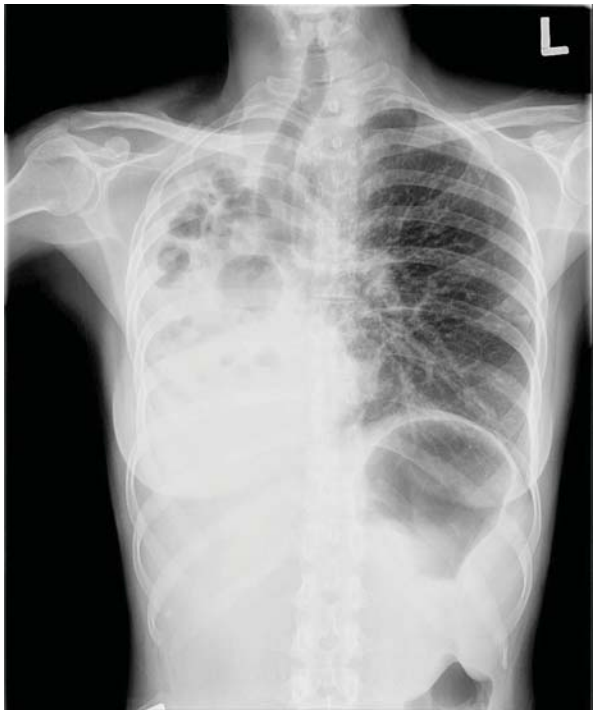
(五)個案於民國80年後，曾經停留在世界衛生組織公布之結核病或多重抗藥性結核病高負擔國家（如附件2）。

Drug-resistant TB ?



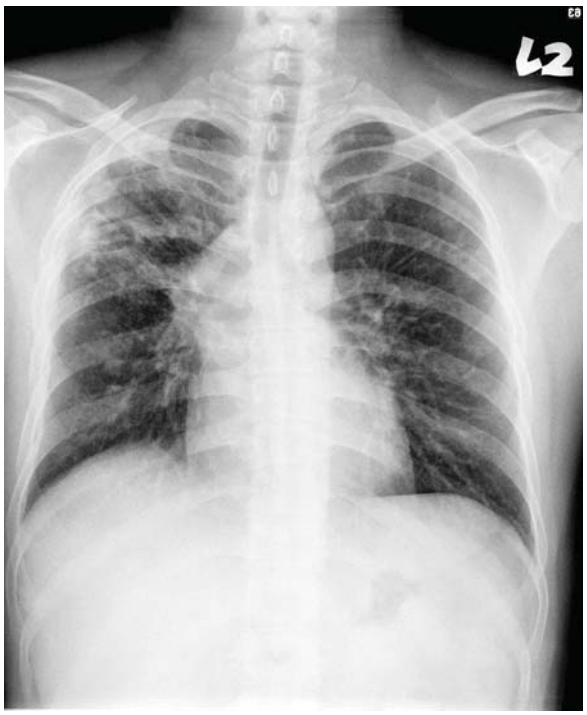
- Female, 16 y/o
- Family Hx of TB: mother , 20 yrs ago
- Xpert: RMP-R
- GenoType: INH-S, RMP-R
- Phenotypic DST: all susceptible

31 y/o, Female



- Xpert
  - R: resistant
- Genotype DST
  - HR: resistant
- Phenotypic DST
  - HE: resistant
  - Rifampin/Rifabutin: susceptible
- Genetic loci
  - *rpoB* L511P (disputed mutation)
  - *KatG* S315T
    - High-level INH resistance

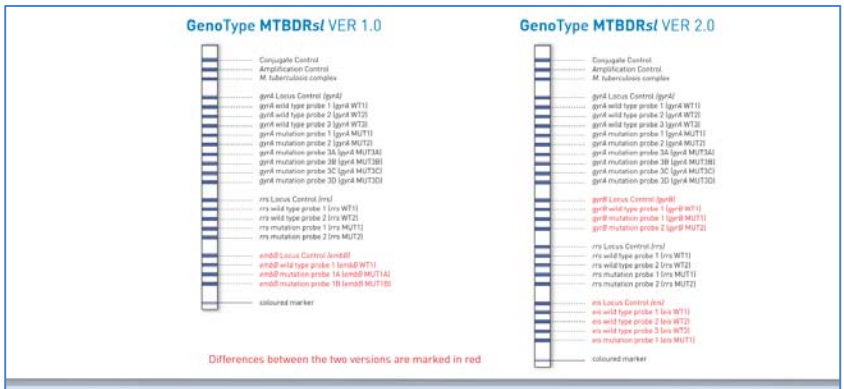
28 y/o, Male



- 2012-4-16
  - 檢體收件
- 2012-4-18
  - 確認MDR-TB
- 2012-5-22
  - 提供第二線抗結核藥物感受性試驗結果

GenoType MTBDR*sl*

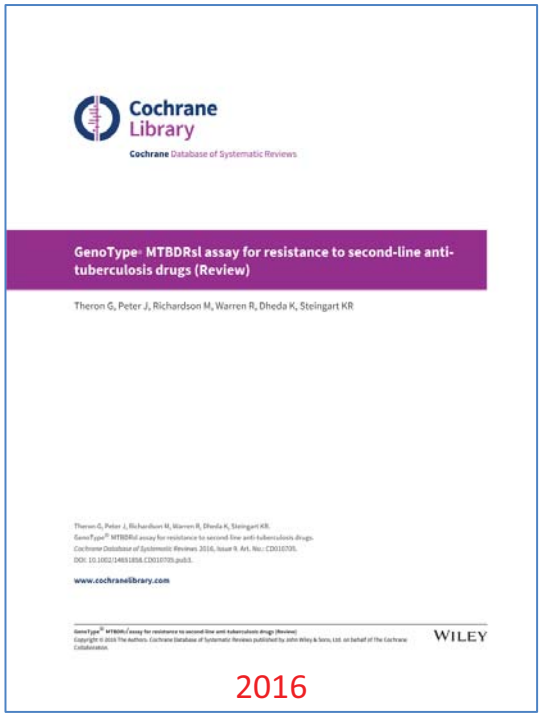
Resistances to fluoroquinolones and aminoglycosides/cyclic peptides (and ethambutol)



	GenoType MTBDR*sl* VER 1.0	GenoType MTBDR*sl* VER 2.0
Detection of	*M. tuberculosis* complex and its resistances to fluoroquinolones, amino-glycosides/cyclic peptides and ethambutol	*M. tuberculosis* complex and its resistances to fluoroquinolones and ami-noglycosides/cyclic peptides
Sample Material	smear-positive pulmonary and cultivated samples	smear-positive and -negative pulmonary and cultivated samples
Ethambutol	Mutations in the *embB* gene that are involved in ethambutol resistance	
	✓	-
Fluoroquinolone	Mutations in the *gyrB* gene that are involved in fluoroquinolone resistance	
	-	✓
Kanamycin	Mutations in the *eis* gene that are involved in kanamycin resistance	
	-	✓

Cochrane Review

GenoType® MTBDRsl assay for resistance to second-line antituberculosis drugs

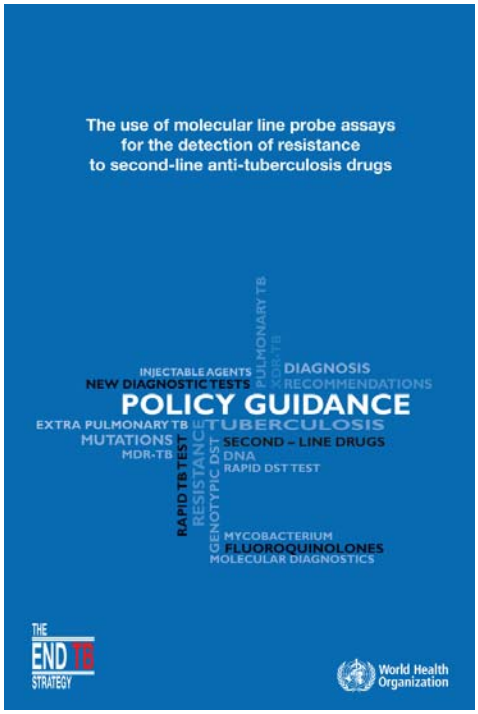


2016

MTBDR*sl version 2.0*

- Fluoroquinolone resistance
  - smear-positive: sensitivity 97% (83% to 100%) and specificity 98% (93% to 100)
  - smear-negative: sensitivity 80% (28% to 99%) and specificity 100% (40% to 100%)
- SLID resistance
  - smear-positive: sensitivity 89% (72% to 98%) and specificity 90% (84% to 95%)
  - smear-negative: sensitivity 80% (28% to 99%) and specificity 100% (40% to 100%)

WHO's Policy Recommendations 2016



- For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones
- For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the second-line injectable drugs

抗藥性結核病檢驗

疾管署參考實驗室

2008年5月始推動MDR-TB需送本署複判

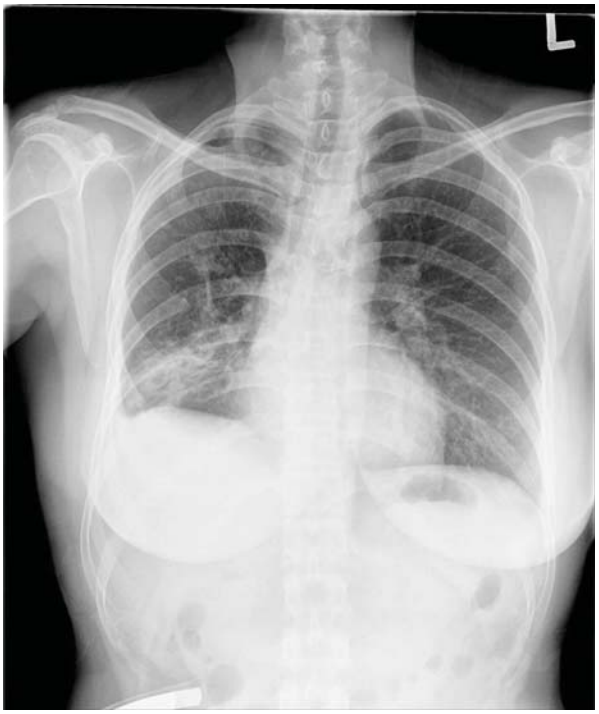
- 複判條件：對INH 且RMP 同時抗藥
- 檢驗方式：
  - 分子鑑定
  - 傳統藥敏 (若分子方法無法判定，再進行傳統藥敏鑑定)
- 經判定為MDR-TB之個案
  - 提供二線藥敏檢測結果

2015年8月1日提供MDR及RMP抗藥個案之二線藥物分子快速檢測

- 抹片陽性痰檢體
  - 檢測FQ、KM、AM及CAP
- 陽性培養菌株
  - 檢測FQ、KM、AM、CAP及PZA

Taiwan CDC 2019.5.8

32y/o, Female



- TB treatment history(+)
- 106-11-23
  - Xpert: RMP-R
- 106-11-29
  - HR: resistant
  - FQ: resistant
  - SLID: susceptible
- 107-2-1: 1st and 2nd DST
  - HRSZ+FQ: resistant

The NEW ENGLAND JOURNAL of MEDICINE

EDITORIAL

The Coming of Age of Drug-Susceptibility Testing for Tuberculosis

Helen Cox, Ph.D., and Valerie Mizrahi, Ph.D.

N Engl J Med 2018;379:1474-5

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 11, 2018 VOL. 379 NO. 15

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

ABSTRACT

BACKGROUND

The World Health Organization recommends drug-susceptibility testing of *Mycobacterium tuberculosis* complex for all patients with tuberculosis to guide treatment decisions and improve outcomes. Whether DNA sequencing can be used to accurately predict profiles of susceptibility to first-line antituberculosis drugs has not been clear.

METHODS

We obtained whole-genome sequences and associated phenotypes of resistance or susceptibility to the first-line antituberculosis drugs isoniazid, rifampin, ethambutol, and pyrazinamide for isolates from 16 countries across six continents. For each isolate, mutations associated with drug resistance and drug susceptibility were identified across nine genes, and individual phenotypes were predicted unless mutations of unknown association were also present. To identify how whole-genome sequencing might direct first-line drug therapy, complete susceptibility profiles were predicted. These profiles were predicted to be susceptible to all four drugs (i.e., pansusceptible) if they were predicted to be susceptible to isoniazid and to the other drugs or if they contained mutations of unknown association in genes that affect susceptibility to the other drugs. We simulated the way in which the negative predictive value changed with the prevalence of drug resistance.

RESULTS

A total of 10,209 isolates were analyzed. The largest proportion of phenotypes was predicted for rifampin (9660 [95.4%] of 10,130) and the smallest was predicted for ethambutol (8794 [89.8%] of 9794). Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity, respectively, and susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity. Of the 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted. Among the 4037 phenotypic profiles that were predicted to be pansusceptible, 3952 (97.9%) were correctly predicted.

CONCLUSIONS

Genotypic predictions of the susceptibility of *M. tuberculosis* to first-line drugs were found to be correlated with phenotypic susceptibility to these drugs. (Funded by the Bill and Melinda Gates Foundation and others.)

1403

The New England Journal of Medicine

- 10,209 isolates
  - Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide
    - Sensitivity: 97.1%, 97.5%, 94.6%, and 91.3%
    - Specificity: 99.0%, 98.8%, 93.6%, and 96.8%
- 7,516 isolates (with complete phenotypic drug-susceptibility profiles)
  - 5,865 (78.0%) (with complete genotypic predictions)
    - Among which 5,250 profiles (89.5%) were correctly predicted
    - Among the 4,037 phenotypic profiles that were predicted to be pansusceptible
      - 3,952 (97.9%) were correctly predicted

N Engl J Med 2018;379:1403-15

Point of Care *Mycobacterium Tuberculosis* Whole Genome Sequencing

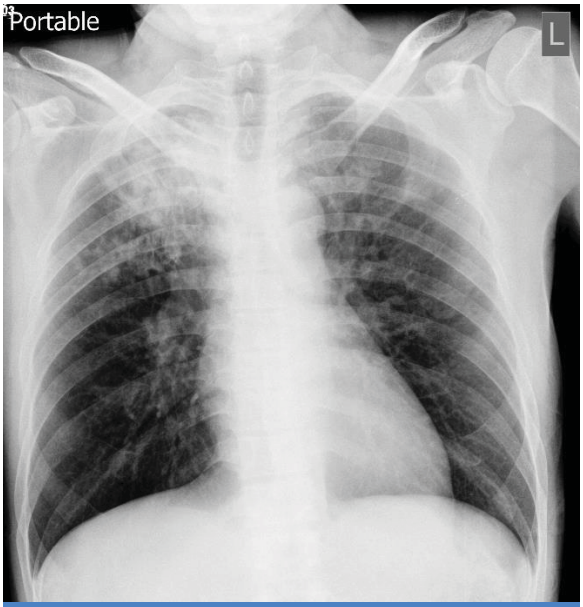
Oxford Nanopore: a New Generation of DNA/RNA Sequencing Technology

Simon Grandjean Lapierre & Niaina Rakotosamimanana - Point of care Mycobacterium tuberculosis whole genome sequencing in remote rural Madagascar

London Calling 2018

Tuberculosis (TB) remains the leading infectious disease killer globally, with 10.4 million new cases in 2016 among which 4.1 million remain undiagnosed. Current approaches to TB control are predicted to fail to meet the World Health Organization objective to eliminate TB by 2030. With the rise of the multi-drug resistant tuberculosis (MDR-TB) epidemic, new innovative TB case finding, diagnosis, and control strategies are needed. Our objective is to achieve real-time TB diagnosis at the point of care, comprehensive genotypic drug susceptibility testing, and molecular epidemiology driven interventions in low resource, high burden settings. In April 2018, the Pasteur Institute of Madagascar, University of Oxford's Modernizing Medical Microbiology group and Stony Brook University's Global Health Institute launched a prospective pilot project which includes methods development for TB sequencing from sputum, and integration of portable TB DNA sequencing within National TB Program (NTP) clinical infrastructures and in collaboration with clinicians and policymakers.

Take Home Messages



結核病的分子藥物敏感性試驗
已經來臨！

- The designated laboratory
  - HR: resistant
  - FQ/SLID: susceptible
- Family Hx of MDR-TB: Mother/Brother
  - Phenotypic DST
    - High-level INH-S
    - Low-level INH-R
    - S/R/Ethionamide: R
  - Genotypic DST
    - HR: R
    - FQ/SLID/PZA: no mutation
  - Genetic loci
    - *rpoB* S531L: high-level resistant to all rifamycins
    - *inhA* C-15T: inhA promoter mutations confer low-level INH resistance, but significantly affect ETH susceptibility
- This patient
  - Phenotypic DST: impending
  - Genetic loci: *rpoB* S531L and *inhA* C-15T