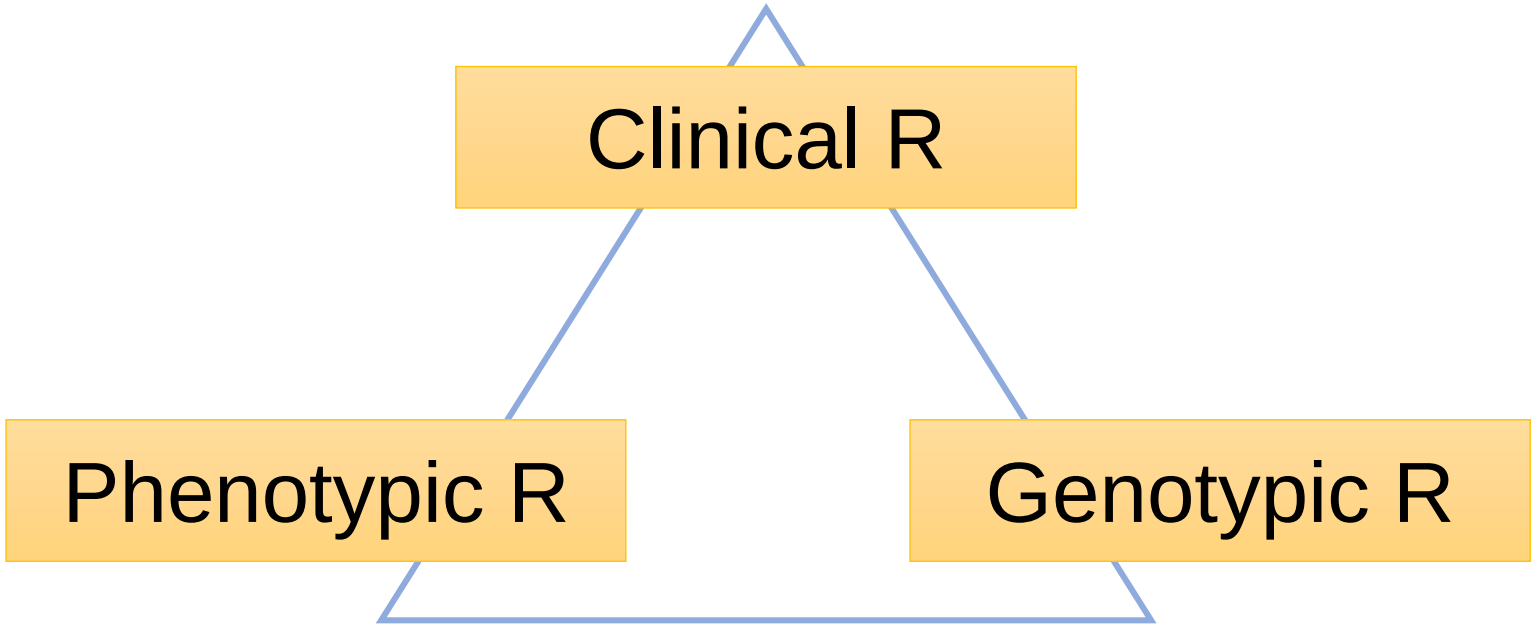
유전자분석을 통한 결핵약제내성 검사 결과의 해석과 적용

양산부산대학교병원 전 두수

What is resistance?

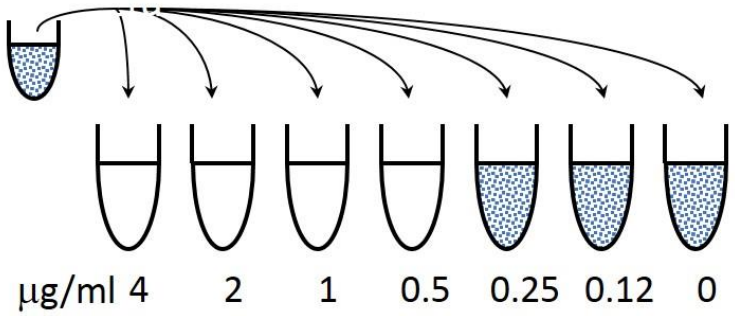


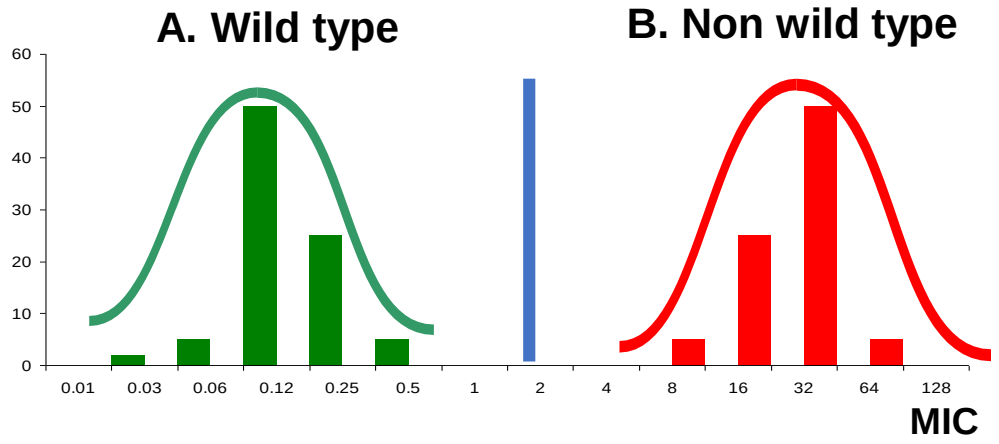
Contents

1. Phenotypic drug resistance testing
2. Genotypic drug resistance testing
    - Rifampin
    - Isoniazid
    - Fluoroquinolones
3. The way forward

Phenotypic R : Discrimination approach

1. Test MIC of
   A. strains from untreated patients
   B. strains from treatment failures
2. What is the best concentration that discriminate A and B?





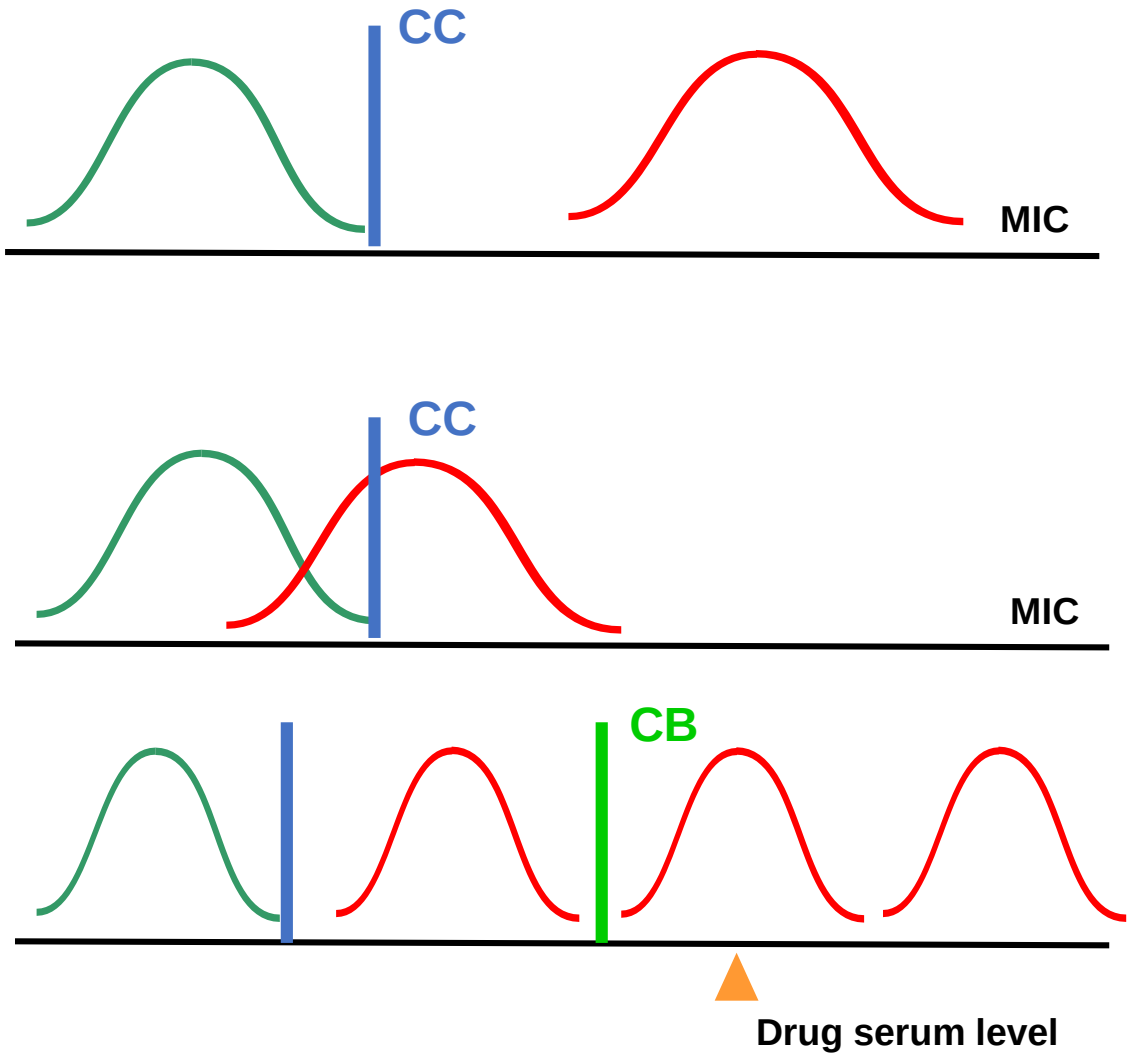
Canetti et al. WHO Bulletin 1969;41:21-43

Phenotypic Resistance

- Epidemiological cut-off value (ECOFF))
  the upper end of the pWT distribution
  (i.e. it typically encompasses 99% of pWT strains).

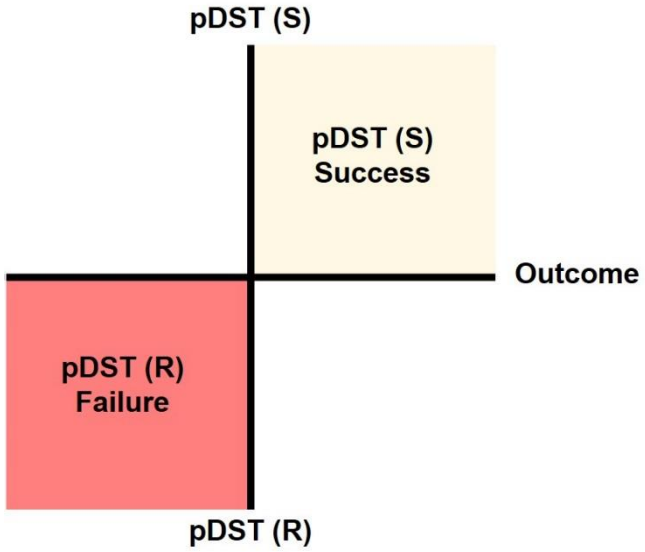
- Critical concentration (CC)
  - MIC value that inhibit the growth of 99% of pWT strains in vitro .

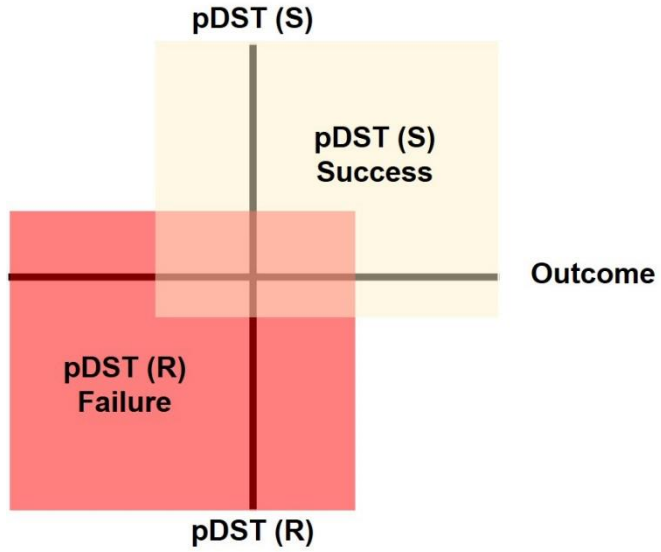
- Clinical breakpoint (CB) : new !
  - MIC value that separates strains that will likely respond to treatment from those will not.
  - Clinical outcome data, MIC distributions, genetic markers, and PK/PD data including drug dose



pDST : imperfect gold standard

Ideal situation



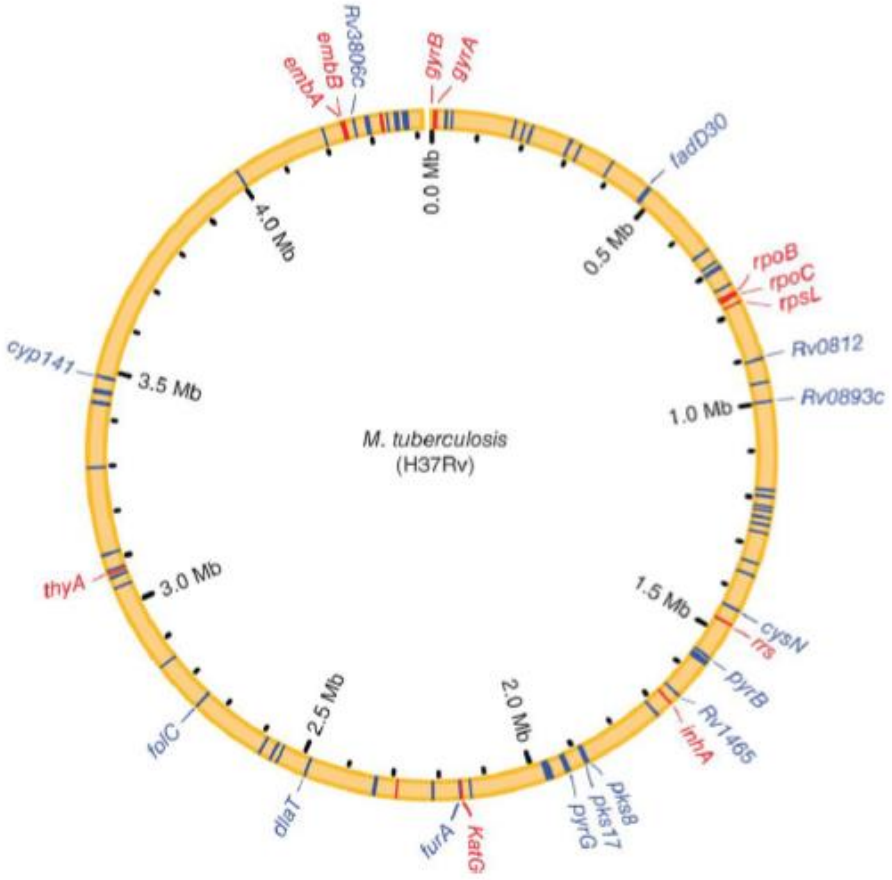


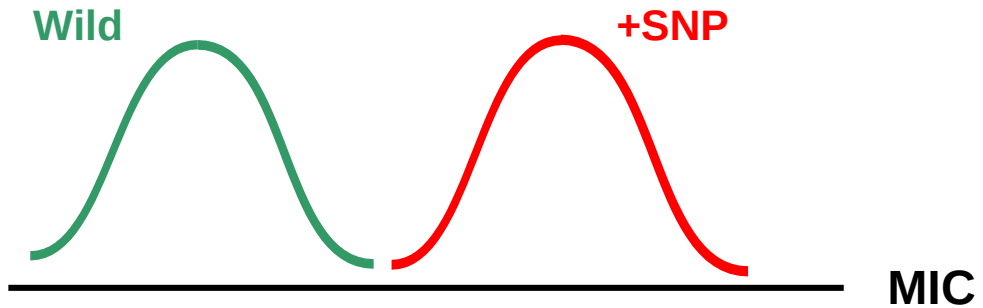
Current situation

- Drug resistance in MTB is heterogeneous (low, mod, high)
- Single CC may not address the biological diversity in MTB
  - do not detect low-level resistant strains clinically meaningful

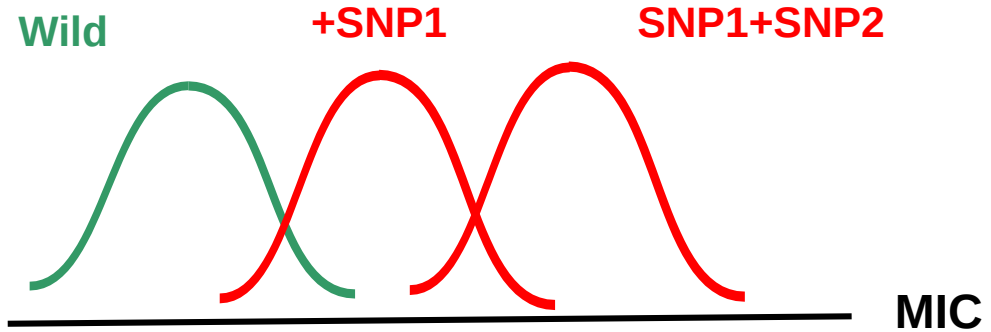
Contents

Drug resistance is mediated by SNPs





1:1 mutation : resistance



Clinical resistance is more complex

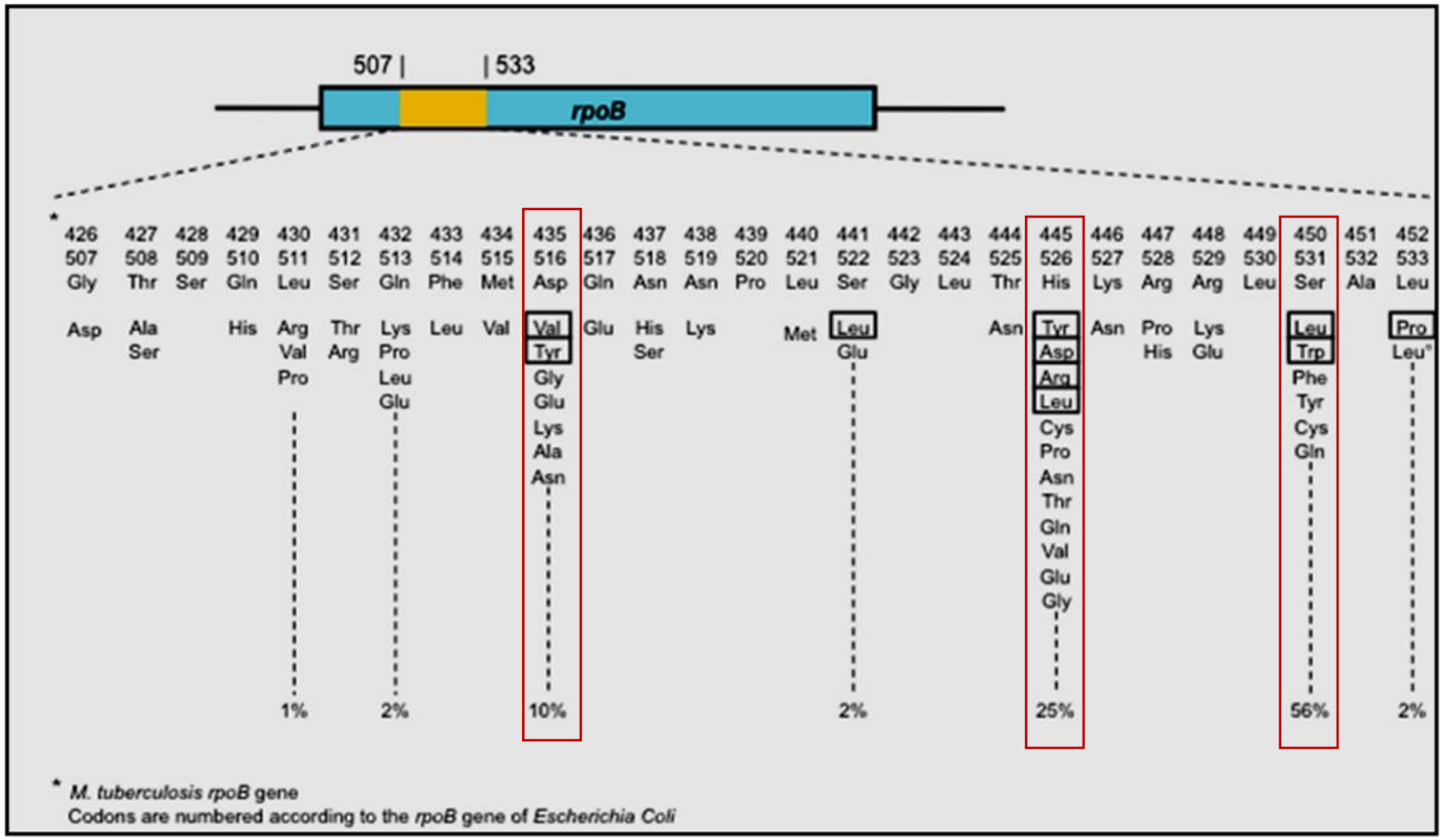
1. Rifampin: Phenotype vs Genotype

- Variable mutations generate various levels of drug resistance
- Discrepancy between phenotypic and genotypic resistance

  (solid ≤ 7%, MGIT ≤ 20%)
- Some RIF-R strains /c rpoB mutations are missed by MIGIT system
- Borderline/low-level RIF-R has been strongly associated treatment failure

Van Deun A et al, J Clin Microbiol 2009;47:3501
Rigouts L et al, J Clin Microbiol 2013;51:2641
Williamson DA et al, Int J Tuberc Lung Dis 2012;16:216
Van Deun A et al, J Clin Microbiol 2013;51:2633
Orcheretina et al, PLoS One 2014;9:e90569
Jamieson FB et al, J Clin Microbiol 2014;52:2157

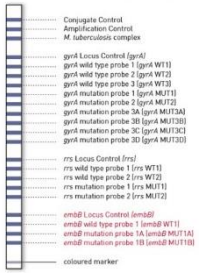
RIF resistance and rpoB mutation



RIF Resistance Determining Region

- 81 base pair core region
- 95% of RIF resistance
- Target of LPA and Xpert test
  : 98% sensitivity

- Xpert MTB/RIF (507-533bp)
- Genotype MTBDRplus (505-533bp)
- rpoB sequencing (462-591bp)



Rifampicin Resistance Determining Region

- High level RIF resistance (MIC > 16 ug/mL)
- 90% of RIF resistance
- Always concordant : phenotypic vs genotypic

What about the remaining 10%?

Discordant results

- pre-analytic, post-analytic and analytic errors
- co-existence of non-tuberculous mycobacteria (NTM)

- Silent mutations : ≈ 2%
- Mutations outside the RRDR: ≈ 5%
- Disputed mutations with low/borderline RIF resistance: ≈ 10%
- Heteroresistance

Hofmann-Thiel S et al. Int J Tuberc Lung Dis 2017;21:721

Silent mutation : false positive

Xpert : RIF-R, pDST : RIF-S

- Do not modify amino acid and protein structure
- Not relevant for drug resistance
- Approximately 2%
- 514 TTT(Phe) mutation ; the most common
- Others: F506, T508, Q510, L511, Q513, F514, T525, A532, L533, P535

Mutation outside the RRDR: false negative

Xpert : RIF-S, pDST : RIF-R

Swaziland. review of 2009 survey

I491F mutation:

38 of 125 (30%) MDR strain

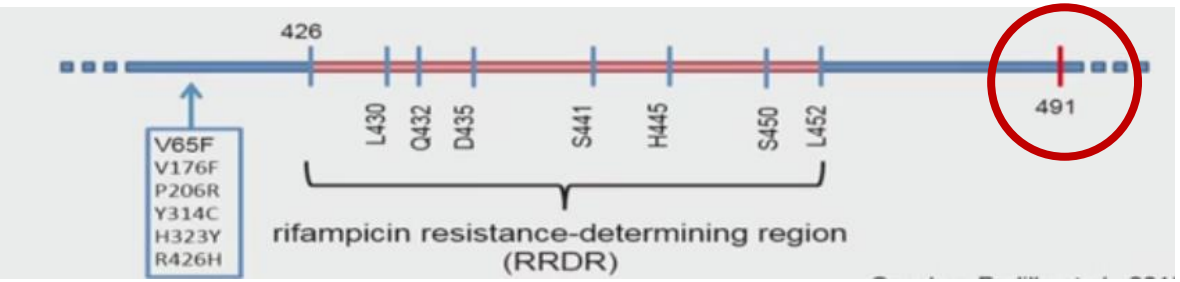


Table 1. Mutations in *rpoB* in 125 Multidrug-Resistant Strains from the 2009 Survey Regarding Tuberculosis-Drug Resistance in Swaziland.*

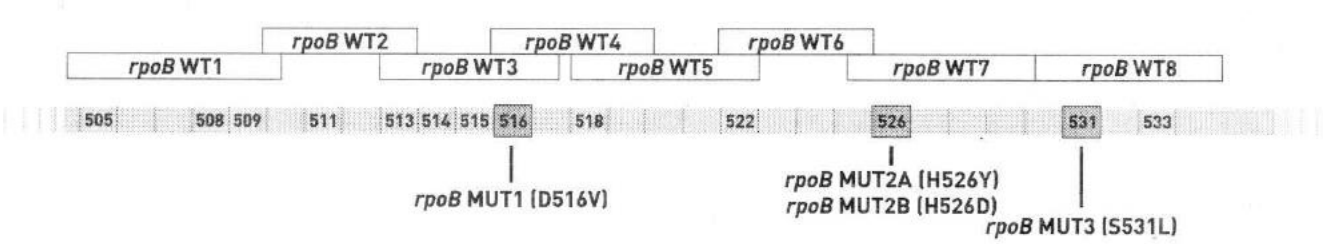
Mutation	Strains with Mutation	Mutation in *rpoB* Hot-Spot Region†
	no. (%)	
D435F	1 (0.8)	Yes
D435F, N437D	3 (2.4)	D435F, yes; N437D, yes
D435V	1 (0.8)	Yes
G442R,‡ I491F	1 (0.8)	G442R, yes; I491F, no
H445D	7 (5.6)	Yes
H445L	6 (4.8)	Yes
H445Y	6 (4.8)	Yes
I491F, R552C	1 (0.8)	I491F, no; R552C, no
I491F	38 (30.4)	No
QF432–433del	1 (0.8)	Yes
S450L	58 (46.4)	Yes
S450W	1 (0.8)	Yes
Unmutated	1 (0.8)	No

Sanchez-Padilla E et al. N Engl J Med 2015;372:1181

Disputed rpoB mutation

Xpert : RIF-R, pDST : RIF-S

- Frequency : not rare
  - 10% of RIF resistance among first-line failure and relapse cases
- Clinical significance
  - Cause low / borderline level RIF resistance
  - **Poor outcome on first-line treatment (failure, relapse)**
- Optimal management : unknown
  - Individualized management
    based on sequencing and measurement of MIC and TDM
  - Potential role of high dose RIF



L511P, D516Y, H526L, L533P…..

Frequency and Type of Disputed *rpoB* Mutations in *Mycobacterium tuberculosis* Isolates from South Korea

- Frequency : **6.9%** (9/130) of RIF resistance strain

Patient No.	Age (yr)	Sex	Initial regimen	Duration of first regimen (mo)	Subsequent regimen	Duration of subsequent regimen (mo)	Treatment outcome	1 Year recurrence
1	43	Male	RIF, EMB, PZA	4	MXF, KM, PTH, CS, PAS	17	Cured	No recurrence
2	50	Male	INH, RIF, PZA	6		-	Cured	No recurrence
3	52	Male	INH, RIF, EMB, PZA	5	MXF, KM, PTH, CS, PAS	13	Cured	No recurrence
4	56	Male	INH, HD-RIF, EMB	1	MXF, HD-RIF, EMB	11	Cured	No recurrence
5	59	Male	INH, RIF, EMB, PZA	2	INH, HD-RIF	8	Treatment completed	No recurrence
6	24	Female	LVFX, KM, PZA, PTH, CS	14	-	-	Cured	No recurrence
7	39	Female	MXF, KM, PZA, PTH, CS	8	-	-	Treatment failed	N/A
8	69	Female	LVFX, KM, PZA, PTH, PAS	3	MXF, RIF, CS, AMOX	9	Cured	No recurrence
9	55	Male	INH, RIF, PZA, CS	0.5	LZD, PZA, CS	-	On treatment	N/A

High dose RIF

Jo KW et al. Tuberc Respir Dis 2017;80:270

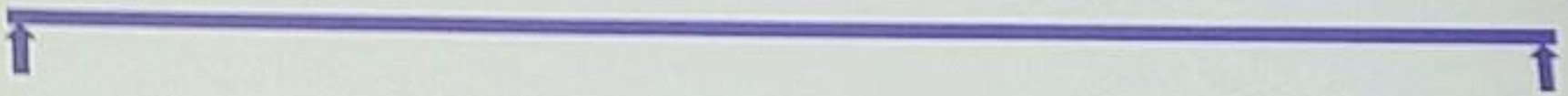
Rifampicin resistance: specificity > sensitivity

Resistance missed

Resistance detected

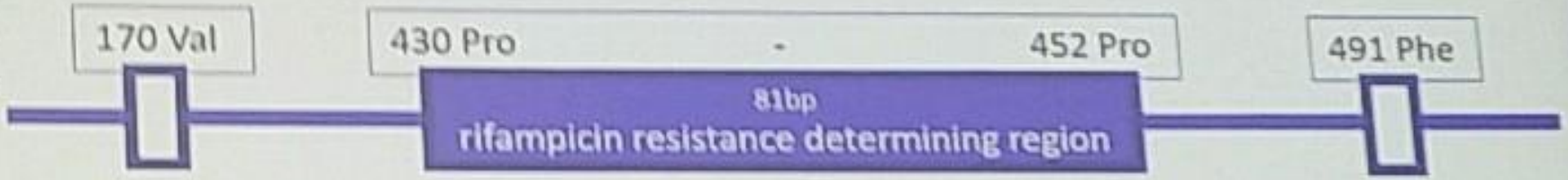
Gold standard: Sequence *rpoB*

- Classical
- NGS



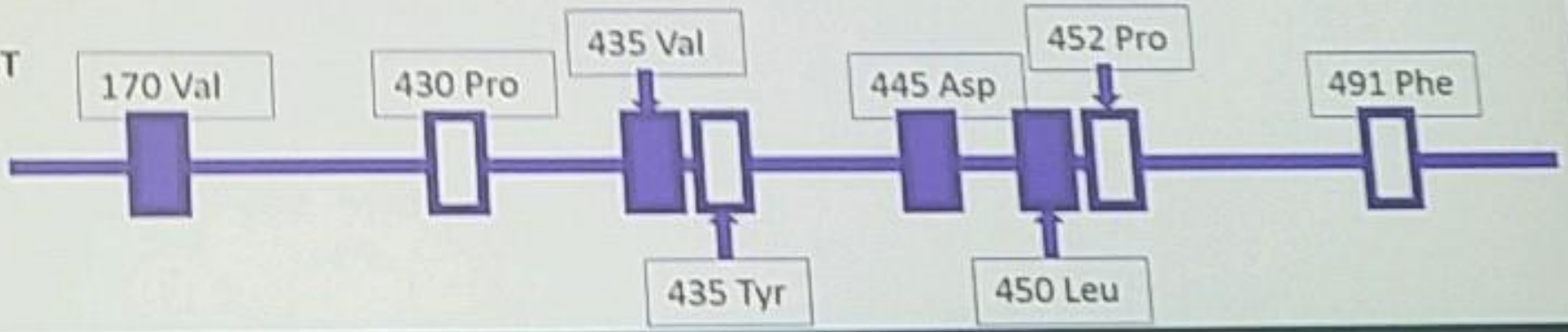
Molecular DST

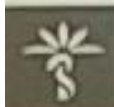
- Line Probe Assays
- Xpert Classic
- Xpert Ultra



Rapid phenotypic DST

- MGIT
- REMA
- MODS





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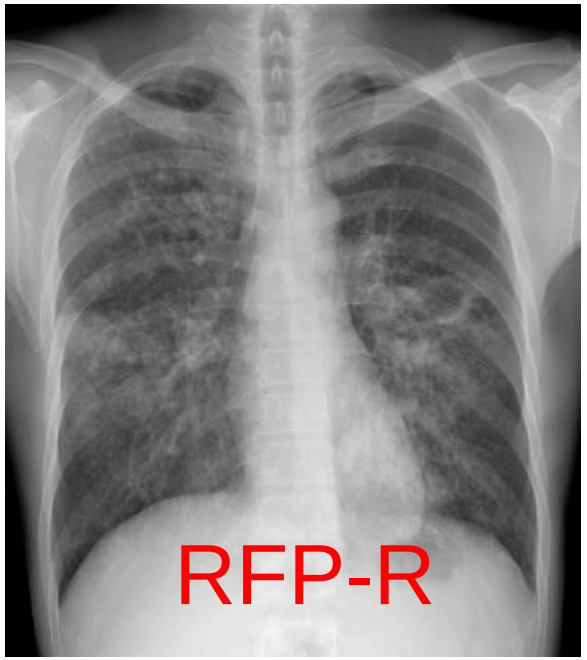
The 49th UNION World Conference, Hague, 2018

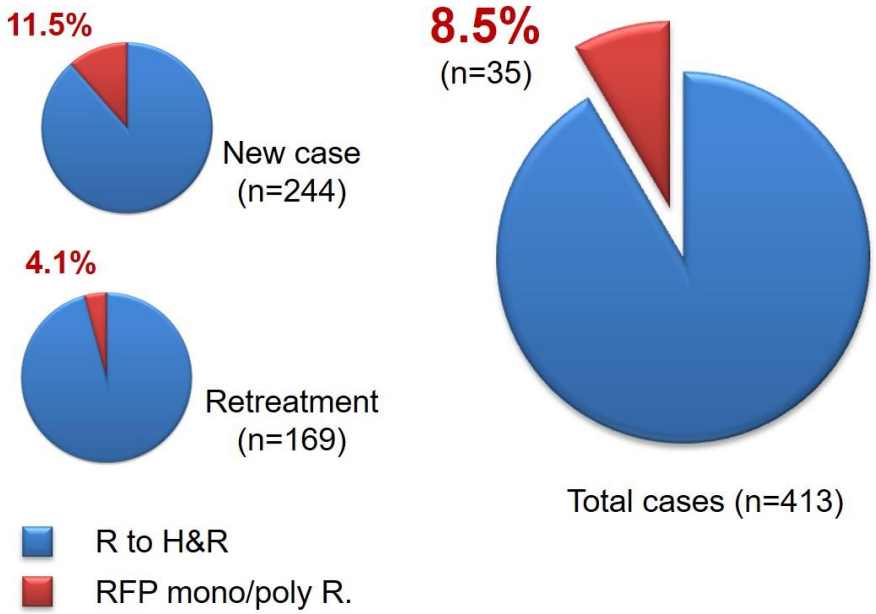
Case 1 M/45. PTB (New case)



- **적절한 처방은?**
  1. HREZ
  2. HEZ + FQ
  3. HEZ + FQ + KM
  4. H + MDR regimen
  5. MDR regimen

Additional Drug Resistance Patterns among Multidrug-Resistant Tuberculosis Patients in Korea: Implications for Regimen Design

RR-TB patients

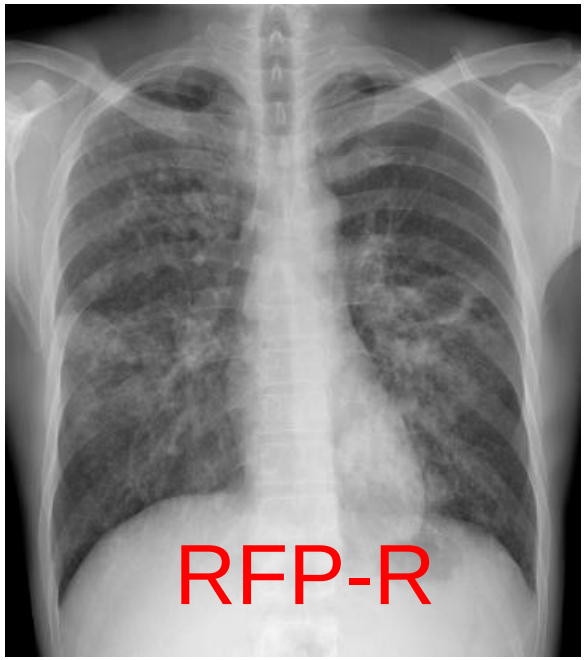


MDR-TB patients

	Resistance, n (%)						
	New	1st line	2nd line	Total	*p*	*P for trend*	
no	216 **57%**	125	37	378			
Ethambutol	146 (67.6)	73 (58.4)	22 (59.5)	241 (63.8)	0.196	0.128	
Pyrazinamide	68 (31.5)	45 (36.0)	22 (59.5)	135 (35.7)	0.004	0.005	**1/3**
Streptomycin	78 (36.1)	33 (26.4)	11 (29.7)	122 (32.3)	0.174	0.139	
Kanamycin	34 (15.7)	22 (17.6)	12 (32.4)	68 (18.0)	0.050	0.045	**1/5**
Amikacin	30 (13.9)	17 (13.6)	9 (24.3)	56 (14.8)	0.244	0.234	
Ofloxacin	39 (18.1)	33 (26.4)	24 (64.9)	96 (25.4)	<0.001	<0.001	**1/4**
Levofloxacin	37 (17.1)	28 (22.4)	23 (62.2)	88 (23.3)	<0.001	<0.001	
Moxifloxacin	30 (13.9)	24 (19.2)	22 (59.5)	76 (20.1)	<0.001	<0.001	
Prothionamide	27 (12.5)	23 (18.4)	16 (43.2)	66 (17.5)	<0.001	<0.001	
Cycloserine	10 (4.6)	12 (9.6)	5 (13.5)	27 (7.1)	0.050	0.024	
PAS	70 (32.4)	35 (28.0)	14 (37.8)	119 (31.5)	0.489	1.000	
Pre-XDR	37 (17.1)	21 (16.8)	20 (54.1)	78 (20.6)	<0.001	<0.001	**1/3**
XDR	20 (9.3)	18 (14.4)	9 (24.3)	47 (12.4)	0.031	0.010	

Mok JH et al. J Korean Med Sci 2017;32:636

Case 1 M/45. PTB (New case)

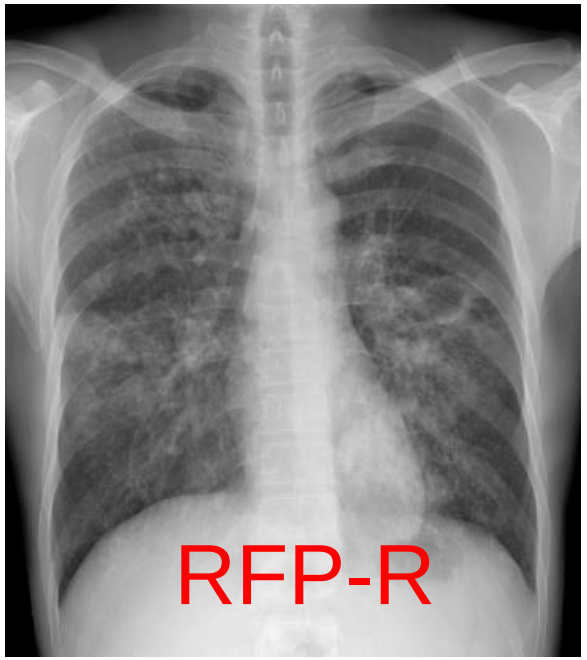


- **적절한 처방은?**
  1. HREZ
  2. HEZ + FQ
  3. HEZ + FQ + KM
  4. H + MDR regimen
  5. MDR regimen

신속내성검사에서 리팜핀 내성 유전자 변이가 확인되면 전통적 약제감수성 검사 결과가 나오기 전까지 다제내성결핵 권고 처방으로 치료한다(IIIA).

- 결핵진료지침 2017 -

Case 1 M/45. PTB (New case)



- MDR 처방으로 치료 중 pDST가 모든 약제에 감수성으로 보고되었다. 조치는?

1. HREZ
2. MDR 처방 지속
3. High dose RIF
4. rpoB sequencing

Rifampin Resistance : Interpretation

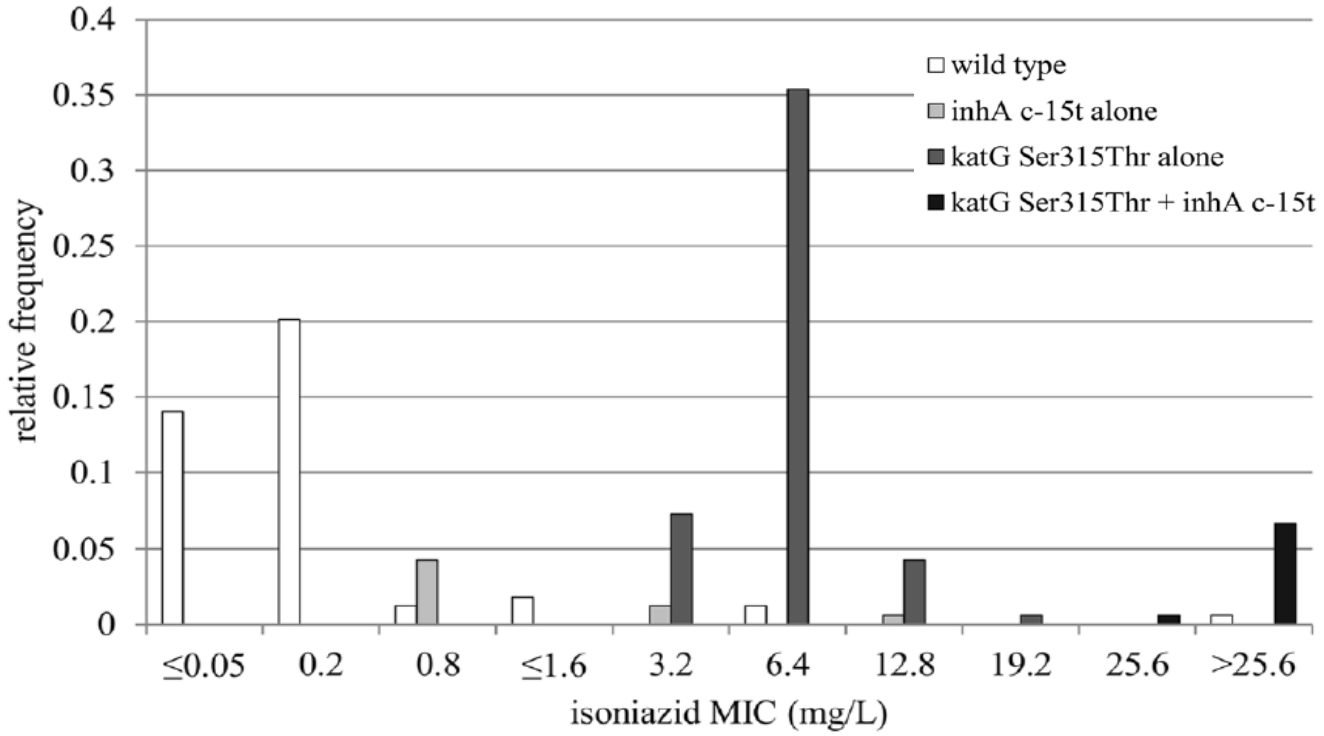
Xpert	LPA	pDST	frequency	Interpretation
S	S	S		Probably RIF susceptible
R	R	R	> 90%	Probably High level RIF-R
R	R	S	< 10%	Disputed mutation (Low level RIF-R) Silent mutation
S	S	R	< 5-10%	Mutation outside the RRDR

rpoB sequencing

Scenario	Interpretation	Management
514phe	Silent mutation	1st line therapy
533pro	Disputed mutation	MIC & TDM High dose RIF MDR regimen

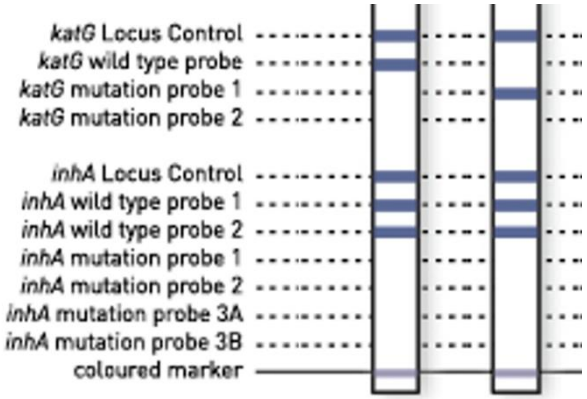
2. Isoniazid: Phenotype vs Genotype

mutation	Freq
katG	≈ 65%
inhA	≈ 20%
Others	≈ 15%
ahpC, fabG1, kasA, ndh, furA, nshA	



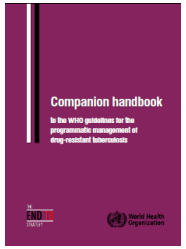
Seifert M et al. PLoS One 2015;10:e0119628
Lempens P et al. Sci Rep 2018;8:3246

Isoniazid Resistance : Interpretation



GenoType MTBDR*plus*

Mutation	Interpertation
katG	INH : High level R, avoid INH Pto : not affect
inhA	INH : low level R, may consider $\mathbf{H}_{\textbf{high dose}}$ Pto : consider cross-resistance
katG+inhA	High level R
No mutation	≠ INH susceptible



If the *inhA* gene mutation is present, ethionamide/prothionamide can still be included in an MDR regimen, but it should not be counted as a “likely effective second-line anti-TB drug”.

3. Fluoroquinolone: Phenotype vs Genotype

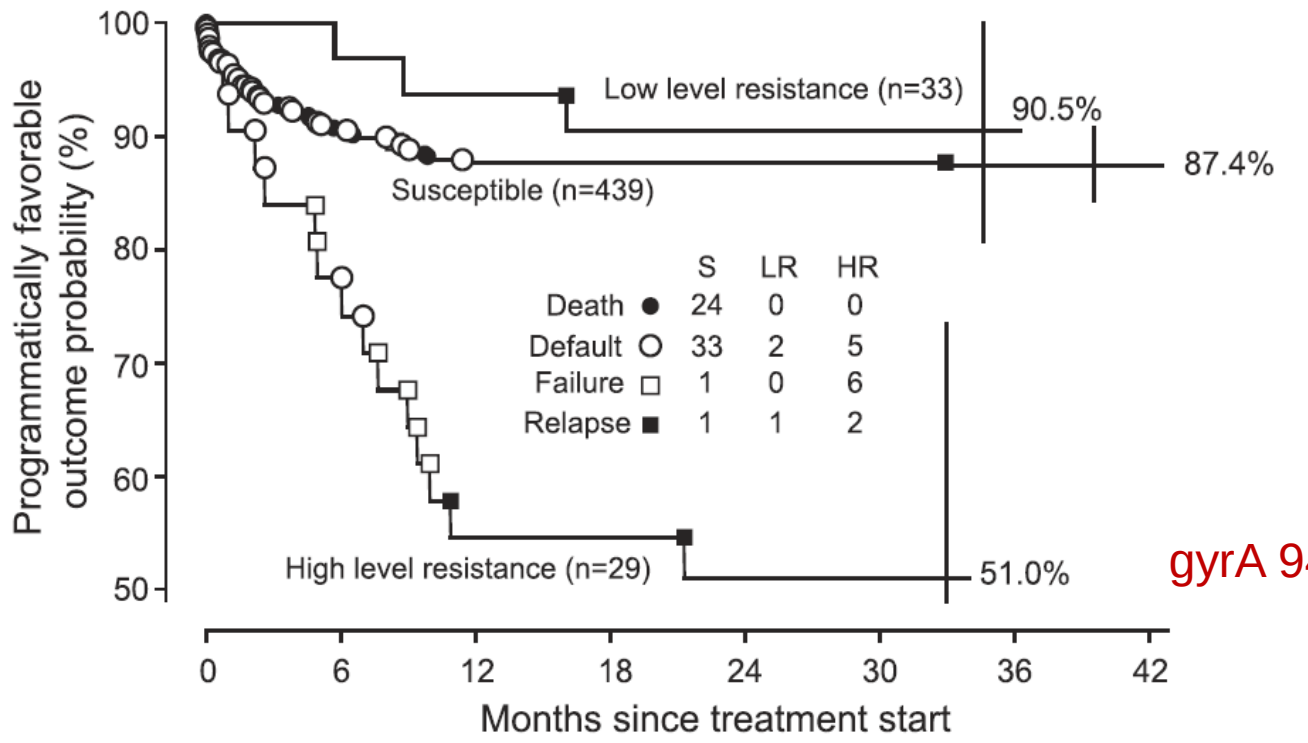
DST			
INH	**R**	OFX	**R**
RIF	**R**	LFX	**S**
EMB	**R**	MFX	**S**
PZA	S	PTO	S
SM	S	CS	S
KM	S	PAS	S

Lfx or Mfx?
Usual or higher dose?

- Cross R between FQs
- Low level FQs resistance
- Optimal dose of FQs

Specific *gyrA* gene mutations predict poor treatment outcome in MDR-TB

L. Rigouts[1,2]*, N. Coeck[1,2], M. Gumusboga[1], W. B. de Rijk[1], K. J. M. Aung[3], M. A. Hossain[3], K. Fissette[1], H. L. Rieder[4], C. J. Meehan[1], B. C. de Jong[1,5,6] and A. Van Deun[1,7]



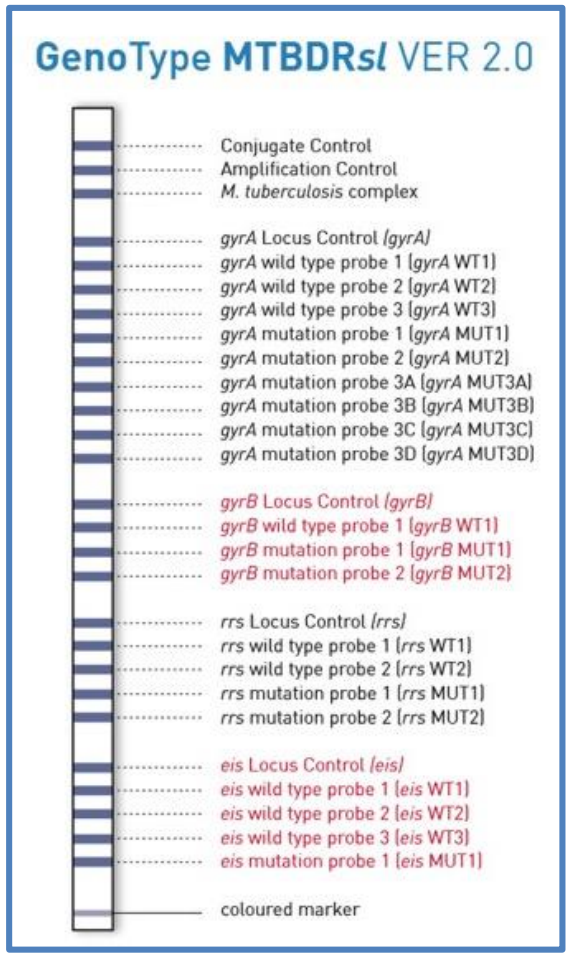
Bangladeshi regimen

High dose Gfx 800mg

gyrA 94 mutation, except 94Ala

J Antimicrob Chemother 2016;71:314

2nd LPA : Guide to the correct regimen



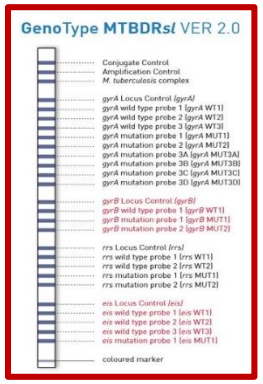
Failing WT band	Developing mutation band	Mutation			Final interpretation for clinicians
			OFX-LFX	MFX	
gyrA WT1	-	G88A	High	Indet	No OFX-LFX use, not informative for MFX
	-	G88C	High	High[#]	No OFX-LFX use, not informative for MFX
gyrA WT2	gyrA MUT1	A90V	High	High	No OFX-LFX use, higher dosage of MFX?
	gyrA MUT2	S91P	High	High	No OFX-LFX use, higher dosage of MFX?
gyrA WT3	gyrA MUT3A	D94A	High	High	No OFX-LFX use, higher dosage of MFX?
	gyrA MUT3B	D94N	High	High	No use of fluoroquinolones
	-	D94Y	High	High	No OFX-LFX use, higher dosage of MFX?
	gyrA MUT3C	D94G	High	High	No use of fluoroquinolones
	gyrA MUT3D	D94H	High	Indet	No OFX-LFX use, not informative for MFX
gyrB WT	gyrB MUT1	N538D[¶]	Indet	Indet	Not informative
	gyrB MUT2	E540V[+]	Indet	Indet	Not informative

Cabibbe AM et al. Eur Respir J 2017;50:1702292

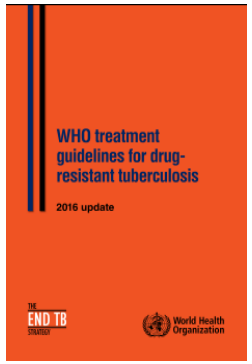
Limitation of current genotypic DST

- Not all mechanisms of resistance are known
  - Lack of mutation ≠ susceptibility
  - Unknown clinical significance for some mutation
  - Commercially available for few drugs and often only hot spots
- Not all mutations are associated with p-resistance
  - Silent mutation
- Not every genetic mutation affects resistance equally
  - Low / borderline / high level resistance
- All tests have limit of detection

Genotypic DST in MDR-TB management



Second-line LPA

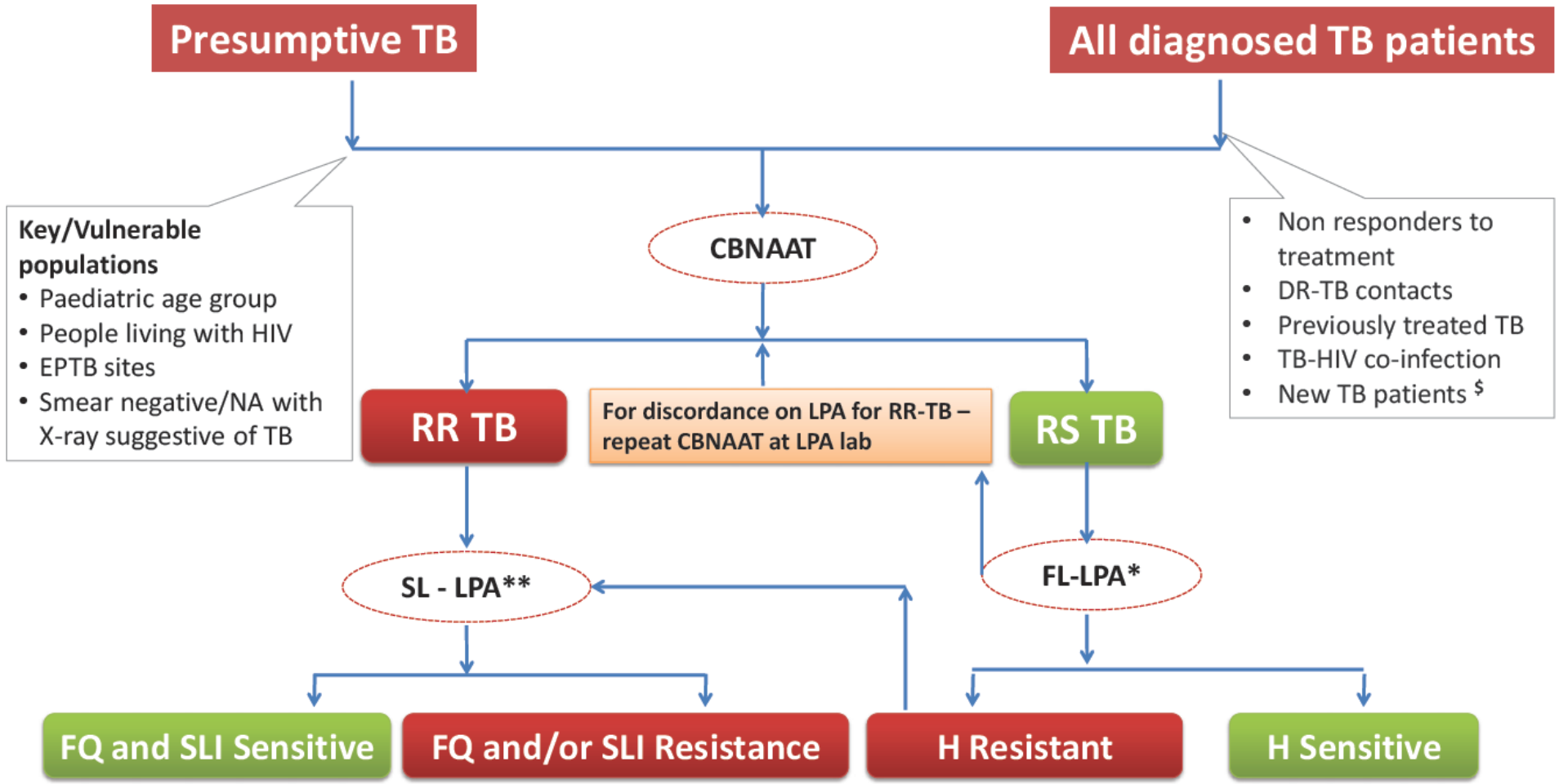


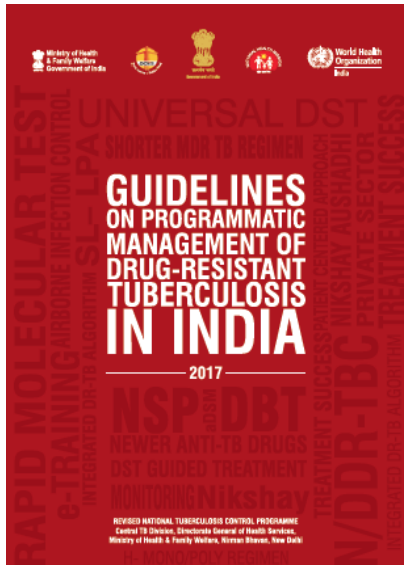
NEW

Shorter Regimen

Longer Regimen

New Regimen


Presumptive TB
All diagnosed TB patients
Key/Vulnerable populations
• Paediatric age group
• People living with HIV
• EPTB sites
• Smear negative/NA with X-ray suggestive of TB
CBNAAT
• Non responders to treatment
• DR-TB contacts
• Previously treated TB
• TB-HIV co-infection
• New TB patients $
RR TB
For discordance on LPA for RR-TB – repeat CBNAAT at LPA lab
RS TB
SL - LPA**
FL-LPA*
FQ and SLI Sensitive
FQ and/or SLI Resistance
H Resistant
H Sensitive


GUIDELINES
ON PROGRAMMATIC
MANAGEMENT OF
DRUG-RESISTANT
TUBERCULOSIS
IN INDIA
2017

MIC vs Mutation vs PK vs Clinical outcome

MIC, Critical concentration, Clinical break point

Genotype vs phenotype

In vitro ≠ in vivo

Xpert. LPA, sequencing, WGS

Mutation

Silent, disputed mutation

Heteroresistance

Low level vs high level resistance

Compensatory mutation
Epistasis

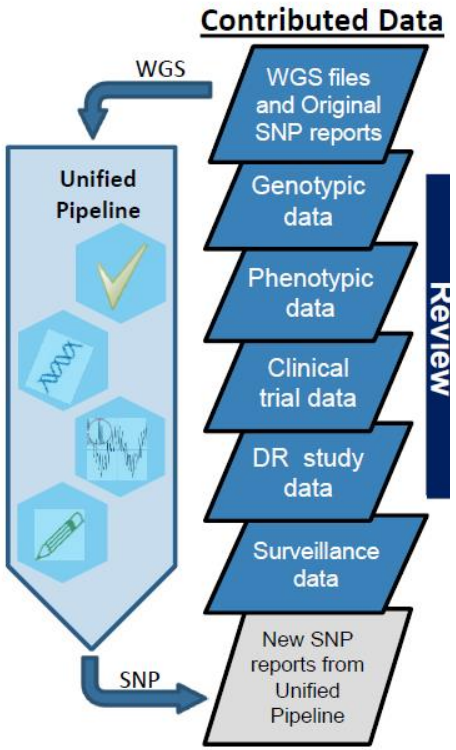
Cross-resistance

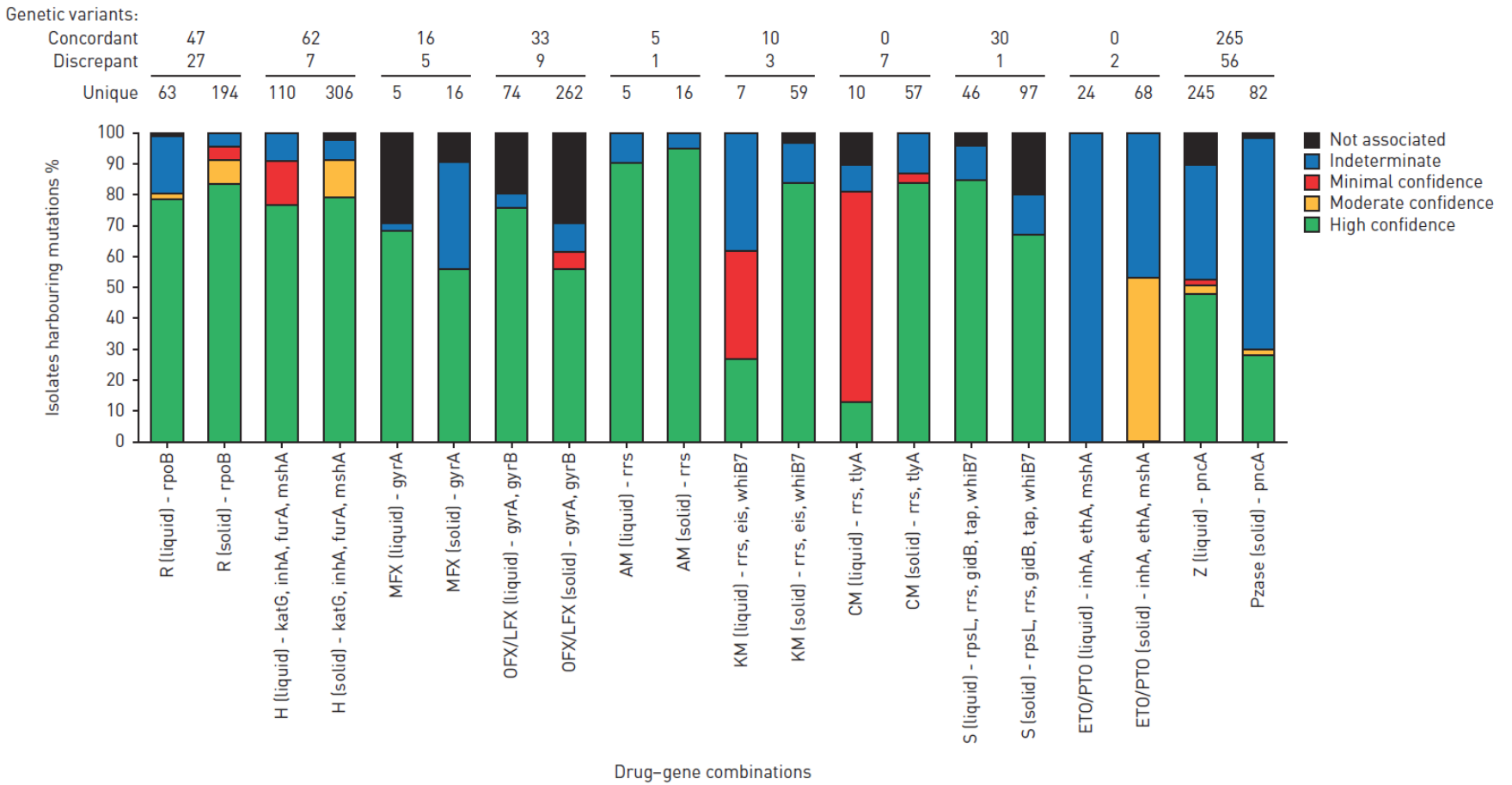
Integrated diagnostic algorithm

Contents

The ReSeqTB Solution: A Standardized System for Grading Mutations

ReSeqTB
Relational Sequencing TB Data Platform





Miotto P, et al. Eur Respir J 2017;50:1701354

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

The CRyPTIC Consortium and the 100,000 Genomes Project

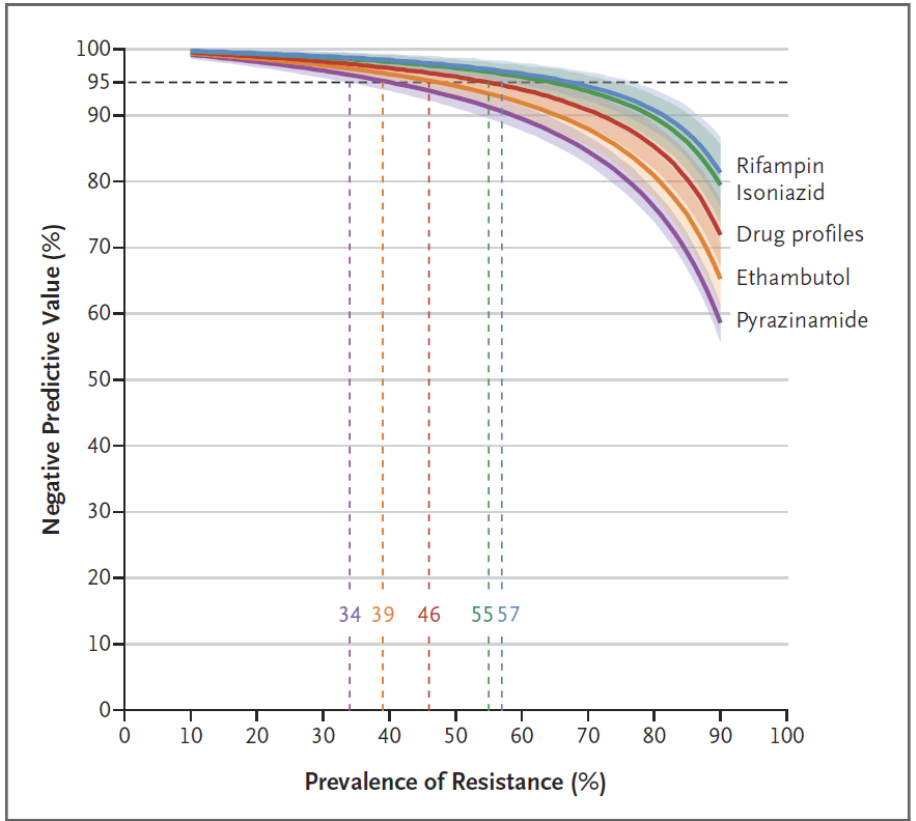
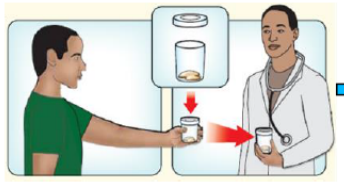


Figure 1. Simulated Negative Predictive Values for Individual Drugs and Complete Drug Profiles.

N Engl J Med 2018;379:1403

Comprehensive Resistance Prediction for Tuberculosis

Personalized Medicine with Genomic Bic Data



Sputum collection

0-1 day

Whole Genome Sequencing

Minutes

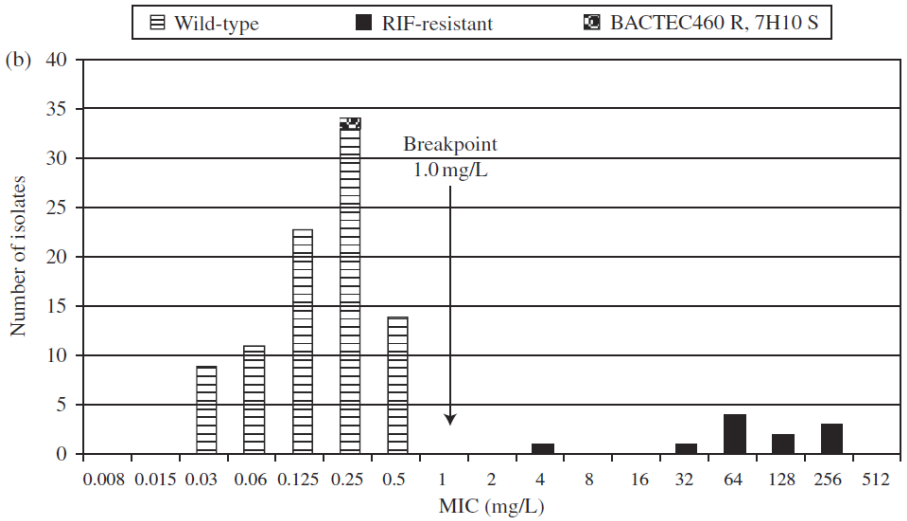
Drug Resistance:

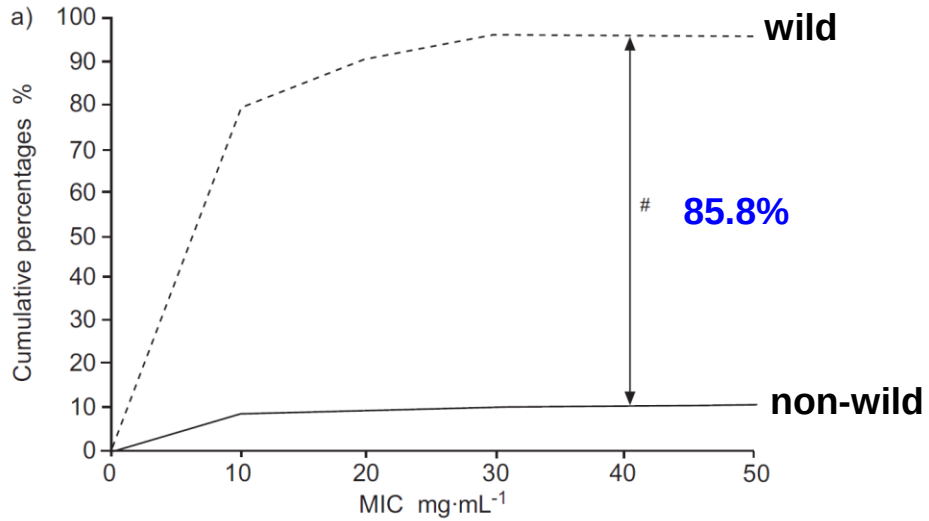
Rifampicin=R
Isoniazid=R
Pyrazinamide=S
Streptomycin=S
Ethambutol=R
Amikacin=S
Capreomycin=S
Kanamycin=S
Ofloxacin=R
Moxifloxacin=S
Ethionamide=R
Bedaquiline=S
Linezolid=S
Cycloserine=S

0-1 day

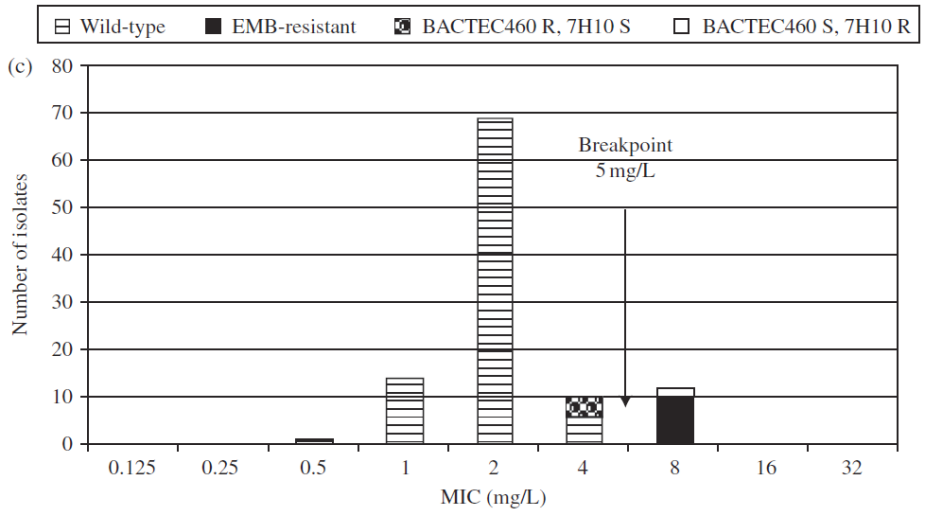
Treatment started

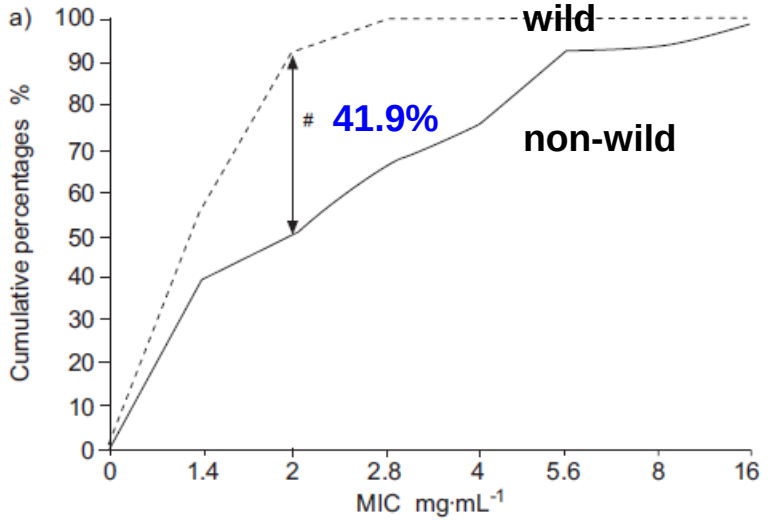
Rifampicin





Ethambutol





Schon T et al. J Antimicrob Chemother 2009;64:786, Kim SJ. Eur Respir J 2005;25:564.