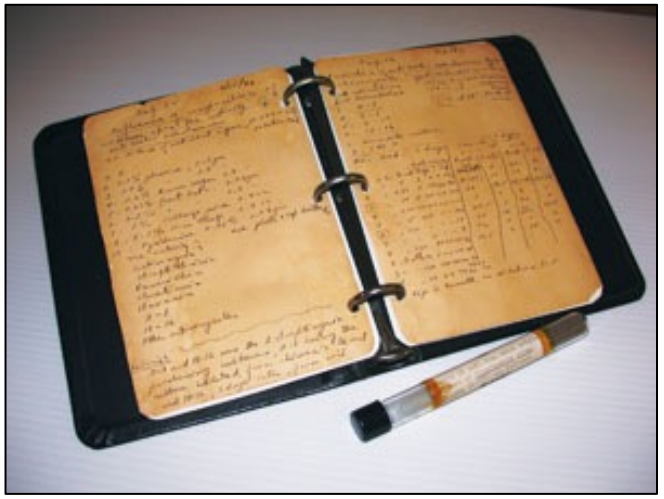
DRUG RESISTANCE IN TB

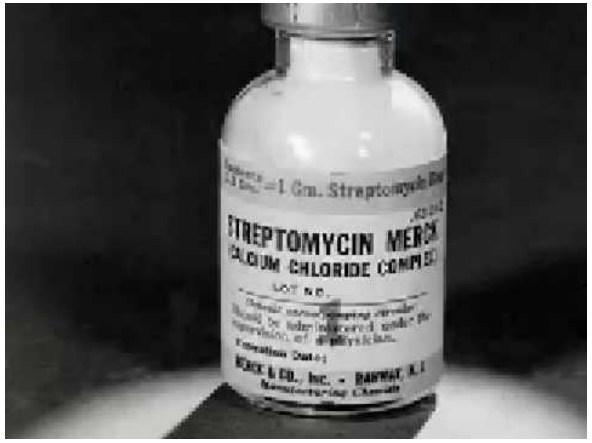
Megan Murray, MD, MPH, ScD
Harvard Medical School
Brigham and Women's Hospital
Harvard School of Public Heath

Dr. Murray has no financial relationships with commercial entities to disclose.

This disease isn't dangerous at my age, and they say the cure is going on quite well, though slowly... We are now sending for some new American drug called streptomycin which they say will speed up the cure.

George Orwell, Hairmyres Hospital, Scotland, February 1948 - Letter to F.J. Warburg




STREPTOMYCIN MERCK
CALCIUM CHLORIDE COMPLEX

BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 30 1948

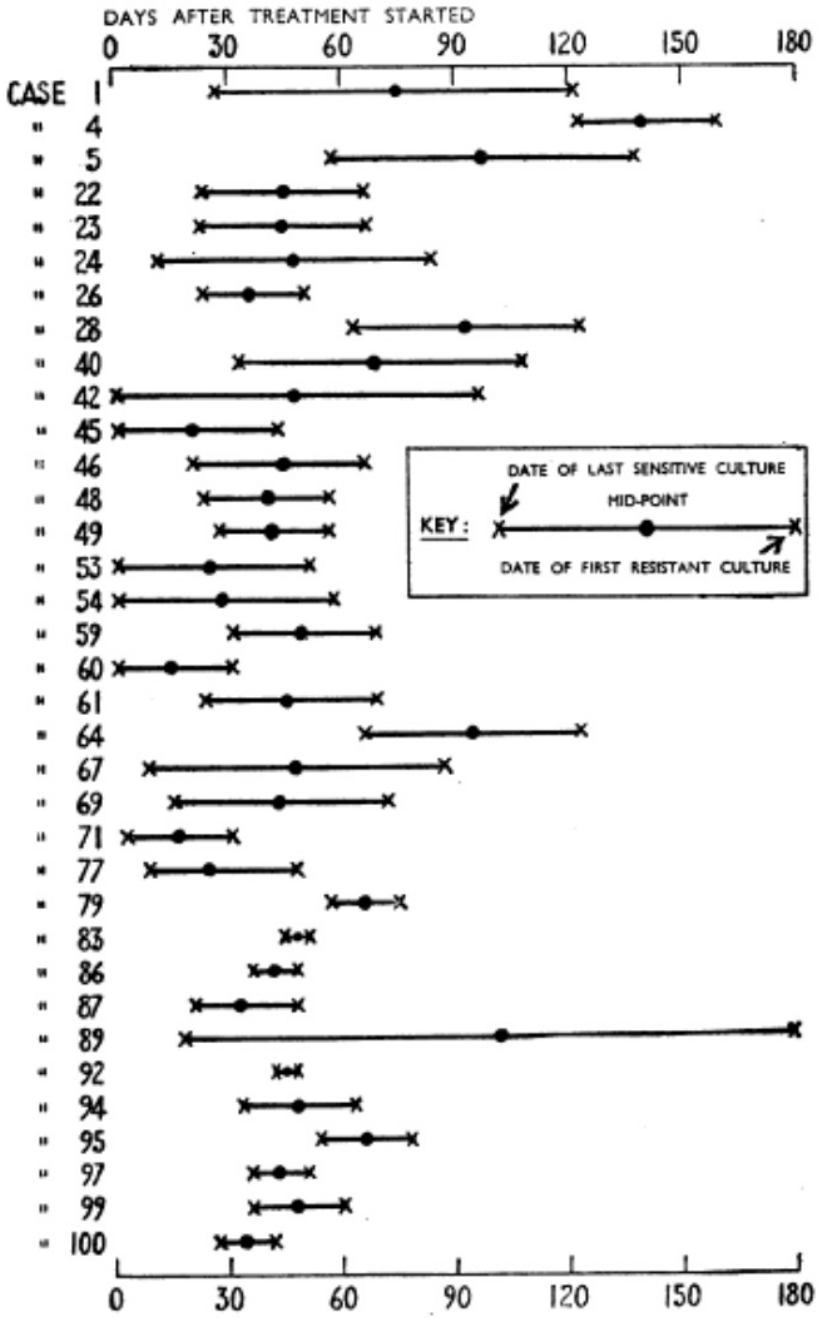
STREPTOMYCIN TREATMENT OF PULMONARY TUBERCULOSIS

A MEDICAL RESEARCH COUNCIL INVESTIGATION

The following gives the short-term results of a controlled investigation into the effects of streptomycin on one

The Control Scheme

Determination of whether a patient would be treated by streptomycin and bed-rest (S case) or by bed-rest alone (C case) was made by reference to a statistical series based on random sampling numbers drawn up for each sex at each centre by Professor Bradford Hill ; the details of the series were unknown to any of the investigators or to the co-ordinator and were contained in a set of sealed envelopes, each bearing on the outside only the name of the hospital and a number. After acceptance of a patient by the panel, and before admission to the streptomycin centre, the appropriate numbered envelope was opened at the central office ; the card inside told if the patient was to be an S or a C case, and this information was then given to the medical officer of the centre.



Despite initial response, large proportion of cases developed resistance, some within very short period after initiatiing treatment.


GEORGE ORWELL
WRITER 1903··1950
LIVED AND WORKED IN
A BOOKSHOP ON THIS SITE
1934··1935..


1944
• Streptomycin used
1950
• PAS, STR combination therapy
1952
• INH, PAS, STR
1957
• Pyrazinamide
1961
• Ethambutol
1960s
• Rifampicin
1990
• Standard short course oral therapy
• Rif, INH, PZA and Ethambutol
2000s
• Bedaquiline and Delaminid discovered

TB: BASIC BIOLOGY

Phylogeny of bacteria

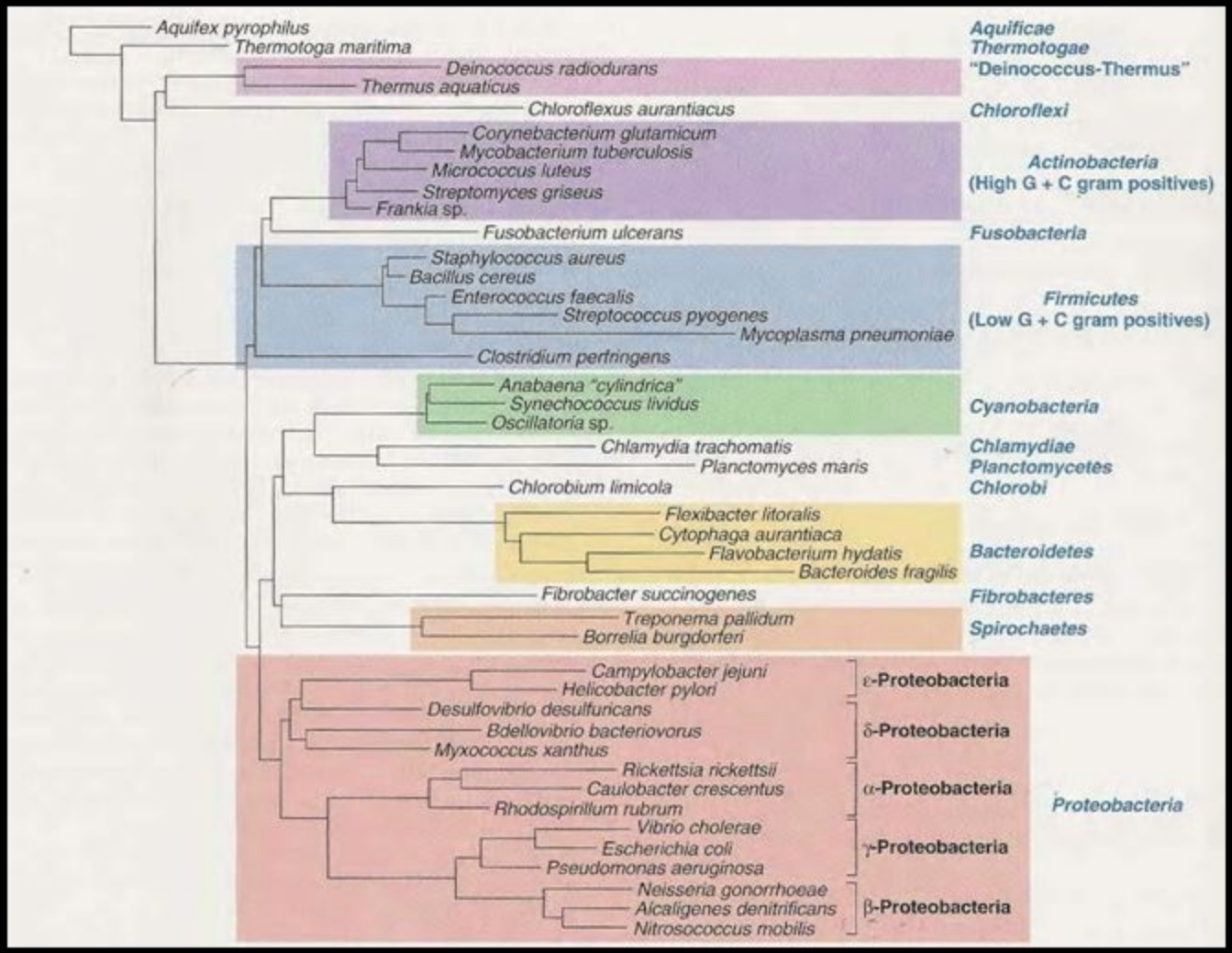


Figure 11. Phylogeny of Bacteria. The tree is based on 16S rRNA comparisons. From Prescott *et al.*, 2005.

Nature **393**, 537-544 (11 June 1998) | doi:10.1038/31159; Received 15 April 1998; Accepted 8 May 1998

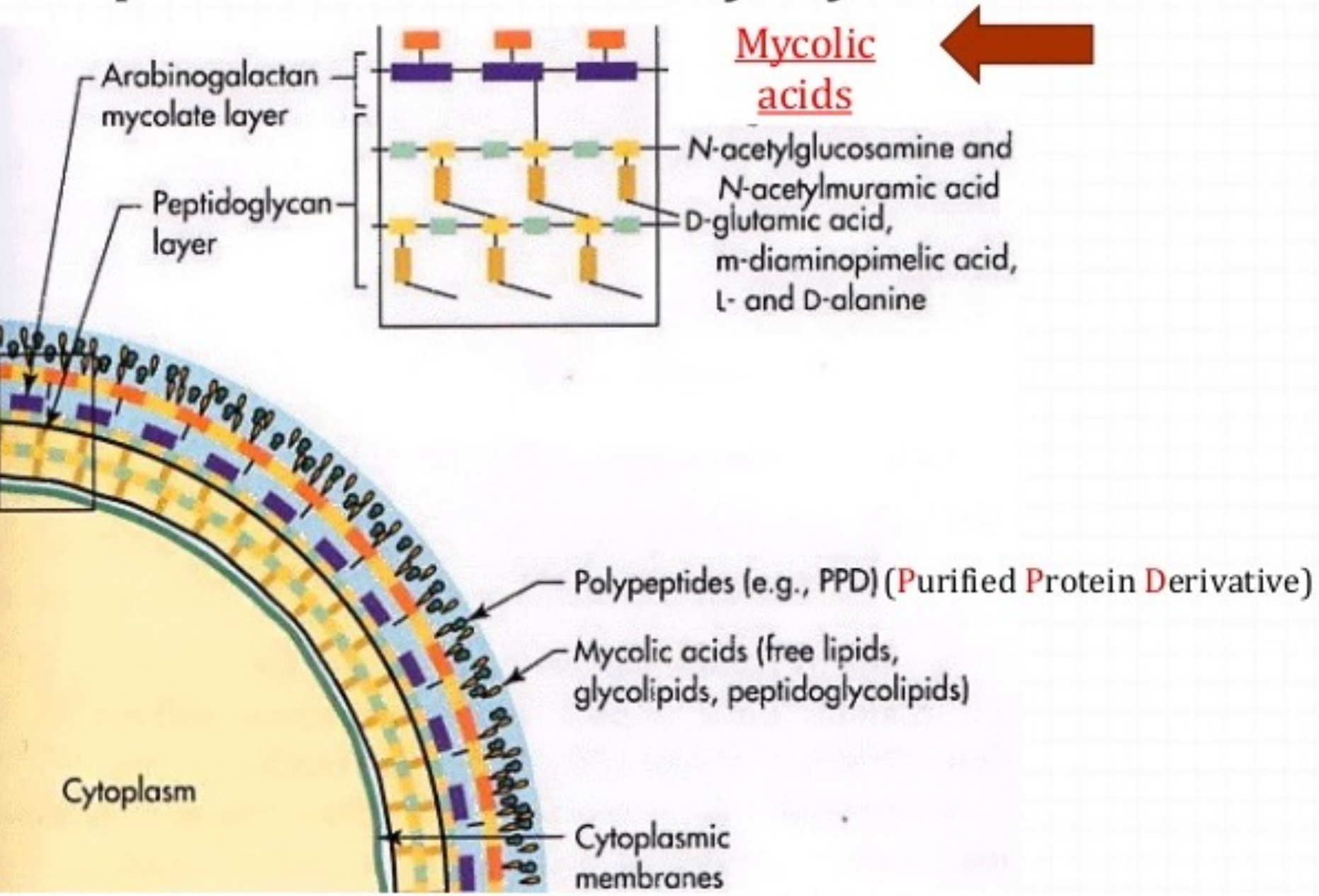
Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence

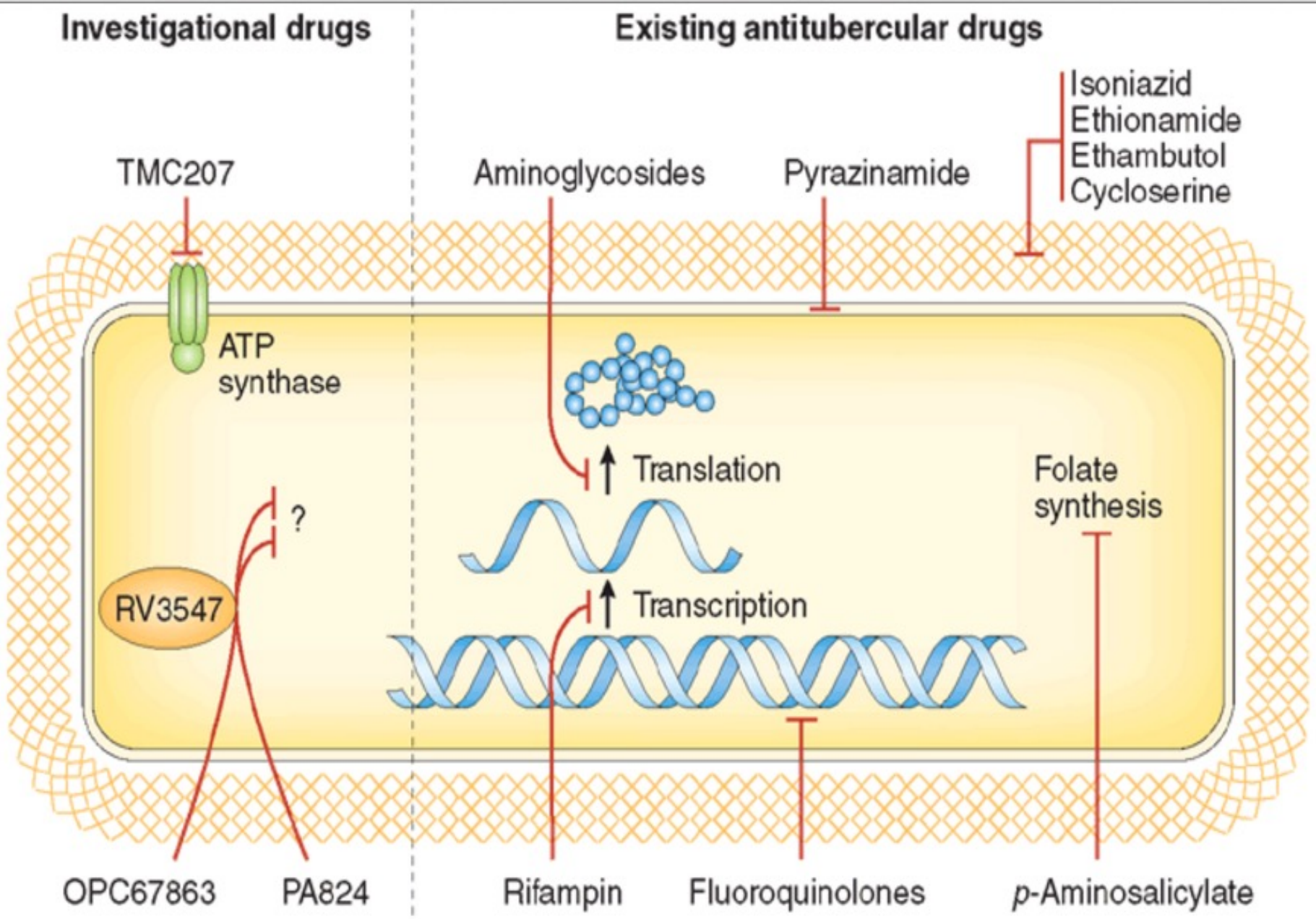
S. T. Cole[2], R. Brosch[2], J. Parkhill[1], T. Garnier[2], C. Churcher[1], D. Harris[1], S. V. Gordon[2], K. Eiglmeier[2], S. Gas[2], C. E. Barry, III[3], F. Tekaia[4], K. Badcock[1], D. Basham[1], D. Brown[1], T. Chillingworth[1], R. Connor[1], R. Davies[1], K. Devlin[1], T. Feltwell[1], S. Gentles[1], N. Hamlin[1], S. Holroyd[1], T. Hornsby[1], K. Jagels[1], A. Krogh[5], J. McLean[1], S. Moule[1], L. Murphy[1], K. Oliver[1], J. Osborne[1], M. A. Quail[1], M.-A. Rajandream[1], J. Rogers[1], S. Rutter[1], K. Seeger[1], J. Skelton[1], R. Squares[1], S. Squares[1], J. E. Sulston[1], K. Taylor[1], S. Whitehead[1] & B. G. Barrell[1]

▲ Top

Countless millions of people have died from tuberculosis, a chronic infectious disease caused by the tubercle bacillus. The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis*, H37Rv, has been determined and analysed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions. The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.

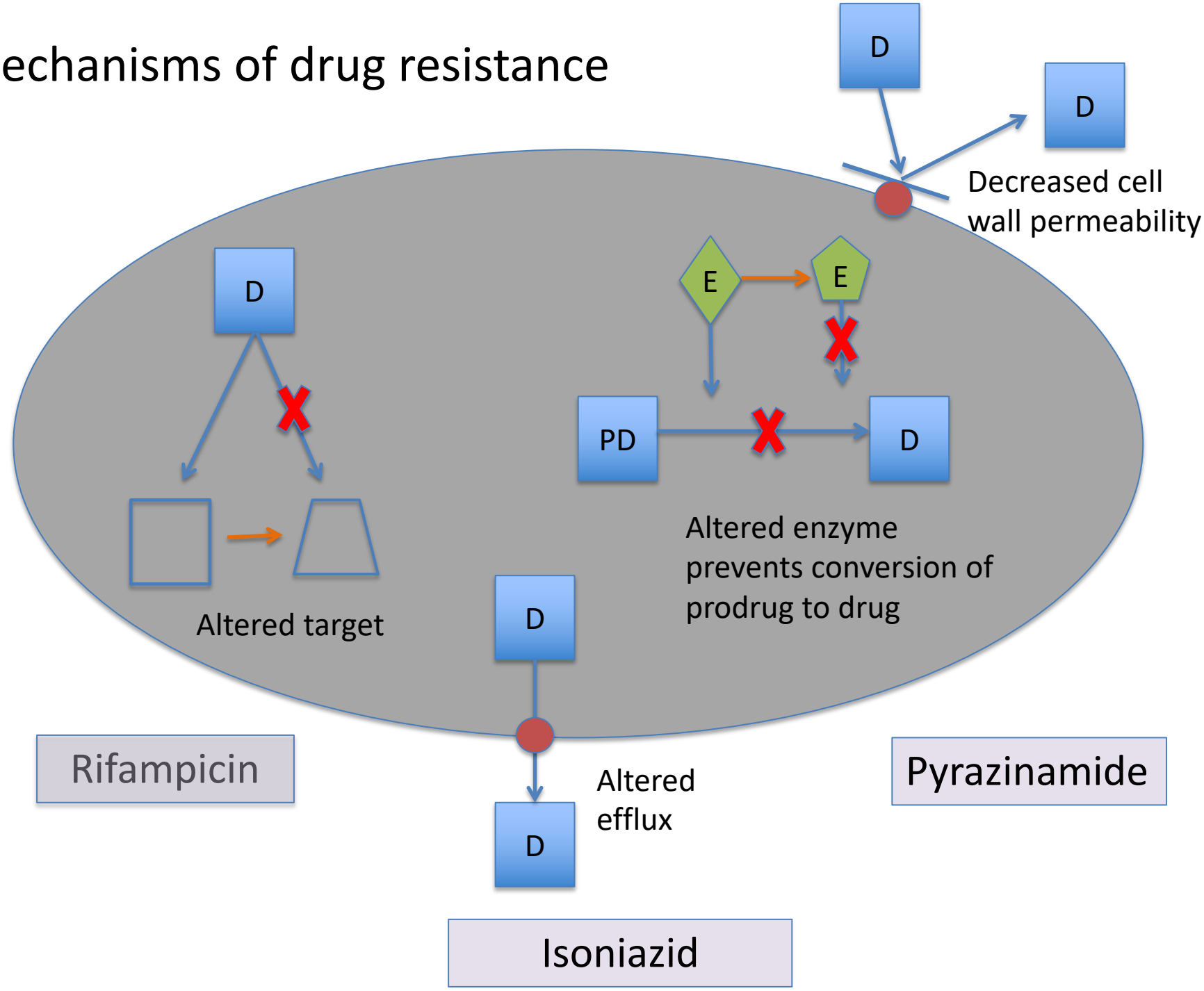
Lipid-Rich Cell Wall of Mycobacterium




Investigational drugs
Existing antitubercular drugs
Isoniazid
Ethionamide
Ethambutol
Cycloserine
TMC207
Aminoglycosides
Pyrazinamide
ATP synthase
Translation
Folate synthesis
?
RV3547
Transcription
OPC67863
PA824
Rifampin
Fluoroquinolones
p-Aminosalicylate

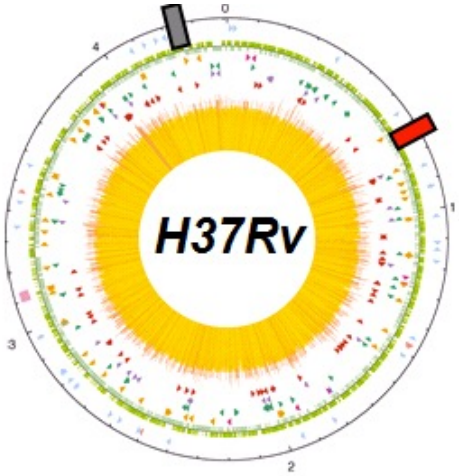
Mechanisms of drug resistance

Mechanisms of drug resistance



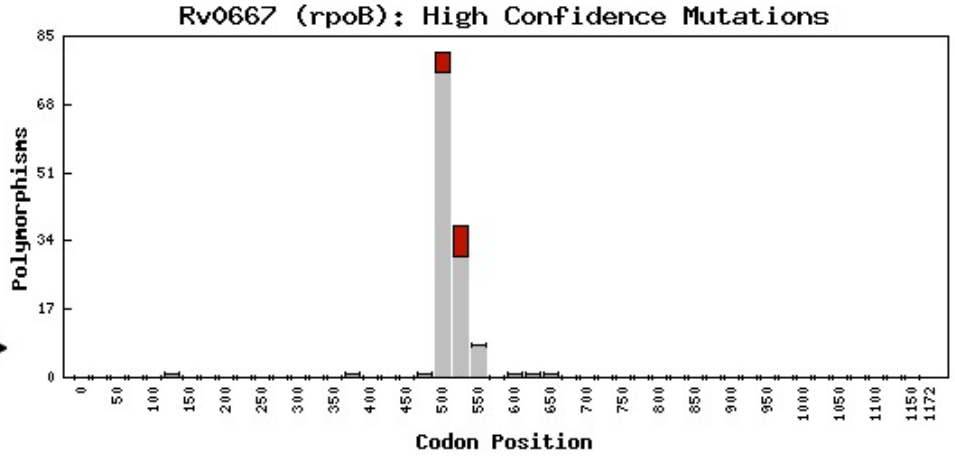
TB Drug Resistance Mutation Database

Rifampicin



Rv0667

NO Promoter Mutations



More about Rv0667 on TBDB.org

More about Rv0667 on TubercuList

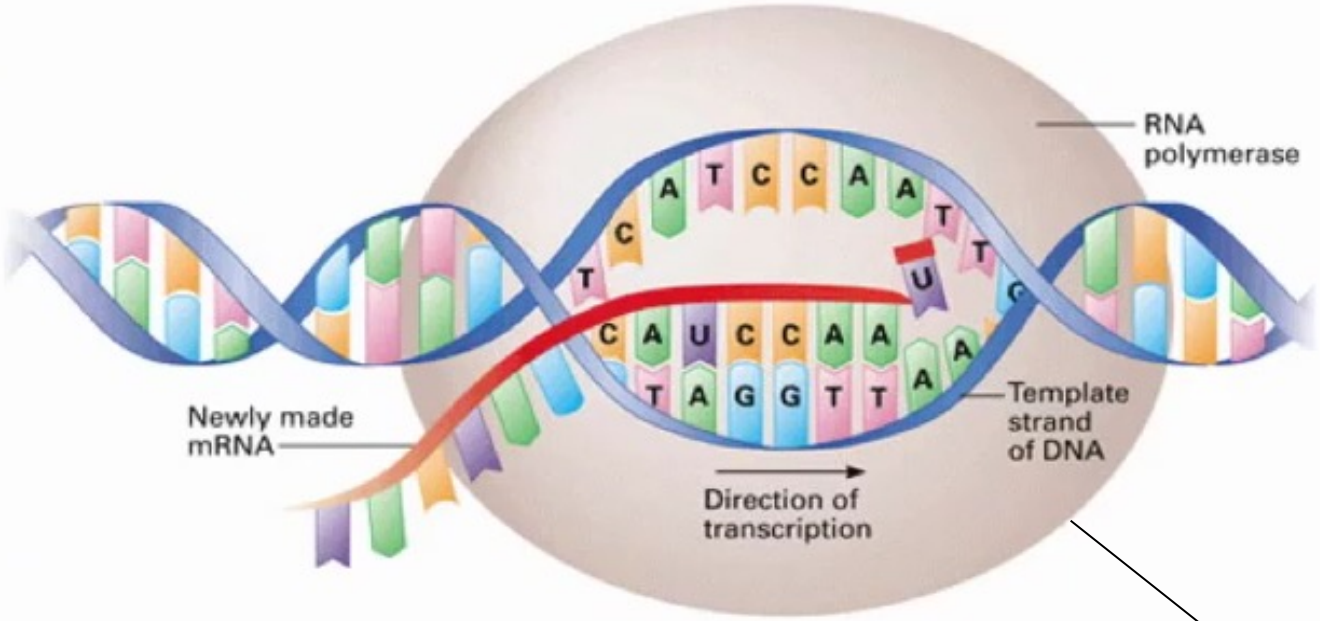
Display all mutations or select a bar from the histogram above to examine in greater detail

Return to gene list

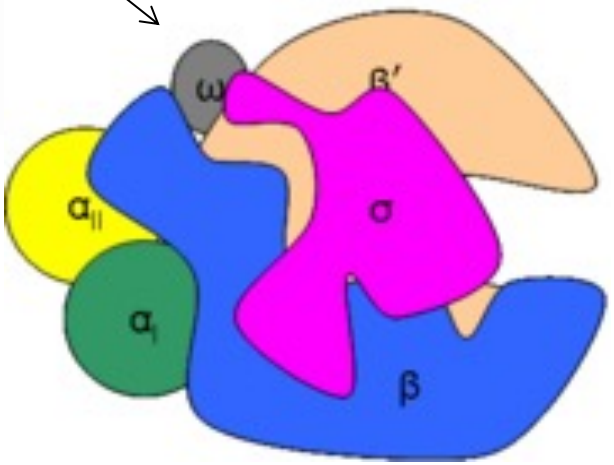
Display high confidence mutations only

Rv0667: high confidence mutations (high confidence mutations are highlighted in yellow)

Primary reference	Polymorphism	Nucleotide position	Codon position	Amino acid	Time period	Origin	Molecular detection method	Gene coverage	Resistance pattern	MIC	Susceptibility testing method	R/Total isolates	R/S isolates with mutation	Additional mutations	High confidence support
Kapur V JCM 1994	CTG/CCG	-	511	Leu/Pro	Published 1994	USA	Sequencing	Whole gene	R	N.S.	Proportion method, BACTEC	121/128	4/0	"One of the strains was also Asp441Glu; another was Asp441Tyr; the third was also Ser437Thr."	
Kapur V JCM 1994	CAA/CTA	-	513	Gln/Leu	Published 1994	USA	Sequencing	Whole gene	R	N.S.	Proportion method, BACTEC	121/128	2/0	None described in the region sequenced.	

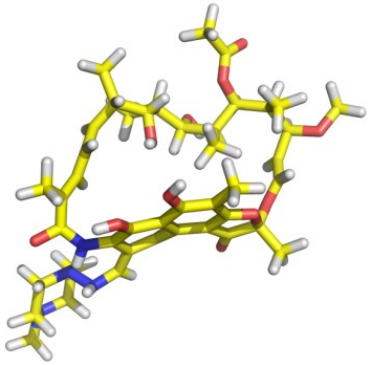


The beta subunit of RNAP is the target of rifampicin.



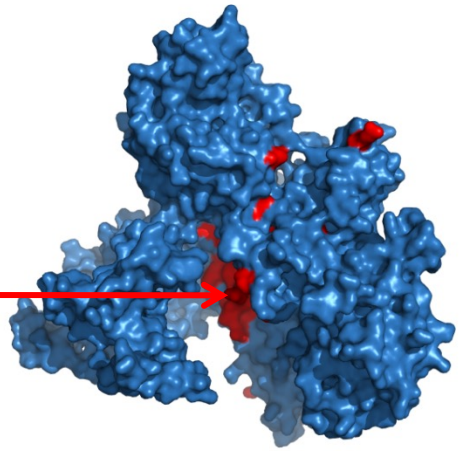
rpoB

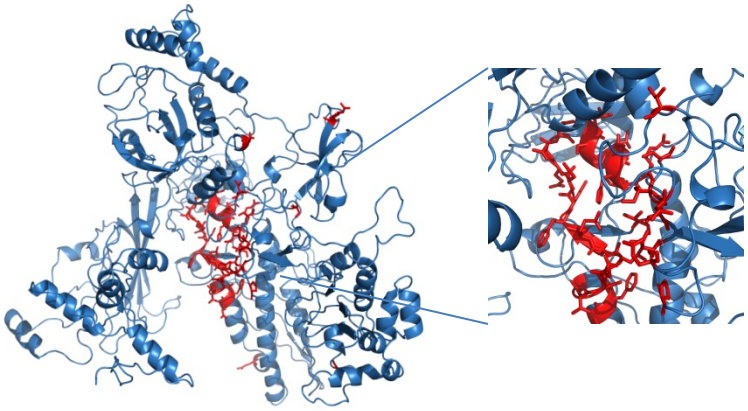
Mechanisms of Rifampin Resistance



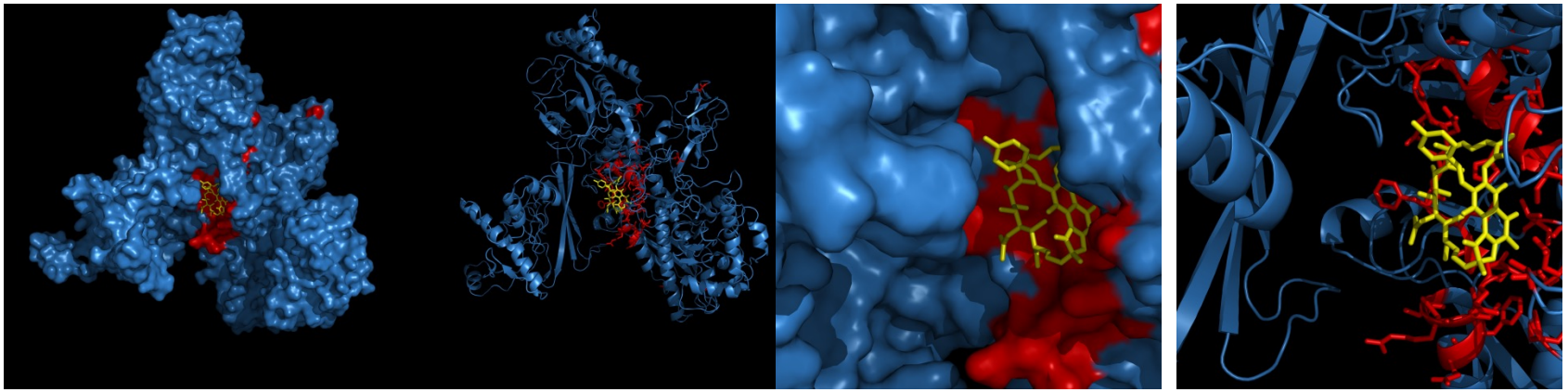
Rifampin

Rifampin Binding Site



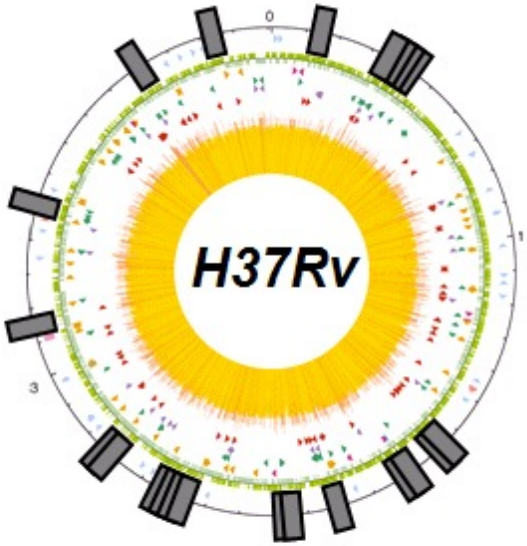


rpoB homology model – target of Rifampin



rpoB homolog from *Thermus aquaticus* with **rifampicin** bound (crystal structure)

TB Drug Resistance Mutation Database

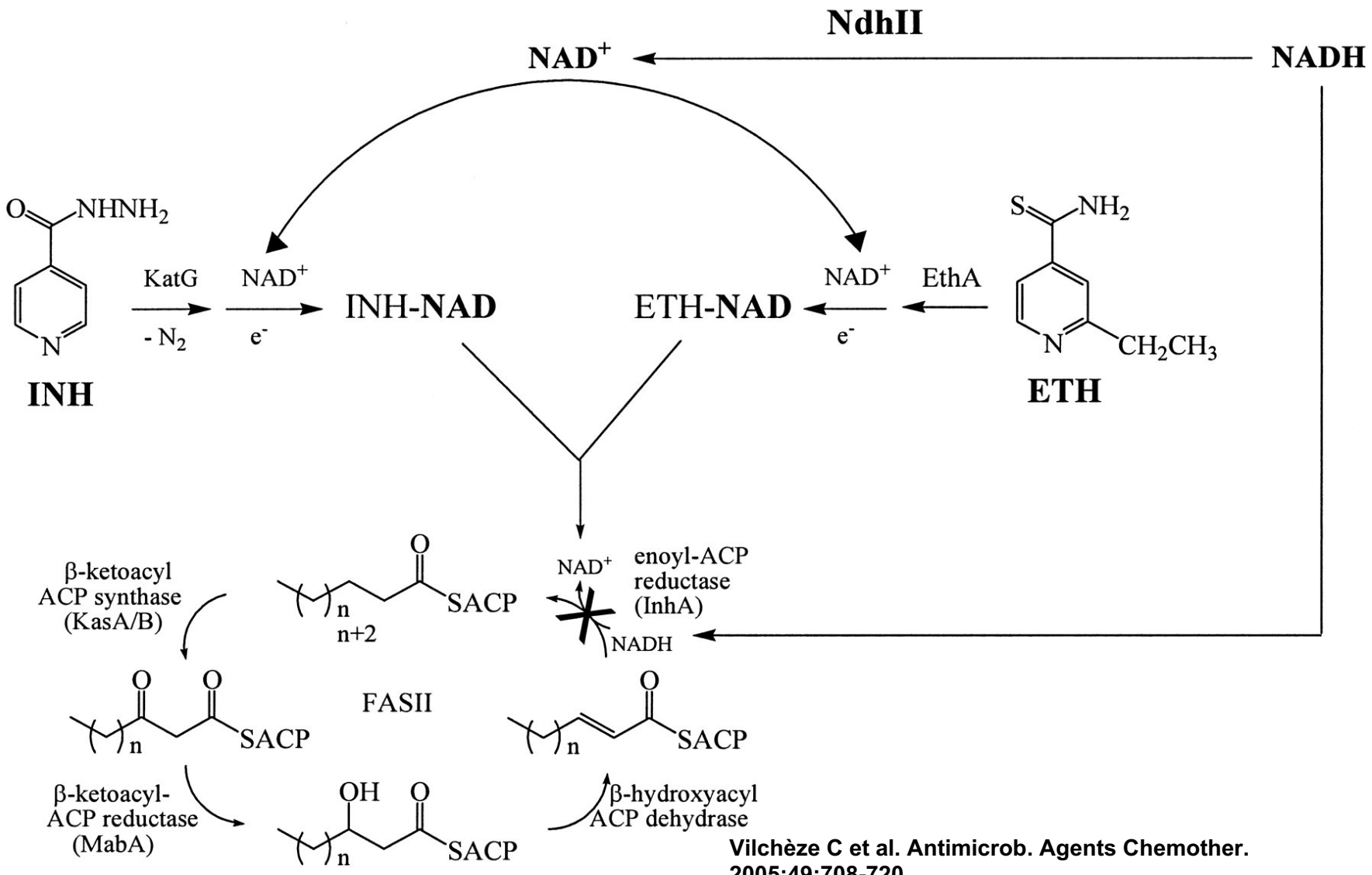


Isoniazid

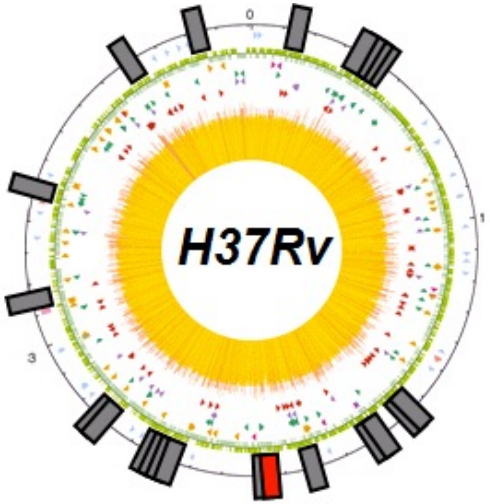
Select gene(s) associated with drug resistance by clicking on the genome or from the list below:

- Rv0129c (fbpC, fbpC2, 85C, mpt45)
- Rv0340 | Rv0341 (iniB) | Rv0342 (iniA) | Rv0343 (iniC) | Rv3795 (embB)
- Rv1483 (mabA, fabG1) | Rv1484 (inhA)
- Rv1592c
- Rv1772
- Rv1854c (ndh)
- Rv1908c (katG) | Rv1909c (furA)
- Rv2242 (srmR homolog) | Rv2243 (fabD, mtFabD) | Rv2245 (kasA) | Rv2247 (accD6)
- Rv2427A (oxyR') | Rv2428 (ahpC)
- Rv2846c (efpA)
- Rv3139 (fadE24)
- Rv3566c (nhoA, nat)
- Rv3795 (embB)

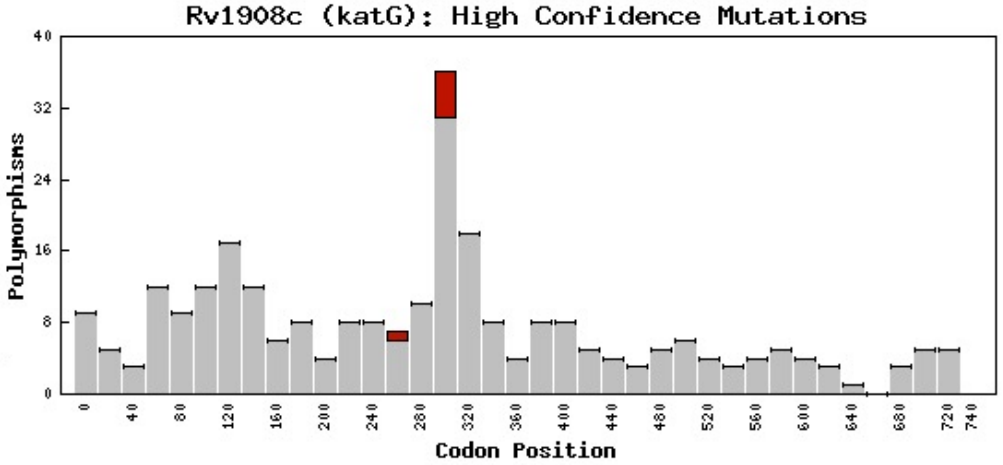
Proposed mechanism of action of KatG
INH is a prodrug activated by the catalase-peroxidase KatG



Vilchèze C et al. Antimicrob. Agents Chemother. 2005;49:708-720



Rv1908c



high confidence mutation | selected high confidence mutation
any mutation | selected mutation

More about Rv1908c on [TBDB.org]

More about Rv1908c on [TuberculList]

[Display all mutations] **or select a bar from the histogram above to examine in greater detail**

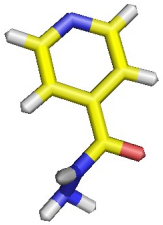
[Return to gene list]

Display high confidence mutations **only**

Rv1908c: high confidence mutations (high confidence mutations are highlighted in yellow)

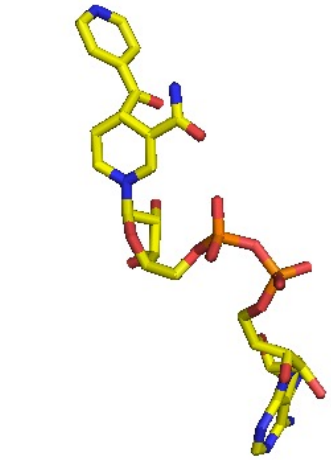
Primary reference	Polymorphism	Nucleotide position	Codon position	Amino acid	Time period	Origin	Molecular detection method	Gene coverage	Resistance pattern	MIC	Susceptibility testing method	R/Total isolates	R/S isolates with mutation	Additional mutations	High confidence support
Morlock GP AAC 2003	GGC/GAC	836	279	Gly/Asp	2003	Brazil	Sequencing	fragment encompassing codons 249 to 342	HEth	>32 I, >200 Eth	Microplate alamar blue assay	17/41	1/0	D55/A ethA,L44L inhA ORF, C(-15)T inhA regulator	
Haas WA AAC 1997	AGC/ATC	944	315	Ser/Ile	1997	Sierra Leone, Lesotho	PCR-RFLP	892 bp central fragment	HRSEth,H	-	-	124/212	4/0	-	

Activation and Binding of Isoniazid Derivatives

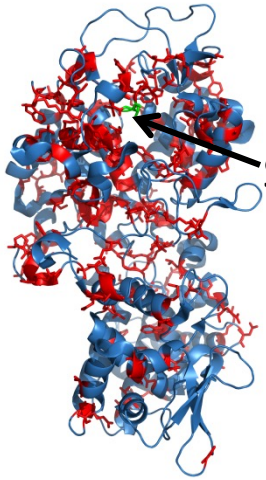


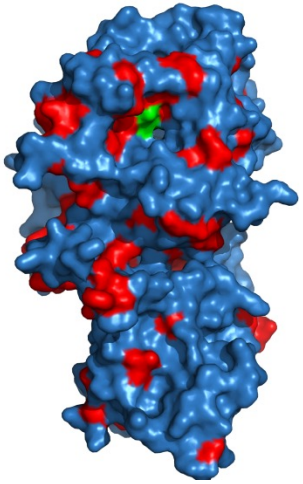
Isoniazid

Activation

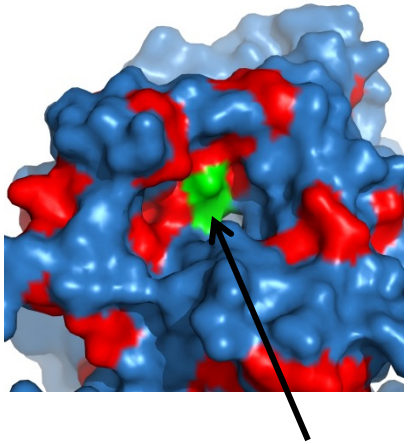


Isoniazid-NADH

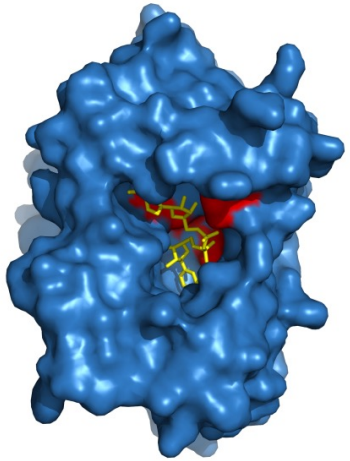


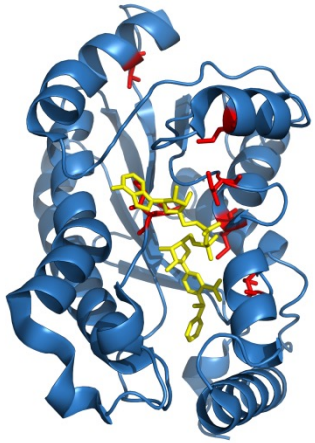


Activation of Isoniazid by KatG

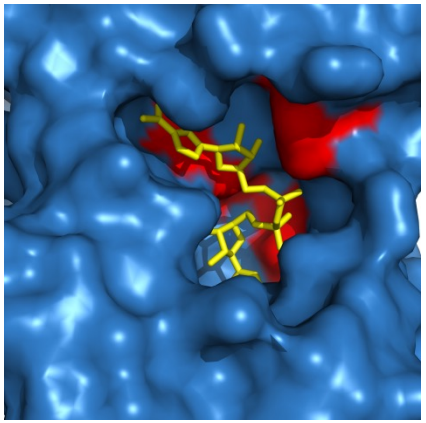


Codon S315T

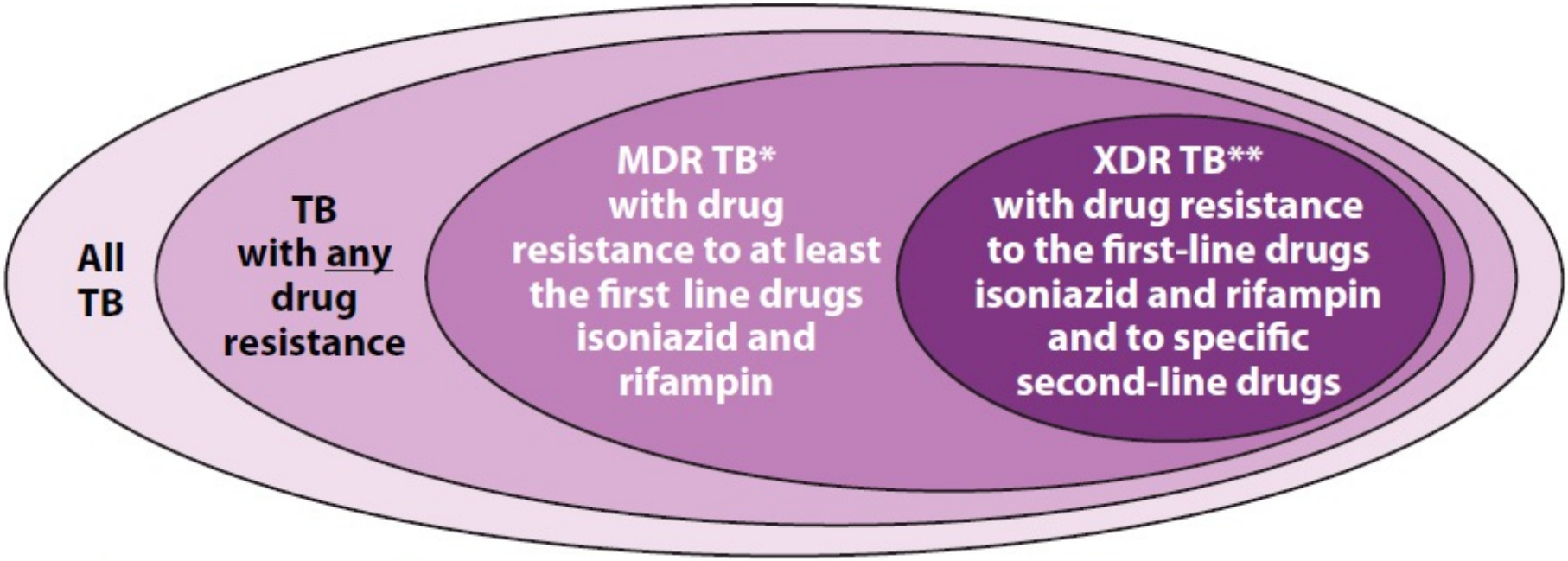




Isoniazid-NADH bound to InhA

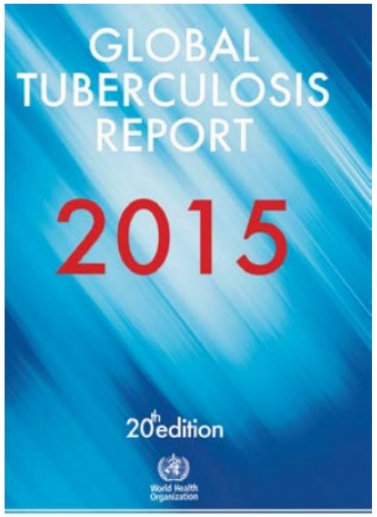


Global Burden of DR TB



* Often resistant to additional drugs

** Resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin)

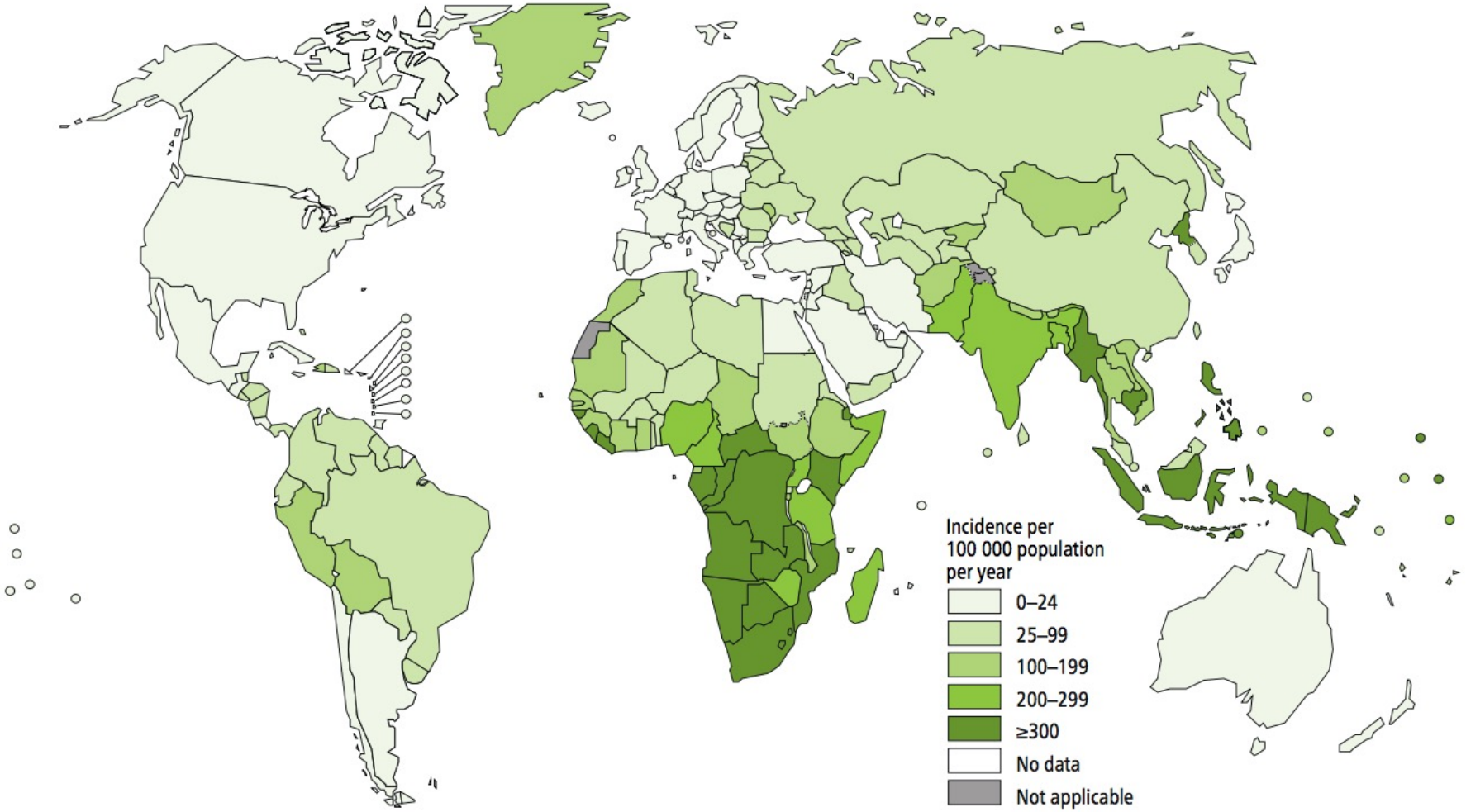


The global TB situation (1)

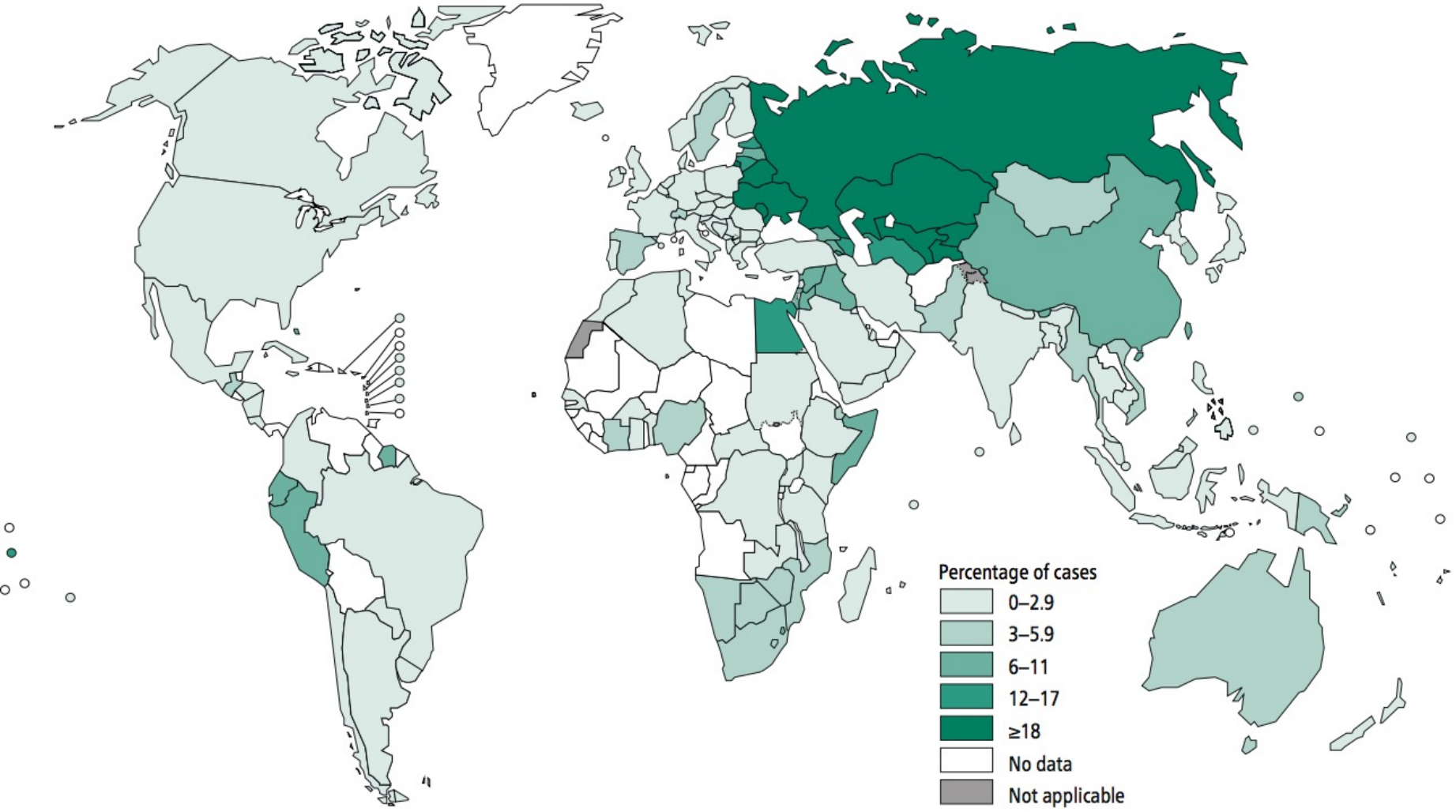
	Estimated incidence, 2014	Estimated number of deaths, 2014
All forms of TB	**9.6 million** (9.1–10.0 million)	**1.1 million*** (1.0–1.3 million)
HIV-associated TB	**1.2 million** (1.0–1.3 million)	**390,000** (350,000–430,000)
Multidrug-resistant TB	**480,000** (360,000–600,000)	**190,000** (120,000–260,000)

FIG. 3.4

Estimated TB incidence rates, 2016

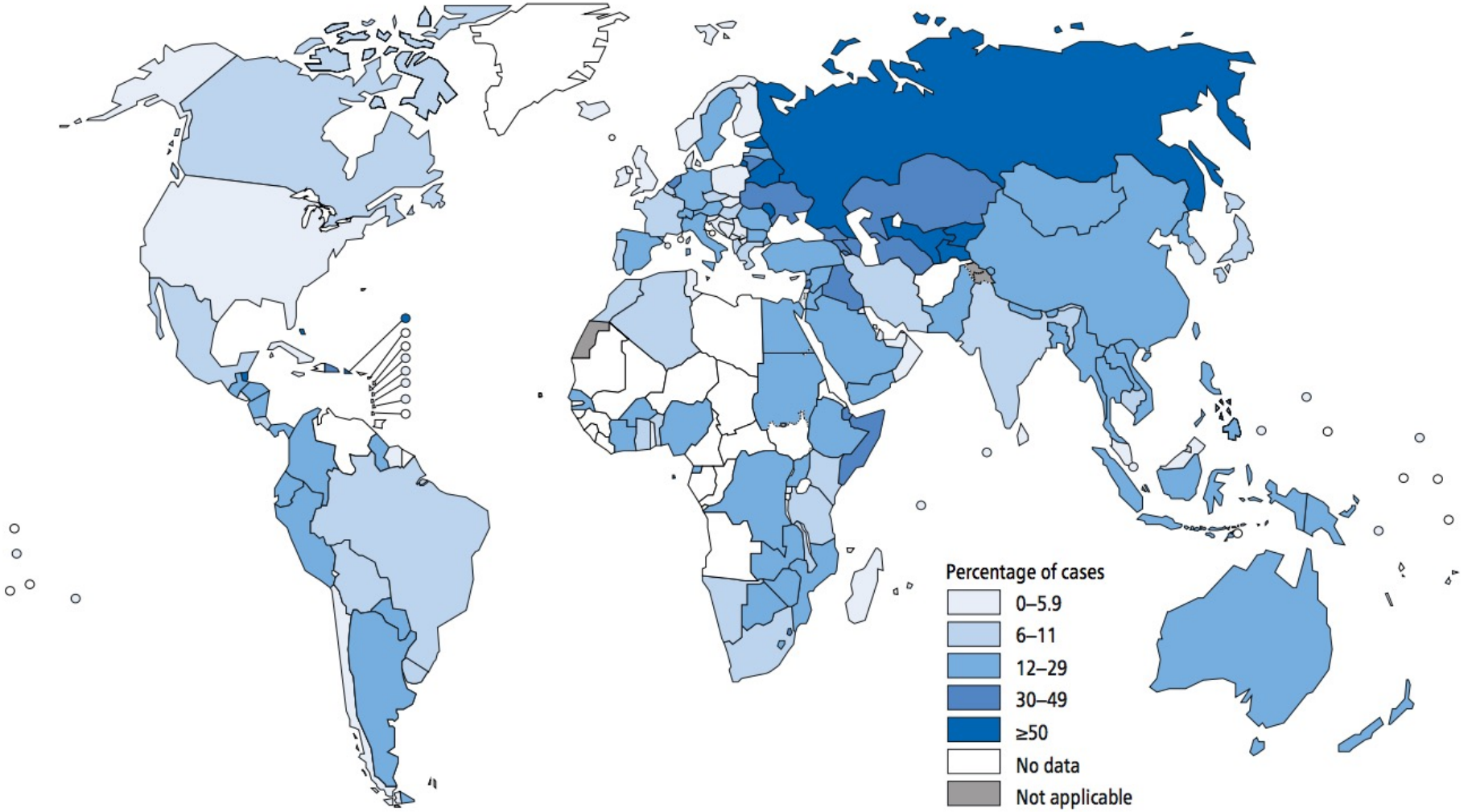


Percentage of new TB cases with MDR/RR-TB[a]



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before 2002 are not shown.

Percentage of previously treated TB cases with MDR/RR-TB[a]



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before 2002 are not shown. The high percentages of previously treated TB cases with MDR-TB in Bahamas, Belize, French Polynesia, Puerto Rico and Sao Tomé and Principe refer to only a small number of notified cases (range: 1–8 notified previously treated TB cases).

Country	Percent of new cases with MDR	Percent of previously treated cases with MDR
Belarus	38%	72%
Kazakstan	26%	44%
Kyrgystan	27%	60%
Moldova	26%	56%
Russia	27%	47%
Ukraine	27%	47%

Countries that notified at least one case of XDR-TB

Incurable TB an epidemic?

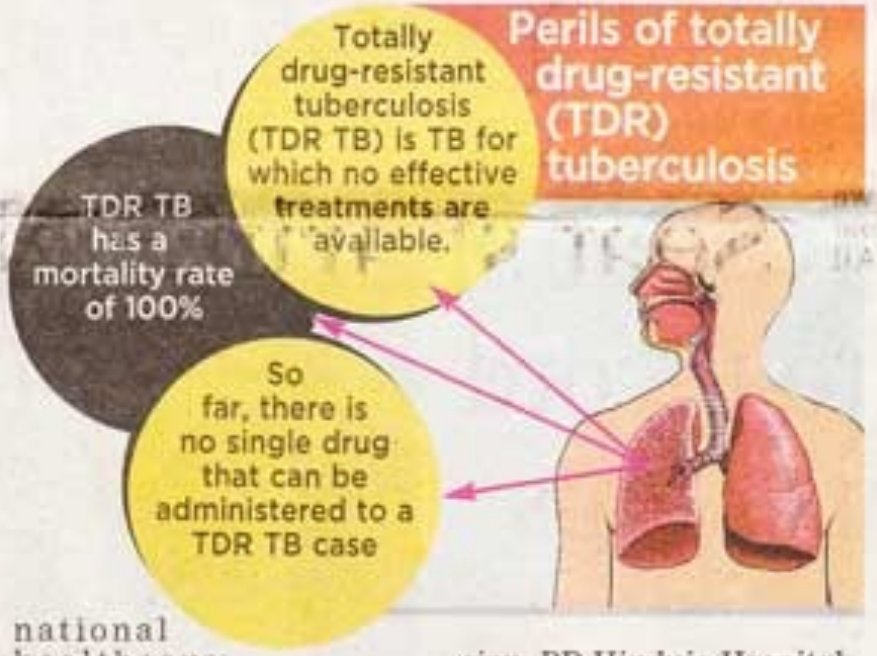
For 1st time, 12 identified with totally drug-resistant TB

SHOBHAN SINGH
MUMBAI, JAN. 6

In what can be considered as the country's first-ever diagnosis of totally drug-resistant (TDR) tuberculosis, PD Hinduja Hospital has identified 12 patients with the alarming disease.

This condition, experts claim, is a result of years of being prescribed heavy antibiotics by doctors with poor knowledge and expertise in treating the disease, which eventually results in resistance to it. According to doctors, TDR TB should ideally be treated as an epidemic.

One of the 12 patients, a 31-year-old woman from Dharavi died a couple of months ago. "We have reached this sorry state because of a complete failure in public, private and national healthcare institutions. Considering any person suffering from TB contracts the disease at least 10-15 persons every year, this is a potential epidemic," said Dr Zarir F. Udwadia, consultant physician, PD Hinduja Hospital.

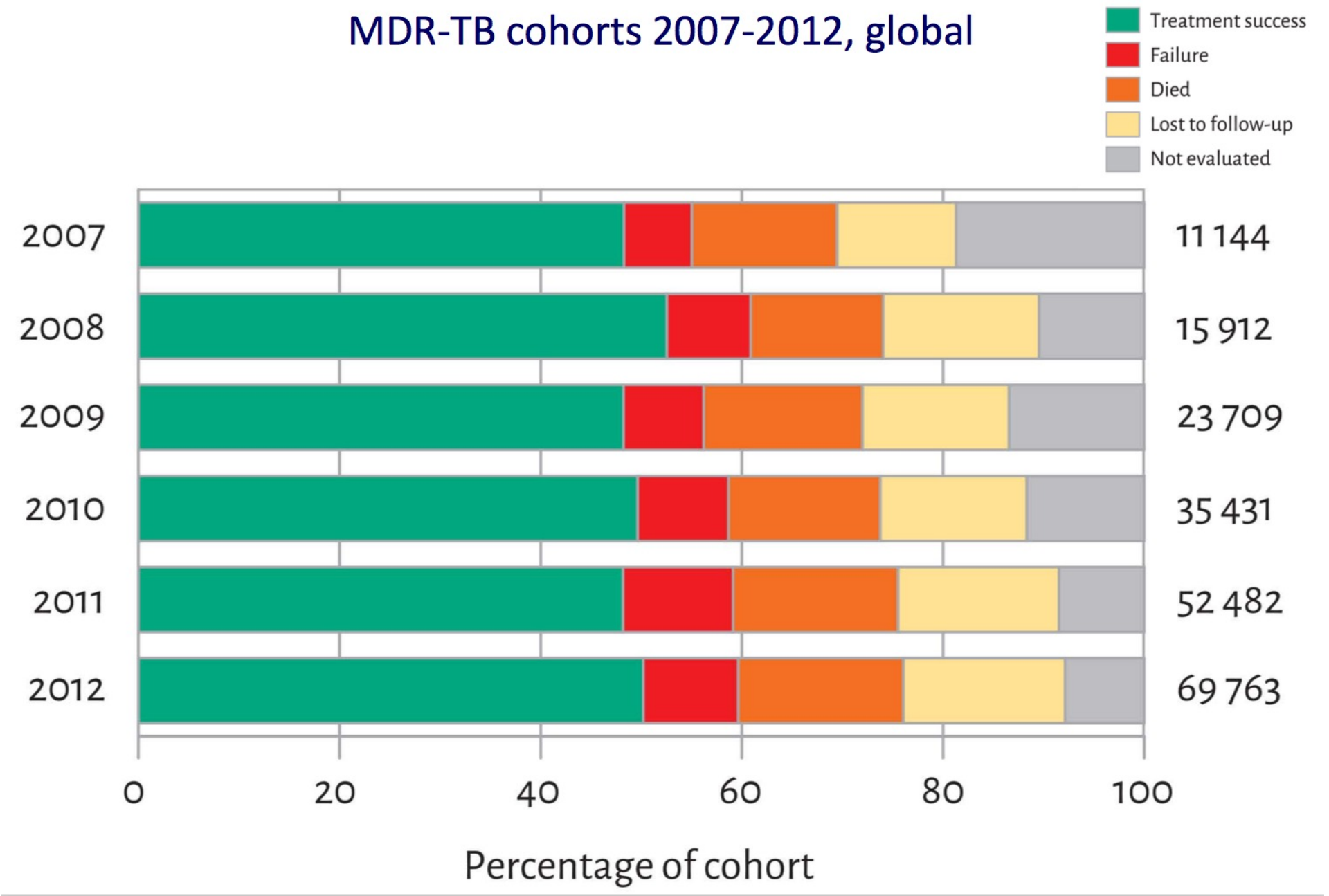


All these patients were resistant to the first line and the second line of TB treatment. While multi-drug resistant (MDR) TB has a mortality rate of 30 per cent, extensively drug resistant (XDR) TB has a mortality rate of 60 per cent, but TDR TB has a mortality rate of 100 per cent, said Dr Udwadia.

So far, there is no single drug that can be administered to a TDR TB case. "Our experience with the 12 cases is disturbing. Each patient on an average, has visited five doctors and subsequently, ended up with TDR, before coming to us," said Dr Udwadia.

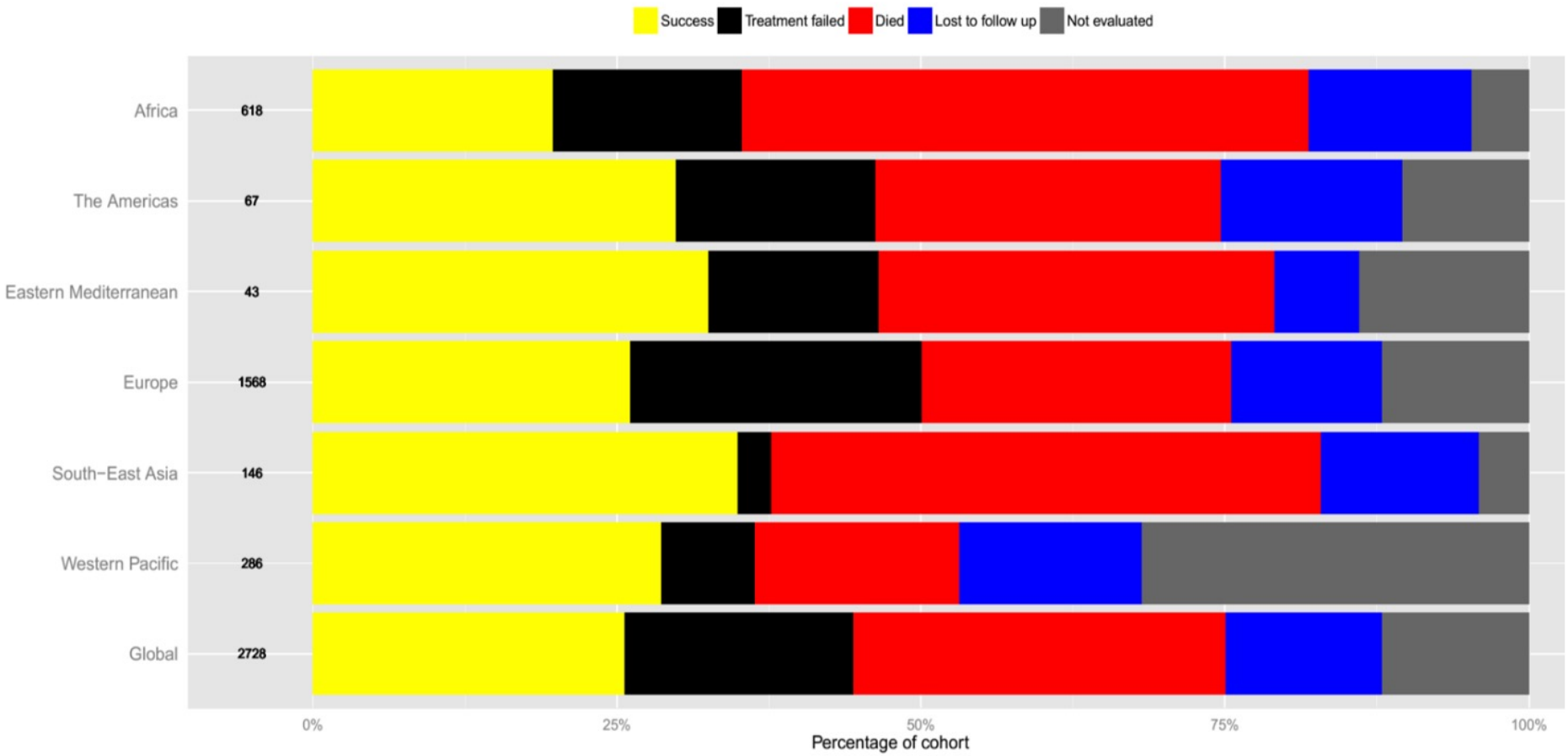
According to him, one of the most callous stages in the TB control programme is Category 2, which is the second line of treatment. "Doctors continue it for months together despite knowing that it hasn't worked in the longest time. Also, during this stage, patients are given some of the most toxic drugs," said Dr Udwadia.

Outcomes of MDR-TB treatment



Outcomes of XDR-TB treatment

XDR-TB cohorts 2012, by WHO Region*



*number of cases observed shown next to the bars

480 000

estimated cases of MDR-TB in 2014

123 000

MDR/RR-TB cases detected in 2014

111 000

TB patients started on MDR-TB treatment in 2014

50% 

of MDR/RR-TB cases started on treatment
in 2012 with successful outcome

FIVE PRIORITY ACTIONS TO ADDRESS THE GLOBAL MDR-TB CRISIS

Prevent the development of drug resistance through high quality treatment of drug-susceptible TB

Expand rapid testing and detection of drug-resistant TB cases

Provide immediate access to effective treatment and proper care

Prevent transmission through infection control

Increase political commitment with financing

How does TB DR emerge in individuals and populations?

The evolution of drug-resistant M. tuberculosis variants has generally been attributed to inadequate implementation of control measures, interrupted drug supply, low-quality drugs and patient non-adherence. However, it is increasingly evident that these factors alone are insufficient to explain the evolution of drug resistance in TB, as resistant M. tuberculosis strains evolve in well-functioning health systems and under strict treatment adherence.

Froma review by Sebastien Gagneux et al.

De novo resistance

- In addition to programmatic failures, "functional mono-therapy" can arise due to:
  - Under-dosing of drugs
  - Low absorption of drugs due to loss of intestinal integrity in chronic disease
  - Poor penetration of drugs into cavitary lesions

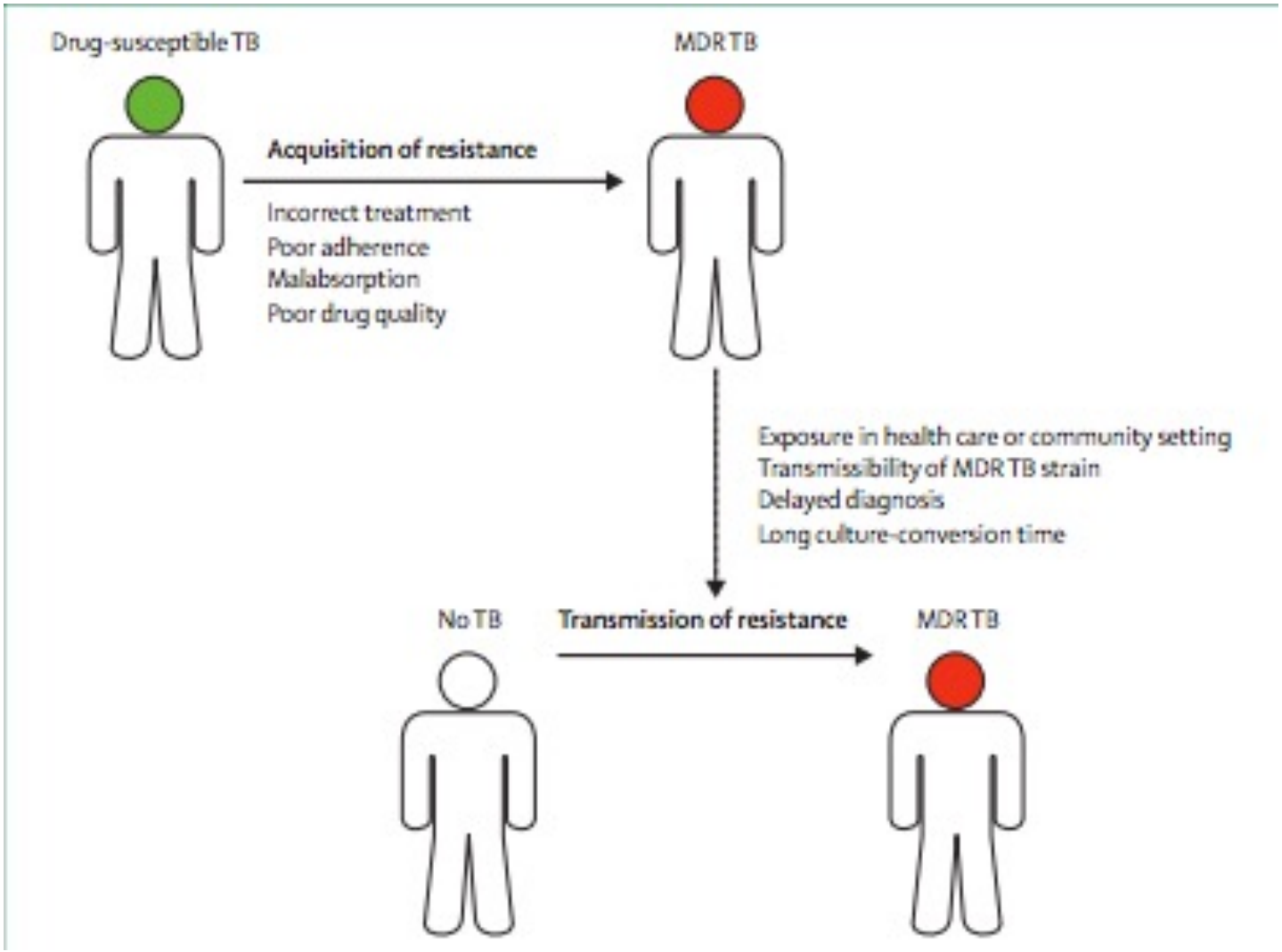
De novo resistance

- Population size
  - Estimated at 10^8-10^10TB bacilli
  - Need to consider cell death as true population size should be binary fission events, not prevalent bacilli at given point.
- Mutation Rate
  - 2-4 x 10^10 - slower than most bacteria.
  - Other factors that affect rate may include
    - Response to stress in macrophage environment
    - Exposure to DNA-damaging drugs
    - Presence of pre-existing mutations

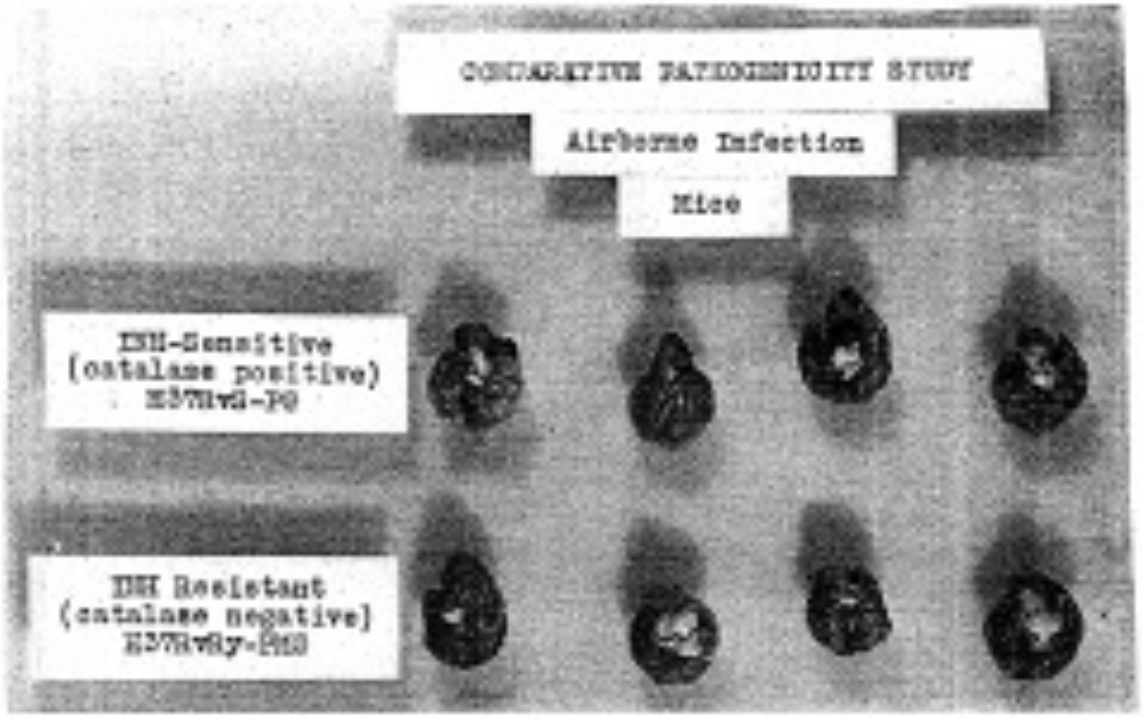
De novo resistance

- Mutational target size varies by drug.
- Much larger target size for pro-drugs that are modified by non-essential enzymes (INH, PZA) than for drug target alterations 9Rif).

How about transmission?

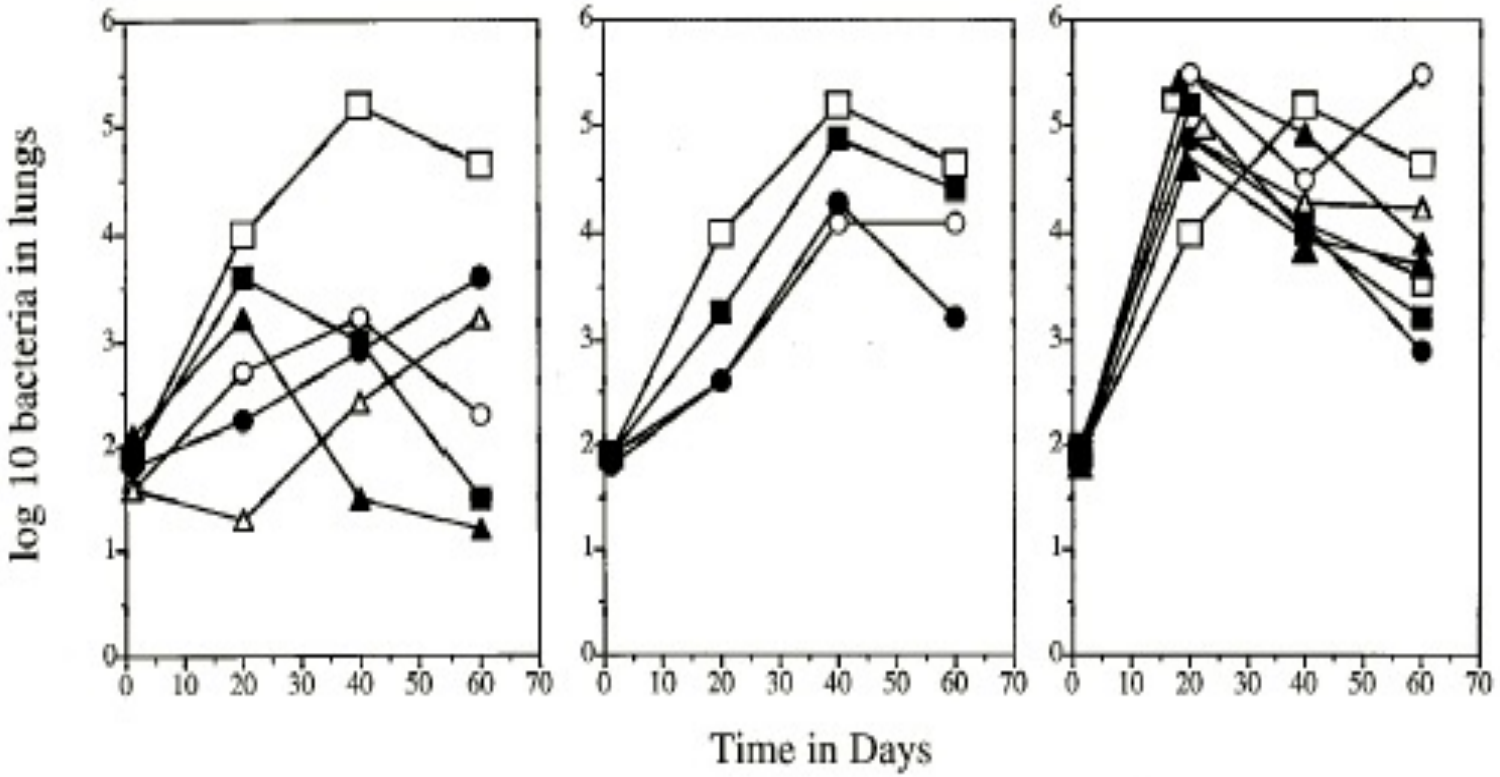


Are there fitness costs to TB drug resistance?



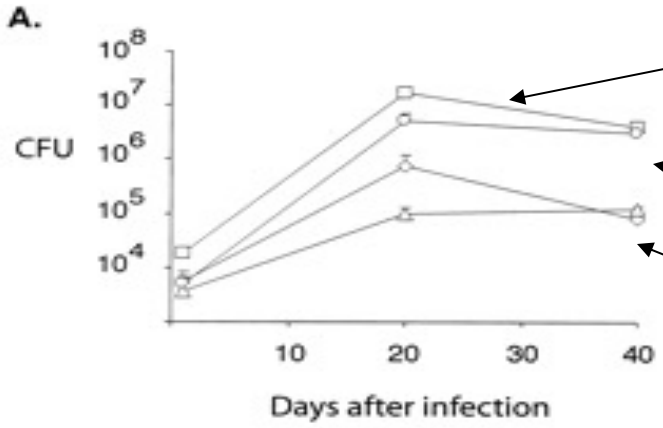
Cohn ML et al, Tubercle, 1954

Mycobacterial growth of INH resistant strains



Ordway et al, *Inf Immun*, 1995

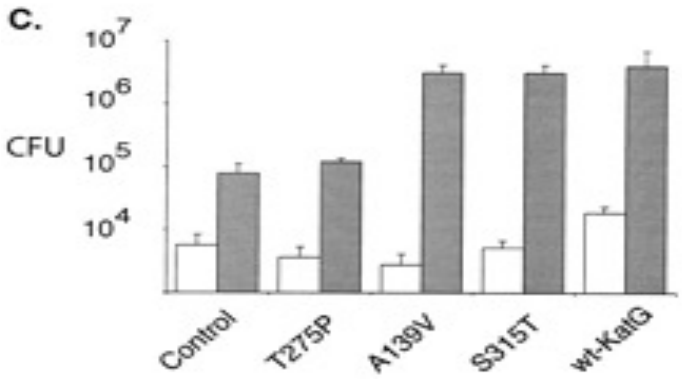
Some mutations that lead to drug resistance do not impair fitness



Wildtype KatG

S315T INH resistant mutant

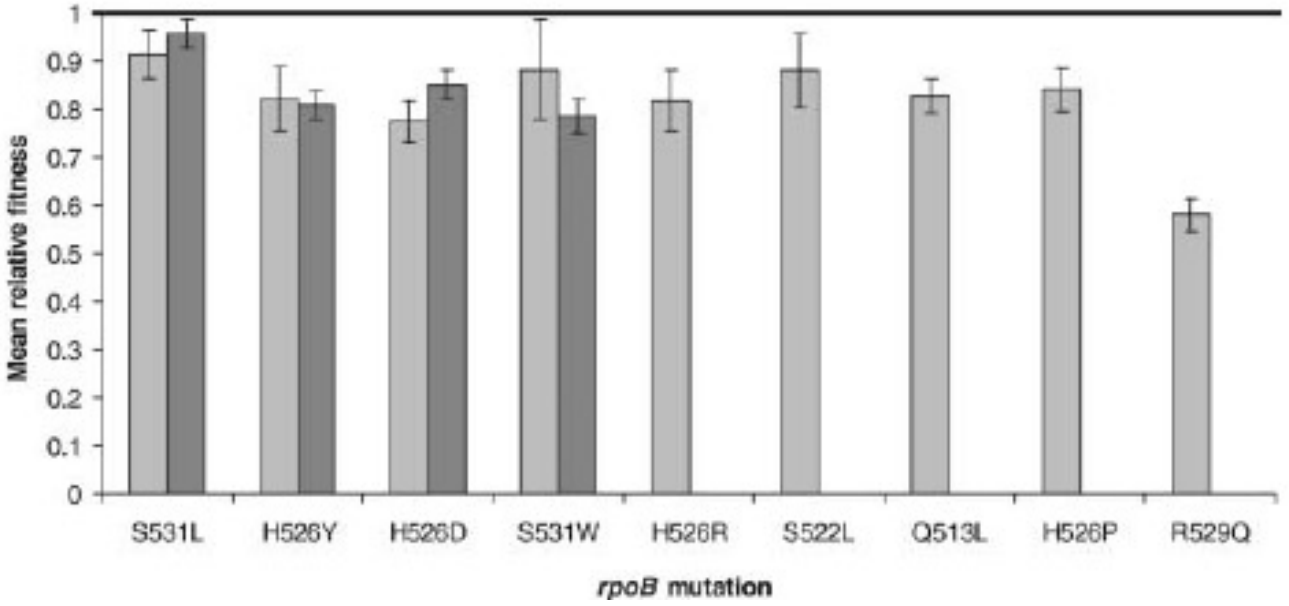
Other INH resistant KatG mutants



The white bars correspond to the CFU after 1 day, and the gray bars correspond to the CFU after 40 days in the lungs.

Pym A et al, *Inf Imm* 2002

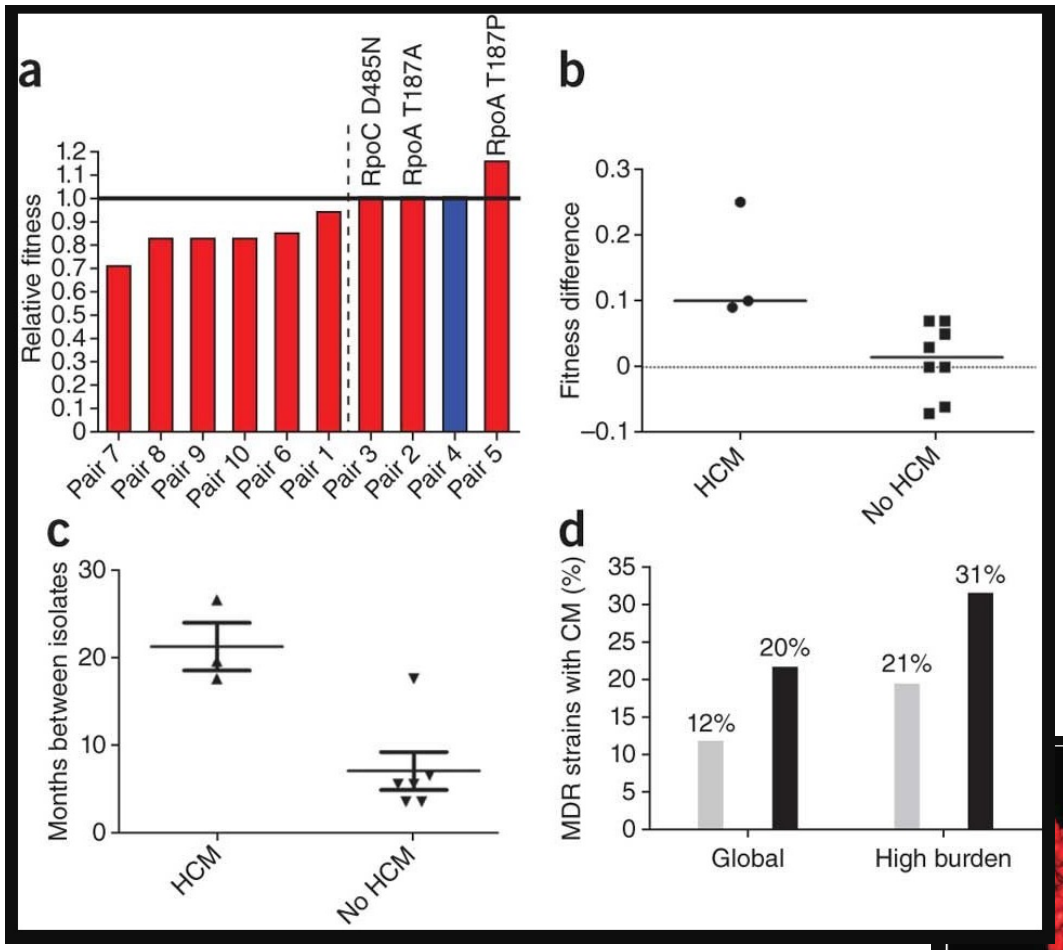
Relative competitive fitness of laboratory-derived rifampin-resistant mutants of M. tuberculosis



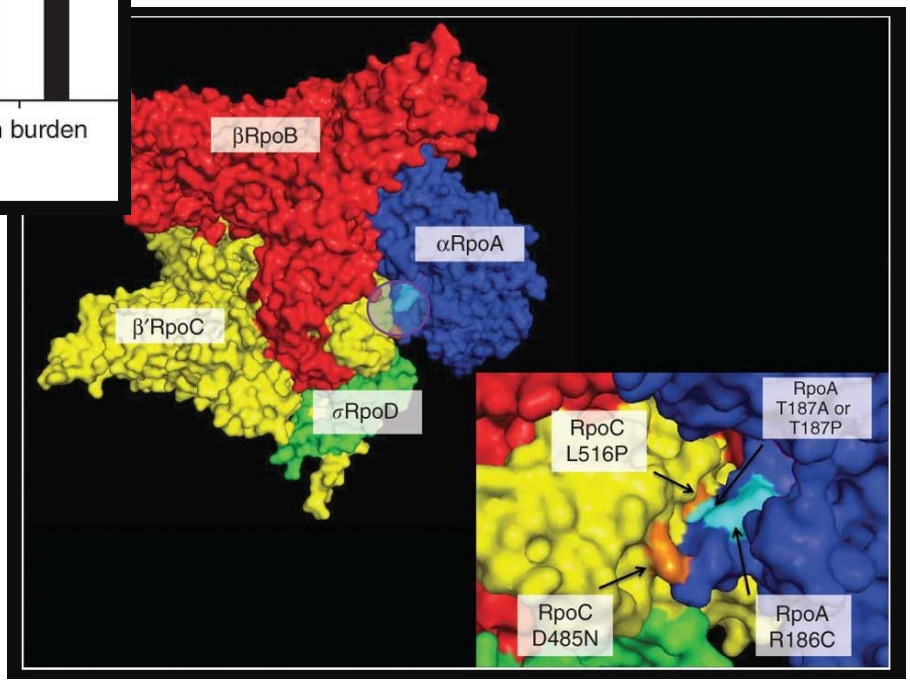
All mutants had a statistically significant fitness cost (error bars indicate 95% confidence intervals). This cost was less in *rpoB* S531L mutants than in other *rpoB* mutants, irrespective of the strain background. Light gray bars, CDC1551 mutants; dark gray bars, T85 mutants. Y, Tyr; W, Trp; P, Pro.

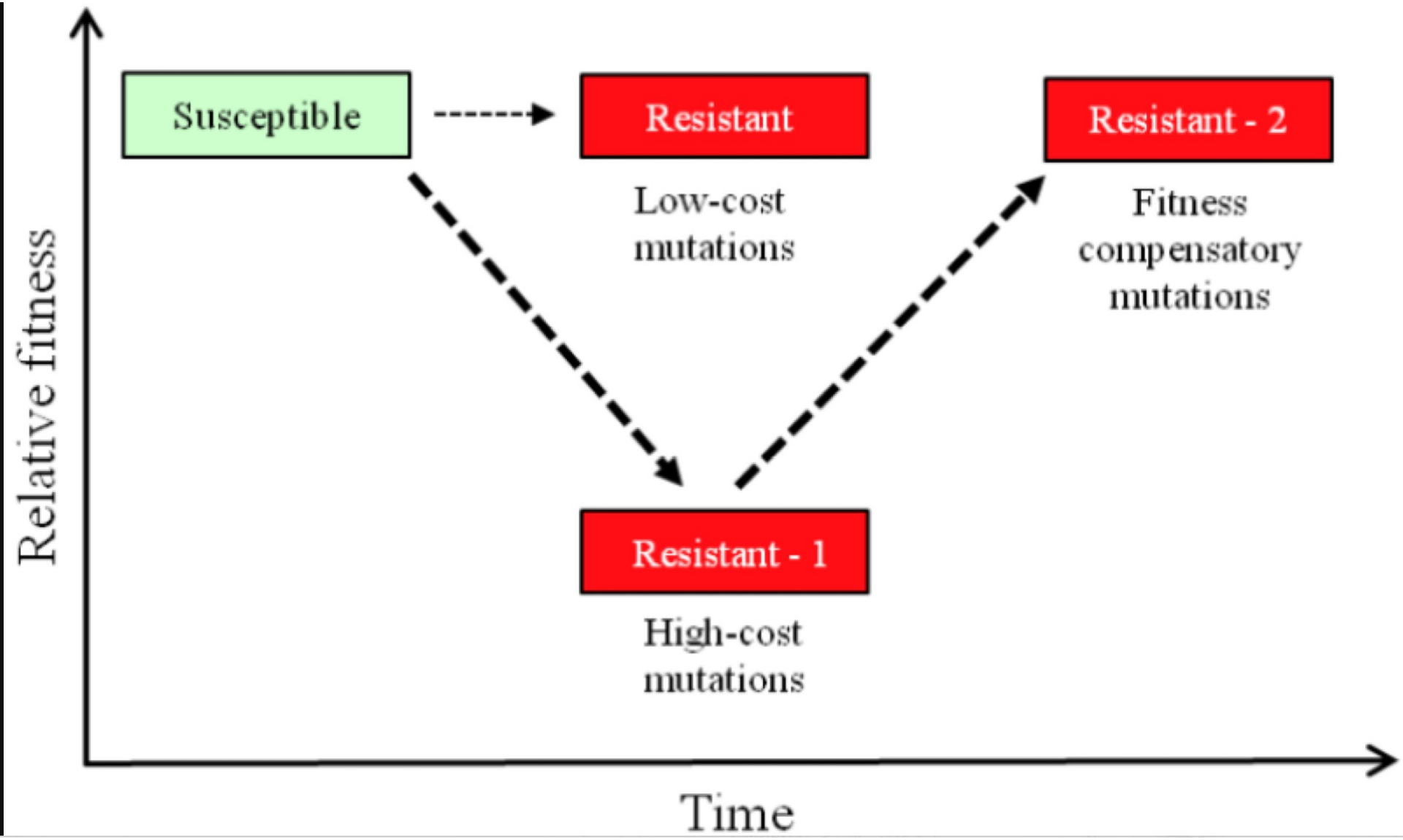
The Competitive Cost of Antibiotic Resistance in *Mycobacterium tuberculosis*

Sebastien Gagneux,[1,4*†] Clara Davis Long,[2*] Peter M. Small,[4,5] Tran Van,[1] Gary K. Schoolnik,[1,3] Brendan J. M. Bohannan[2]



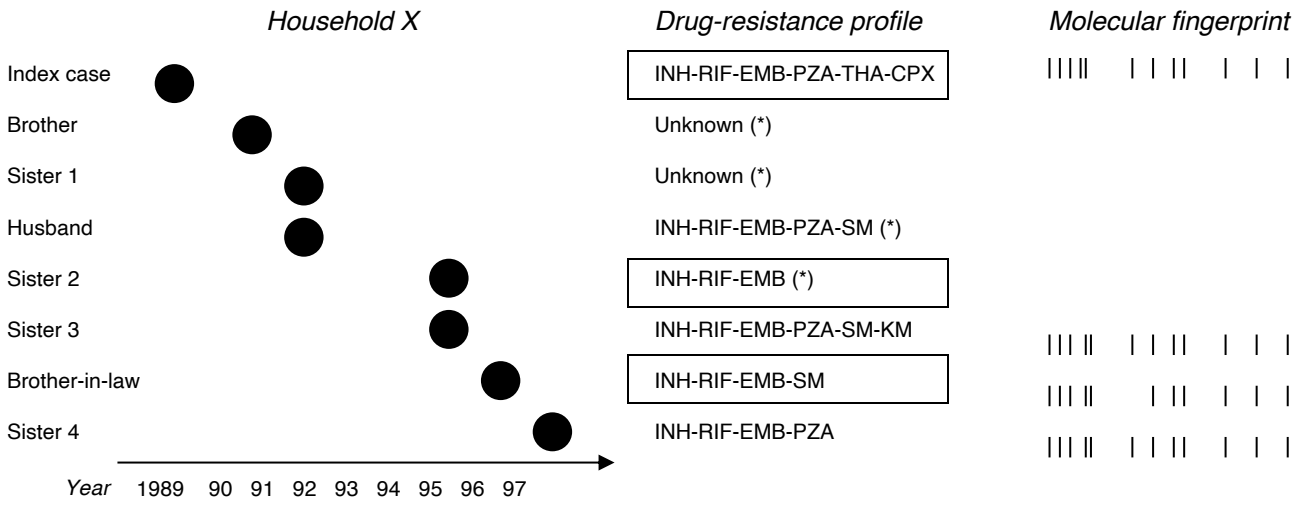
Strains with HCMs in RpoA and C were more fit, reached higher prevalence and were associated with longer disease duration in patients.




Susceptible
Resistant
Low-cost mutations
Resistant - 2
Fitness compensatory mutations
Resistant - 1
High-cost mutations
Relative fitness
Time

Epidemiologic studies | Epidemic modeling | Within-host modeling | Sequencing

Pulmonary TB, drug-resistance profiles and molecular fingerprints in the household of a patient with infectious MDR TB



Mercedes Becerra
Department of Social Medicine
HMS

Other epidemiological studies

Odds ratio for clustering of M. tuberculosis

	Odds Ratio (95% Confidence Interval)	*P*
Resistance to isoniazid and rifampin*	0.16 (0.4-0.6)	.008
Other resistance*	1.14 (0.5-2.7)	.80
Pleural effusion†	15.28 (1.6-147.9)	.02
Primary school or less education	3.41 (1.6-7.4)	.002
Cavitary disease†	2.18 (1.0-4.6)	.04

** Compared with fully susceptible strains.*
† Compared with other radiographic appearances.

Garcia-Garcia et al, *Arch Intern Med*, 2000

Impact of drug resistance on infection

DR profile	Baseline TST all	95% CI	Baseline TST kids	95% CI	Conversion	95% CI
Pan-S	1 (ref)		1 (ref)		1 (ref)	
Mono-R	1.06	.99-1.13	1.19	1.01-1.28	1.04	.85-1.27
Poly-R	1.08	1.01-1.17	1.02	.78-1.35	1.1	.81-1.5
MDR	1.09	1.01-1.17	1.07	.90-1.28	1.6	1.15-2.22

Adjusting for multiple possible confounders including SES, co-morbidities, BCG vaccination, index host HIV status, smoking, and alcohol use.

Impact of drug resistance on disease

DR profile	HR for disease progression	95% CI
DS	1 (ref)	
Mono-R	1.24	.9-1.72
Poly-R	.68	.38-1.21
MDR	1.24	.9-1.72

Adjusting for multiple possible confounders including SES, co-morbidities, BCG vaccination, index host HIV status, smoking, and alcohol use.

What is to be done?

- Early diagnosis and appropriate treatment of MDR will reduce ongoing transmission.
- Calls for
  - Programmatic interventions such as active case finding
  - Rapid diagnostics that allow selection of appropriate drug regimens at time of diagnosis. This requires identification of full compendium of DR mutations and development of rapid diagnostic tests to identify them.