Unit of Medical Microbiology - IHMT/UNL

RiCC
RePORT INTERNATIONAL COORDINATING CENTER

Mycobacterium tuberculosis genetics network in Brazil

Miguel Viveiros,

João Perdigão, Diana Machado, Jorge Ramos, Isabel Couto, Isabel Portugal.

Institute of Hygiene and Tropical Medicine
Universidade Nova de Lisboa, Lisboa, Portugal

3RD Annual RePORT International Meeting, Rio de Janeiro, Brazil
September 13th 2017

INSTITUTO DE HIGIENE E MEDICINA TROPICAL DESDE 1902

UNIVERSIDADE NOVA DE LISBOA

REDE-TB

The old example of Portugal

Always present with TB incidence greater than **300/100.000 in 1900!!!**

Dona Amélia, Ex-Empress of Brazil, ordered the construction, in 1853, a hospital-sanatorium, in memory of his daughter**, Princess D. Maria Amelia,** who died the same year in the city of Funchal (Madeira islands), with 22-year-old mowed by phthisis, "to what is believed", infected by his father**, D. Pedro IV (Pedro I of Brazil),** also killed by the disease. In 1862, the Hospital Princess D. Maria Amelia, "*intended to treat patients affected with consumption, and other chronic lung diseases, which may still have hope of improvement*" received their first patients.

Dona Amélia Augusta Eugênia Napoleona de Beauharnais, founder of the 1st Hospital-Sanatórium Princesa D. Maria Amélia (Funchal)

http://www.saudepublica.web.pt/TrabFrada/TBsecXX_JFrada.htm
Da A.N.T. ao S.L.A.T.: história sumária de uma instituição (1899-1979), Lisboa, 1979, p.7

The old example of Portugal

Incidence higher than 300/100.000 in 1900 reduced to 250/100.000 in 1940 with basic hygiene measures!!!

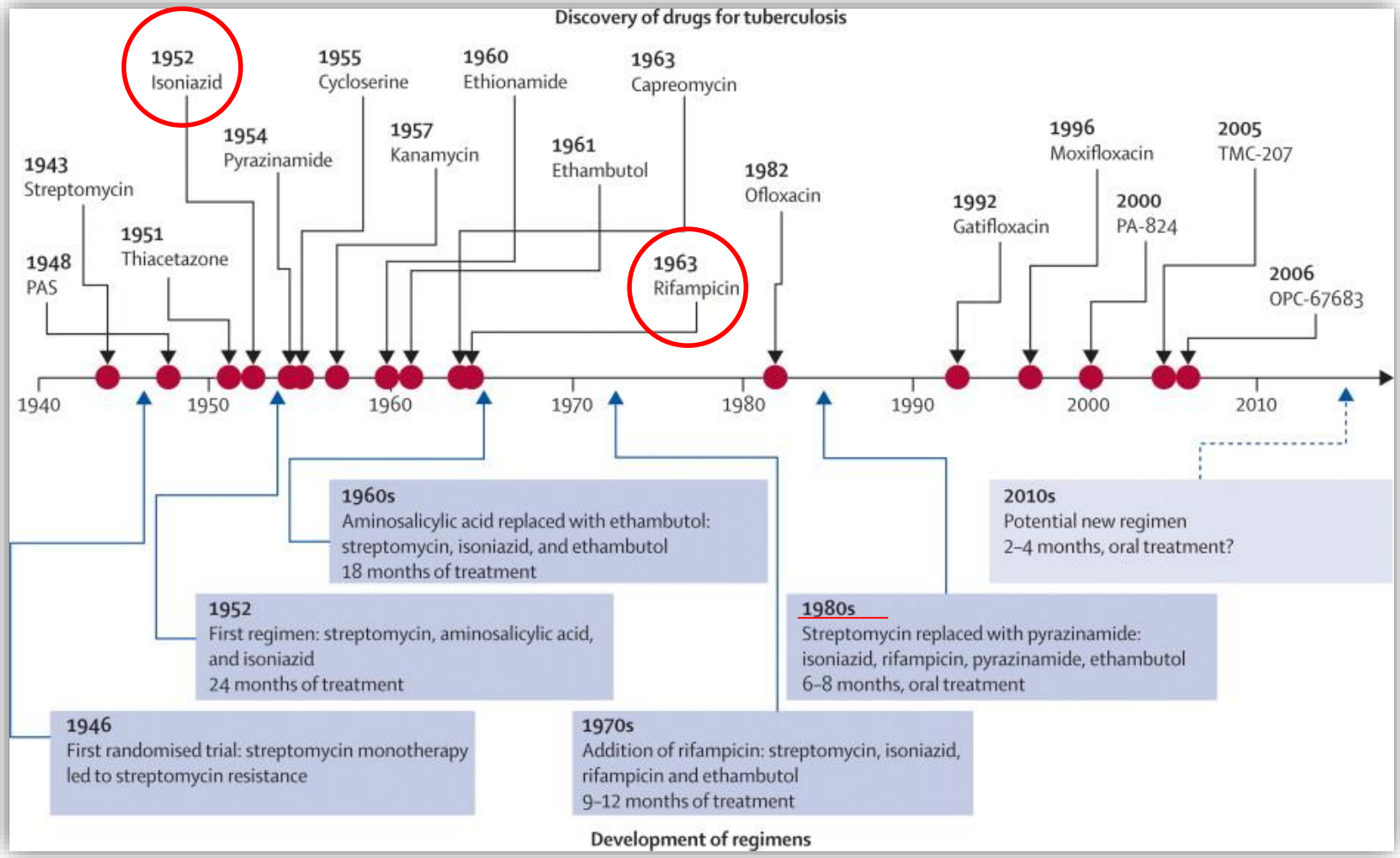
Sanatório Marítimo do Norte em Francelos

Preventório de Penacova

O Sanatório de Sant'Ana na Parede

http://www.saudepublica.web.pt/TrabFrada/TBsecXX_JFrada.htm
Da A.N.T. ao S.L.A.T.: história sumária de uma instituição (1899-1979), Lisboa, 1979, p.7

Tuberculosis, "consumption " or pthisis –
CAN BE CURED WITH APPROPRIATE TREATMENT



Zhenkun Ma, Christian Lienhardt, Helen McIlleron, Andrew J Nunn, Xiexiu Wang. (2010) Global tuberculosis drug development pipeline: the need and the reality. *The Lancet*, Volume 375, Issue 9731, Pages 2100-2109

The old example of Portugal

Incidence of TB in Portugal (>300/100.000) in 1900!
1945 antibioterapy associated with the maintenance of the National Assistance Against Tuberculosis (SLAT)
Incidence of TB in Portugal (80/100.000) in 1970

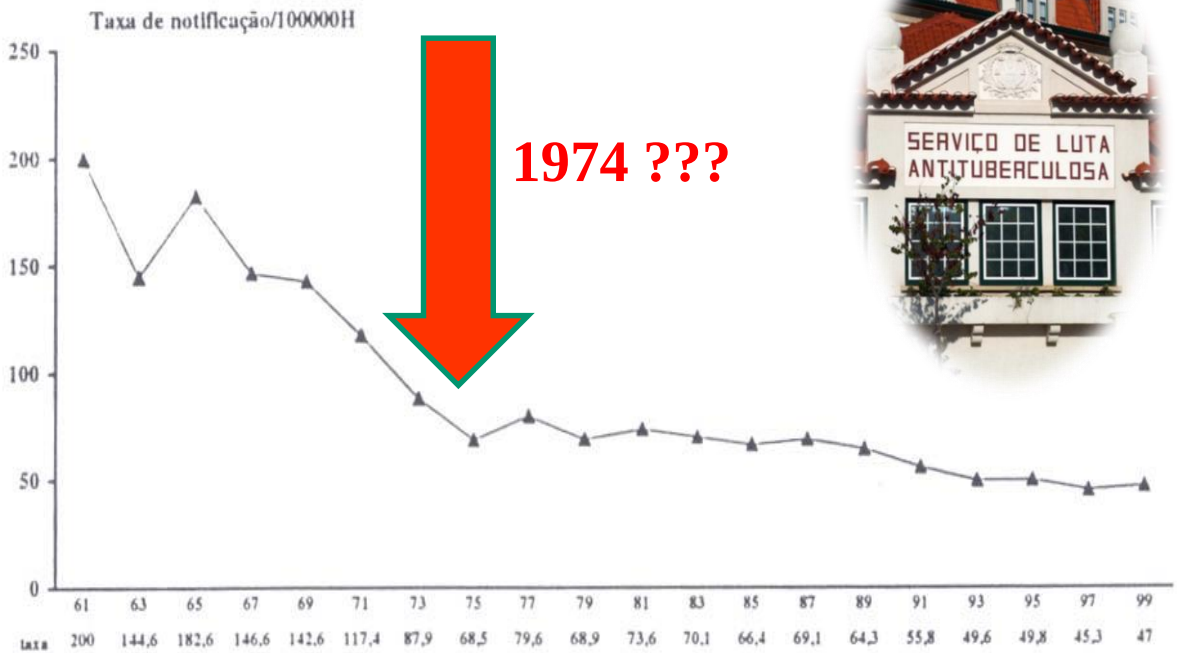
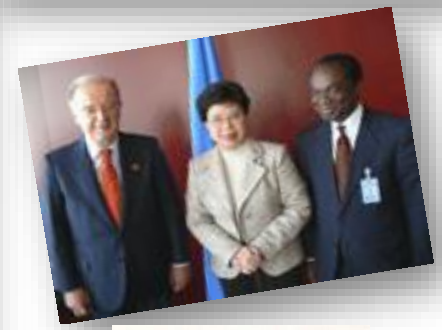


Fig 1. Casos novos de tuberculose por 100 mil habitantes de 1960 a 1999.

http://www.saudepublica.web.pt/TrabFrada/TBsecXX_JFrada.htm
Da A.N.T. ao S.L.A.T.: história sumária de uma instituição (1899-1979), Lisboa, 1979, p.7




Tuberculose. Cinco alunos e uma professora da Escola EB 2/3 de Alijó foram infectados com a doença. Subdirector regional de Saúde de Vila Real garante que não há motivos para alarme, pois os doentes estão a ser tratados

Aluno com tuberculose está a ser discriminado

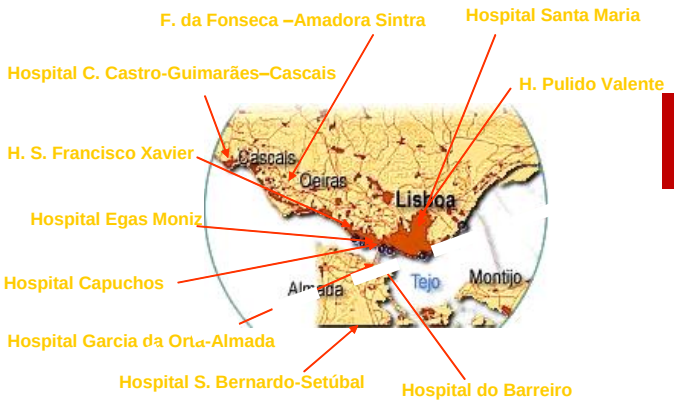
But beyond TB

there was now a new problem!!!

MDR-TB above 35% in 1997-2000 in Lisboa!!!!

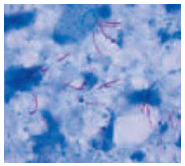
FUNDAÇÃO CALOUSTE GULBENKIAN

The example of Portugal - TB-Task Force for Lisbon (2003, 2005, 2009)



Descontamination & homogenization

Ziehl-Neelsen staining



Culture

System BACTEC MGIT 960

Identification

System *Accu*probe

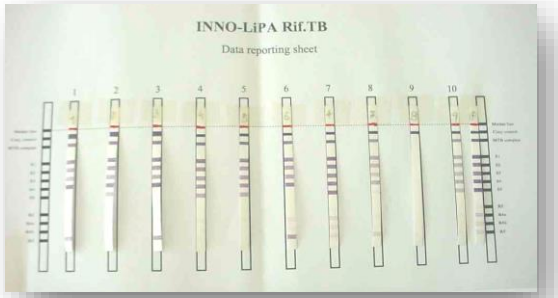
M. tuberculosis ID +

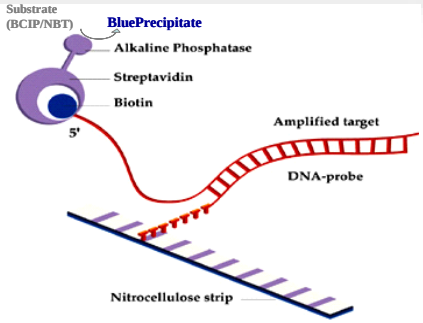
Susceptibility tests

(BACTEC MGIT 960)

Extracction of DNA

INNOLiPA Rif. TB





Trends in Drug Resistance Rates

Any Resistance Rate: 22.3 – 26.0%

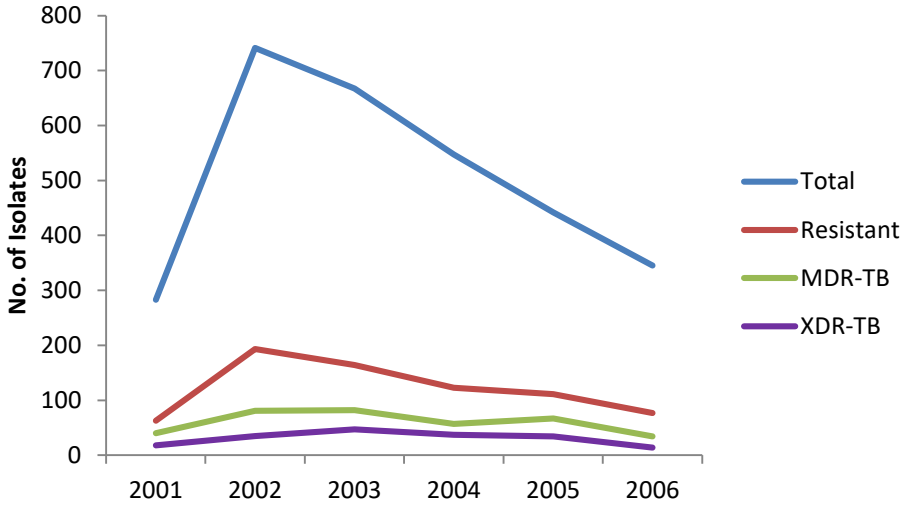
MDR-TB Rate: 9.9 – 15.3%

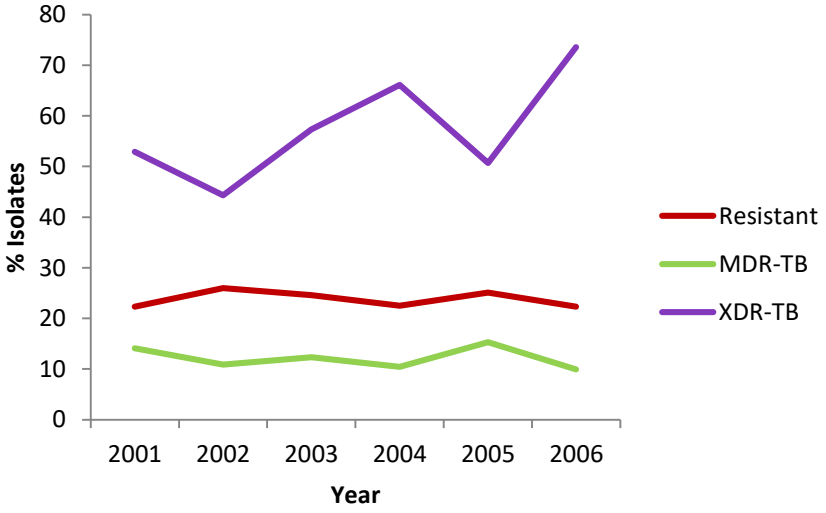
XDR-TB Rate: 44.3 – 73.6%

Resistance	No. of isolates (%)					
	2001	2002	2003	2004	2005	2006
Total	**283**	**741**	**667**	**547**	**442**	**345**
Resistant[a]	63 (22.3)	193 (26.0)	164 (24,6)	123 (22.5)	111 (25.1)	77 (22.3)
MDR-TB[b]	40 (14.1)	81 (10.9)	82 (12.3)	57 (10.4)	67 (15.3)	34 (9.9)
XDR-TB[c]	18 (52.9)	35 (44.3)	47 (57.3)	37 (66.1)	34 (50.7)	14 (73.6)

MDR-TB isolates resistant to all first-line drugs show a steady increase between 2001-2006 -> 25.9 – 52.9%

The proportion of M/XDR-TB does not follow the decreasing trend of the total number of cases !!??
ON THE CONTRARY THEY INCREASED STEADILY





The example of Portugal - TB-Task Force for Lisbon (2003, 2005, 2009)

FUNDAÇÃO CALOUSTE GULBENKIAN

2003
n = 360 samples

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2005, p. 4880–4884 Vol. 43, No. 9
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.9.4880–4884.2005

Direct Application of the INNO-LiPA Rif.TB Line-Probe Assay for Rapid Identification of *Mycobacterium tuberculosis* Complex Strains and Detection of Rifampin Resistance in 360 Smear-Positive Respiratory Specimens from an Area of High Incidence of Multidrug-Resistant Tuberculosis

Miguel Viveiros,[1] Clara Leandro,[1] Liliana Rodrigues,[1] Josefina Almeida,[1] Rosário Bettencourt,[1] Isabel Couto,[1,2] Lurdes Carrilho,[3] José Diogo,[4] Ana Fonseca,[5] Luís Lito,[6] João Lopes,[7] Teresa Pacheco,[8] Mariana Pessanha,[9] Judite Quirim,[10] Luísa Sancho,[11] Max Salfinger,[12] and Leonard Amaral[1]*

Unidade de Micobacterias, UPMM, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal[1]; Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Lisboa, Portugal[2]; Laboratorio de Micobacterias, Hospital Pulido Valente, Lisboa, Portugal[3]; Laboratorio de Microbiologia, Hospital Garcia da Orta, Lisboa, Portugal[4]; Laboratorio de Microbiologia, Hospital Condes de Castro Guimarães, Cascais, Portugal[5]; Laboratorio de Microbiologia, Hospital de Santa Maria, Lisboa, Portugal[6]; Laboratorio de Microbiologia, Hospital de Nossa Senhora do Rosário, Barreiro, Portugal[7]; Laboratorio de Microbiologia, Hospital Egas Moniz, Lisboa, Portugal[8]; Laboratorio de Microbiologia, Hospital São Francisco Xavier, Lisboa, Portugal[9]; Laboratorio de Microbiologia, Hospital de São Bernardo, Setúbal, Portugal[10]; Laboratorio de Microbiologia, Hospital Fernando da Fonseca, Amadora-Sintra, Portugal[11]; and Clinical Mycobacteriology Laboratory, Wadsworth Center, New York State Department of Health, Albany, New York 12208[12]

Received 11 June 2005/Accepted 15 June 2005

Review

EXPERT REVIEWS

Molecular tools for rapid identification and novel effective therapy against MDRTB/XDRTB infections

Expert Rev. Anti Infect. Ther. 8(4), 465–480 (2010)

Miguel Viveiros, Marta Martins, Isabel Couto, Liliana Rodrigues, Diana Machado, Isabel Portugal and Leonard Amaral†
*†Author for correspondence
Unit of Mycobacteriology, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Rua da Junqueira, 100, 1349-008 Lisbon, Portugal
Tel.: +351 213 652 600
Fax: +351 213 632 105
lamaral@ihmt.unl.pt*

Tuberculosis (TB) is mainly an intracellular infection of the lung alveolar macrophages, and any anti-TB agent must therefore be active at the macrophage. Among the available therapies, isoniazid and rifampicin are the most effective drugs against susceptible *Mycobacterium tuberculosis*, but they are ineffective against multidrug-resistant TB (MDRTB) strains. Rates of MDRTB in Portugal are the highest in Western Europe, demanding effective measures for their control. Our application of molecular techniques for the early identification of MDRTB assisted in the reduction of these rates. Further examination revealed that a large number of MDRTB cases were extensively-drug resistant (XDRTB), providing evidence for the urgent need of new and effective anti-MDRTB/XDRTB therapeutic strategies. This review describes in detail: the characteristics of the main *M. tuberculosis* strains circulating in Portugal; the creation of a Task Force for TB control, based on molecular tools that allow 1-day identification of an MDRTB patient; the usefulness of evaluating the *ex vivo* activity of anti-tubercular agents against the *M. tuberculosis* isolated from the patient's sputum; and the mode of action by which phenothiazines have been shown to promote the killing of intracellular MDRTB/XDRTB by nonkilling macrophages.

KEYWORDS: macrophages • MDRTB • multidrug resistance • phenothiazines • tuberculosis • XDRTB

2005
n = 630 samples

INT J TUBERC LUNG DIS 3(3):207–213

Outbreak of multiple drug-resistant tuberculosis in Lisbon: detection by restriction fragment length polymorphism analysis

I. Portugal,* M. J. Covas,†‡ L. Brum,§ M. Viveiros,‡§ P. Ferrinho,†‡ J. Moniz-Pereira,* H. David‡

*Departamento de Microbiologia, Faculdade de Farmácia de Universidade de Lisboa, †Unidade de Sistemas de Saúde, ‡Centro de Malária e Doenças Tropicais, e §Departamento de Microbiologia, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Portugal

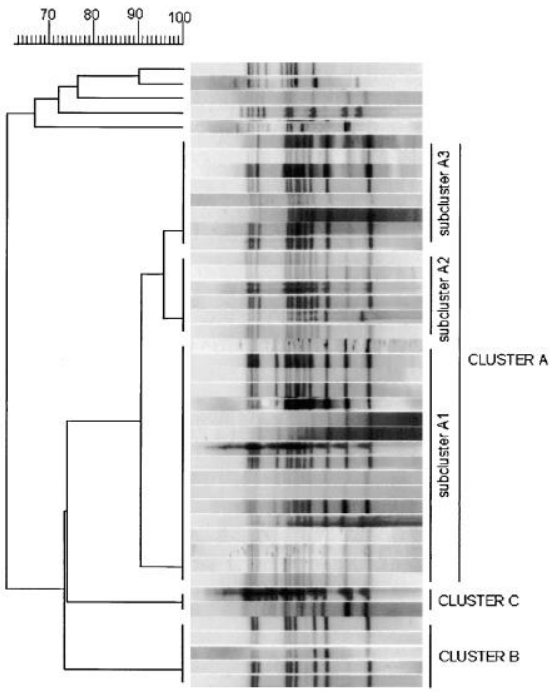


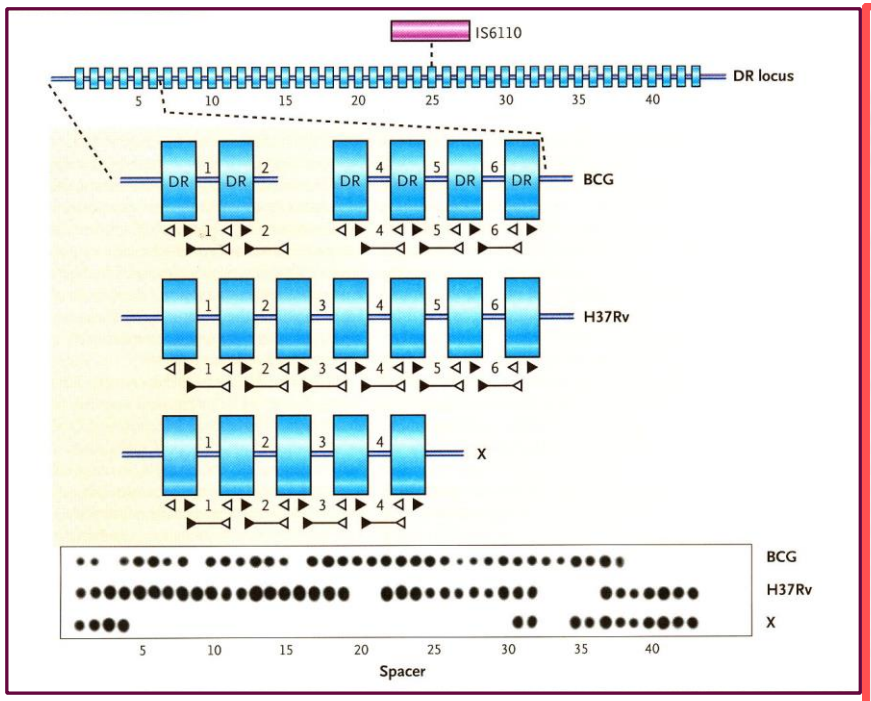
Figure Dendrogram based on computer-assisted comparison of DNA fingerprints from 43 MDR-TB strains isolated in Lisbon, Portugal. The clustered strains are termed clusters A (subcluster A1, A2 and A3), B and C.

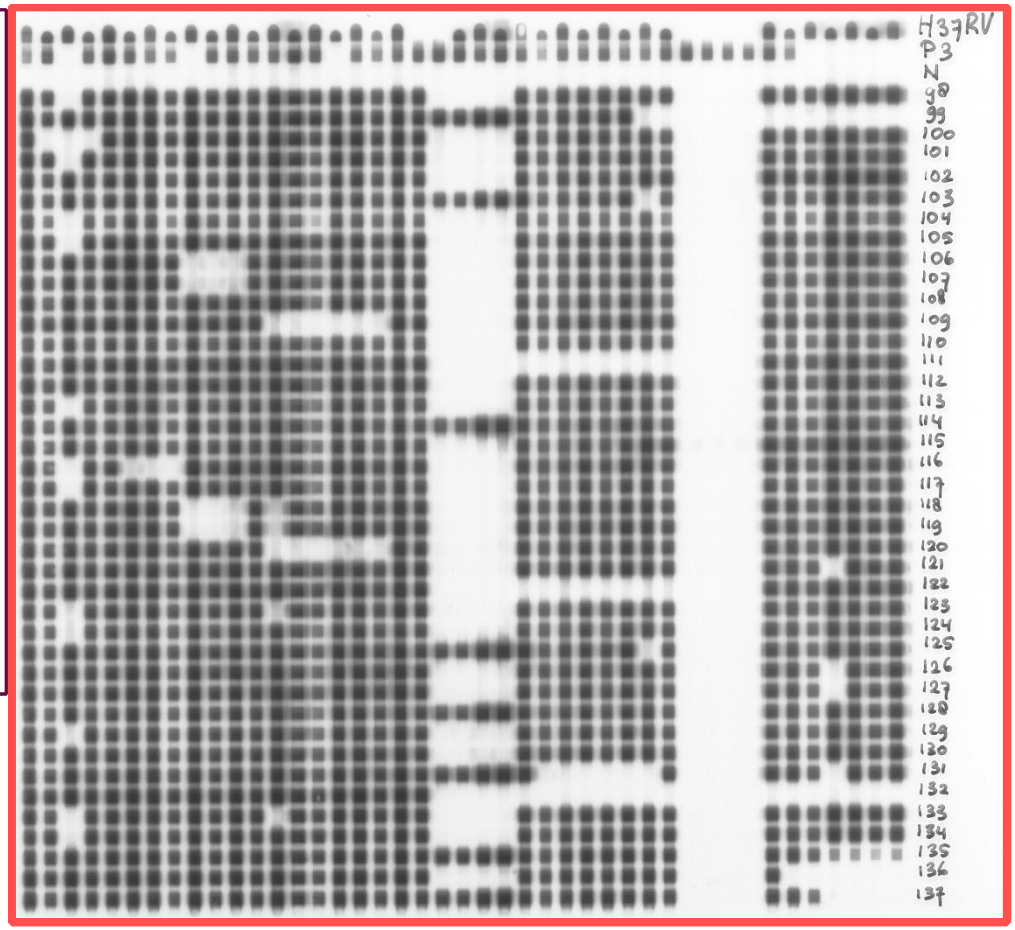
Table 6 Patients' trajectory across health institutions

	1994												1995												1996												1997											
Case	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N
1																																				A	A	A	A	A	A	A	A	A	A	A	A	A
2																															B	B	B															
3					C				M	F	M	M	M					C	M	M																												
4																					C	M	M	M	M	M	M			C	M	M	C	C														
5																															B	B			G	M	M	N										
6																											A	A		A	A																	
7																											C	C	M	M																		
8																														A	A	A	A	A														
9														J	J	J	J	J	J	J																												
10																																A	A	A		A	A	A										
11										B	B	B	M	M																																		
12			F	F						F	M	M	M	M	M												M	M	M																			
13									A			A	A	A	A	A	A	A																														
14																									A	A			A	A	A		A	A	A	A	A	A	A	A								
15																																	A	A	A													
16																																	F	F	L	L												
17																			A	A	A								A	A	A	A	A															
18																											B	M	M	M			B	B														
19																																	A	A														
20							M	M	M	C			C	M	M	M																																
21																																	A	A	A	A	A	A										
22																														D	D	D			D	M	M											
23																															A	A	A															
24																					A	A	A						A		A	A	A	A	A													
25																									A	A	A								A	A	A	A	A	A	A	A			A	A		
26																																							A	A	A	A	A	A	A	A	A	A
27																																			Q													

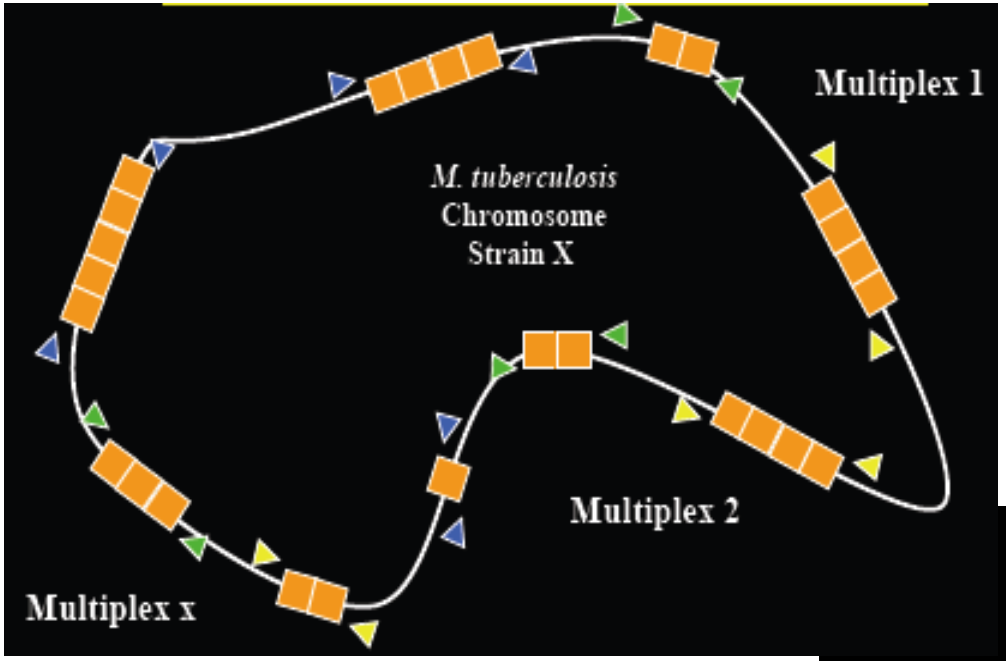
Letters A–D, F, G, J and L indicate hospital units where strains were obtained. M and N indicate additional hospital units where patients were hospitalised. Letter 'O' indicates an out-patient.

Spoligotyping





SITVIT2 database comparison: spoligotype pattern 477777607760771
belongs to shared-international-type **SIT 753**.

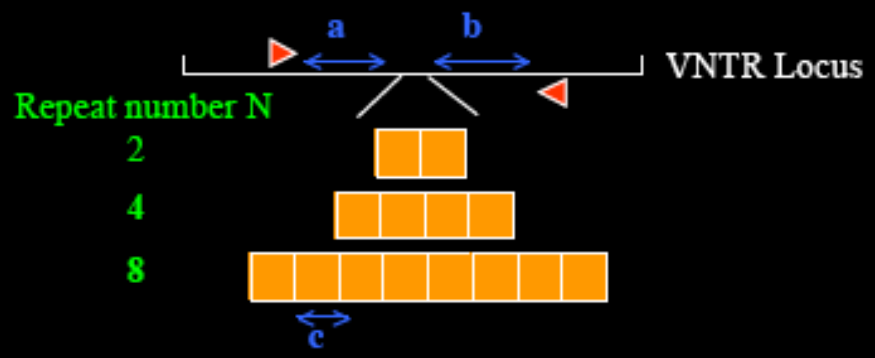


MIRU-VNTR

mycobacterial interspersed repetitive units - (MIRU)

variable number of tandem repeats (VNTR)

MIRU-VNTR genotyping



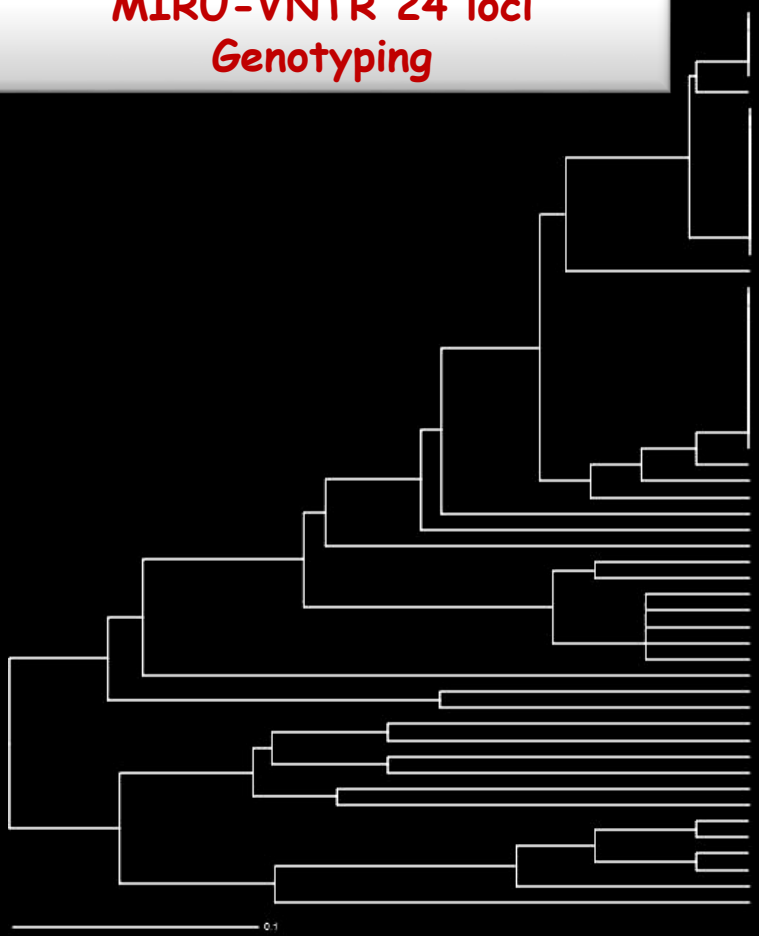
PCR product size (pb) = a + b+ N x c

$$N = \frac{\text{size} - (a + b)}{c}$$

MIRU-VNTR unit

http://www.miru-vntrplus.org/MIRU/index.faces

MIRU-VNTR 24 loci Genotyping



Isolate	154	424	577	580	802	960	1644	1955	2059	2163b	2165	2347	2401	2461	2531	2687	2996	3007	3171	3192	3690	4052	4156	4384	Drug-Resistance		Cluster
FF181_97	2	4	4	2	1	3	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRS	na	Lisboa3-A
FF291_98	2	4	4	2	1	3	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRS	na	
ARS10348	2	4	4	2	1	3	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRS	ETH	
IHMT134_09	2	4	4	2	1	3	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSP	RFB ETH	
IHMT288_05	2	4	4	2	1	3	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSP	RFB ETH	
ARS6559	2	4	4	2	1	4	2	3	2	3	2	4	1	1	6	1	4	3	5	3	2	8	2	2	I	nd	
FF359_98	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRS	na	Lisboa3-B
ARS1900	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSEP	CAP KAN OFX ETH	
ARS2202	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSP	OFX ETH CS	
ARS3649	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSEP	KAN OFX ETH	
ARS6483	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSEP	OFX ETH	
ARS6539	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IS	nd	
ARS7884	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSEP	OFX ETH	
ARS8437	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSP	CAP ETH	
HCC1470_11	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRSEP	CAP AMK OFX MOX ETH CS PAS	
IHMT82_09	2	4	4	2	1	3	2	3	2	4	2	4	1	1	6	1	4	3	5	3	2	8	2	2	IRS	RFB CAP ETH	
HPV70_09	2	4	4	2	1	4	2	3	2	3	2	4	1	1	6	1	5	3	5	3	2	4	2	2	susceptible	nd	
ARS11131	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSP	CAP AMK OFX MOX ETH	Q1
ARS11661	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IS	nd	
ARS12740	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSP	ETH	
HCC1276_11	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	CAP AMK OFX MOX ETH	
HCC916_10	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	CAP AMK KAN OFX ETH	
HPV115_08	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	CAP AMK KAN OFX ETH	
IHMT149_09	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	RFB CAP AMK OFX MOX ETH	
IHMT194_11	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	RFB CAP AMK ETH	
IHMT308_08	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRP	RFB ETH	
IHMT361_08	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IREP	CAP ETH	
IHMT80_11	2	4	4	2	1	3	1	3	2	3	2	4	1	1	4	1	4	2	5	3	2	8	2	2	IRSEP	RFB CAP AMK ETH	
ARS4857	2	4	4	2	1	3	1	3	2	3	2	4	1	1	5	1	4	2	5	3	2	8	2	2	IRP	na	
ARS2061	2	4	4	2	1	2	1	3	2	3	2	4	1	1	4	1	6	2	5	3	2	8	2	2	IRP	CAP AMK KAN OFX ETH CS PAS	
ARS3806	2	4	4	2	1	2	1	3	2	4	2	4	1	1	4	1	4	2	5	3	2	7	2	2	I	nd	
ARS1930	2	5	4	2	1	4	2	3	2	4	2	4	1	1	6	1	5	3	5	3	3	8	2	2	IRSP	na	
ARS7860	1	4	4	2	1	2	1	3	2	3	2	4	1	1	6	1	3	1	5	3	2	9	2	2	IS	nd	
HPV157_06	2	4	4	2	1	3	1	3	2	2	1	4	1	1	5	1	6	3	3	2	2	6	2	2	IS	nd	
ARS2573	2	4	4	2	1	3	2	3	2	2	2	5	1	2	6	1	8	3	1	3	2	4	2	2	I	nd	
ARS8600	2	4	4	2	1	3	2	3	2	2	2	5	1	2	6	1	7	3	3	3	2	4	0	2	I	nd	
ARS5858	2	1	4	2	1	3	2	3	2	2	2	5	1	2	7	1	5	3	3	3	2	4	2	2	IREP	OFX	
HPV105_09	2	1	4	2	1	3	2	2	2	2	2	5	1	2	6	1	5	3	3	3	2	4	2	2	S	nd	
HPV50_09	2	1	4	2	1	3	2	3	2	2	2	5	1	2	6	1	5	3	3	3	2	2	2	2	susceptible	nd	
HVNGT	2	1	4	2	1	3	1	3	2	2	2	5	1	2	6	1	5	3	3	3	2	4	2	2	IRSEP	CAP AMK KAN	
IHMT359_03	2	1	4	2	1	3	2	3	2	2	2	5	1	2	6	1	6	3	3	3	2	4	2	2	R	nd	
ARS7571	2	3	2	2	3	3	2	3	2	4	2	3	2	2	6	1	5	3	1	3	1	9	2	2	I	nd	
ARS11463	1	3	2	2	4	2	3	3	2	2	4	4	1	1	6	1	5	3	3	2	3	7	2	2	I	nd	
ARS1760	1	3	4	2	4	2	3	3	2	1	2	4	1	2	6	1	5	3	3	2	2	5	2	2	I	nd	
FF674_96	2	2	3	2	4	4	3	2	2	2	3	4	4	2	4	1	5	3	3	3	3	3	0	2	susceptible	na	
HCC1095_10	2	2	3	2	3	5	3	2	1	4	3	2	4	2	4	1	5	3	3	3	3	7	2	2	IRE	nd	
ARS11285	2	2	3	2	5	3	3	4	2	3	3	4	4	2	5	1	5	3	3	3	5	5	3	2	IRS	AMK OFX MOX	
HPV51_09	2	1	4	2	3	4	3	4	2	3	3	4	4	2	5	1	5	3	3	3	6	9	0	2	susceptible	nd	
ARS1717	2	2	2	2	3	5	4	2	2	1	2	4	2	2	5	1	5	3	3	3	4	5	2	2	IRP	OFX ETH	
HPV65_08	2	2	4	2	2	3	3	2	1	5	3	4	2	2	5	1	5	3	3	3	3	5	2	2	susceptible	nd	
ARS7496	2	4	4	2	3	3	3	4	2	6	4	4	4	2	5	1	6	3	3	5	3	7	2	3	IS	nd	
IHMT69_11	2	4	4	2	3	3	3	4	2	6	2	4	4	2	5	1	6	3	3	5	3	7	2	3	IRSEP	RFB CAP AMK ETH	
HPV113_08	2	4	4	2	3	3	3	5	2	6	4	4	4	2	5	1	7	3	3	1	3	7	2	3	IRSEP	ETH	
IHMT295_08	2	4	4	2	3	3	3	5	2	6	4	4	4	2	5	1	7	3	3	5	3	7	2	3	IRSEP	RFB ETH	
HCC759_09	2	4	4	2	3	3	2	5	2	4	4	4	4	2	5	1	7	3	3	5	3	8	2	3	IR	ETH	
ARS9427	2	4	2	2	3	3	5	4	2	2	4	4	2	2	5	1	6	3	2	3	3	8	3	3	I	nd	

- Three major clusters: Lisboa3-A, Lisboa3-B and Q1;
- **XDR-TB isolates belonged to either Lisboa3-B or Q1 cluster;**
- No XDR-TB isolate was found to belong to Lisboa3-A cluster.

Perdigão et al, BMC Genomics 2014

Microevolution within Lisboa3 and Q1 clades: resistance acquisition

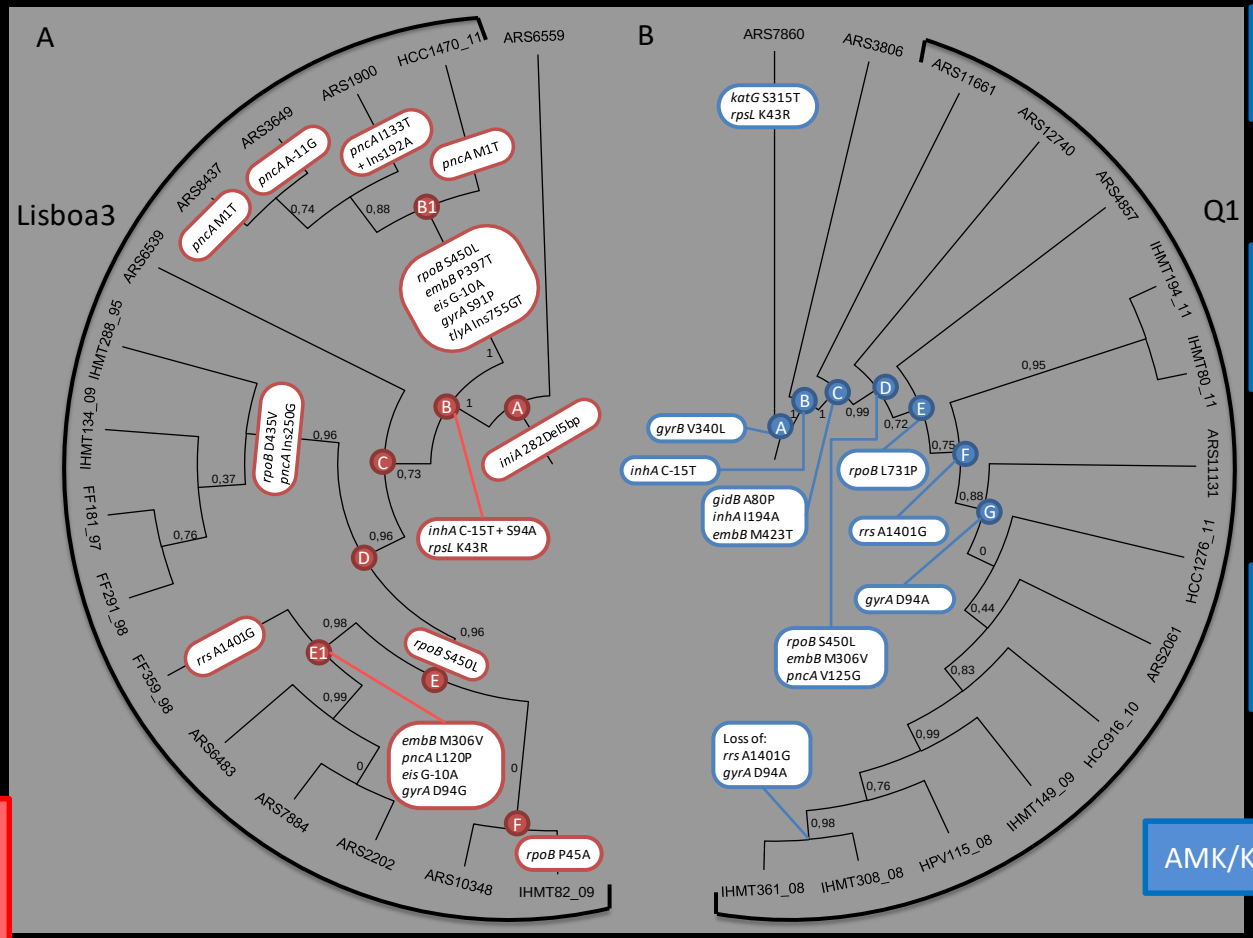
INH: *inhA* C-15T (LL) + S94A (HL)
STP: *rpsL* K43R

RIF: *rpoB* D435V
PZA: *pncA* Ins250G

RIF: *rpoB* S450L

EMB: *embB* M306V
PZA: *pncA* L120P
KAN: *eis* G-10A
FQ: *gyrA* D94G

AMK/KAN/CAP: *rrs* A1401G



INH: *inhA* C-15T (LL)

STP: gidB A80P
INH (HL): *inhA* I194A
EMB: *embB* M423T

RIF: *rpoB* S450L
EMB: *embB* M306V
PZA: *pncA* V125G

AMK/KAN/CAP: *rrs* A1401G

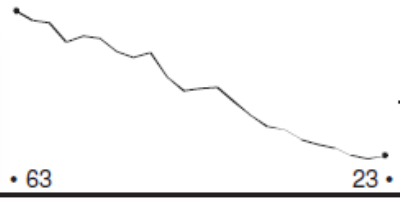
FQ: *gyrA* D94A

Whole-genome sequencing

Perdigão et al, BMC Genomics 2014

The more we detect ! – the more we treat! – the more we cure ! – but we also select DR-TB !

We have seen this in Portugal !



1990	6 214
1995	5 577
2000	4 227
2005	3 308
2010	2 487
2011	2 406
2012	2 490

J Antimicrob Chemother 2013; **68**: 27–33
doi:10.1093/jac/dks371 Advance Access publication 10 October 2012

Journal of Antimicrobial Chemotherapy

From multidrug-resistant to extensively drug-resistant tuberculosis in Lisbon, Portugal: the stepwise mode of resistance acquisition

João Perdigão[1], Rita Macedo[1,2], Carla Silva[1], Diana Machado[3], Isabel Couto[3,4], Miguel Viveiros[3], Luisa Jordao[5] and Isabel Portugal[1]*

[1]Centro de Patogénese Molecular, URIA, Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal; [2]Public Health Laboratory: Mycobacteriology/Tuberculosis, Public Health Department, Administração Regional de Saúde de Lisboa e Vale do Tejo, I.P., Lisboa, Portugal; [3]Grupo de Micobactérias, Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Lisboa, Portugal; [4]Centro de Recursos Microbiológicos (CREM), Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal; [5]Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal

We have reduced TB incidence from 63/10^5 in 1990 to 19/10^5 in 2016
We have reduced from 35% MDR in 2000 to less then 1.5% in 2016
In 2016 - 75% MDRs are XDR-TB in Lisbon now are less than 5%

Source: Direcção Geral de Saúde. 2017. Programa Nacional de Luta Contra a Tuberculose (PNT). Ponto da situação epidemiológica e de desempenho ano 2015/2016 , p. 1-33. DGS, Lisboa, Portugal ; Perdigão *et al J Antimicrob Chemother*. 2013 Jan;68(1):27-33; WHO Global Tuberculosis Report, 2016, WHO 2017

HOW M/X/TDR-TB WAS GENERATED AND CONTROLED IN PORTUGAL?

Home | Training | Clinical | MUR Zone | Business | OTC | Blogs | Jobs | My C

09 Jul 2012

Poor compliance blamed for surge in drug-resistant tuberculosis

By James Waldron

Perdigão *et al. BMC Genomics* 2014, **15**:991
http://www.biomedcentral.com/1471-2164/15/991

BMC Genomics

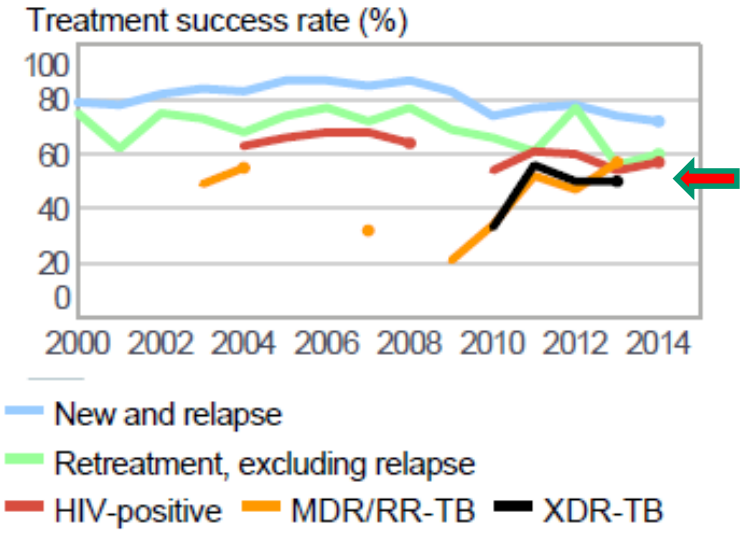
RESEARCH ARTICLE — Open Access

Unraveling *Mycobacterium tuberculosis* genomic diversity and evolution in Lisbon, Portugal, a highly drug resistant setting

João Perdigão[1], Hugo Silva[1], Diana Machado[2], Rita Macedo[3], Fernando Maltez[4], Carla Silva[1], Luisa Jordao[5], Isabel Couto[2,6], Kim Mallard[7], Francesc Coll[7], Grant A Hill-Cawthorne[8,9], Ruth McNerney[7], Arnab Pain[8], Taane G Clark[7], Miguel Viveiros[2] and Isabel Portugal[1*]

BLAME THE TB PATIENT !

TRUE BUT IT'S ONLY ONE PART OF THE PROBLEM AS WE HAVE SEEN IN PORTUGAL!



WHO Global Tuberculosis Report, 2016, WHO 2017

Ignorance on the molecular biology of the *M. tuberculois* infecting strains adds allot to this !!!

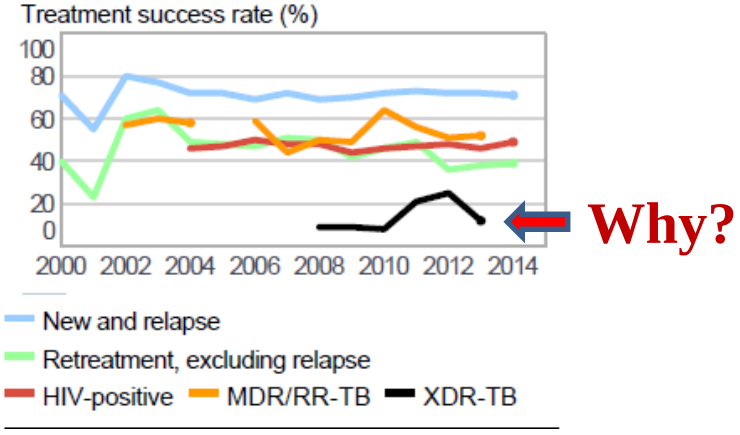
Blame also the Health System and Public Health control policies AND THE INAPROPRIATE/UNADJUSTED 2ND LINE THERAPEUTICS USED FOR THE LOCAL "*M. tuberculosis* genetic background".. - THIS REALITY CHANGED IN PT AFTER 2010

Examples from Brasil #1 – What is going on with the Brazilian *M. tuberculosis* strains and the evolution of M/XDR-TB?

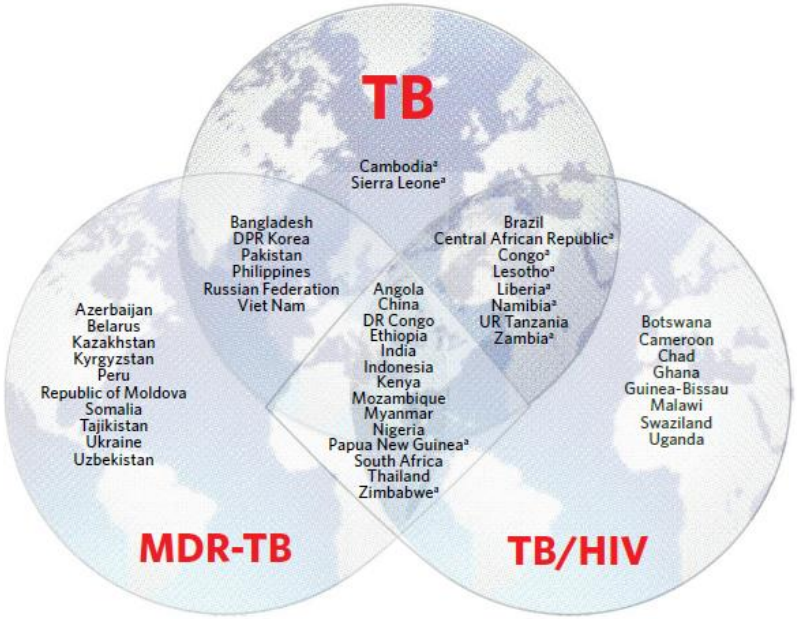
Brazil

Population 2015 — **208 million**

Estimates of TB burden*, 2015	Number (thousands)	Rate (per 100 000 population)
Mortality (excludes HIV+TB)	5.5 (5.2–5.9)	2.7 (2.5–2.8)
Mortality (HIV+TB only)	2.2 (1.2–3.6)	1.1 (0.56–1.7)
Incidence (includes HIV+TB)	84 (72–97)	41 (35–47)
Incidence (HIV+TB only)	13 (11–15)	6.3 (5.3–7.3)
Incidence (MDR/RR-TB)**	2.3 (1.9–2.8)	1.1 (0.91–1.3)



:: FIG. 2.2
Countries in the three TB high-burden country lists that will be used by WHO during the period 2016–2020, and their areas of overlap



DPR Korea, Democratic People's Republic of Korea; DR Congo, Democratic Republic of the Congo; HIV, human immunodeficiency virus; MDR, multidrug resistant; TB, tuberculosis; UR Tanzania, United Republic of Tanzania; WHO, World Health Organization

* Indicates countries that are included in the list of 30 high-burden countries for TB on the basis of the severity of their TB burden (i.e. TB incidence per 100 000 population), as opposed to the top 20, which are included on the basis of their absolute number of incident cases per year.

Examples from Brasil #1 – What is going on with the Brazilian *M. tuberculosis* RD^{Rio} ? Known to be very virulent and causing severe disease, is it associated with M/XDR-TB in Brazil? Does it have particular SNPs for resistance?

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2008, p. 1259–1267 Vol. 46, No. 4
0095-1137/08/$08.00+0 doi:10.1128/JCM.02231-07

Application of Sensitive and Specific Molecular Methods To Uncover Global Dissemination of the Major RD^{Rio} Sublineage of the Latin American-Mediterranean *Mycobacterium tuberculosis* Spoligotype Family▽‡

Andrea L. Gibson,[1]† Richard C. Huard,[1,2]† Nicolaas C. Gey van Pittius,[3] Luiz Claudio Oliveira Lazzarini,[1,4] Jeffrey Driscoll,[5] Natalia Kurepina,[6] Thierry Zozio,[7] Christophe Sola,[7,8] Silvana Miranda Spindola,[9] Afrânio L. Kritski,[4] Daniel Fitzgerald,[1,10] Kristin Kremer,[11] Helmi Mardassi,[12] Poonam Chitale,[1] Jessica Brinkworth,[1,13] Dario Garcia de Viedma,[14,15] Brigitte Gicquel,[8] Jean W. Pape,[10] Dick van Soolingen,[11] Barry N. Kreiswirth,[6] Robin M. Warren,[3] Paul D. van Helden,[3] Nalin Rastogi,[7] Philip N. Suffys,[16] Jose Lapa e Silva,[4] and John L. Ho[1]*

JOURNAL OF CLINICAL MICROBIOLOGY, July 2008, p. 2175–2183 Vol. 46, No. 7
0095-1137/08/$08.00+0 doi:10.1128/JCM.00065-08

RD^{Rio} *Mycobacterium tuberculosis* Infection Is Associated with a Higher Frequency of Cavitary Pulmonary Disease▽

Luiz Claudio Oliveira Lazzarini,[1,3] Silvana Miranda Spindola,[4] Heejung Bang,[2] Andrea L. Gibson,[1] Scott Weisenberg,[1] Wania da Silva Carvalho,[5] Claudio José Augusto,[6] Richard C. Huard,[1,7] Afrânio L. Kritski,[3] and John L. Ho[1]*

Division of International Medicine and Infectious Diseases, Department of Medicine,[1] and Division of Biostatistics and Epidemiology, Department of Public Health,[2] Weill Medical College of Cornell University, New York, New York; Tuberculosis Research Unit, Medical School of Federal University of Rio de Janeiro, Rio de Janeiro, Brazil[3]; Departamento de Clinica Medica/Pneumologia da Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil[4]; Faculdade de Farmacia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil[5]; Fundação Ezequiel Dias, Minas Gerais, Belo Horizonte, Brazil[6]; and Clinical Microbiology Service and the Department of Pathology, New York-Presbyterian Hospital, Columbia University Medical Center, New York, New York[7]

JCM Journals.ASM.org April 2013 Volume 51 Number 4 Journal of Clinical Microbiology p. 1071–1077

Mycobacterium tuberculosis of the RD^{Rio} Genotype Is the Predominant Cause of Tuberculosis and Associated with Multidrug Resistance in Porto Alegre City, South Brazil

Elis Regina Dalla Costa,[a] Luiz Claudio Oliveira Lazzarini,[b] Paulo Fernado Perizzolo,[a] Chyntia Acosta Díaz,[c] Fernanda S. Spies,[a] Lucas Laux Costa,[a] Andrezza W. Ribeiro,[a] Caroline Barroco,[a] Sandra Jungblut Schuh,[e] Marcia Aparecida da Silva Pereira,[c] Claudia F. Dias,[e] Harrison M. Gomes,[c] Gisela Unis,[e] Arnaldo Zaha,[f] Pedro E. Almeida da Silva,[d] Philip N. Suffys,[c] Maria L. R. Rossetti[a,g]

Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS), Porto Alegre, Brazil[a]; Tuberculosis Research Unit, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil[b]; Laboratory of Molecular Biology Applied to Mycobacteria, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil[c]; Fundação Universidade de Rio Grande, Rio Grande, Brazil[d]; Hospital Sanatório Partenon (HSP), Porto Alegre, Brazil[e]; Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil[f]; Universidade Luterana do Brazil (ULBRA/RS), Porto Alegre, Brazil[g]

Examples from Brasil #2– What is going on with the most prevalent *M. tuberculosis* lineages in Brazil..? Which ones are associated with M/XDResistance?

March 2011 | Volume 6 | Issue 3 | e18256

OPEN ACCESS Freely available online

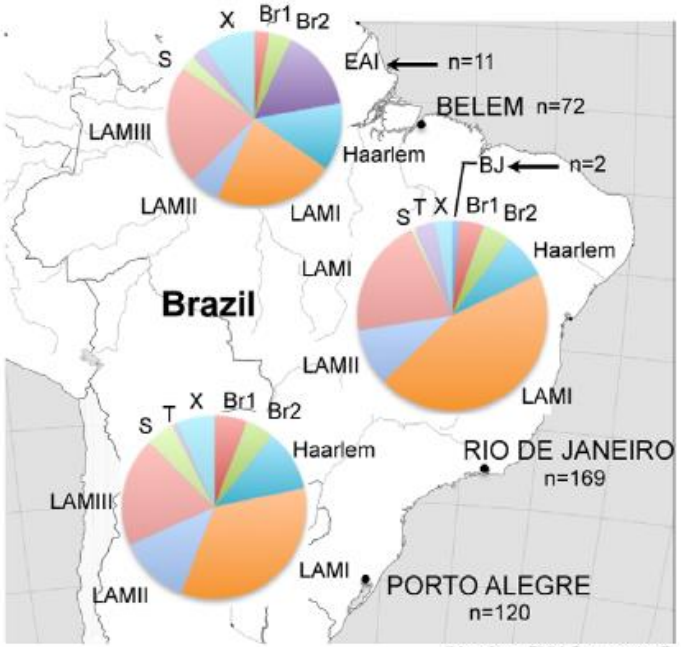
PLoS one

The Forest behind the Tree: Phylogenetic Exploration of a Dominant *Mycobacterium tuberculosis* Strain Lineage from a High Tuberculosis Burden Country

Maranibia Cardoso Oelemann[1], Harrison M. Gomes[1], Eve Willery[2,3,4,5], Lia Possuelo[6], Karla Valéria Batista Lima[7], Caroline Allix-Béguec[2,3,4,5¤a], Camille Locht[2,3,4,5], Yves-Olivier L. Goguet de la Salmonière[8], Maria Cristina Gutierrez[2,3,4,5,8¤b], Philip Suffys[1], Philip Supply[2,3,4,5]*

1 Laboratory of Molecular Biology Applied to Mycobacteria, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil, **2** INSERM U1019, Lille, France, **3** CNRS UMR 8204, Lille, France, **4** Univ Lille Nord de France, Lille, France, **5** Institut Pasteur de Lille, Center for Infection and Immunity of Lille, Lille, France, **6** Center of Scientific and Technological Development, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, Brazil, **7** Institute Evandro Chagas, Belém, Brazil, **8** Department of Infection and Epidemiology, Institut Pasteur, Paris, France

Mycobacterium tuberculosis genetics network in Brazil – we need to update and expand this network!

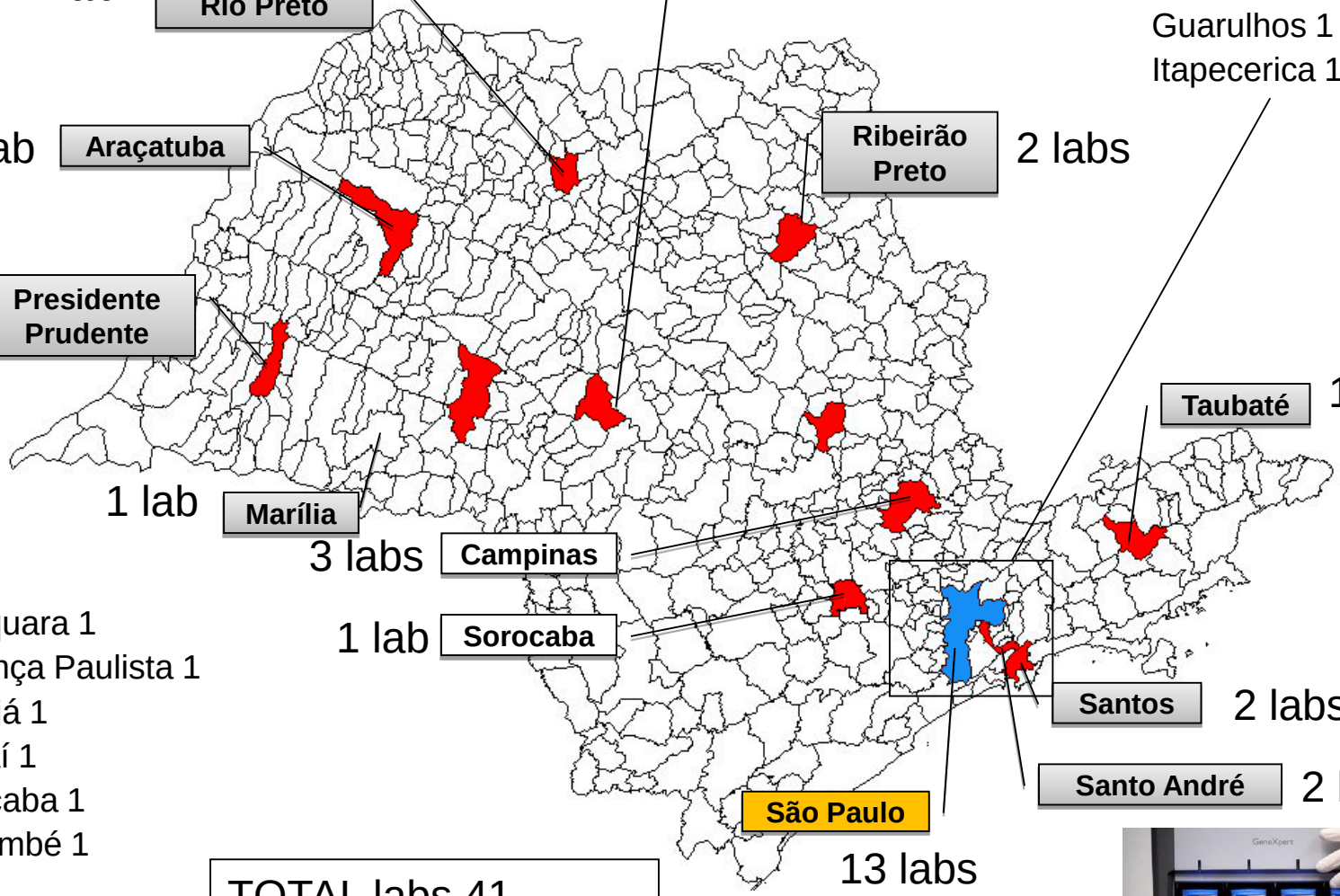


(Map from BYU Geography Dept)

Figure 4. ***M. tuberculosis strain*** **lineage distribution among three Brazilian regions.** Lineage distributions result from the congruence analyses shown in Fig. 1 and 3. One isolate from Rio De Janeiro was of an unknown lineage/branch. Arrows indicate region-specific lineages. Br1 and Br2, Brazil 1 and 2, respectively.

Example from Brazil #3 - São Paulo network of Fast Molecular Test (TRM-SP NET)

Grande São Paulo
Barueri 1
Carapicuíba 1
São Bernardo 1
Franco da Rocha 1
Guarulhos 1
Itapecerica 1



Araraquara 1
Bragança Paulista 1
Guarujá 1
Jundiaí 1
Piracicaba 1
Tremembé 1

TOTAL labs 41
Total equipments 63

Rifampicin resistance by molecular test (GeneXpert)

- From January to May 2017:
- 53 rifampicin resistant cases by molecular test
  - 7 rifampicin susceptible cases by MGIT
  - **13.2% discrepant results**

- 2016
  - **30% discrepant results**

What is the reason for so high percentage of discrepant results? - Pre-analytic problems? Cross contamination? Problems with DST?

No. Isolates	DST RIF	*rpoB* Xpert	*rpoB* RRDR sequencing	Relation with RIF resistance Critical concentration (fluorometric method) = 1 µg/ml	Notes	References
1	S	R	T508A	Non-related **MIC < 1 µg/ml**	Both results are correct	Ocheretina et al., 2014
1	S	R	T508G	Non-related	-	Not described
1	S	R	T508T	Silent - Non-related	Both results are correct	Ocheretina et al., 2014
1	S	R	L511P	Non-related* **MIC < 0,25 µg/ml**	Both results are correct	Berrada *et al*., 2016 Ocheretina et al., 2014 Aubry et al., 2014
2	S	R	L511R	Questionable	-	
6	S	R	F514F	Silent - Non-related **MIC 0,125 µg/ml**	Both results are correct	Alonso et al., 2011 Berrada *et al*., 2016
1	S	R	D516Y	Non-related* **MIC 0,25 µg/ml**	Both results are correct	Berrada *et al*., 2016 Andres et al., 2014 Aubry et al., 2014
1	S	R	D516F	Questionable **MIC 2 µg/ml**		Berrada *et al*., 2016
1	S	R	L524L	Silent - Non-related	Both results are correct	
4	S	R	H526N	Non-related **MIC 0,125-0,25 µg/ml**	Both results are correct	Berrada *et al*., 2016 Jamieson *et al*., 2014
1	S	R	nd	-	-	-
2	S	R	H526N + WT (MIX)	Non-related **MIC 0,125-0,25 µg/ml**	Both results are correct	Berrada *et al*., 2016
1	S	R	H526S	Non-related **MIC 0,25 µg/ml**	Both results are correct	Berrada *et al*., 2016
1	S	R	L533P	Non-related* **MIC 0,5 µg/ml**	Both results are correct	Ma et al., 2006; Berrada *et al*., 2016
1	S	R	L533Q	Non-related* **MIC <1 µg/ml**	Both results are correct	Ma et al., 2006
1	S	R	L533Q + WT (MIX)	Non-related **MIC <1 µg/ml**	Both results are correct	Ma et al., 2006
1	S	R	P535S	Non-related	Can appears in combination with drug resistant mutations as H526Y	Tracevska et al., 2002
6	S	R	WT	-	**Problem with GeneXpert or samples?**	-
N= 33						

Blame the Gene-Expert, but why so many silent mutations in rpoB in São Paulo?

*Detected also in resistant isolates; nd, not done

nature COMMUNICATIONS

ARTICLE

Received 11 Apr 2014 | Accepted 25 Jul 2014 | Published 1 Sep 2014

DOI: 10.1038/ncomms5812 OPEN

A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains

Francesc Coll[1], Ruth McNerney[1], José Afonso Guerra-Assunção[2], Judith R. Glynn[2], João Perdigão[3], Miguel Viveiros[4], Isabel Portugal[3], Arnab Pain[5], Nigel Martin[6] & Taane G. Clark[1,2]

Coll *et al. Genome Medicine* (2015) 7:51
DOI 10.1186/s13073-015-0164-0

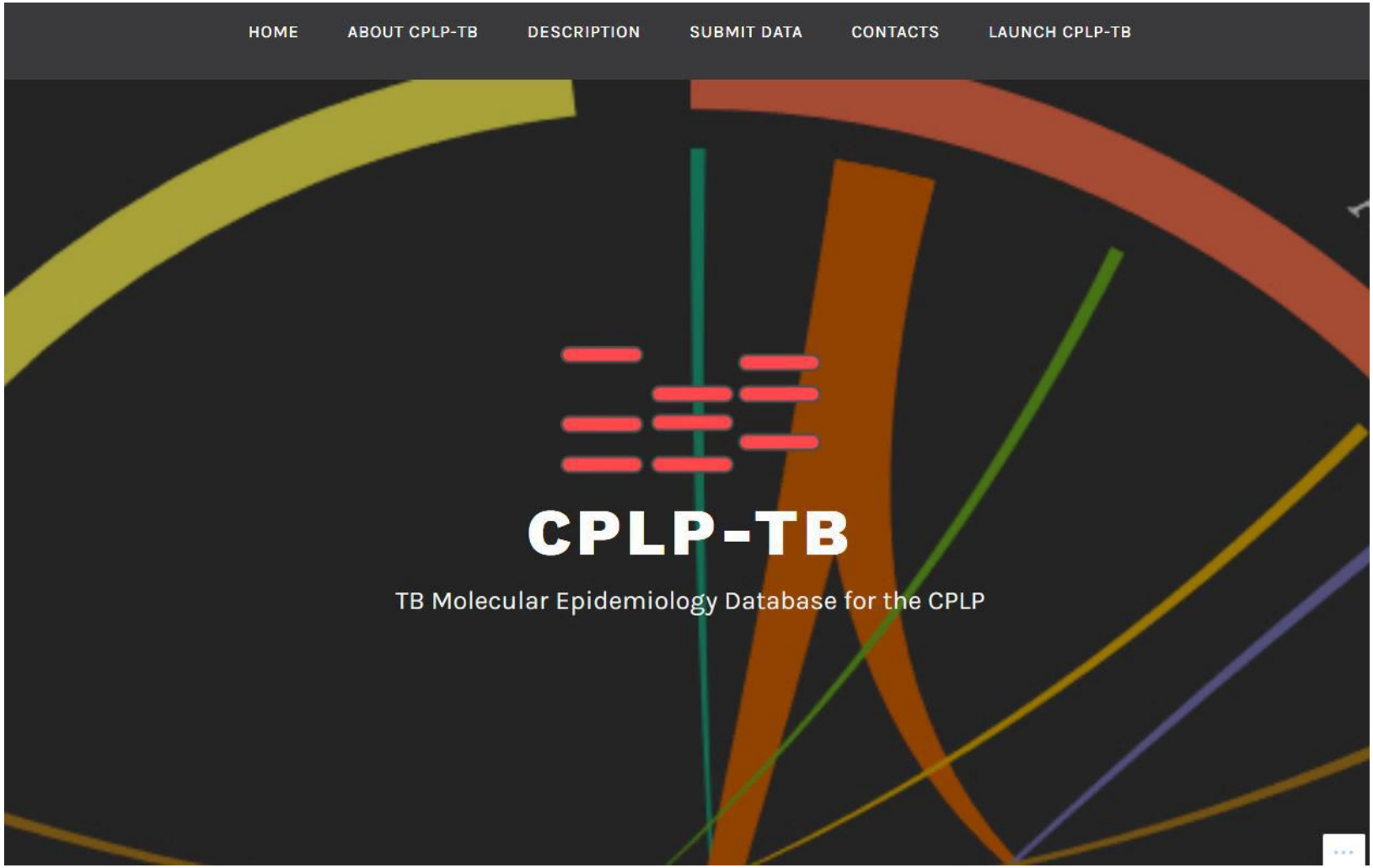
Genome Medicine

METHOD Open Access

Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences

Francesc Coll[1], Ruth McNerney[1], Mark D Preston[1], José Afonso Guerra-Assunção[1], Andrew Warry[2], Grant Hill-Cawthorne[3,4], Kim Mallard[1], Mridul Nair[3], Anabela Miranda[5], Adriana Alves[5], João Perdigão[6], Miguel Viveiros[7], Isabel Portugal[6], Zahra Hasan[8], Rumina Hasan[8], Judith R Glynn[1,9], Nigel Martin[10], Arnab Pain[3*] and Taane G Clark[1*]

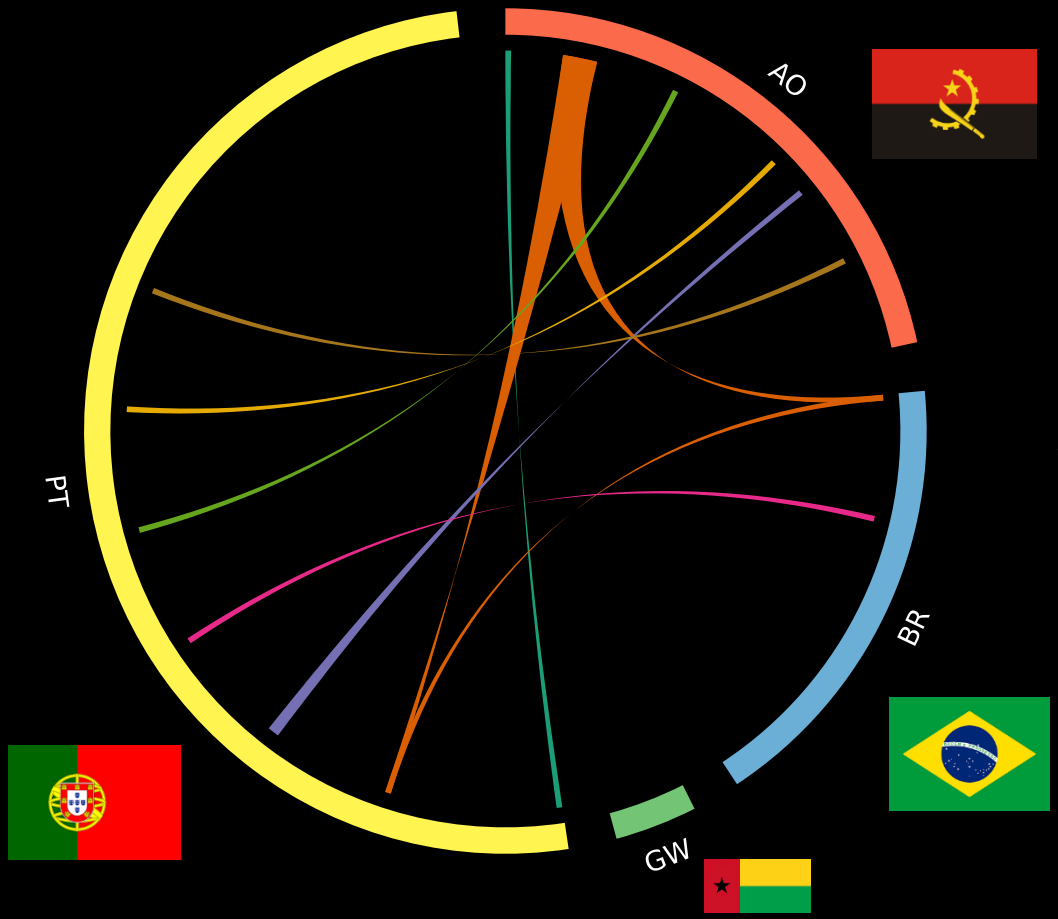
CPLP-TB: Database and Analyis Tool for the Lusophone Space



Available at: www.ff.ulisboa.pt/cplp-tb

www.ff.ulisboa.pt/cplp-tb

Transnational clustering: an overview of the ancestral voyage of *M. tuberculosis* among lusophone countries



Multiple transnational clustering highlights the complex interactions between Lusophone countries and are potentially associated with migratory events.

:: 24-*loci* MIRU-VNTR

: N=381 CPLP clinical isolates

Perdigão et al, Sci Reports 2017 (submitted)

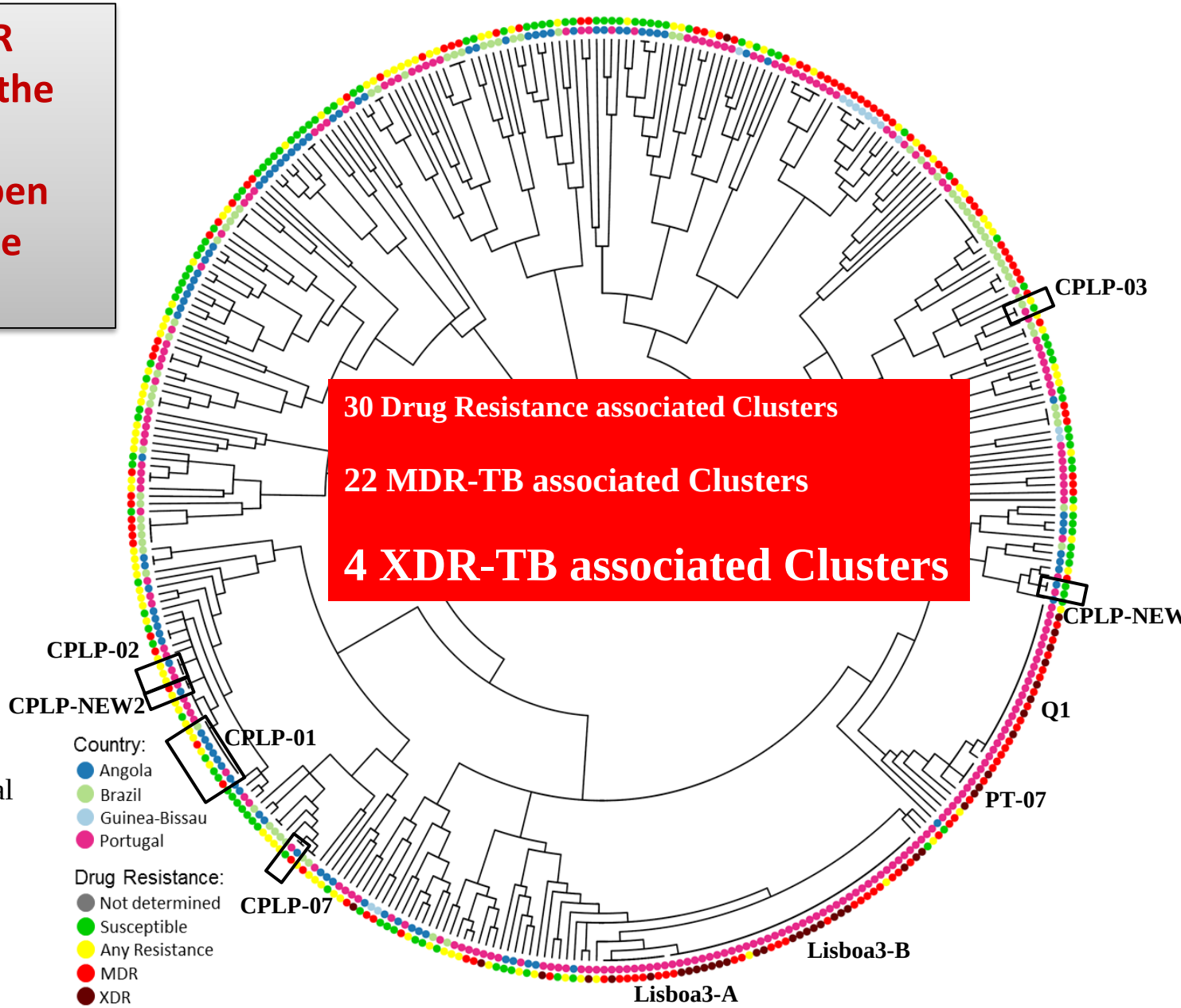
A MIRU-VNTR Framework for the CPLP : The CPLP-TB open acess database

:: 24-*loci* MIRU-VNTR

: N=381 clinical isolates

33 genetic clusters
153 isolates
(40.2%)

6 confirmed transnational clusters



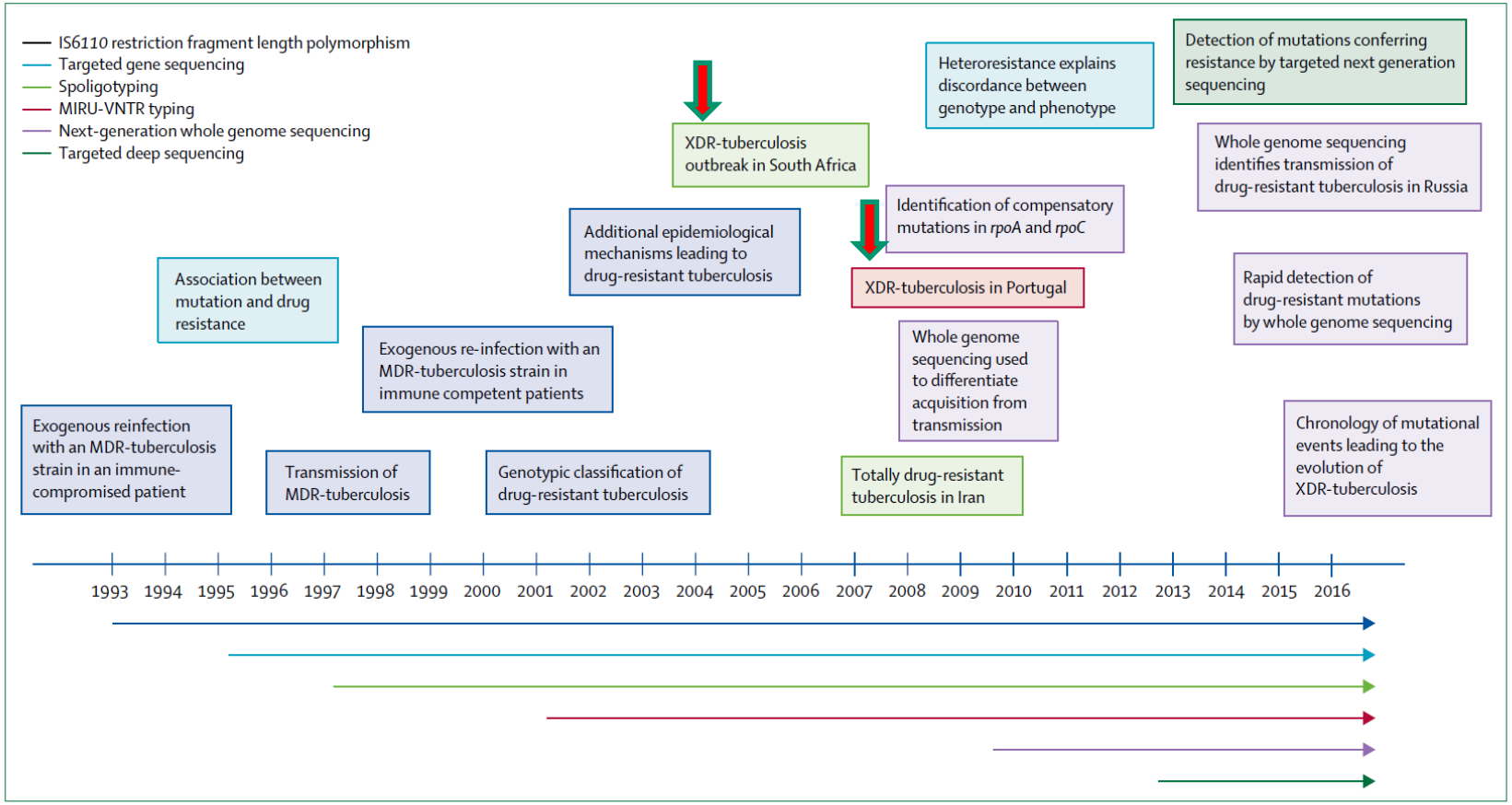


Figure 2: **Timeline of key molecular epidemiological findings using different genotyping tools**
Genotyping tools used for each finding are indicated by different colours. MDR=multidrug resistant. MIRU–VNTR=mycobacterial interspersed repetitive units–variable numbers of tandem repeat. XDR=extensively drug-resistant.

The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis

Keertan Dheda, Tawanda Gumbo*, Gary Maartens*, Kelly E Dooley*, Ruth McNerney*, Megan Murray*, Jennifer Furin*, Edward A Nardell*, Leslie London*, Erica Lessem*, Grant Theron, Paul van Helden, Stefan Niemann, Matthias Merker, David Dowdy, Annelies Van Rie, Gilman K H Siu, Jotam G Pasipanodya, Camilla Rodrigues, Taane G Clark, Frik A Sirgel, Aliasgar Esmail, Hsien-Ho Lin, Sachin R Atre, H Simon Schaaf, Kwok Chiu Chang, Christoph Lange, Payam Nahid, Zarir F Udwadia, C Robert Horsburgh Jr, Gavin J Churchyard, Dick Menzies, Anneke C Hesseling, Eric Nuermberger, Helen McIlleron, Kevin P Fennelly, Eric Goemaere, Ernesto Jaramillo, Marcus Low, Carolina Morán Jara, Nesri Padayatchi, Robin M Warren**

Lancet Respir Med 2017;
5: 291–360
Published Online
March 23, 2017
http://dx.doi.org/10.1016/S2213-2600(17)30079-6

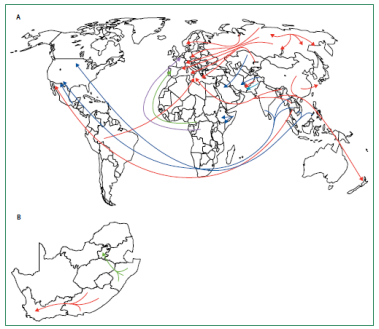


Figure 3: Inter-country and intra-country spread of drug-resistant tuberculosis according to *M tuberculosis* genotype
(A) Worldwide spread of drug-resistant strains of *M tuberculosis*. Red=Beijing strain.[76,78,79,81,82] Green=LAM6 strain.[77] Light blue=Haarlem1 strain.[79] Purple=T1 strain.[81] Dark blue=unique strains.[80,82,83] (B) Ongoing intra-country spread of extensively drug-resistant tuberculosis strains in South Africa. Red=atypical Beijing strains. Green=LAM4 strain.[77] Inter-country spread has also been reported in Brazil,[84] and Russia.[85]

HOW TO FIGHT M/X/TDR-TB?

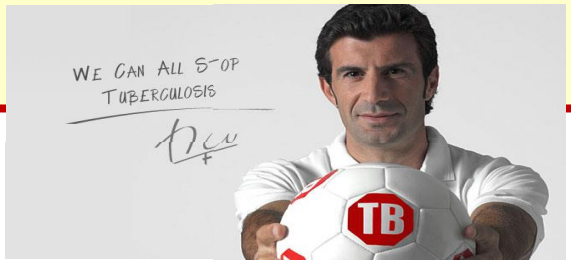
World Health Organization

World Health Organization | THE STOP TB STRATEGY

THE END TB STRATEGY

"Early" detection of TB + M/XDR-TB
(Laboratory)
+
DOT strategy + "Molecular Epidemiology"
(Ministry of Health)

WE WILL END TB | END TB



Conclusions

- **Molecular epidemiology was/is essential to control TB and especially M/XDR-TB – Need for centralized detection (Reference Centers and Labs for early detection) plus Nationally centralized molecular epidemiology databases (nowdays internet based)**

- This strategy allows **the description of a considerable host/setting adaptation phenomena with the identification of well adapted variants/clades that are prone to develop infection and resistance** plus early detection of outbrakes of these strains in specific settings (prisons/ hospitals/ cities/ etc…)

  - Genetic markers for strain typing/tracking
  - Better, more efficient and tolerable treatments (eg: only a subset of the Lisboa3 strains is associated with XDR-TB and requires a specific 2nd line treatment regimen)
  - Study of long term host-pathogen adaptation
  - Identification of the **LOCAL** most prevalent mutations for resistance and its microevolution path…Helps in the development of novel and locally adapted molecular tests – **very important to control the "iatrogenicity" of the XDR-TB! – WE NEED A BRAZILIAN MTB GENETICS NETWORK !**

World Health Organization

REDE-TB

Acknowledgments

Stop TB Partnership

THE END TB STRATEGY

FUNDAÇÃO CALOUSTE GULBENKIAN

THANK YOU!

CAPES

ESGMYC ESCMID STUDY GROUP FOR MYCOBACTERIAL INFECTIONS
European Society of Clinical Microbiology and Infectious Diseases

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

https://www.escmid.org/research_projects/study_groups/mycobacterial_infection/