

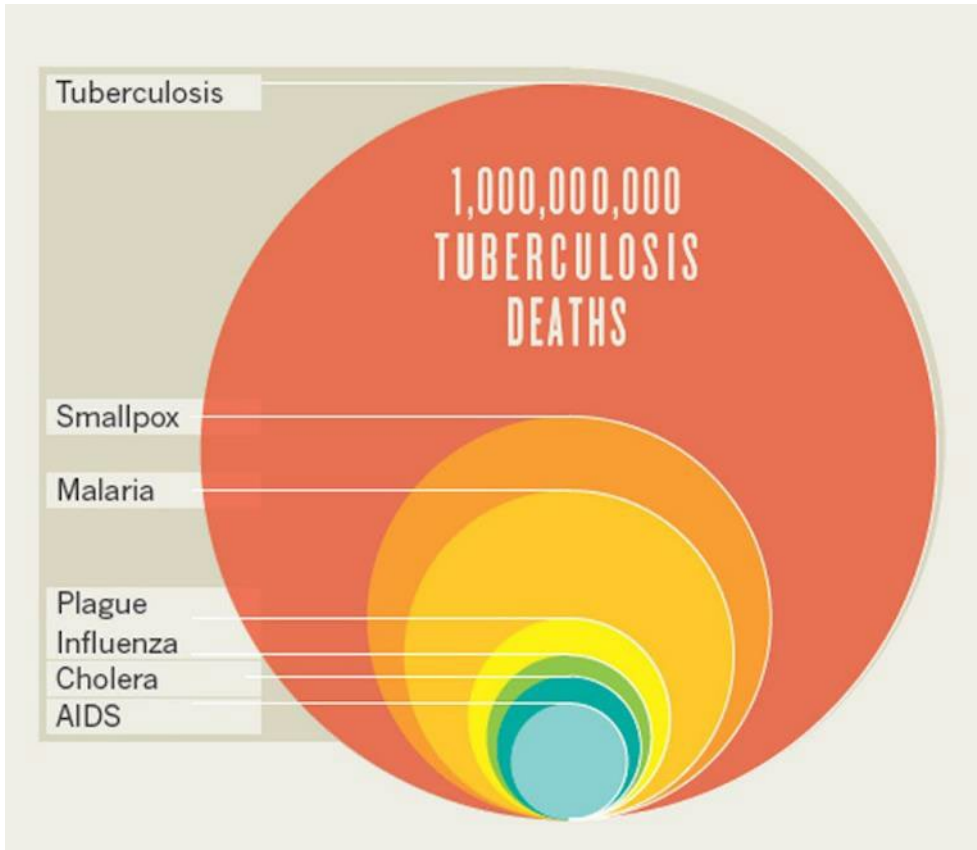
Advances in *Mycobacterium tuberculosis* Genomics to Inform TB Control

Basel, 21th March 2023

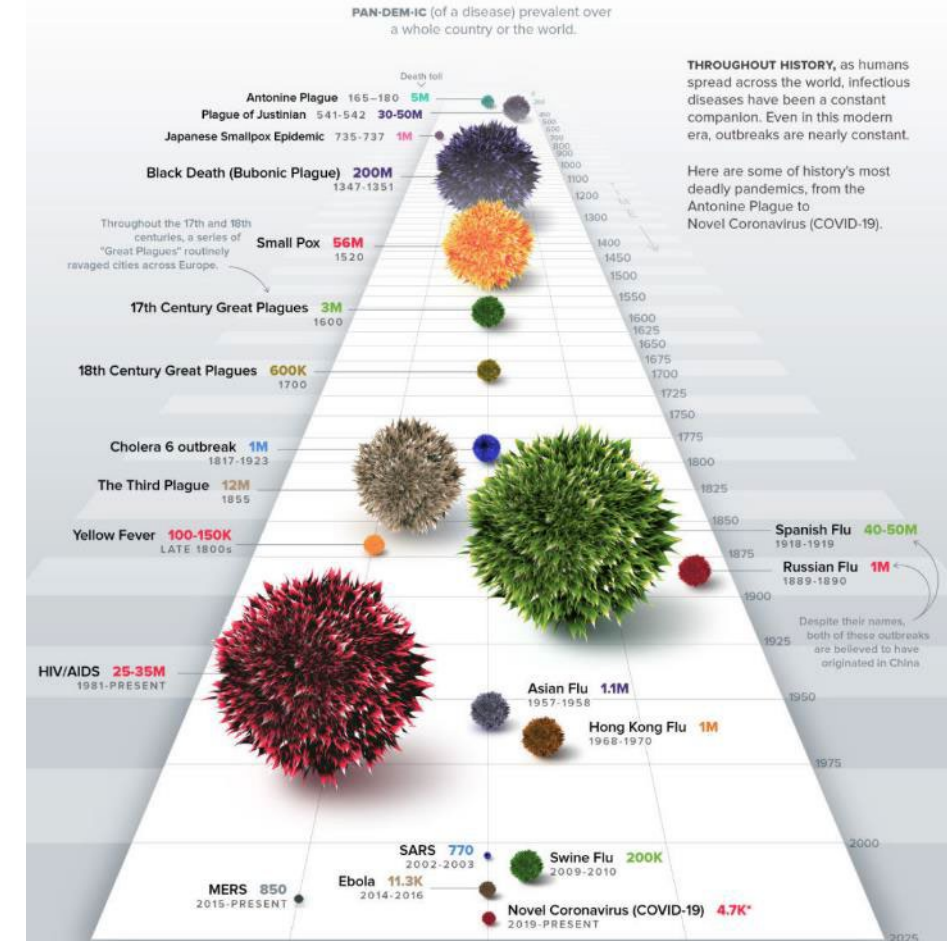
The Tuberculosis Pandemic – A Call to Action – Swiss TPH Symposium

Iñaki Comas, PhD
icomas@ibv.csic.es
Institute of Biomedicine of Valencia (CSIC-IBV)
Valencia, Spain

Image: Visual Capitalist
HISTORY OF PANDEMICS

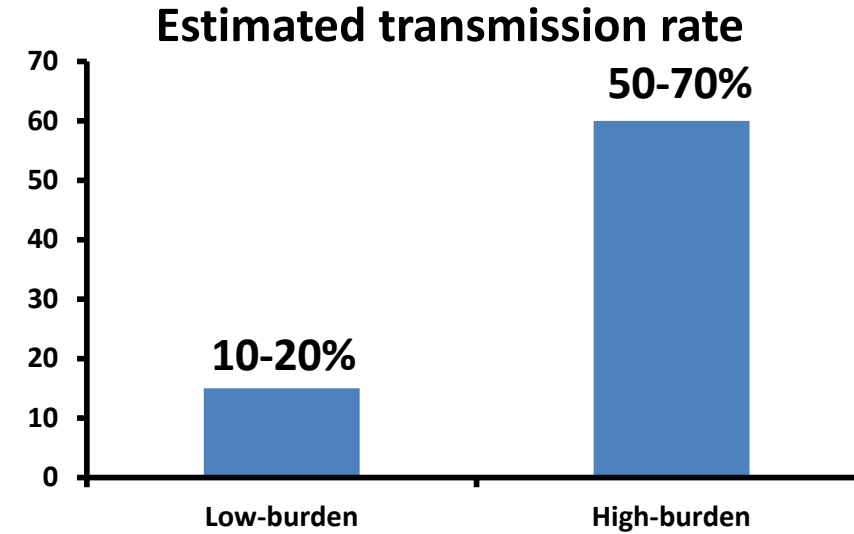
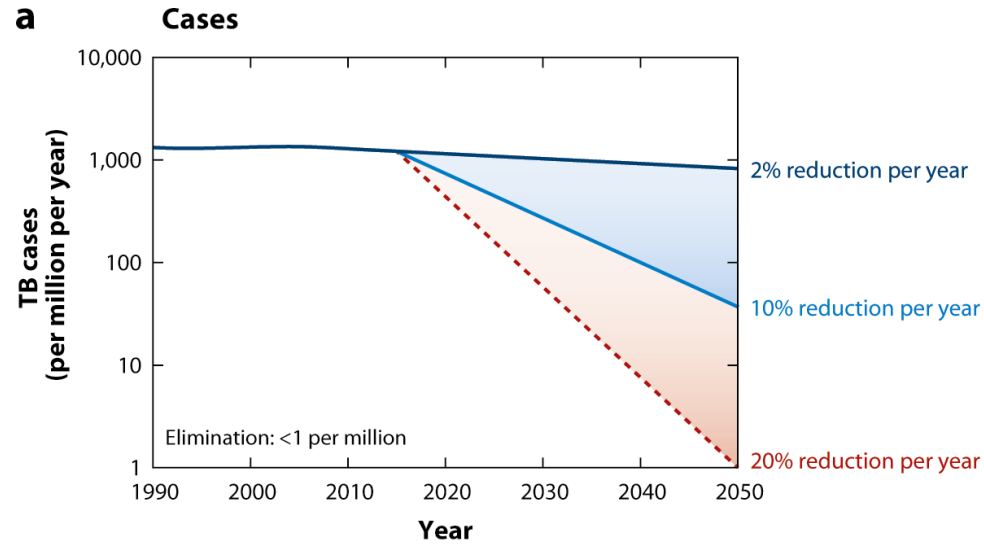


Paulson T. Epidemiology: a mortal foe. Nature 2013;502(7470):S2-3

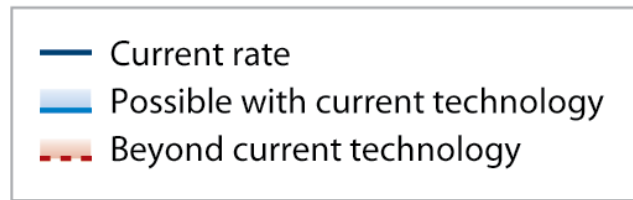


Endemic = harmless in the Global North

Slow and Steady is not an option!



Dye 2013



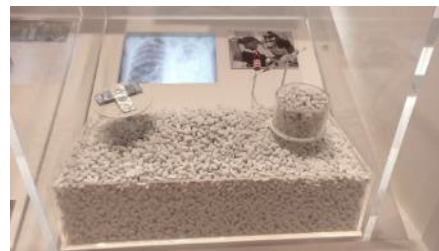
• **Decades old tools**



DIAGNOSTICS
~120 y/o



Vaccine
100 y/o



Drugs
~50 y/o

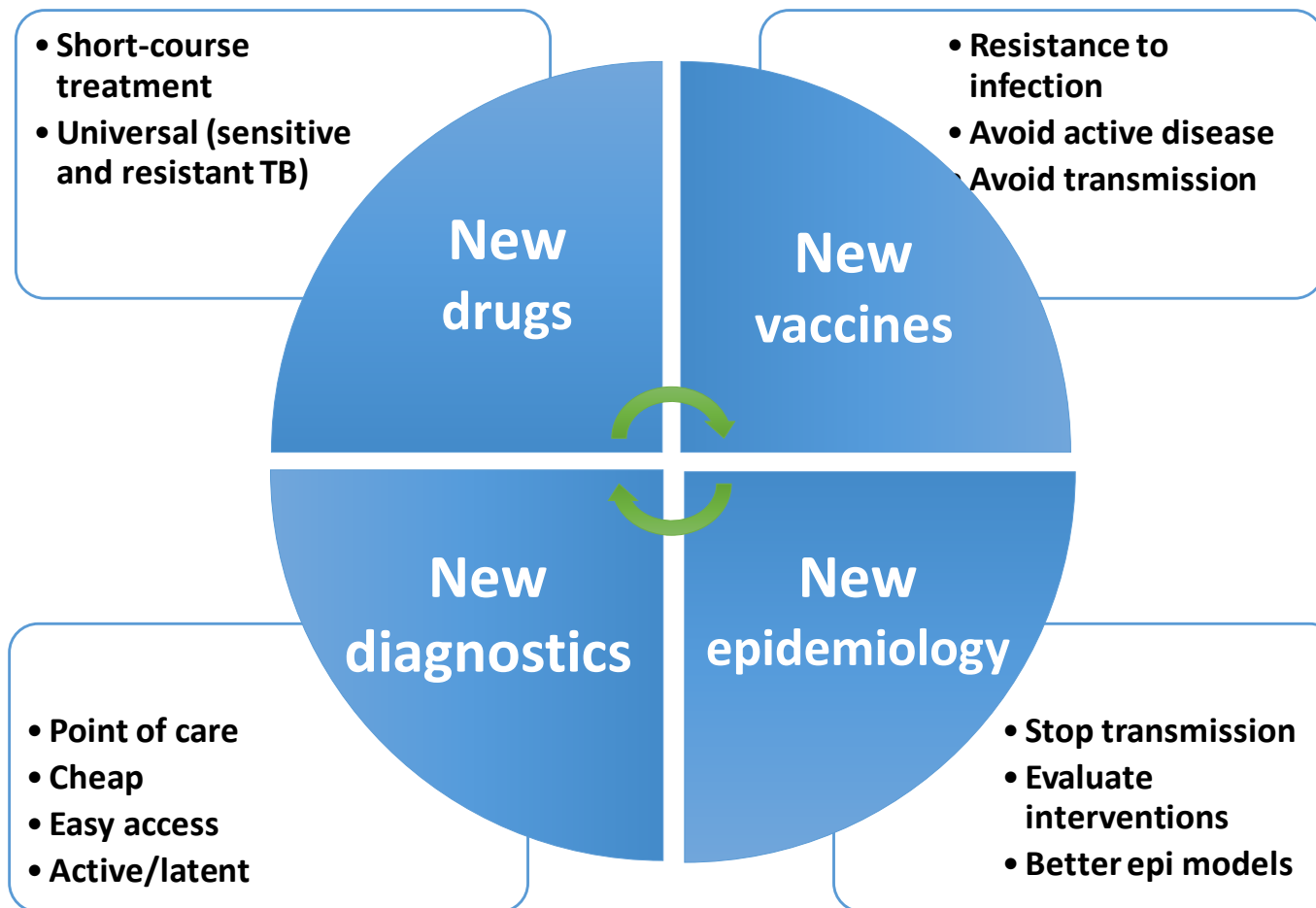


Epidemiology
~100 y/o

Modernizing DOTS to accelerate TB eradication

2013 – Bedaquiline/Delamanid

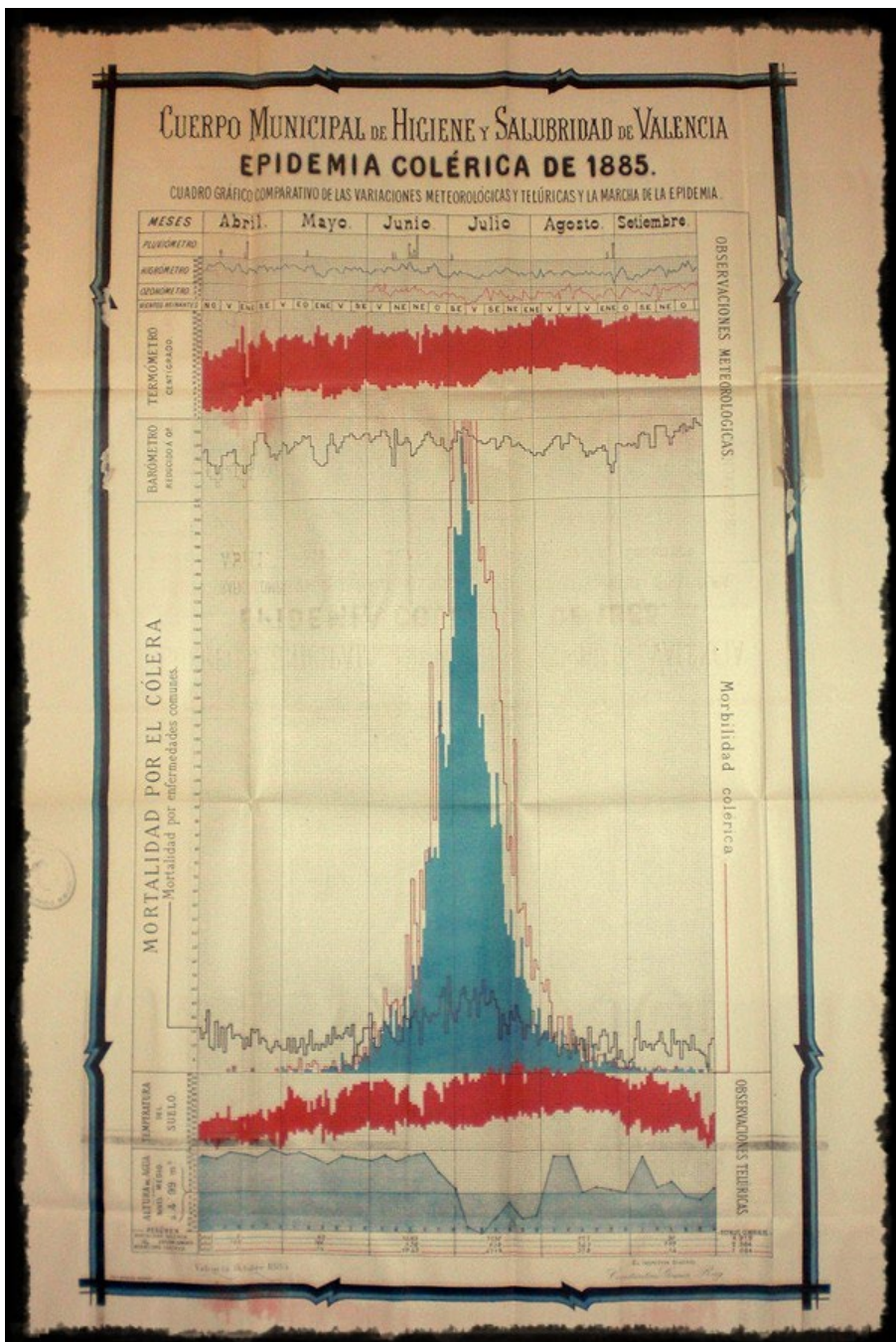
2013 – MVA85A



2010 - Expert MTB/RIF

2010- Whole genome

- We have been tracking and treating diseases for centuries...with very little knowledge about the pathogen behind



Valencia cholera vaccination

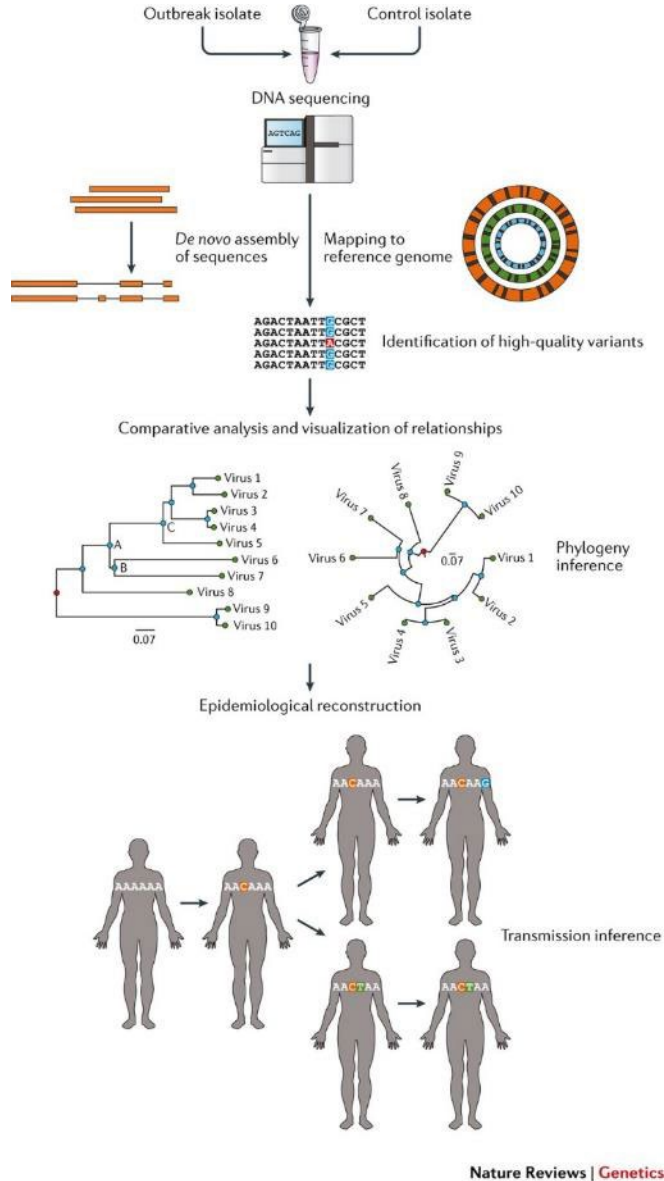


Valencia children TB sanatorium



Streptomycin

Genomic Epidemiology for Infectious Diseases



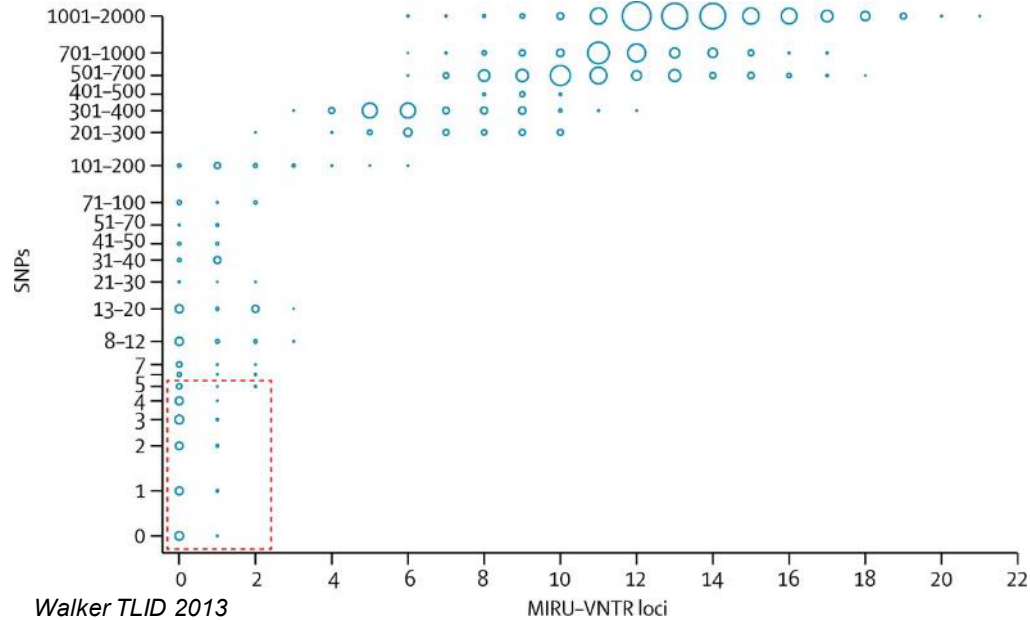
1. Thanks to sequencing advances we can now sequence **hundreds** of samples at **low prices**, almost **real-time if needed**
2. We have **computer power** to genetically compare thousand, **even millions of samples** and to share globally
3. We have tools to **convert genetic similarities** to local, regional and global **patterns of transmission** of the disease **and impact of genetic diversity**

All this is particularly true for SARS-CoV-2 but it was already advanced in TB!

Role of *Mtb* genomics in public health

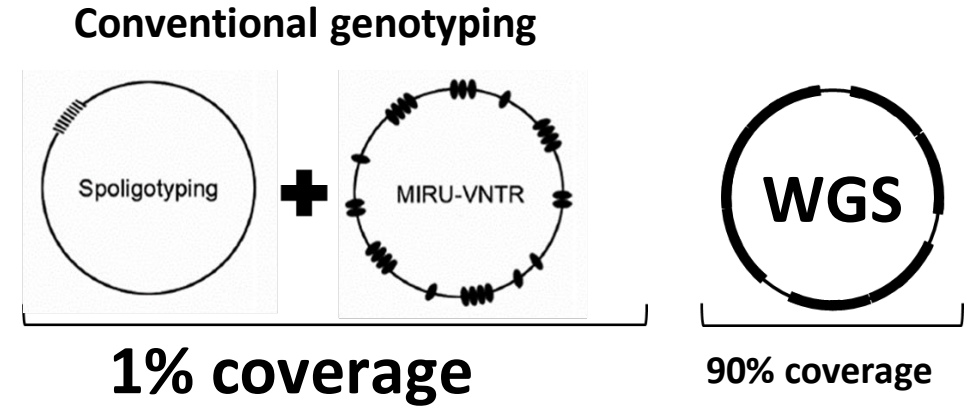
1. To investigate (on-going)
2. To evaluate (control programmes, interventions)
3. To understand TB transmission

Genome sequencing reveals limitations of typing tools



- Identical MIRU-VNTR not a marker of recent transmission (Wyllie EBioMedicine 2018)
- Identical MIRU pairs separated by 10-125 years! (Meehan EBioMedicine 2018)

Why WGS?

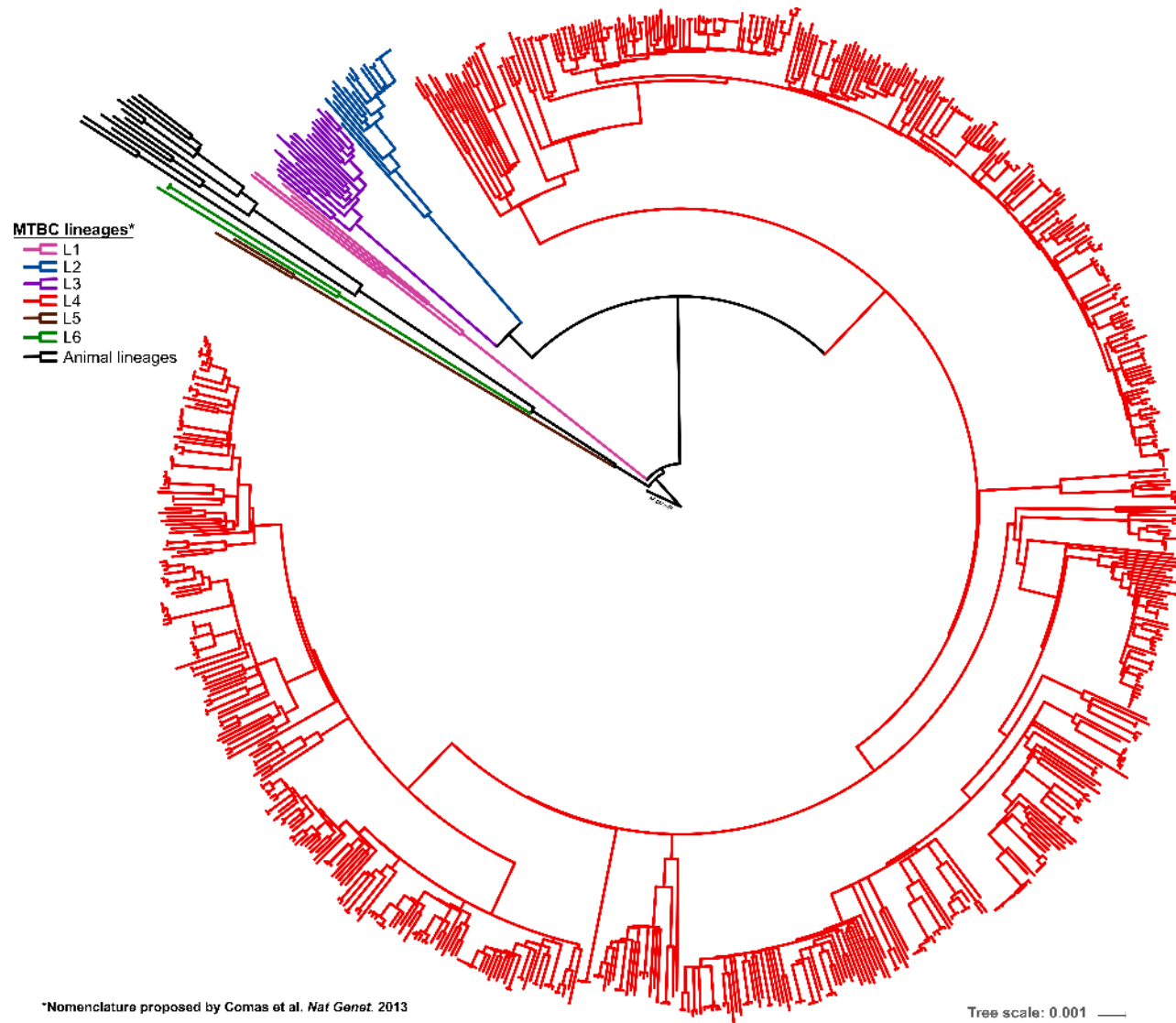


Use of genome for recent transmission:

0-5-12 SNPs (genetic differences)

(Walker LID 2013, Jajou Plos One 2018, Guerra-Asunção 2015)

Valencia Region MTBC genome Phylogeny (2014-2016)



Mariana G. López
Senior Scientist

Low burden setting

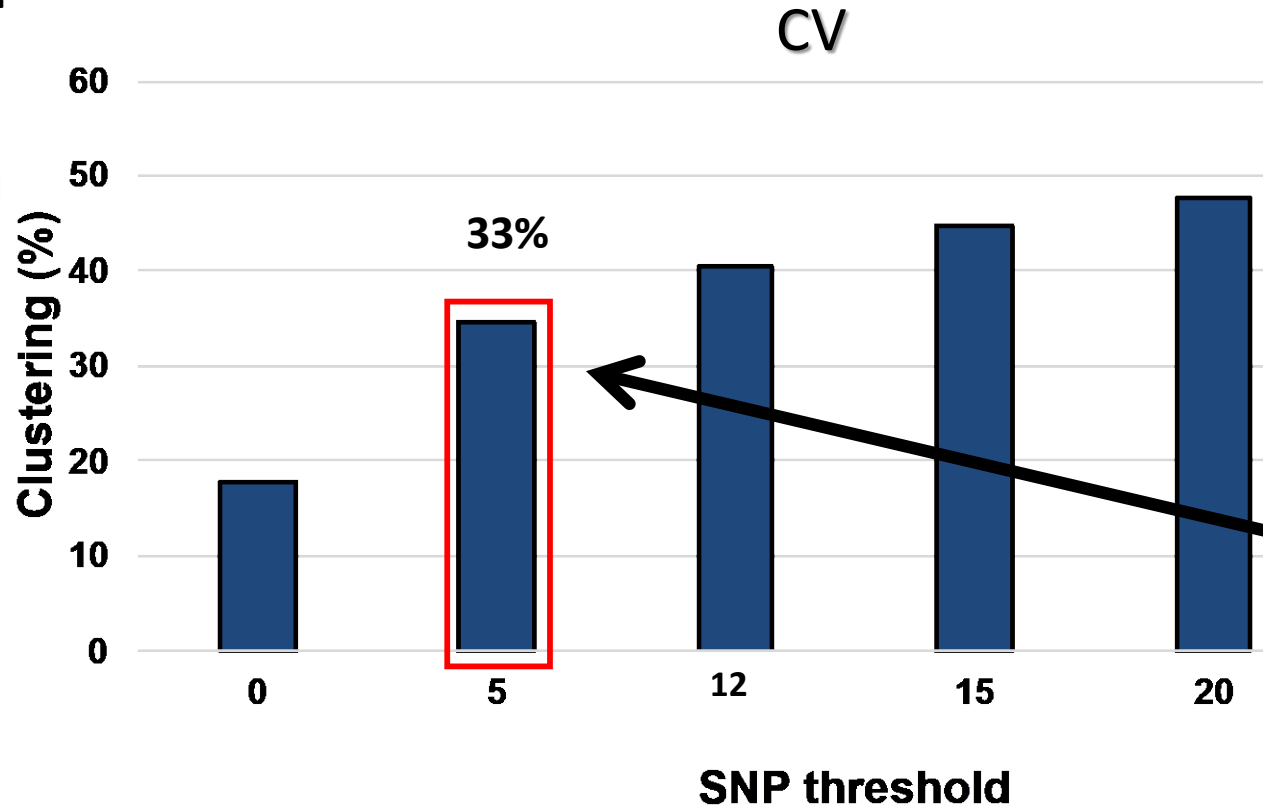
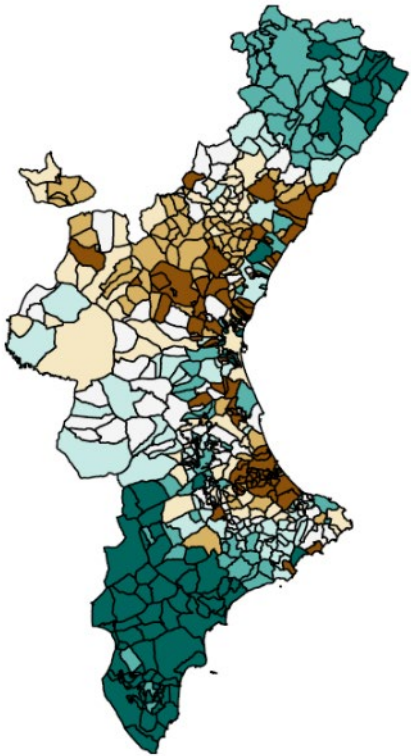
8/100,000

Transmission vs long-term reactivation vs imported

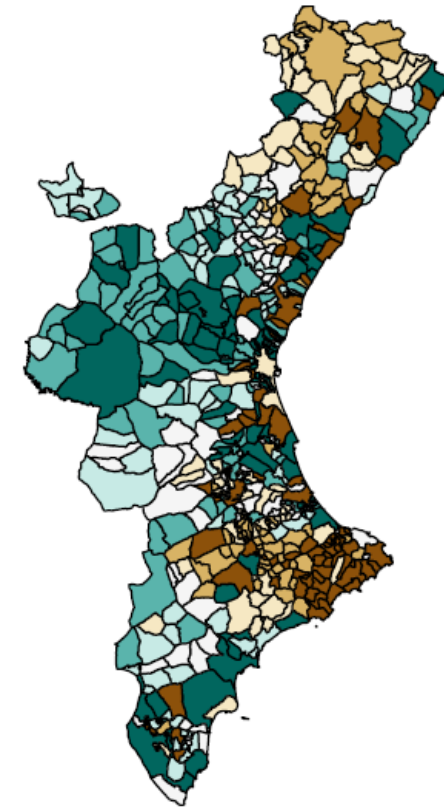


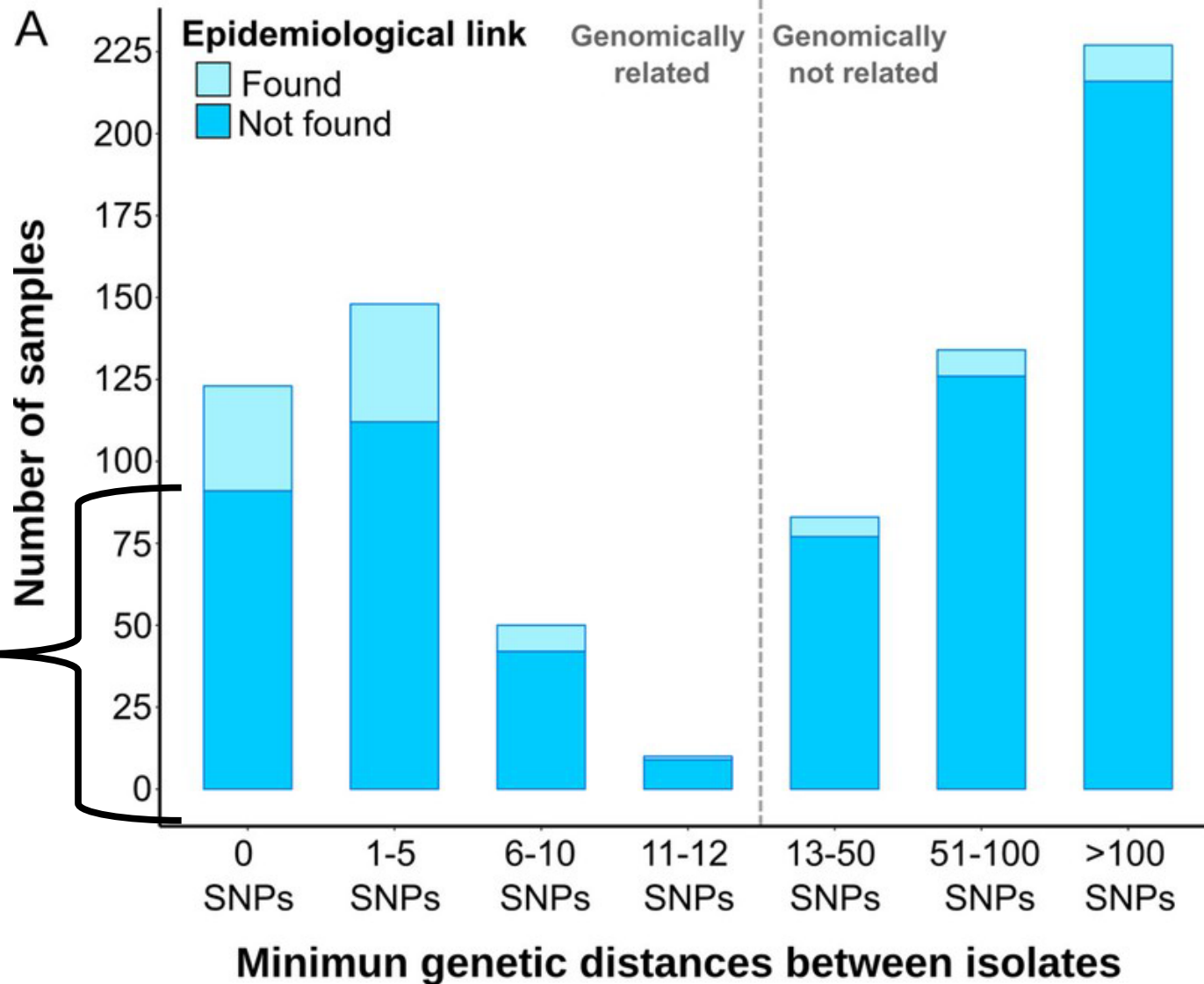
Recent transmission is a major contributor to TB burden in

Risk of transmission
using genomics



Risk of transmission
using contact-tracing

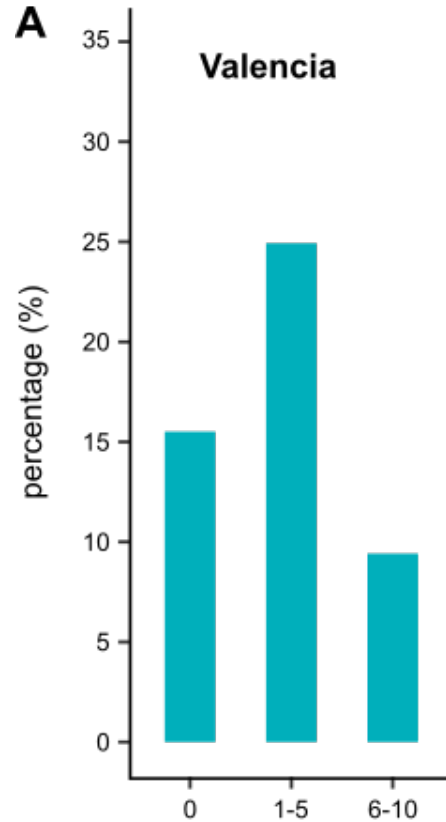




Community transmission is common even in a low-burden country

Most genomic links are missed by contact tracing

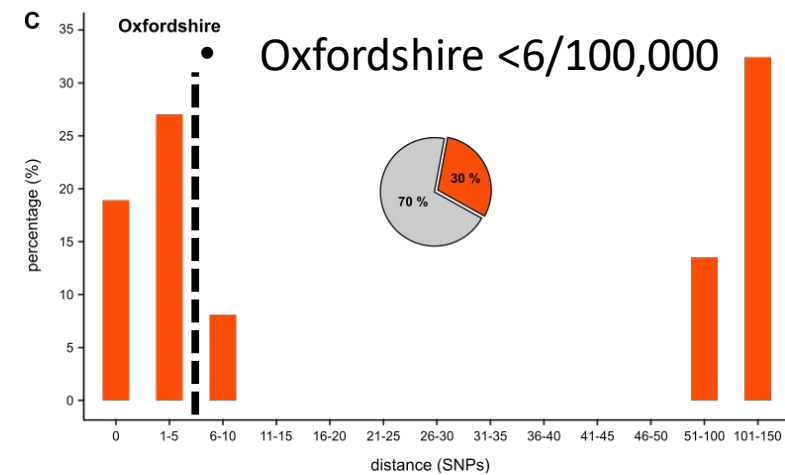
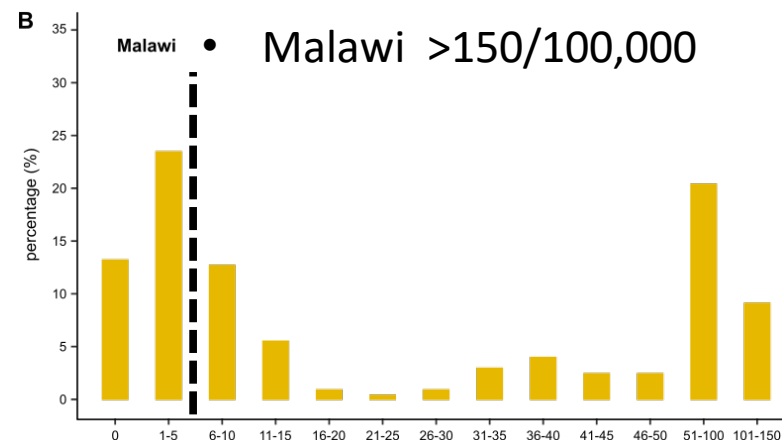
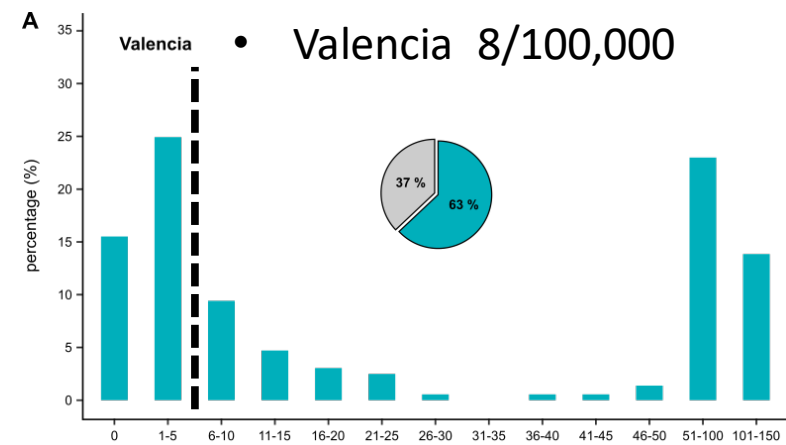
Beyond SNP threshold: genomic epidemiology for tailor-made strategies in TB



Role in Public Health

INTERVENTIONS

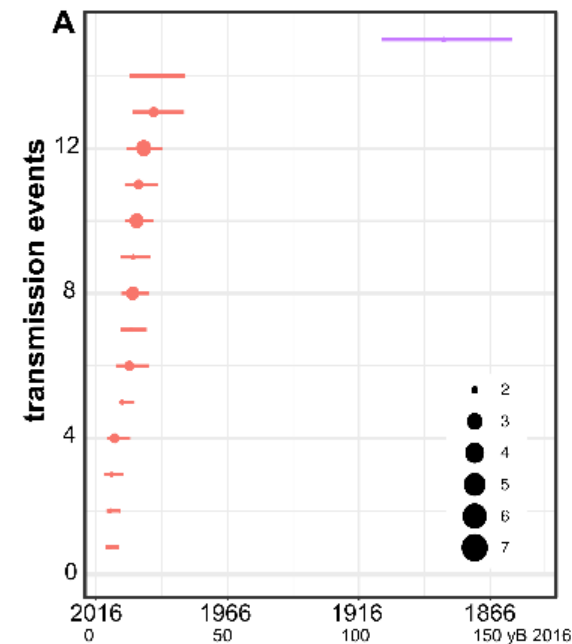
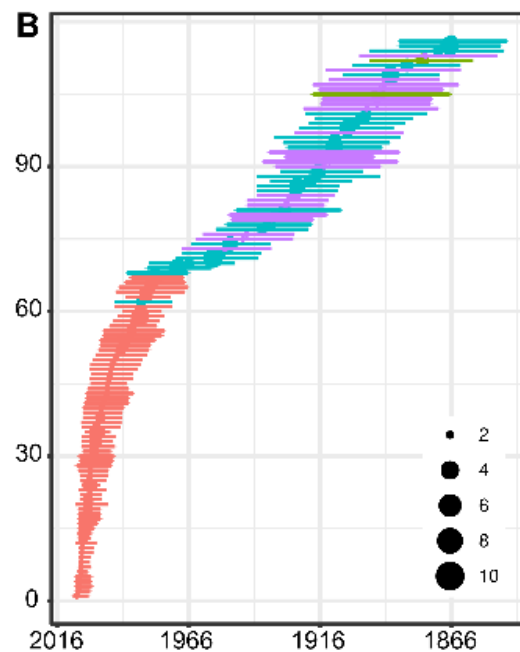
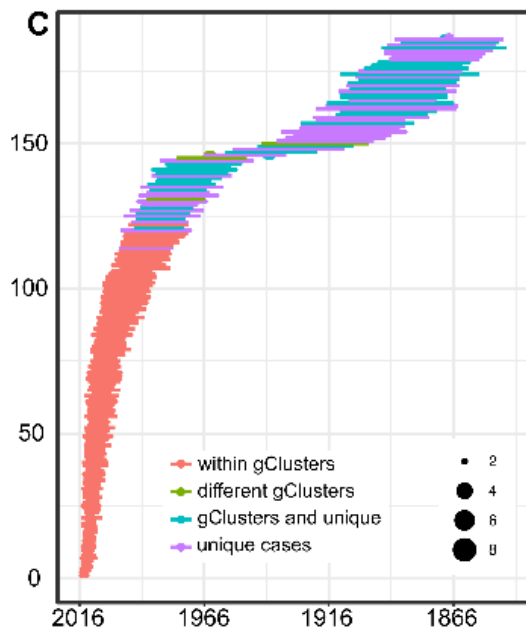
EVALUATIONS



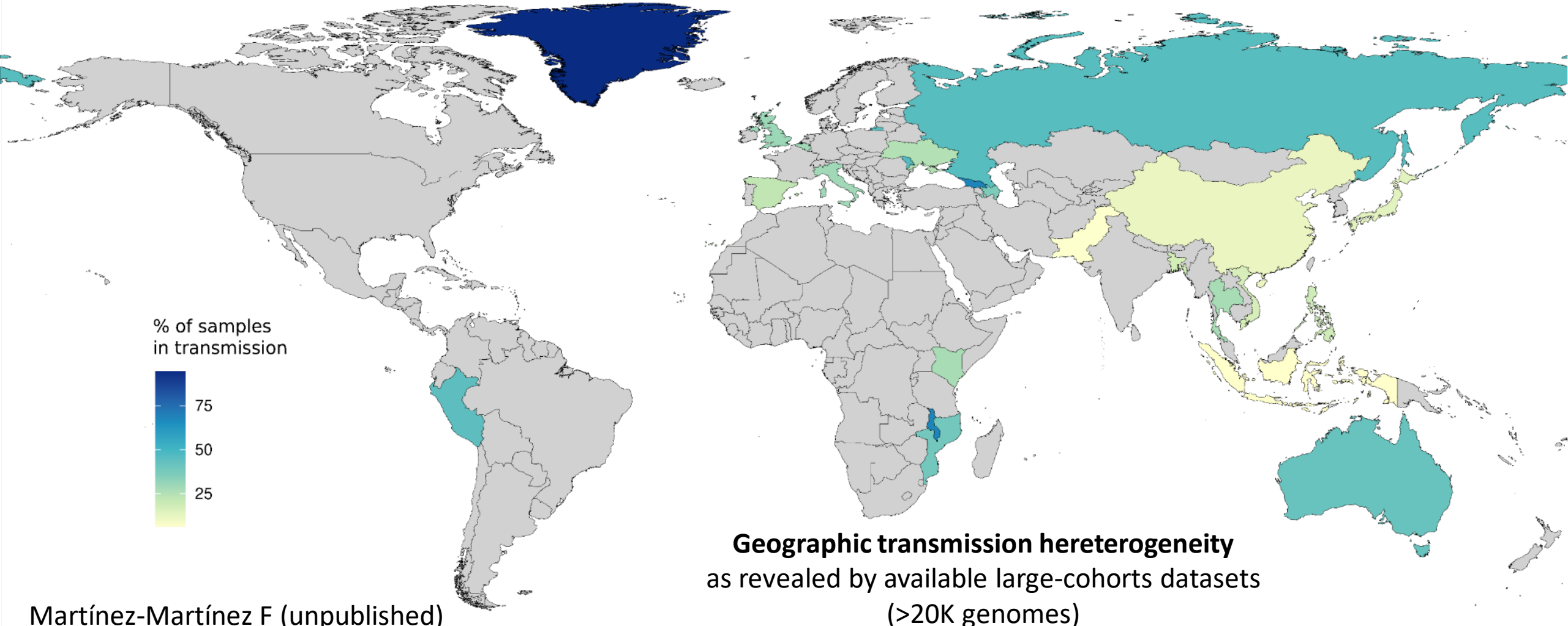
1. Burden does not correlate with transmission dynamics

2. TRANSMISSION is much more complex, goes beyond recent transmission

3. SNP distributions reflect uninterrupted transmission, limited impact of infection control

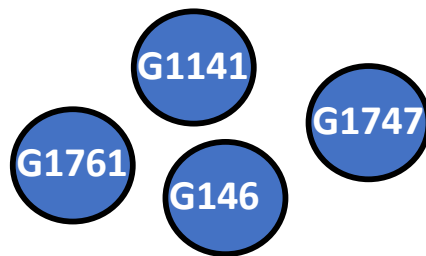


Need for population-based genomic epidemiology studies

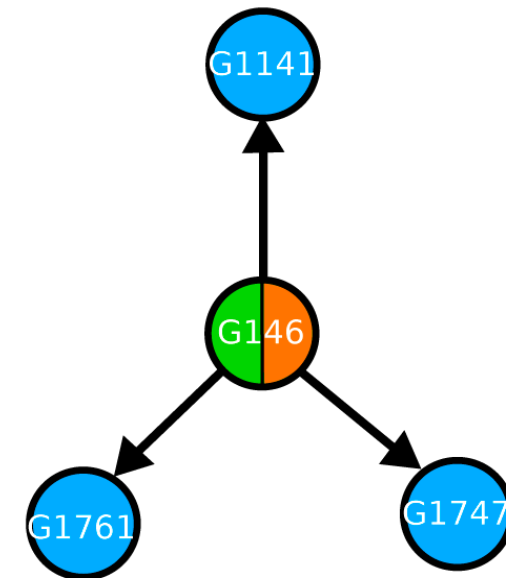


Can we improve understanding of TB transmission?

- Most studies measure clusters
 - We need to **measure individual events**
- Develop high resolution phylogenetic mapping based on TransPhylo



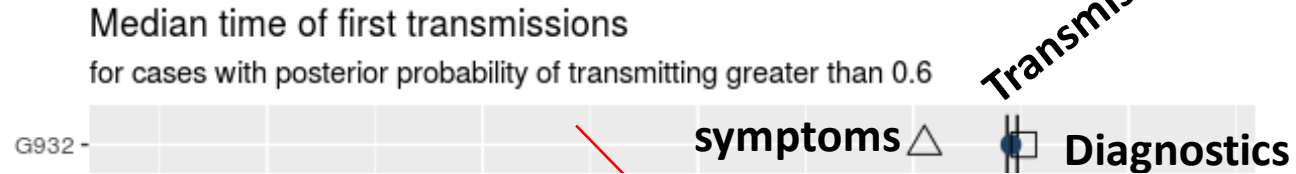
When?
Who is the index case?
Missing cases?
Risk factors associated to transmitters?





Caroline Colijn

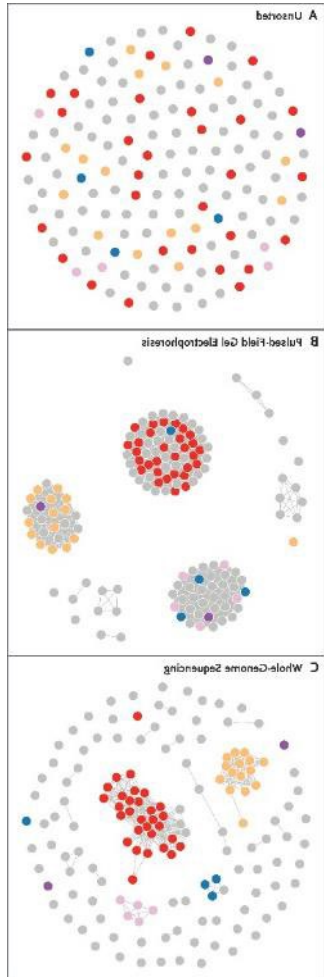
Phylogenetic mapping to infer individual transmission events



- Evidence of **presymptomatic transmission**

- Impact of subclinical TB in Transmission?

Beyond epidemiology: comprehensive nature of genomic data

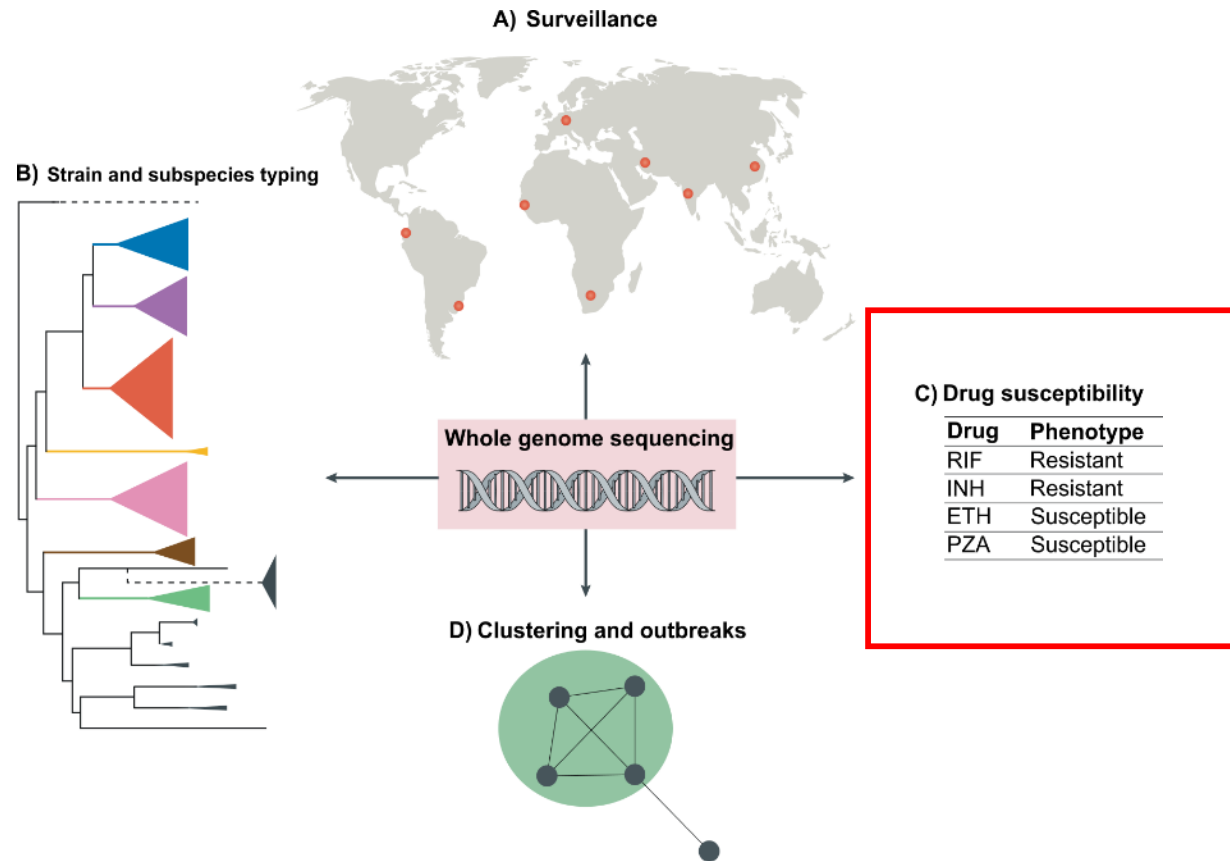


NO MARKER

LOW RESOLUTION

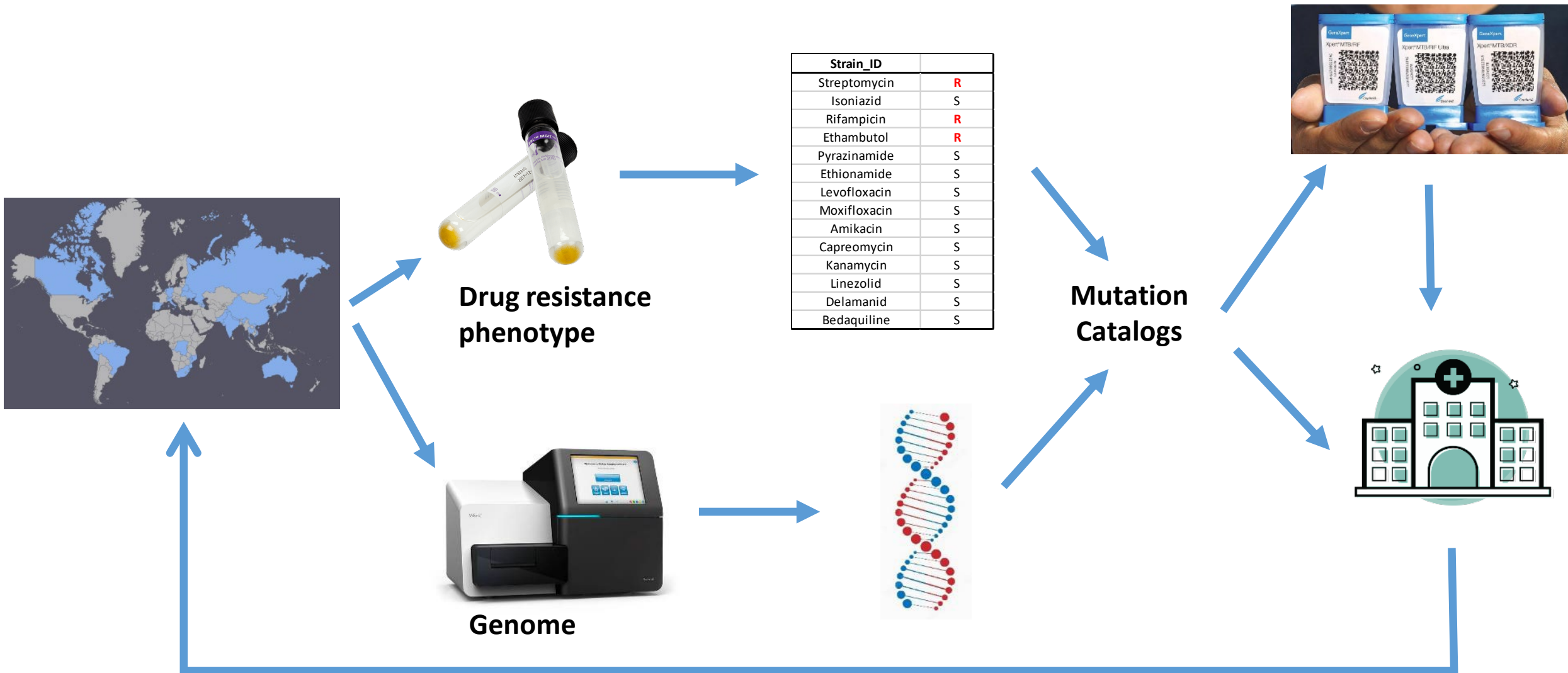
GENOME (HIGH RESOLUTION)

Armstrong et al. NEJM 2019



Meehan et al. Nat Rev. Mic. 2019

Towards the genome as a drug resistance molecular test



From bench to bedside

13 drugs – 47 genomic regions

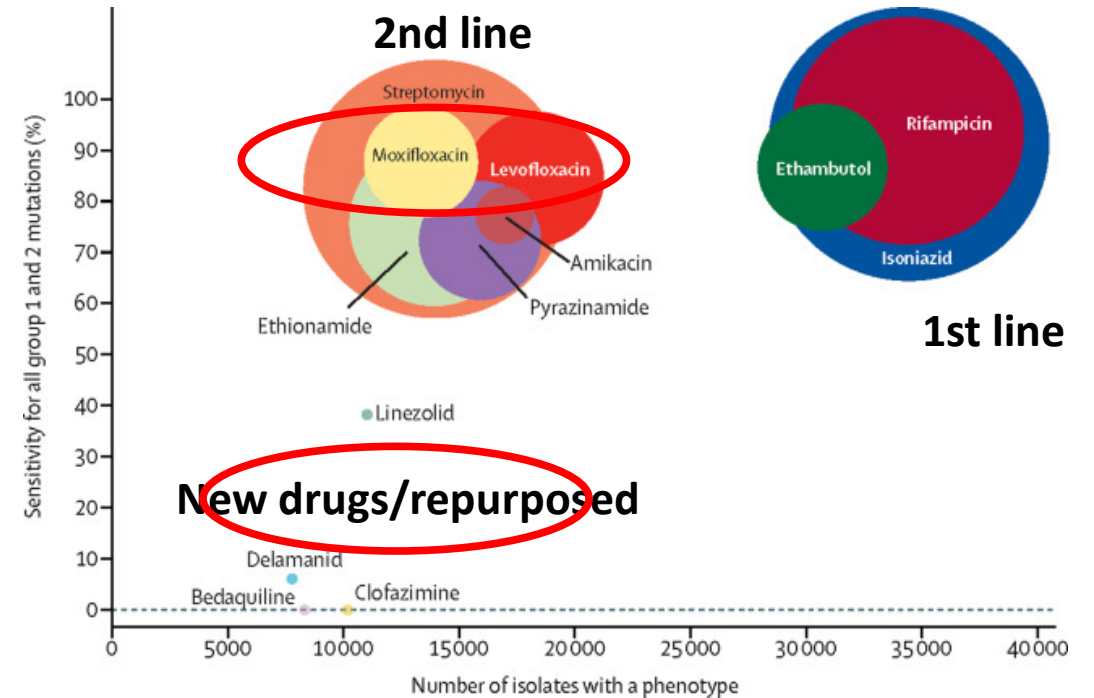
Categoría	Mutaciones
Asociada a R	196
Asociada a R Interim	1004
Incierto	15910
No asociada a R interim	33
No asociada a R	213

- **Hain MTBDRplus** – 9 mutations – 3 genes – 2 drugs
- **Xpert MTB/RIF** – 23 mutations – 1 gene – 1 drug

38K genotype/phenotype isolates

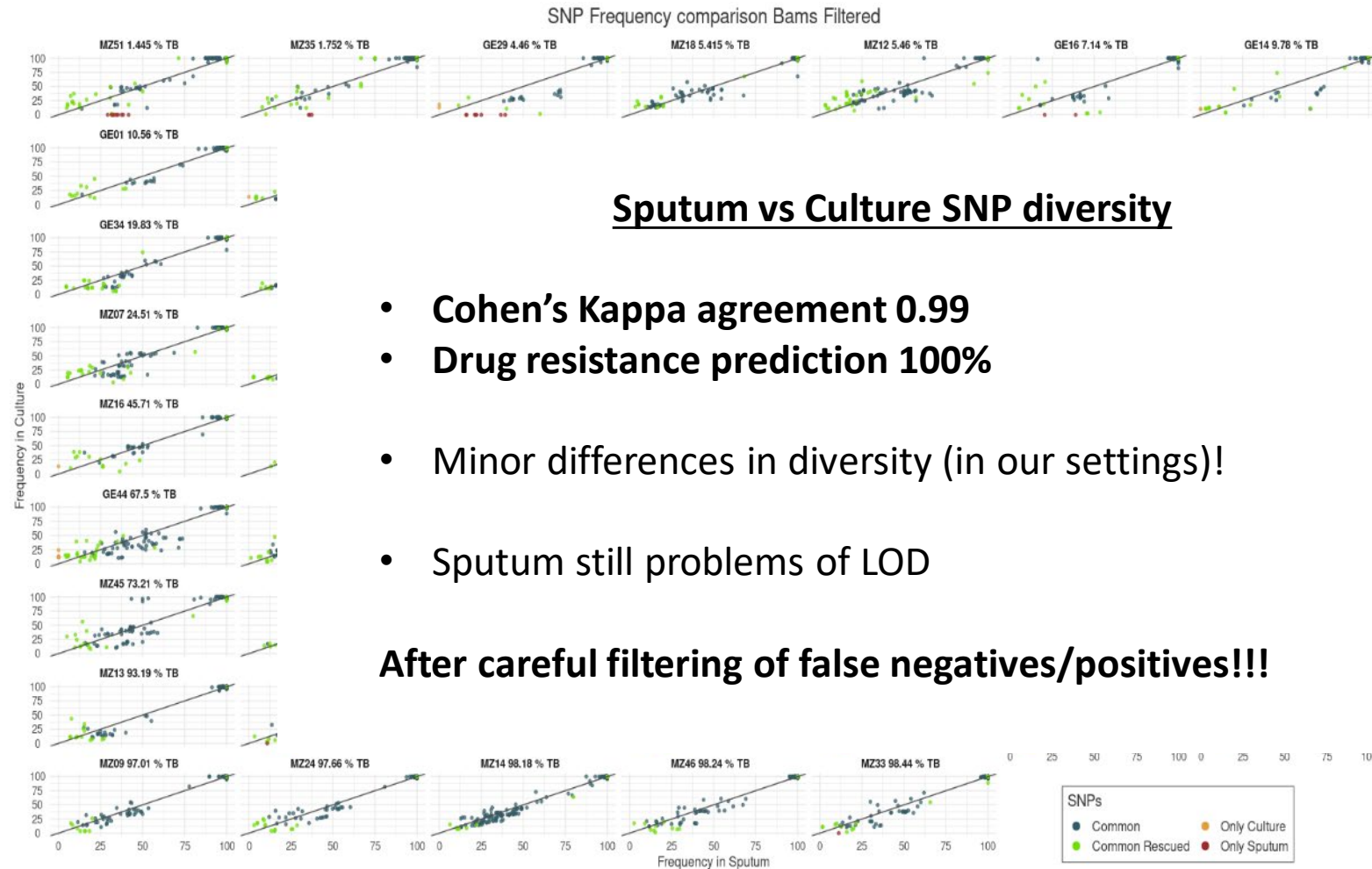
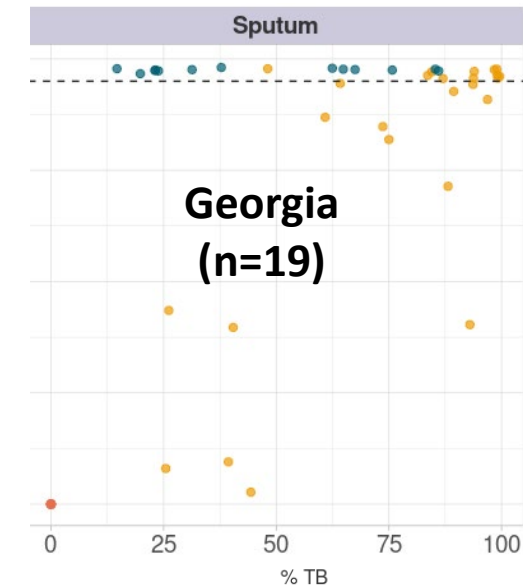
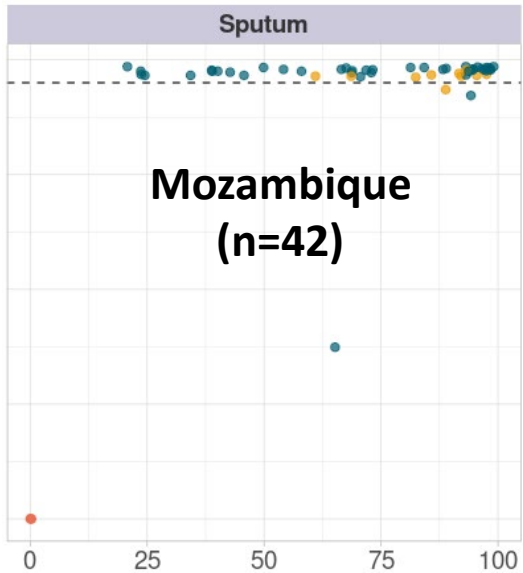


High sensitivity/specificity for 1st line
(WHO consortium, The Lancet Microbe 2022)



Limited in new and repurposed, geographic and bacterial diversity -> New versions

Final frontier: genome sequencing directly from Dx samples



Sputum vs Culture SNP diversity

- Cohen's Kappa agreement 0.99
- Drug resistance prediction 100%
- Minor differences in diversity (in our settings)!
- Sputum still problems of LOD

After careful filtering of false negatives/positives!!!

Drug resistance is a more complex phenomenon than just diagnostic mutations:
three examples

1. The case of unnoticed mutations
2. The case of polyclonal infections
3. The case of early responses to treatment

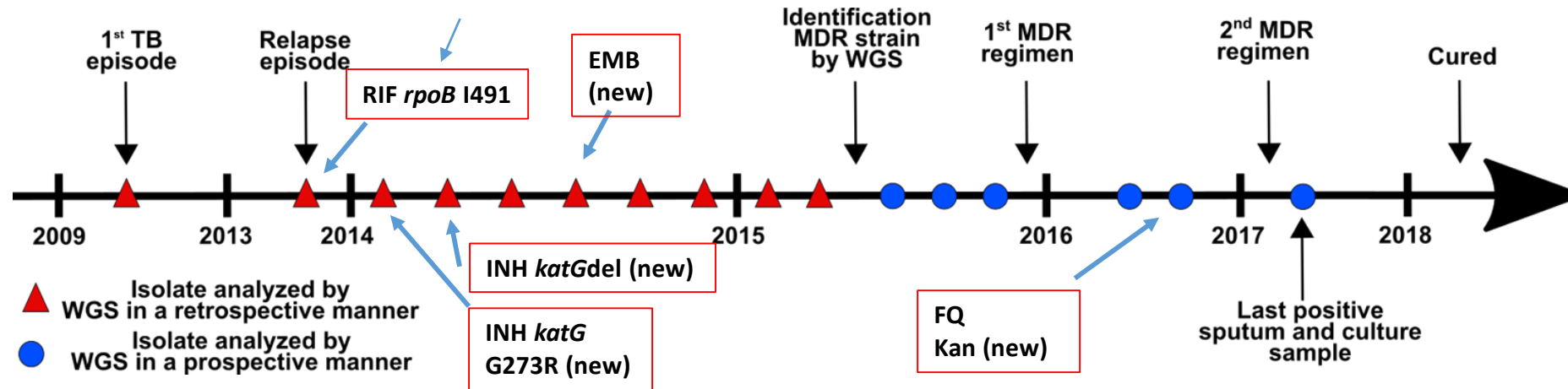
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CONSORCI
HOSPITAL GENERAL
UNIVERSITARI
VALÈNCIA

False negative DST (eg. Torrea et al. Sci Rep 2019)
Driver of hidden MDR in Eswatini (Beckert et al. Genome Med 2020)

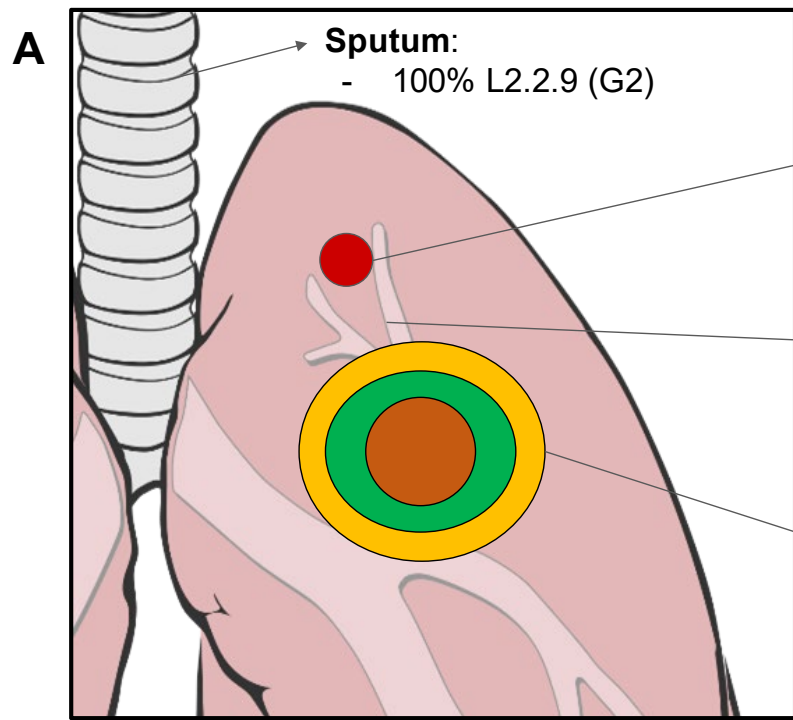


Sample	G1480	G1479	G516	G520	G249	G252	G535	G841	G842	G993	G1003	G1257	G1478	G1720	G1721	G1928
Date	27/04/09	20/09/13	21/01/14	17/03/14	27/06/14	11/08/14	13/10/14	09/12/14	27/01/15	15/04/15	11/06/15	09/11/15	11/12/15	02/06/16	14/10/16	09/01/17
Sputum/Culture	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Hospital DST (RIF/INH)	-/-	-/-	-/-	-/-	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/+	-/+	-/-	-/+	-/+

↑ ↑
RIF-R MDR-TB

Drug resistance is a more complex phenomenon than just diagnostic mutations:
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1. The case of unnoticed mutations
- 2. The case of polyclonal infections**
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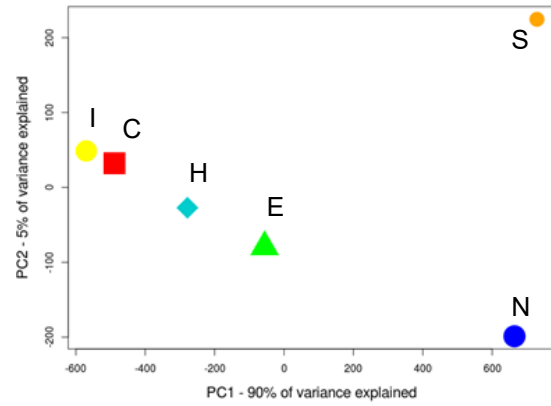
PATIENT G039

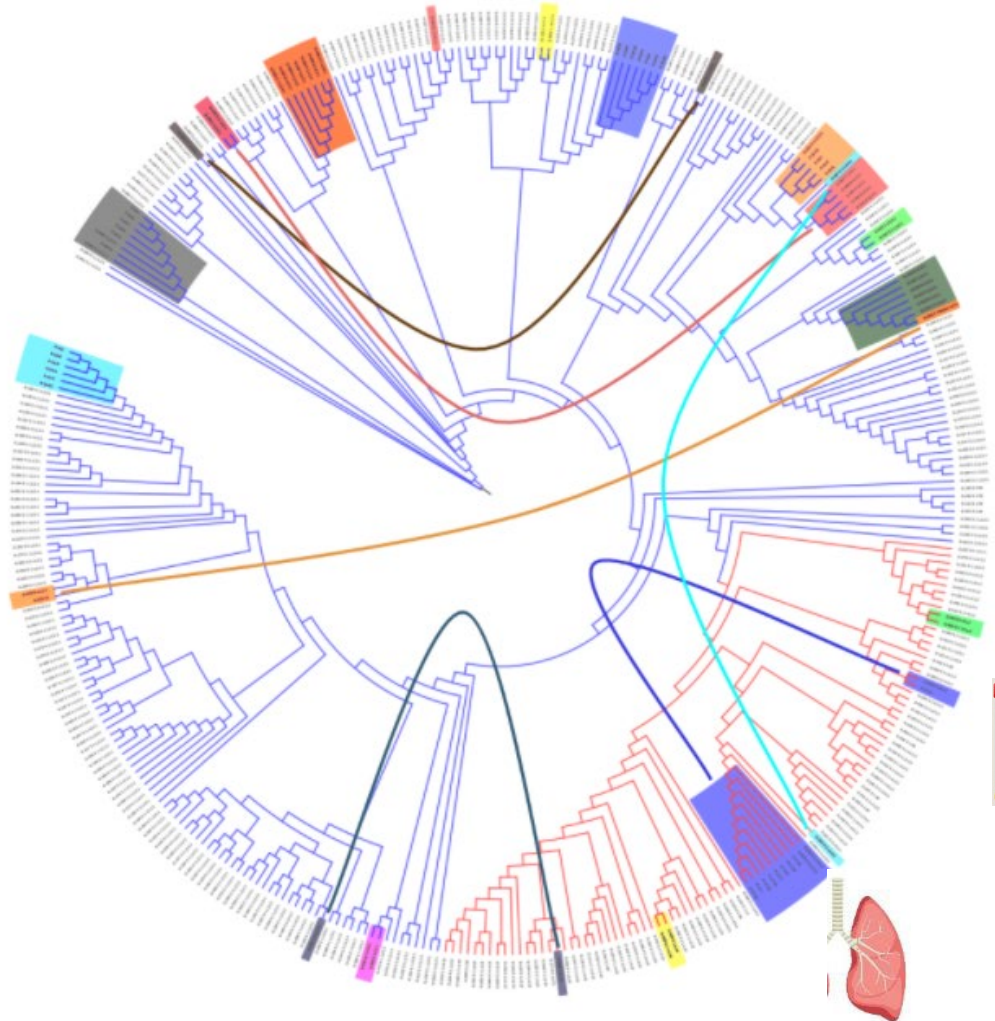
Nodulus:
100% L2.2.9 (G2)

Healthy tissue:
~75% L2.2.10 (G1)
~25% L2.2.9 (G2)

Granuloma:

C:	~80% L2.2.10 (G1)	~20% L2.2.9 (G2)
I:	~85% L2.2.10 (G1)	~15% L2.2.9 (G2)
E:	~50% L2.2.10 (G1)	~50% L2.2.9 (G2)





1) Polyclonal infections (>1 genotype) better reveal in lung

40% in patients with surgery samples available
5% in patients when only sputum examined
18% when using serial sputum samples

2) Most “lungs” were part of transmission clusters suggesting **superinfections** common

3) 71,4% cases of polyclonal infection **reverted DST profile** -> TREATMENT FAILURE

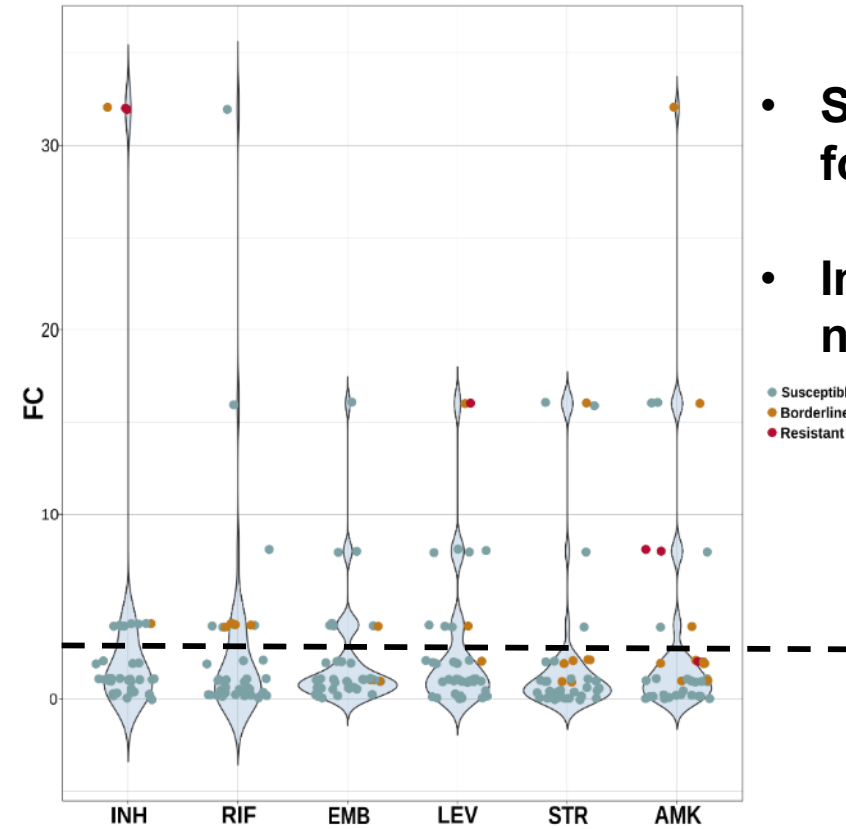
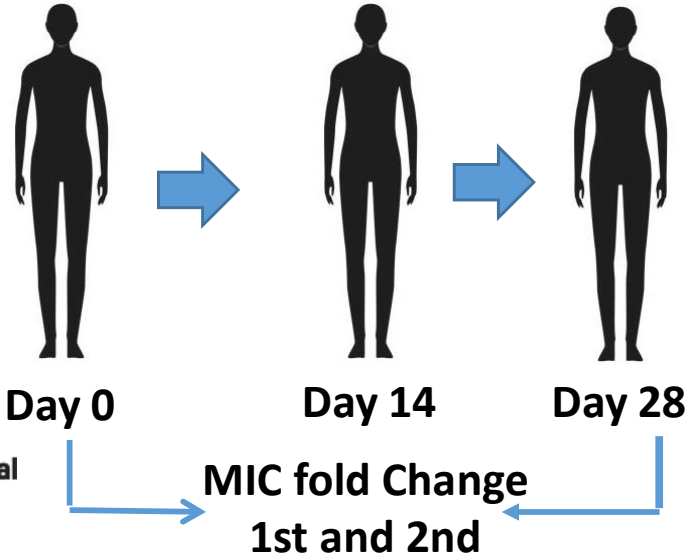
4) Nobody knows the **global extend** and impact of polyclonal infections

Drug resistance is a more complex phenomenon than just diagnostic mutations:
three examples

1. The case of unnoticed mutations
2. The case of polyclonal infections
- 3. The case of early responses to treatment**

MOZAMBIQUE

40 Patients
Susceptible
HIV+ and HIV-



- Significant increases for several patients
- Increases also in drug not in the regimen

1) Shifts in Minimum inhibitory concentrations at the end of treatment associated to relapse -> Colangeli et al. NEM 2018

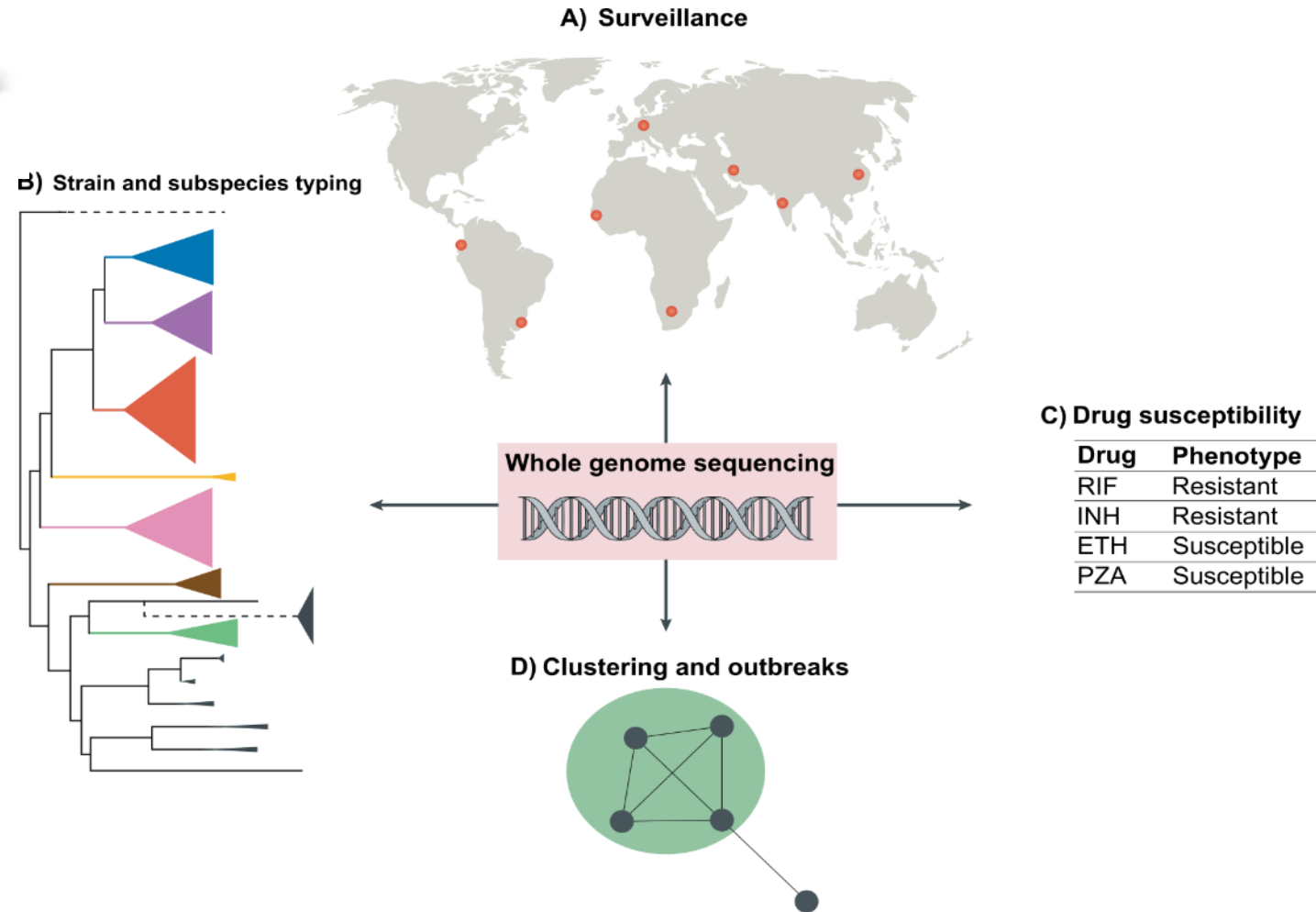
2) Can we identify similar shifts during the first month of treatment?

3) Are they associated to worst outcomes?

Our data. 10 out of 12 patients with MIC shifts remained culture-positive after a month of treatment. All cured at the end



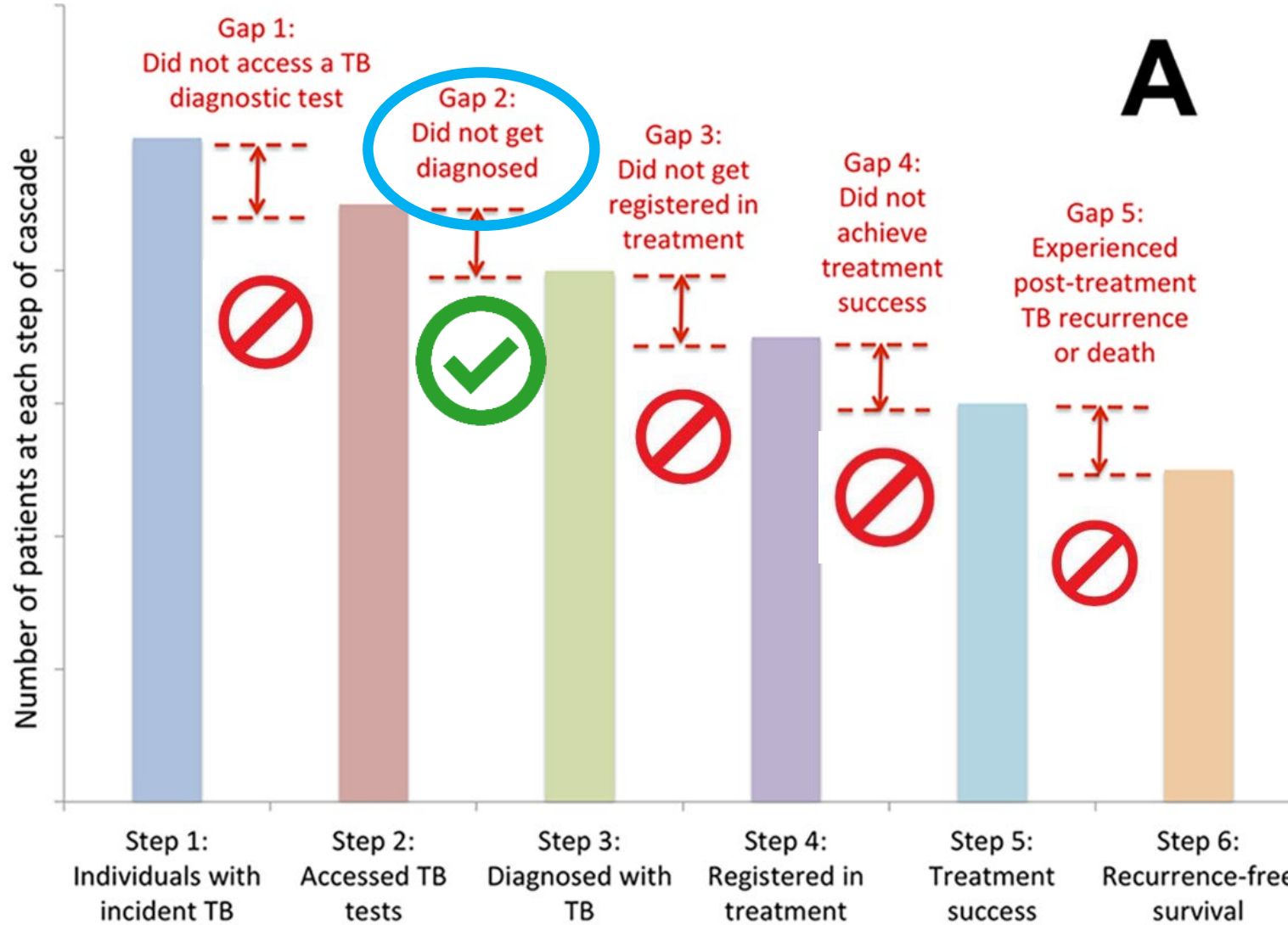
Genome sequencing is very powerful but...



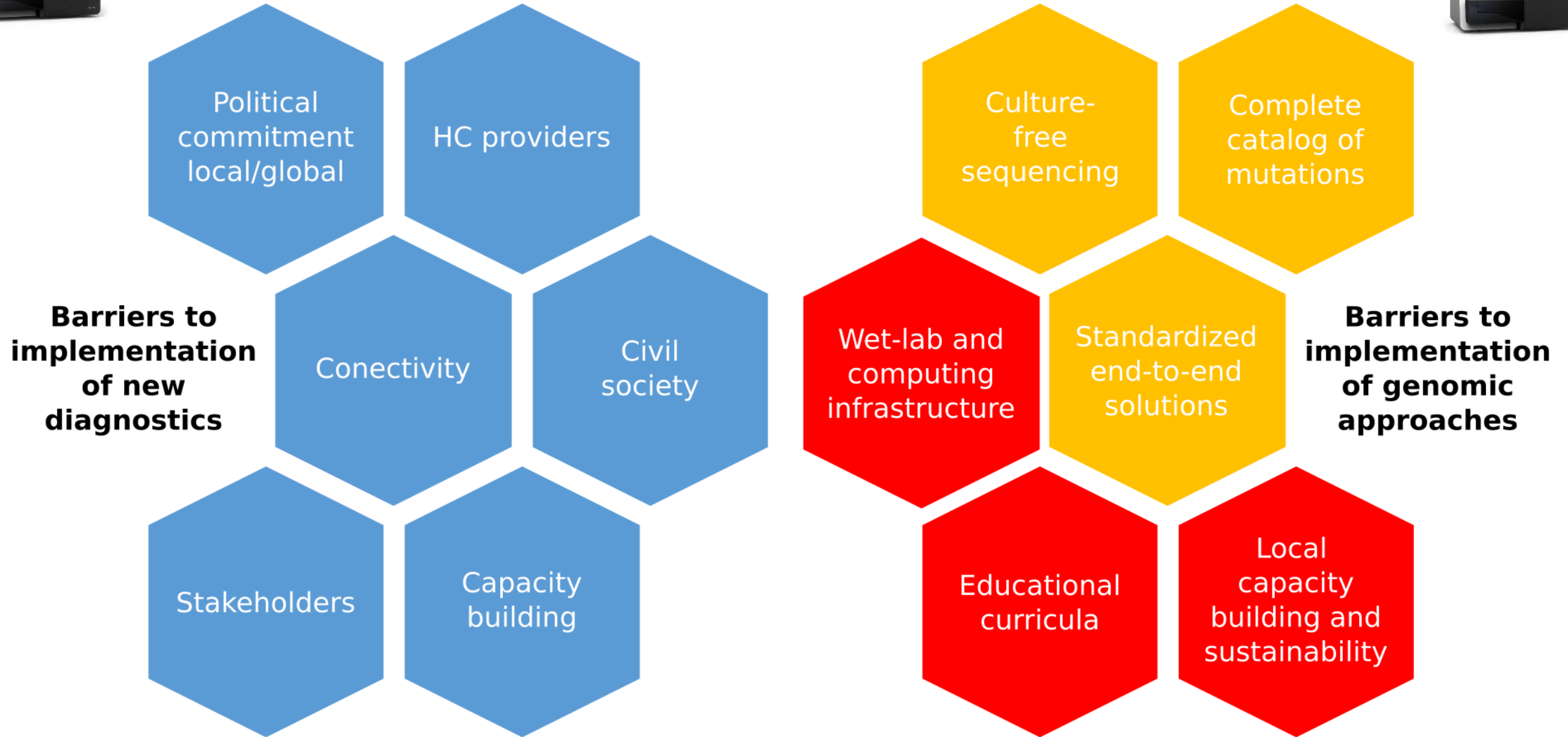
Lessons from the past on the role of new technologies



Xpert/RIF



TB CARE ECOSYSTEM IS AS IMPORTANT AS DISRUPTIVE TECHNOLOGIES



Tuberculosis Genomics Unit Institute of Biomedicine of Valencia (IBV-CSIC)



Funding



Mozambique work:



Thanks:

- Health professionals contributing to our studies
- TB and COVID-19 patients involved in our studies

