Emerging Bacterial
Pathogens Unit

OSPEDALE SAN RAFFAELE

New diagnostics for MDR/XDR-TB

Riccardo Alagna

WHO Collaborating Centre for TB Laboratory Strengthening
Supranational Reference Laboratory Milan
Emerging Bacterial Pathogens Unit,
San Raffaele Scientific Institute, Milan

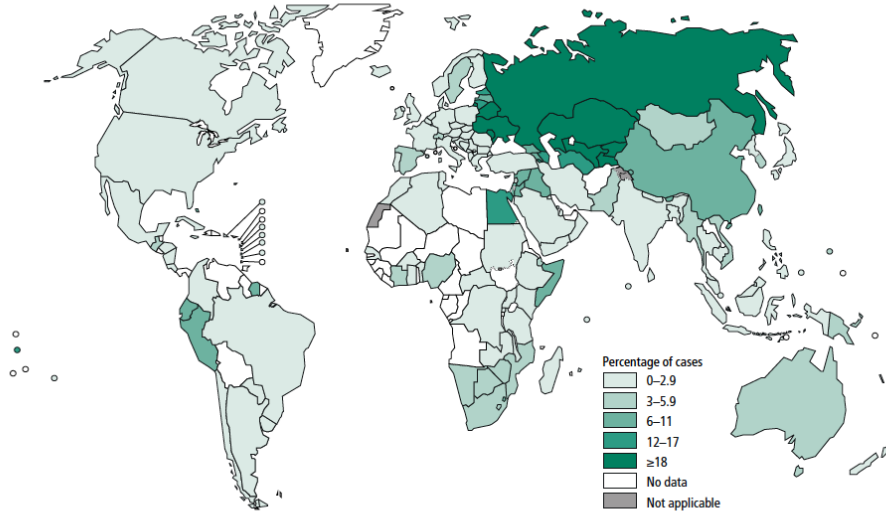
The Global burden of DR-TB

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

FIG. 3.20

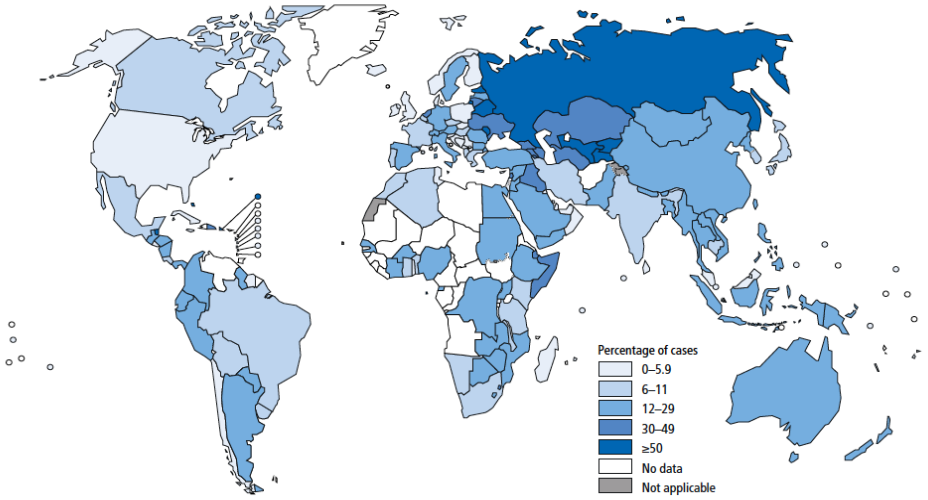
Percentage of new TB cases with MDR/RR-TB[a]



[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.

FIG. 3.21

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).

The DR-TB problem is getting bigger…

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Estimating the future burden of multidrug-resistant and extensively drug-resistant tuberculosis in India, the Philippines, Russia, and South Africa: a mathematical modelling study

% MDR-TB among all incident cases of TB to increase to 12·4% in India, 8·9% in the Philippines, 32·5% in Russia, and 5·7% in South Africa in 2040

Figure 2: Projected trends of the proportion of individuals with MDR tuberculosis of those with incident tuberculosis, and the proportion with XDR tuberculosis of those with incident MDR tuberculosis

DR-TB diagnosis

Emerging Bacterial Pathogens Unit

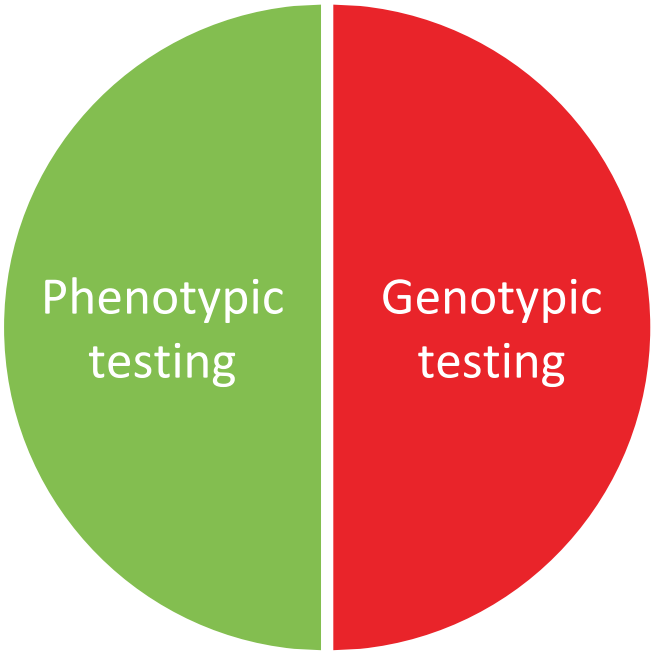
OSPEDALE SAN RAFFAELE

Culture-based methods, "Gold standard"

Expensive, time-consuming and requires a specialized biosafety laboratory

DST for many of the second-line drugs have not yet been fully standardized

Outdated definition of phenotypic susceptibility/resistance



Sensitivity close to culture-based methods

Cheap, rapid and easy-to-use

Detection of most common mutations

Probe-based: Can only detect what you are looking for

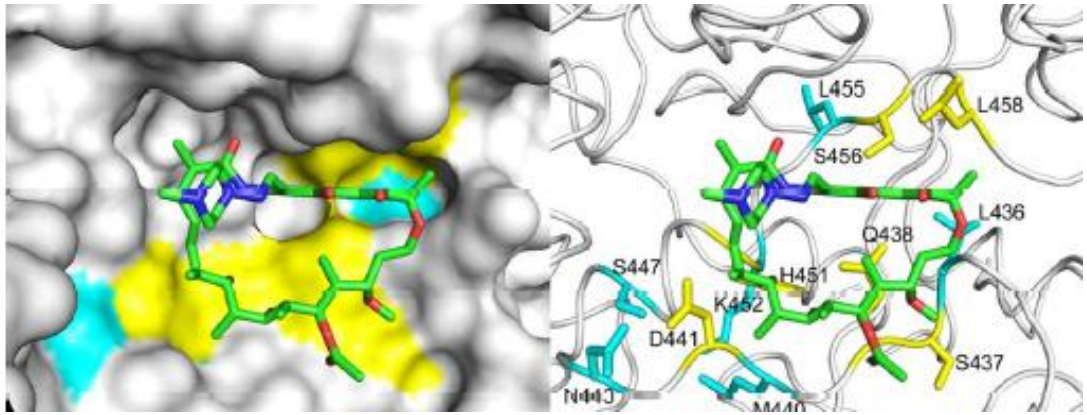
Is pDST always a Gold standard? (Rifampicin)

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Some Rifampin mutants are associated to sensitive MGIT results

"Disputed mutations" cause DISCREPANT genotypic/phenotypic results



in silico analysis of the effect of disputed mutations on the structural interaction between the RpoB protein and rifampin

The binding affinity towards rifampin is affected

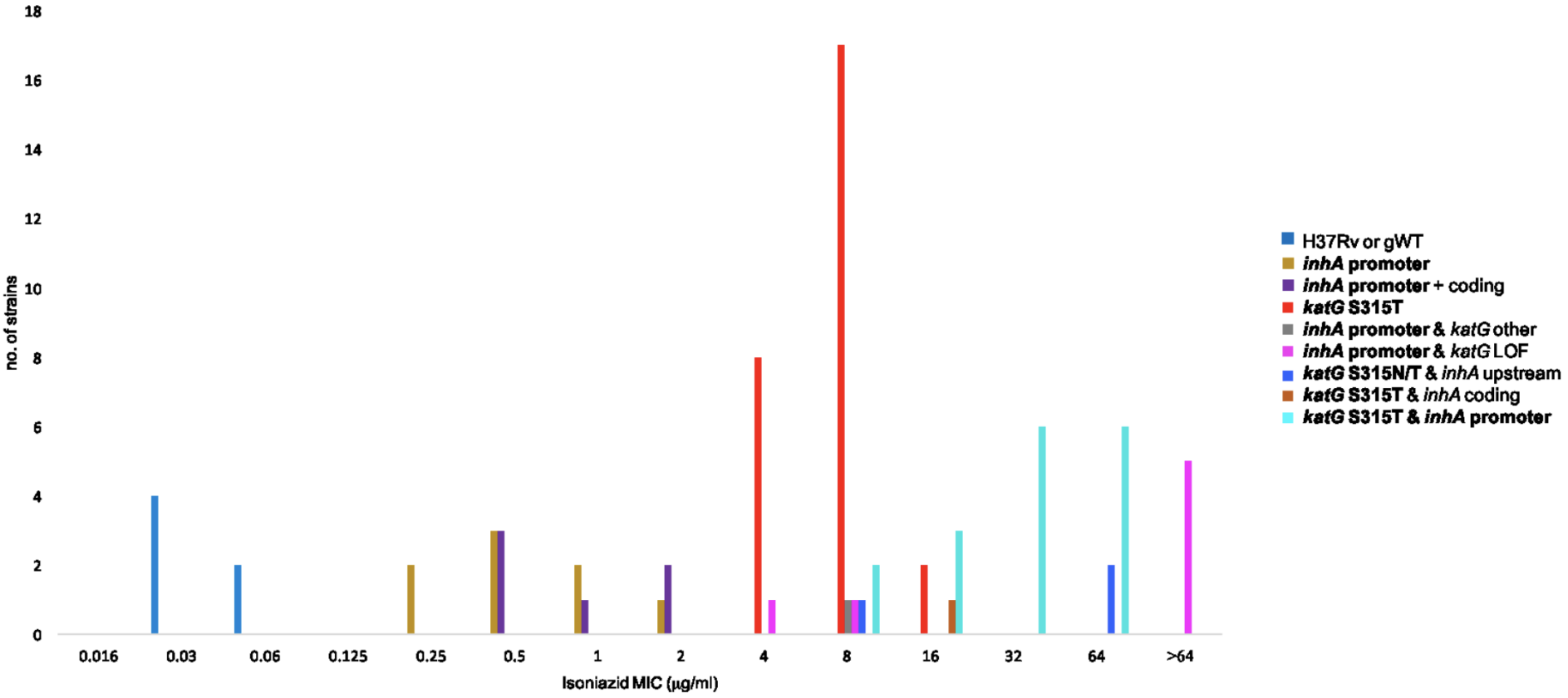
➢ Strains should be considered RESISTANT to rifampin

Miotto P, et al. J Clin Microbiol. 2018 Apr 25;56(5).

Is pDST always a Gold standard? (Isoniazide)

Emerging Bacterial Pathogens Unit

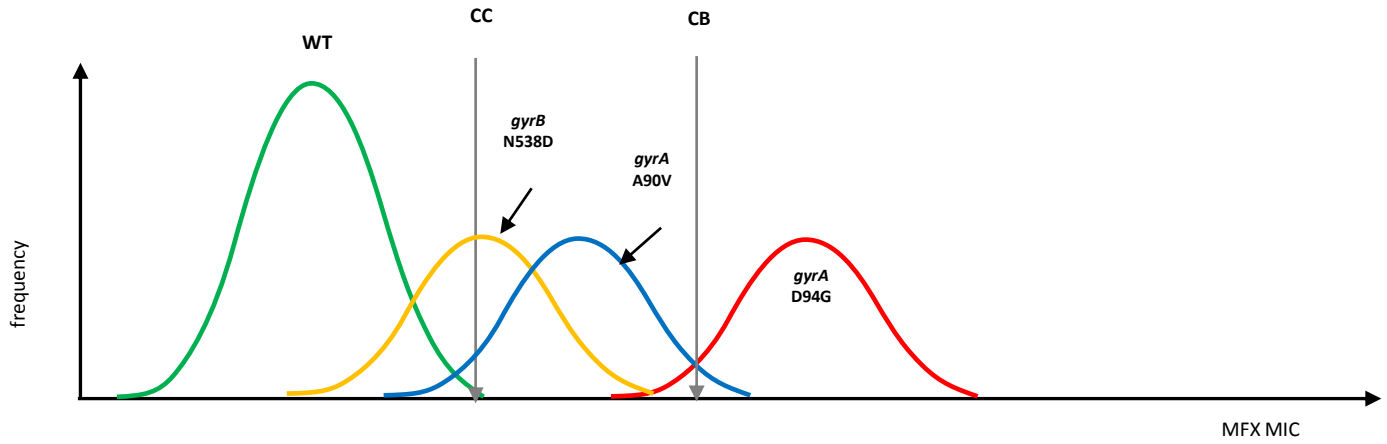
OSPEDALE SAN RAFFAELE



Is pDST always a Gold standard? (Fluoroquinolones)

Emerging Bacterial Pathogens Unit
OSPEDALE SAN RAFFAELE

For FQs pDST is not always a reliable confirmatory test

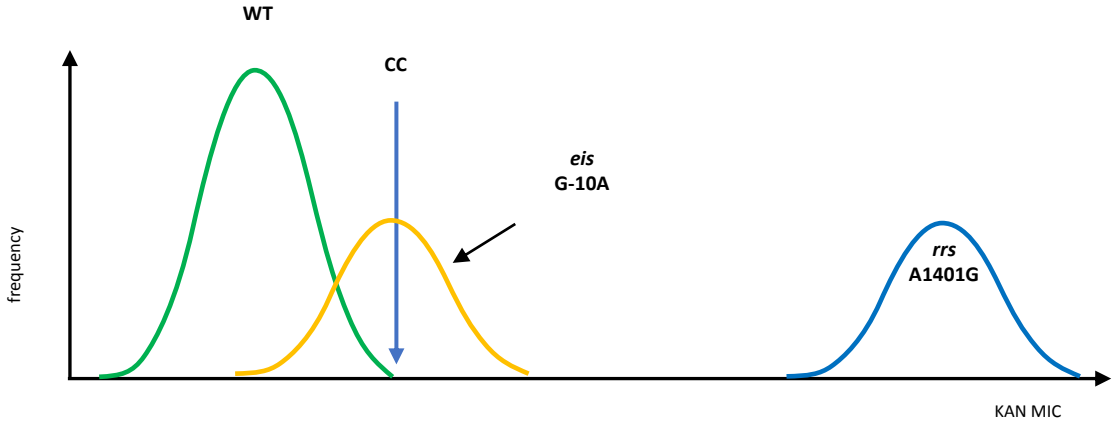


- MIC distributions of LLR mutations (e.g. *gyrB* N538D and *gyrA* A90V) overlap with WT (S) population:
  - pDST at CC will misclassify a proportion of LLR strains as S
  - Detection of LLR mutation should be reported as "at least LLR" and overrule any S pDST result (pDST only at CB needed to exclude HLR)
- MIC distribution of LLR mutations overlaps with HLR mutations (e.g. *gyrA* D94G):
  - pDST at CB will misclassify some LLR mutations as HLR and vice versa
  - Detection of HLR mutation should be reported as HLR and no pDST needed (unless a genotypic test does not confirm presence of mutation)

Is pDST always a Gold standard? (SLIDs)

Emerging Bacterial Pathogens Unit
OSPEDALE SAN RAFFAELE

For SLIDs pDST is not always a reliable confirmatory test



- *rrs* A1401G leads to high MIC increase, pDST should be in concordance no need to confirm
- By contrast, even if tested in the same laboratory, an isolate with *eis* G-10A will test susceptible about 50% of the time because of the inherent variation in pDST:
  - Sequencing can be used to confirm mutation
  - Detection of mutation should overrule a susceptible pDST result

DR-TB diagnosis

Emerging Bacterial Pathogens Unit

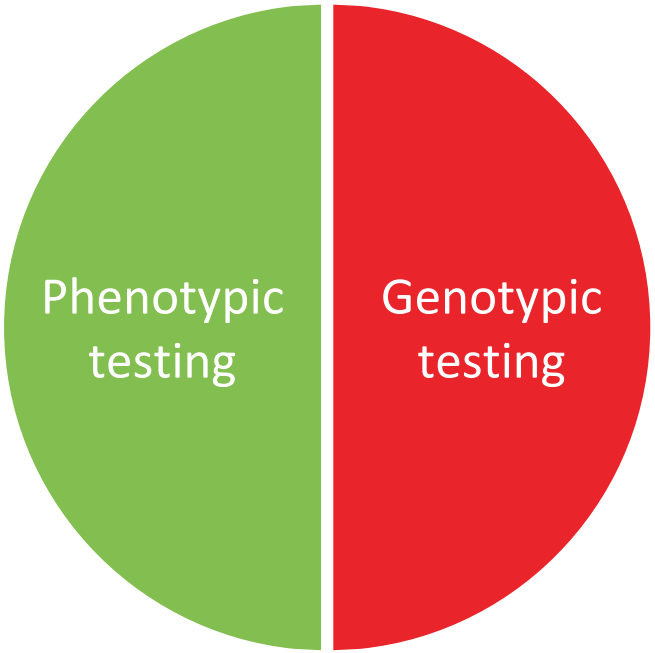
OSPEDALE SAN RAFFAELE

Culture-based methods, "Gold standard"

Expensive, time-consuming and requires a specialized biosafety laboratory

DST for many of the second-line drugs have not yet been fully standardized

Outdated definition of phenotypic susceptibility/resistance



Sensitivity close to culture-based methods

Cheap, rapid and easy-to-use

Detection of most common mutations

Probe-based: Can only detect what you are looking for

Rapid detection of rifampicin resistance

- Xpert MTB/RIF assay has revolutionized the detection of RR/MDR-TB allowing detection of cases to be started on treatment
- Test detects with high sensitivity the MTB genomes and detect mutations in a DNA fragment of 81bp in *rpoB* responsible for 95% of RR cases

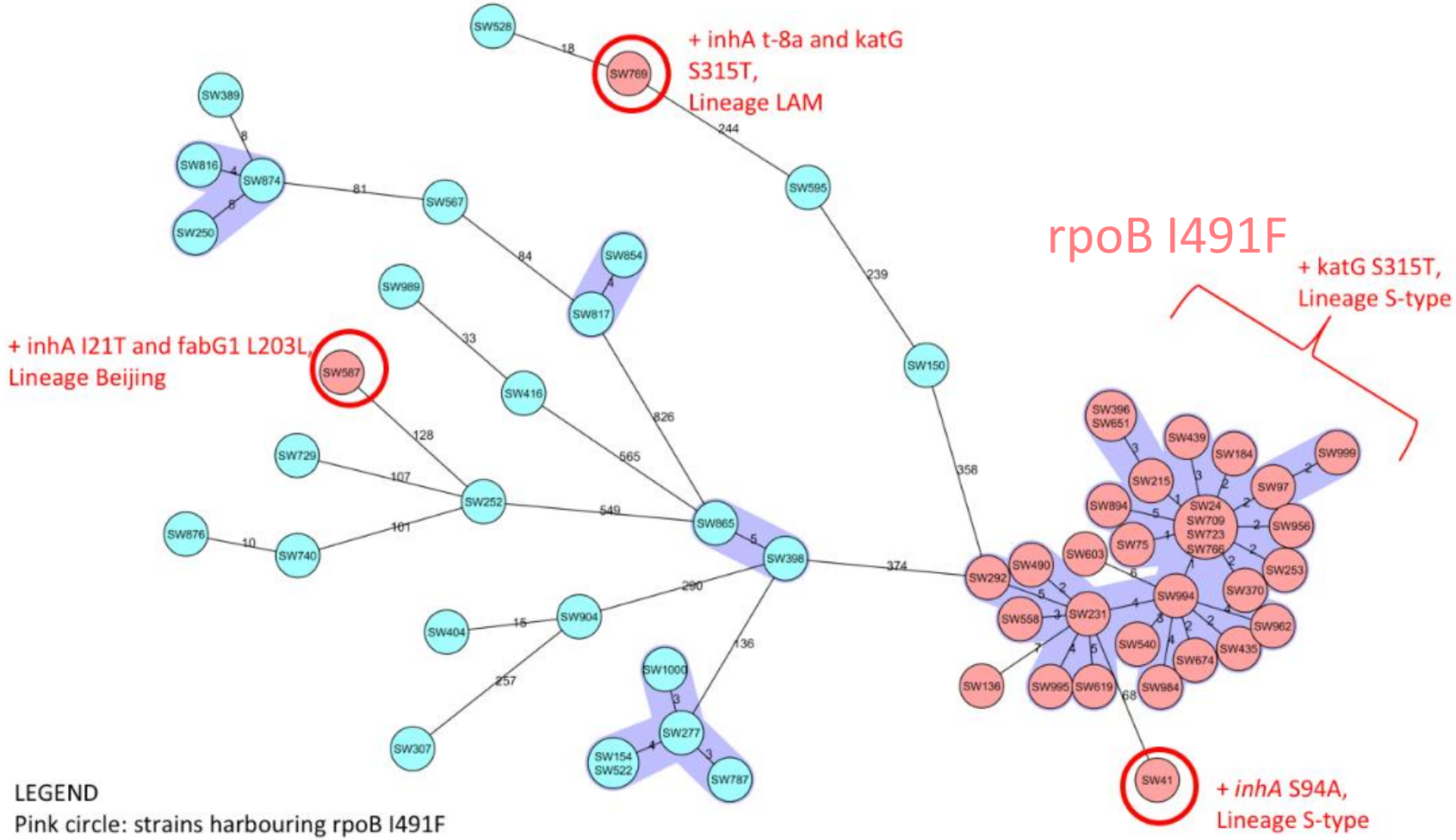
WHO approved molecular diagnostics can target **a limited number of genes and a limited number of specific mutations**

Improper use of diagnostics is not "risk free"

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

cgMLST analysis: Minimum spanning tree of DRS strains carrying rpoB mutations conferring resistance to RIF



LEGEND

Pink circle: strains harbouring rpoB I491F

Light blue circle: strains harbouring rpoB mutations other than I491F

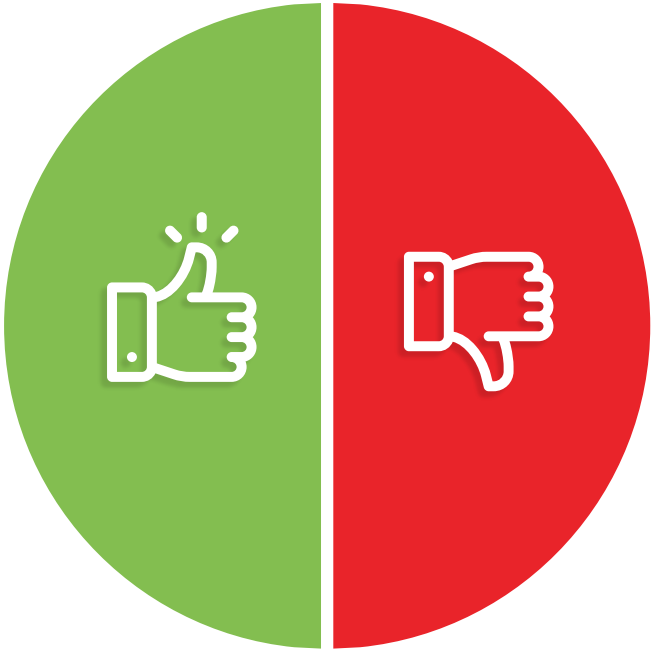
Purple shadow: clustered strains (≤5 alleles)

Public health consequences of dichotomous results

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Contributed to expanded access to care



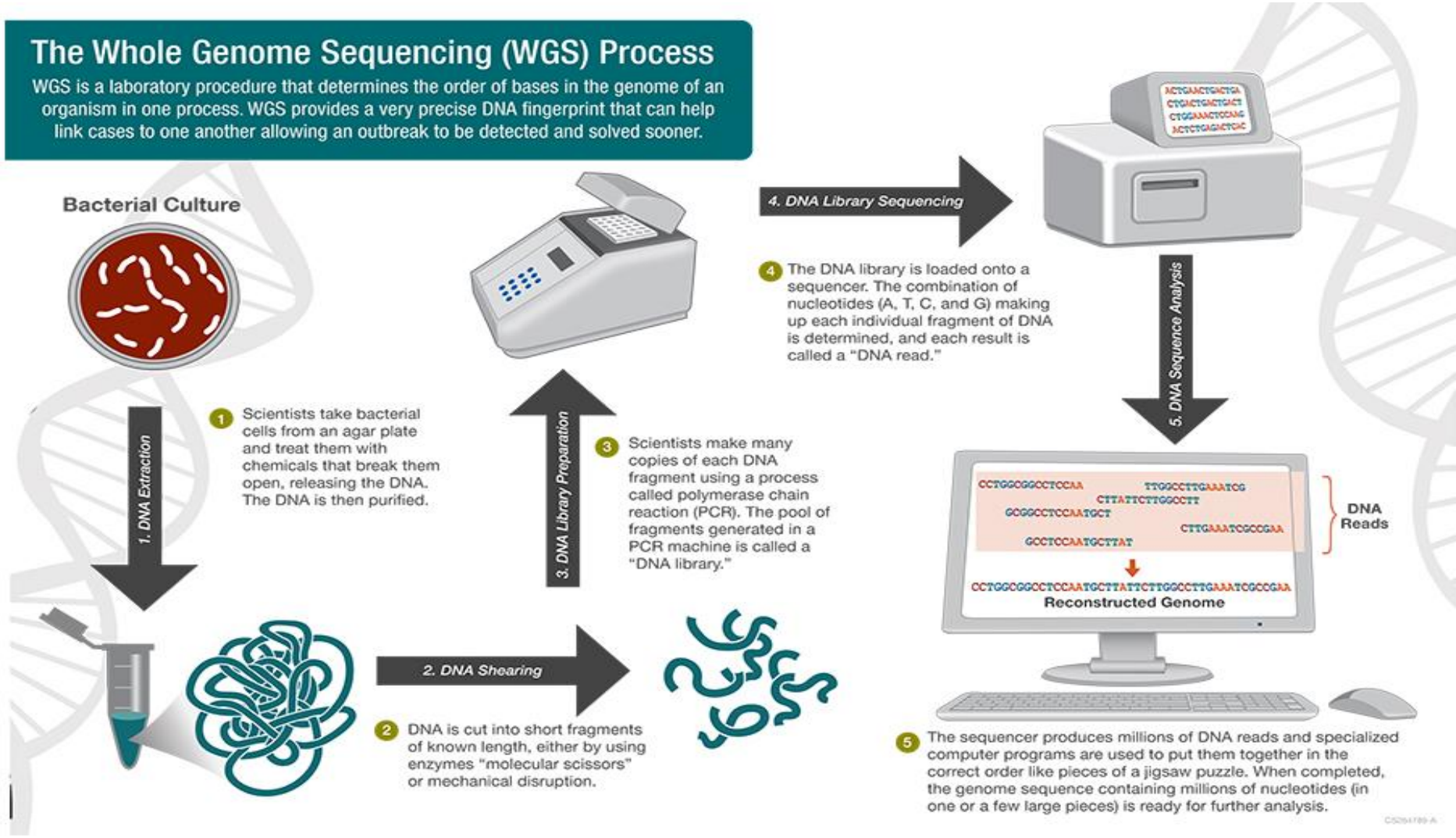
Contributed to resistance emergence

Can we do better?

Whole Genome Sequencing

Emerging Bacterial Pathogens Unit

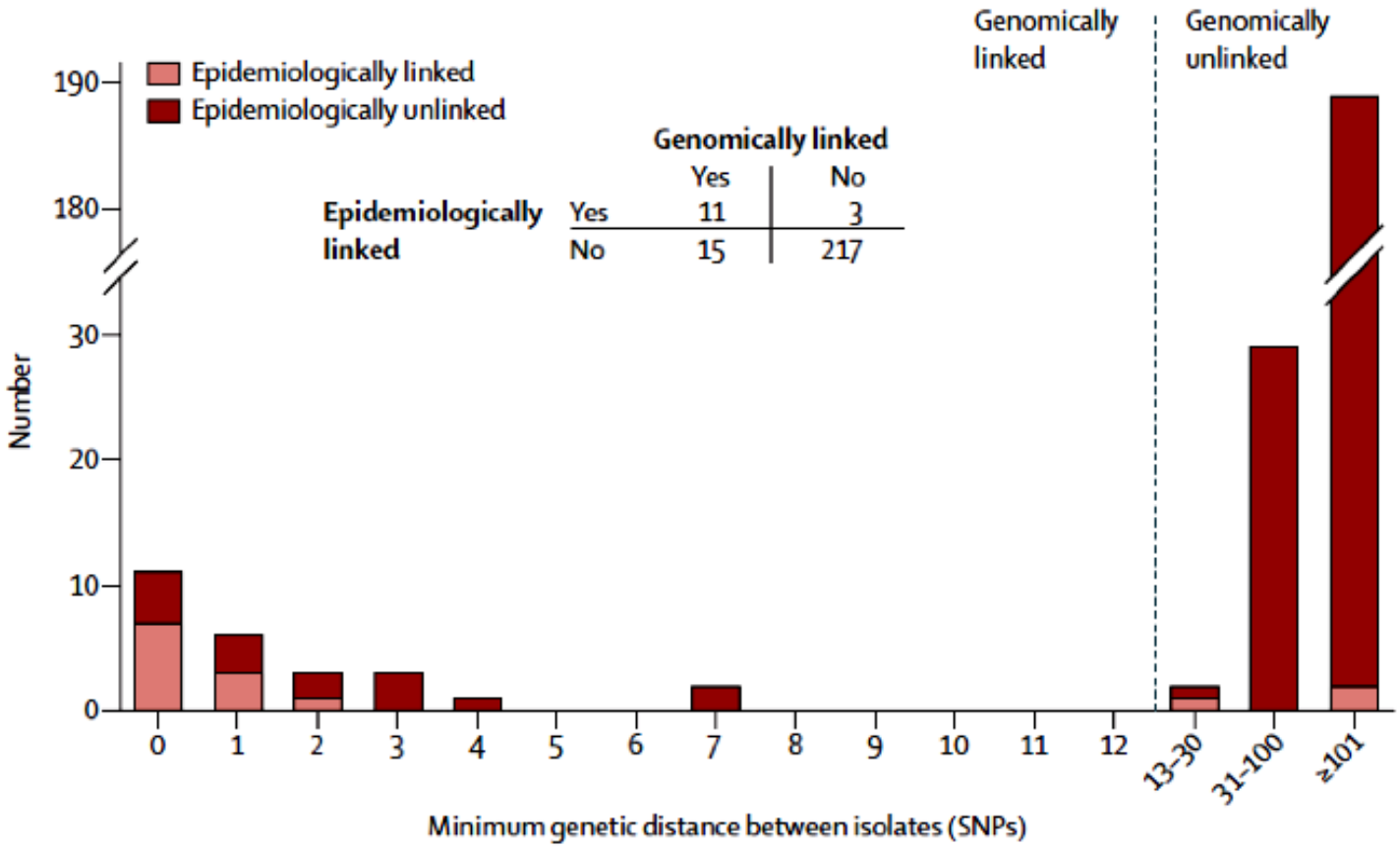
OSPEDALE SAN RAFFAELE



Role of WGS in TB epidemiology

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



- Walker et al., Lancet ID 2013
- Walker et al., Lancet Respir Med 2014

A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study

Timothy M Walker, Matthias Merker*, Astrid M Knoblauch*, Peter Helbling, Otto D Schoch, Marieke J van der Werf, Katharina Kranzer, Lena Fiebig, Stefan Kröger, Walter Haas, Harald Hoffmann, Alexander Indra, Adrian Egli, Daniela M Cirillo, Jérôme Robert, Thomas R Rogers, Ramona Groenheit, Anne T Mengshoel, Vanessa Mathys, Marjo Haanperä, Dick van Soolingen, Stefan Niemann†, Erik C Böttger†, Peter M Keller†, and the MDR-TB Cluster Consortium‡*

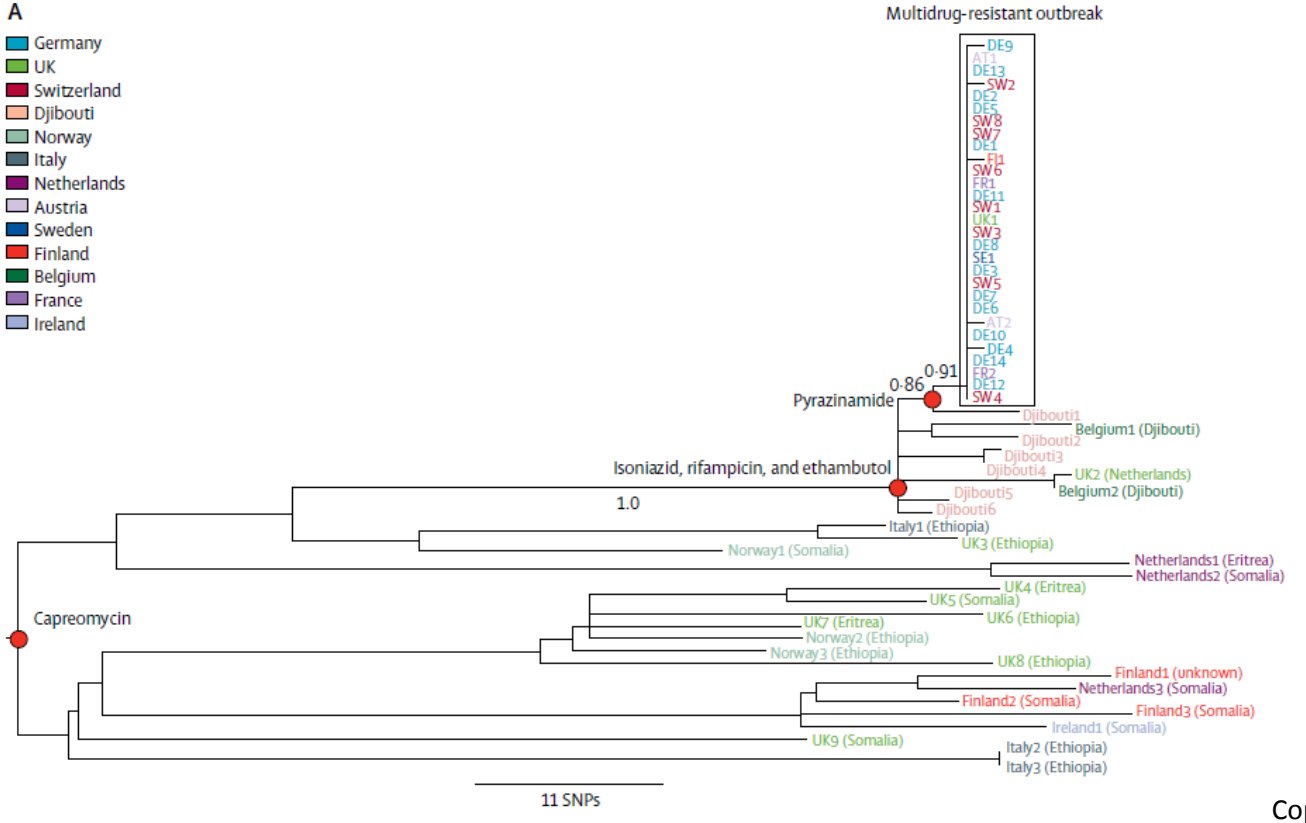
Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Case Definition Proposal

- Resistance phenotype as on table 3.; i.e. INH high-level R, RIF R, CAP R, PZA R, quinolones S, amikacin S.
- Specific set of resistance mutations: see table 3
- MIRU-VNTR: 2-2-4-2-4-3-3-3-2-4-2-4-2-2-5-1-4-3-3-3-4-3-2-2 and/or WGS core genome MLST with <4 SNP (to be discussed)

WGS is ready to be incorporated into national and European programmes for cross-border identification, management, and prevention of tuberculosis outbreak scenarios.



Use of WGS for drug resistance detection

Emerging Bacterial Pathogens Unit

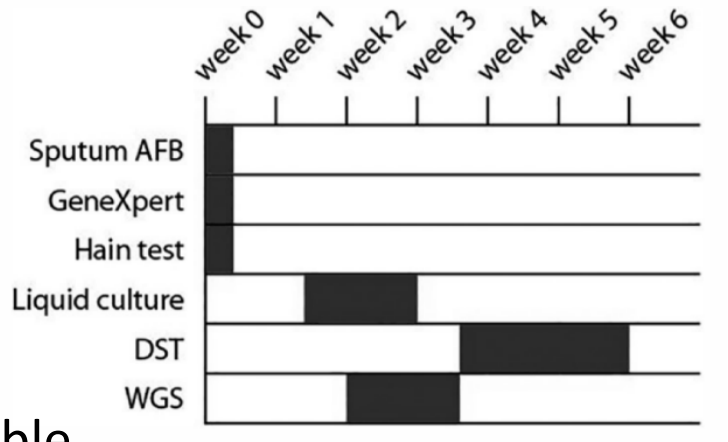
OSPEDALE SAN RAFFAELE

Time to results: 10 days-3 weeks.

Costs saving: allows to replace several different molecular assays.

Diagnostic power: WGS allows to screen for any mutations in any gene.

Expected time frame for receiving results for each test following sample collection.



WGS performances are accurate and reliable compared to conventional phenotypic diagnostics:

- species identification accuracy 93-99%,
- drug susceptibility accuracy 93-96%

Concordance of genotypic prediction and phenotypic resistance:

- High for some drugs (RIF, INH)
- Far from 100% for others (PZA, EMB) due to issues with current DST methods, gaps in understanding the genetic basis of drug resistances, sampling heterogeneous populations etc.…

A standardized system for grading mutations

Drug (phenotypic testing)		Gene	High confidence mutations	Moderate confidence mutations	Minimal confidence mutations	No association with resistance
First-line	RIF	*rpoB*	D516A, D516F, D516G, D516G+L533P, D516ins, D516N, D516V, D626E, Del N518, F505V+D516Y, F514dupl, H526C, H526D, H526F, H526G, H526L, H526R, H526Y, M515I+D516Y, Q513-F514ins, Q513H+L533P, Q513K, Q513L, Q513P, S522Q, S531F, S531L, S531Q, S531W	D516Y, H526P, L533P, S522L	H526N, I572F, L511P	
	INH	*inhA-mabA*	g-102a$^{G\text{-}NC}$	c-15t		L68F, g-47c, t-80g, T4I
		katG	S315I, S315N, S315T, Pooled frameshifts and premature Stop codons			A110V, L499M, R463L
		mshA		A187V$^{G\text{-}NC}$		N111S
Second-line (group A)	MOX	*gyrA*	A90V, D94A, D94G, D94N, D94Y, G88C, S91P			E21Q, G247S, G668D, S95T, V712L
	OFX/LEV	*gyrA*	A90V, D94A, D94G, D94H, D94N, D94Y, G88A, G88C, S91P	D89N		E21Q, G247S, G668D, S95T, T80A, V712L
		gyrB	A504V, E459K			
Second-line (group B)	AMK	*rrs*	a1401g, g1484t			
	KAN	*eis*	c-14t, g-10a		c-12t, g-37t	a1338c
		rrs	a1401g, a514c^{NC}, c1402t, g1484t			
		rrs+eis	*rrs* c517t^{NC} + *eis* g-37t			
	CAP	*rrs*	a1401g, c1402t, g1484t			c517t
		tlyA	N236K, Pooled frameshifts and premature Stop codons			D149H
	STR	*rpsL*	K43G, K43R, K43T, K88Q, K88R, T40I			
		rrs	a1401g^{NC}, a514c, a514t, c462t, c513t, c517t			
		gidB		E92D$^{G\text{-}NC}$		L16R, V110G, Pooled frameshifts and premature Stop codons
Second-line (group C)	ETH/PTH	*inhA*	c-15t+I194T, c-15t+S49A	c-15t		
		ethA				Q347Stop
Second-line (group D)	PZA	*pncA*	a-11g, A134V, A3E, A46V, C138Y, C14R, C72R, D12A, D12N, D49G, D49N, D63G, D8E, D8G, D8N, F94L, F94S, G108R, G132A, G132D, G132S, G162D, G17D, G24D, G97C, G97D, G97S, H137P,	A171E, K96E, K96T, M175I, P54L, Q10R, W68G	D12G, F58L, H71R, I133T, V139A	I31T, I6L, indel - c-125del, K48T, L35R, T114M, T47A

Targeted NGS on clinical specimens

Deeplex MycTB (Genoscreen) drug resistance-associated targets

Species ID
Mycobacterial species (hsp65, rrs, rplC, rrl)

MTBC genotyping
Spoligotyping
CRISPR/DR region
Phylogenetic SNPs

Drug	Gene target
Rifampicin	rpoB
Isoniazid	inhA, fabG1, katG, ahpC
Ethionamide	ethA, inhA
Pyrazinamide	pncA
Ethambutol	embB
Streptomycin	rpsL, rrs, gidB
Amikacin	rrs
Kanamycin	rrs, eis
Capreomycin	rrs, tlyA
Fluoroquinolones	gyrA, gyrB
Linezolid	rrl, rplC
Bedaquiline	Rv0678
Clofazimine	Rv0678

Mutations detected with Deeplex Myc TB assay	Mutations detected with WGS on corresponding isolates: Mutation detected	Mutations detected with WGS on corresponding isolates: No mutations	Performance (% [95%CI*]): Correlation	Performance (% [95%CI*]): Sensitivity	Performance (% [95%CI*]): Specificity
INH					
Mutation detected	5	0	100 (95.3 - 100)	100 (56.6 - 100)	100 (95 - 100)
No mutations	0	73			
RIF					
Mutation detected	4	1[a]	98.7 (93.1 - 99.8)	100 (51.0 - 100)	98.7 (92.7 - 99.8)
No mutations	0	73			
PZA					
Mutation detected	1	3[b]	96.2 (89.3 - 98.7)	100 (20.7 - 100)	96.1 (89.2 - 98.7)
No mutations	0	74			
AMK / KAN					
Mutation detected	3	0	100 (95.3 - 100)	100 (43.9 - 100)	100 (95.1 - 100)
No mutations	0	75			
CAP					
Mutation detected	7	0	100 (95.3 - 100)	100 (64.6 - 100)	100 (94.9 - 100)
No mutations	0	71			
MOX					
Mutation detected	0	1[c]	98.7 (93.1 - 99.8)	na	98.7 (93.1 - 99.8)
No mutations	0	77			

a One sample had a mixture with 2,3% resistant at Ser450Leu
b Three samples had a mixture with <7% resistant mutations
c One sample had a mixture with 6,3% resistant at Asp94Ala

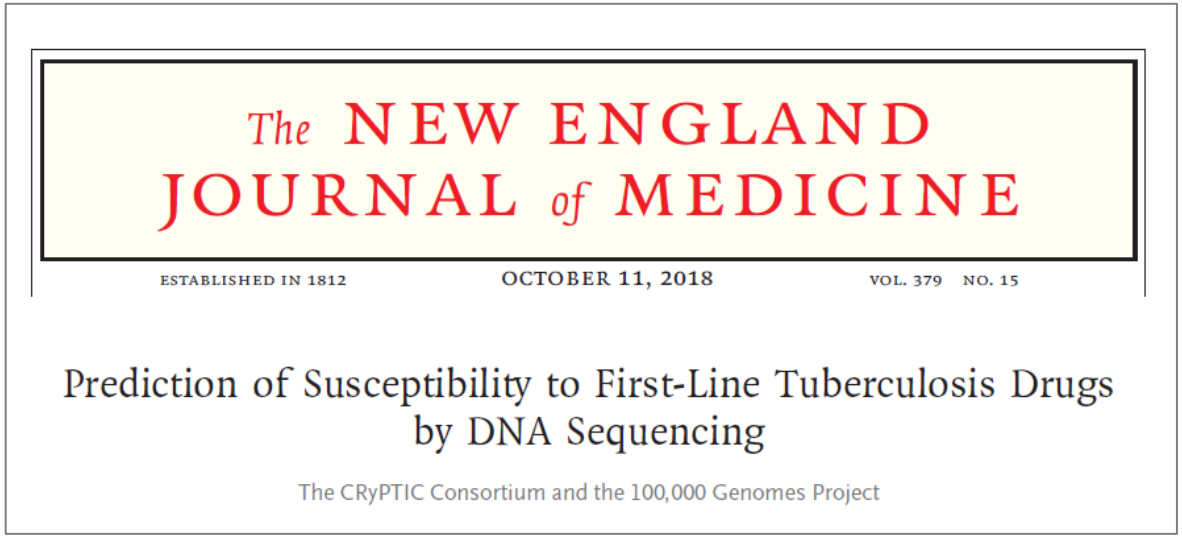
Tagliani E *et al* , Sci Rep. 2017 Dec 15;7(1):17672

Shifting the paradigm: can we use WGS to predict sensitivity avoiding the cost of unnecessary DST?

Can we use WGS to predict sensitivity?

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



The NEW ENGLAND JOURNAL of MEDICINE

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

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

The CRyPTIC Consortium and the 100,000 Genomes Project

Analysis of 10.209 MTB isolates
16 Countries, 6 continents
All major lineages represented

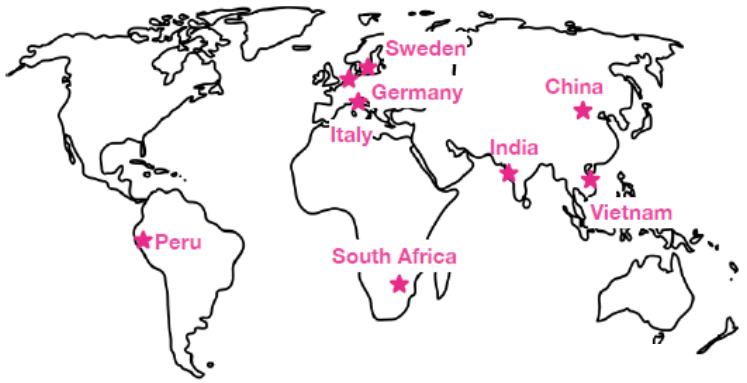
- Resistance to H, R, E, Z was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity,
- Susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity.
- 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted.
- On the 4037 phenotypic profiles predicted to be pan-susceptible, 3952 (97.9%) were correctly predicted.

Design and validation of MIC plates for MIC of 14 drugs

Emerging Bacterial Pathogens Unit

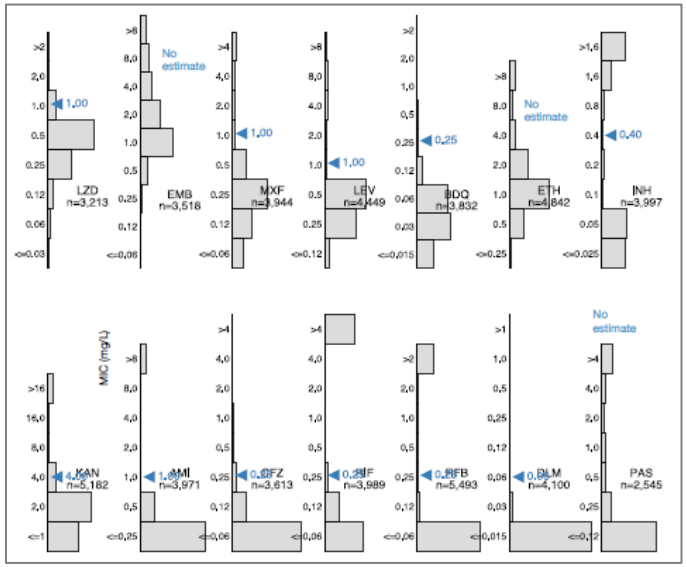
OSPEDALE SAN RAFFAELE

CRyPTIC laboratories in this study



96-well broth microtitre plate for high-throughput research antibiotic susceptibility of MTB

UKMYC6 plate design



ECOFF definition of individual drugs

Drug	Abbreviation	Tentative ECOFF
Isoniazid	INH	0.4 mg/L
Rifampicin	RIF	0.25 mg/L
Ethambutol	EMB	4 mg/L
Amikacin	AMI	1 mg/L
Kanamycin	KAN	4 mg/L
Moxifloxacin	MXF	1 mg/L
Levofloxacin	LEV	1 mg/L
Ethionamide	ETH	4 mg/L
Rifabutin	RFB	0.25 mg/L
Linezolid	LZD	1 mg/L
Clofazimine	CFZ	0.25 mg/L
Bedaquiline	BDQ	0.25 mg/L
Delamanid	DLM	0.06 mg/L
para-aminosalicylic acid	PAS	–

Still to be addressed …or ongoing

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- Make WGS accessible to most settings
- Low cost platforms, reagents
- Capacity building for wet and data analysis
- Standardization of analysis and reporting
- Moving to culture free tests

Acknowledgements

Emerging Bacterial Pathogens Unit

Daniela M. Cirillo

Simone Battaglia
Claudia Boin
Emanuele Borroni
Andrea M. Cabibbe
Matteo Chiacchiaretta
Arash Ghodousi
Floriana Gona
Giovanna Graziano
Paola Mantegani
Paolo Miotto
Viriginia Quaresima
Camilla Riva
Marco Rossi
Andrea Spitaleri
Elisa Tagliani
Enrico Tortoli
Alberto Trovato

Alagna.Riccardo@hsr.it