Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Genome sequencing for the surveillance of drug-resistant TB

Wolfheze Workshops 2019; Thursday 16, May 2019; The Hague, The Netherlands

Andrea M. Cabibbe, PhD

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

Outline

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- Global Surveillance of Antituberculosis-Drug Resistance
- Challenges of conventional (phenotypic) tools
- Transition to molecular screening
- Role of genome sequencing
- Incorporating sequencing into surveys
- DRS algorithms including sequencing
- Implementation of NGS: challenges and advantages
- Global data platform at WHO
- Conclusions and next steps

Global Surveillance of Antituberculosis-Drug Resistance

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Objectives: to estimate the magnitude and determine trends in drug resistance

- Statistically representative of the study population
- New/previously treated cases
- Quality-assured results
- Periodic or continuous

- 160 countries with >97%

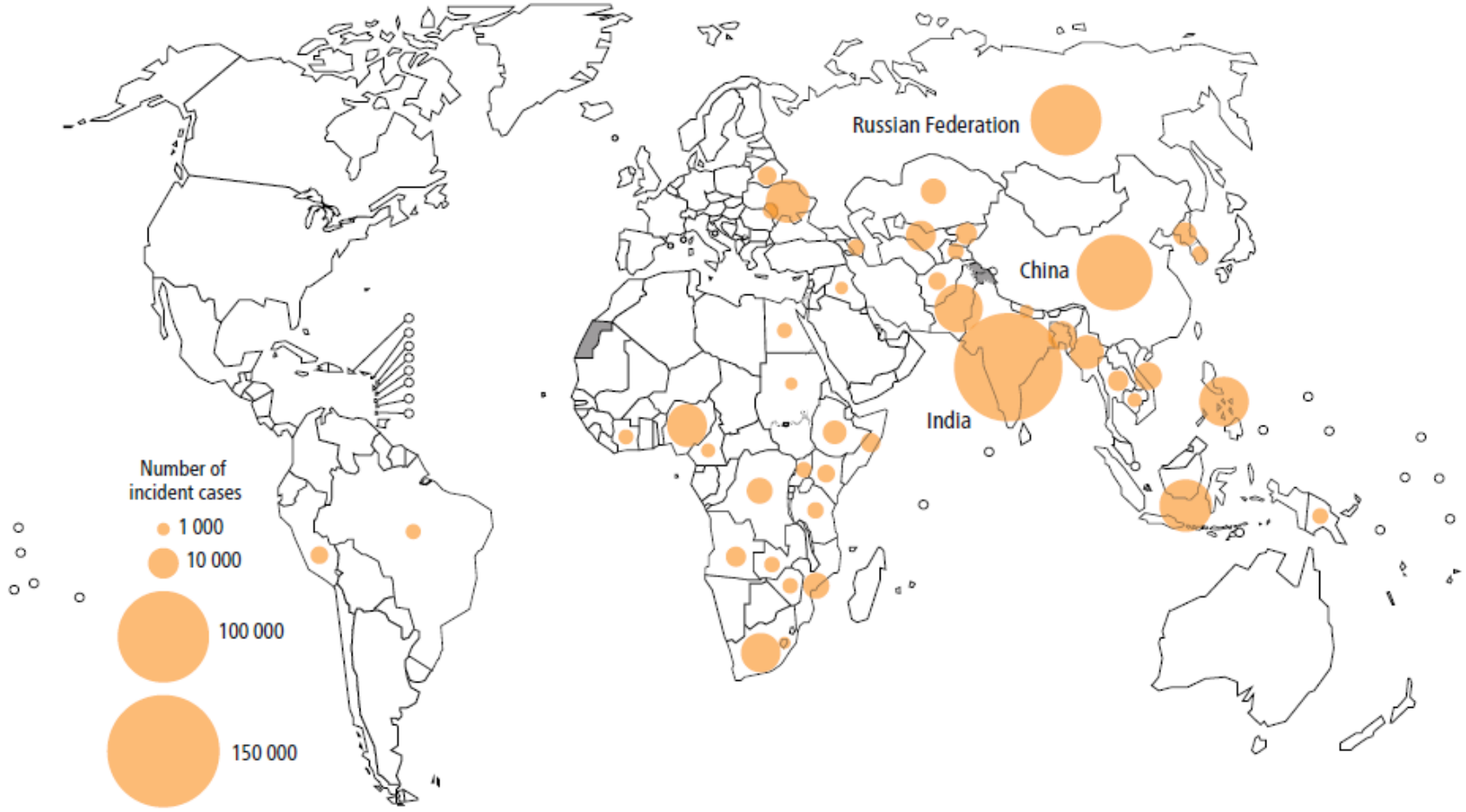
of world's population and TB cases

- Global estimates:

% new cases with MDR/RR: 4.1

% previously treated with MDR/RR: 19

Estimated incidence of MDR/RR-TB in 2016, for countries with at least 1000 incident cases



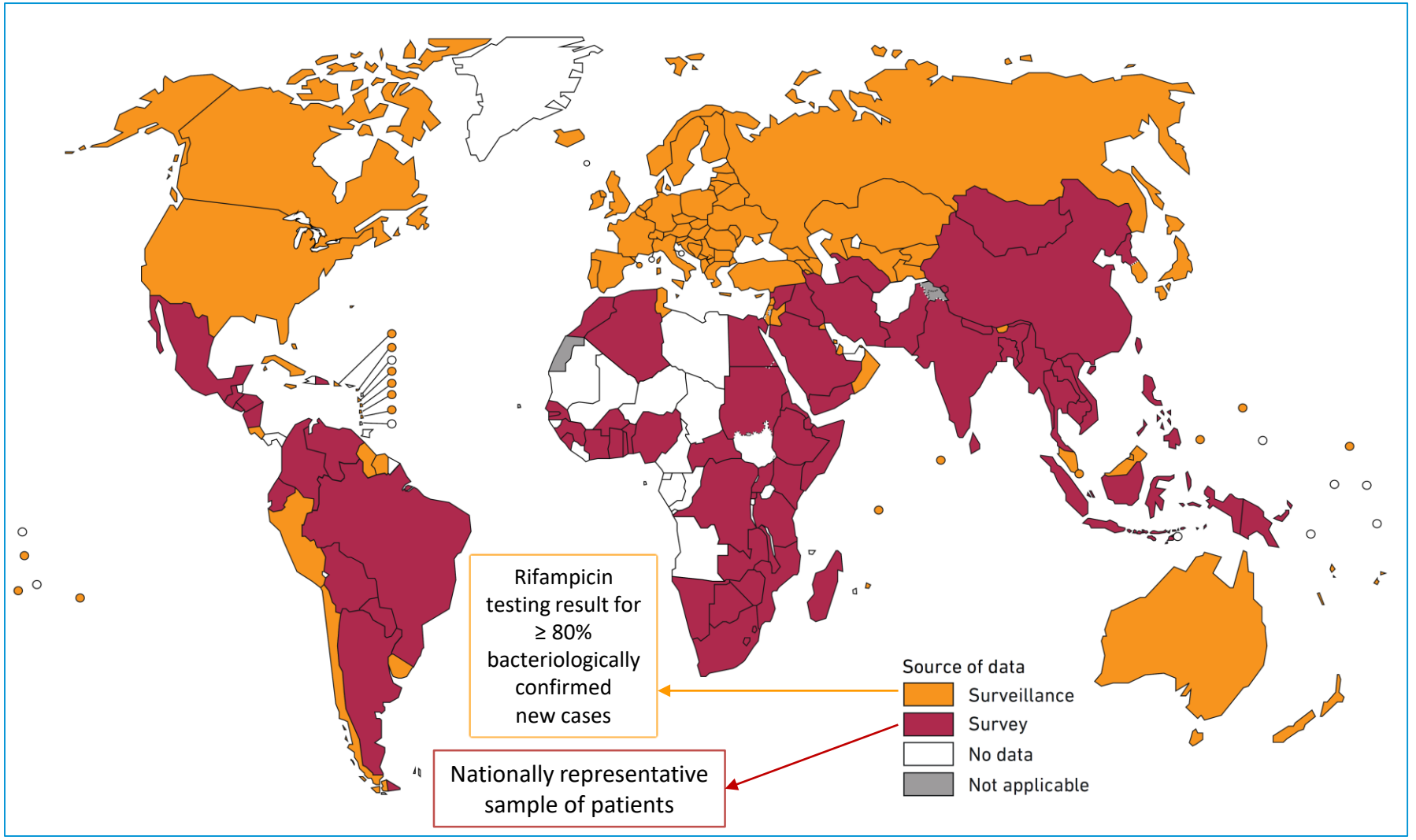
From: Geneva, World Health Organization

Challenges:

Periodic surveys versus continuous surveillance systems

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



Challenge: moving from periodic surveys to continuous surveillance systems

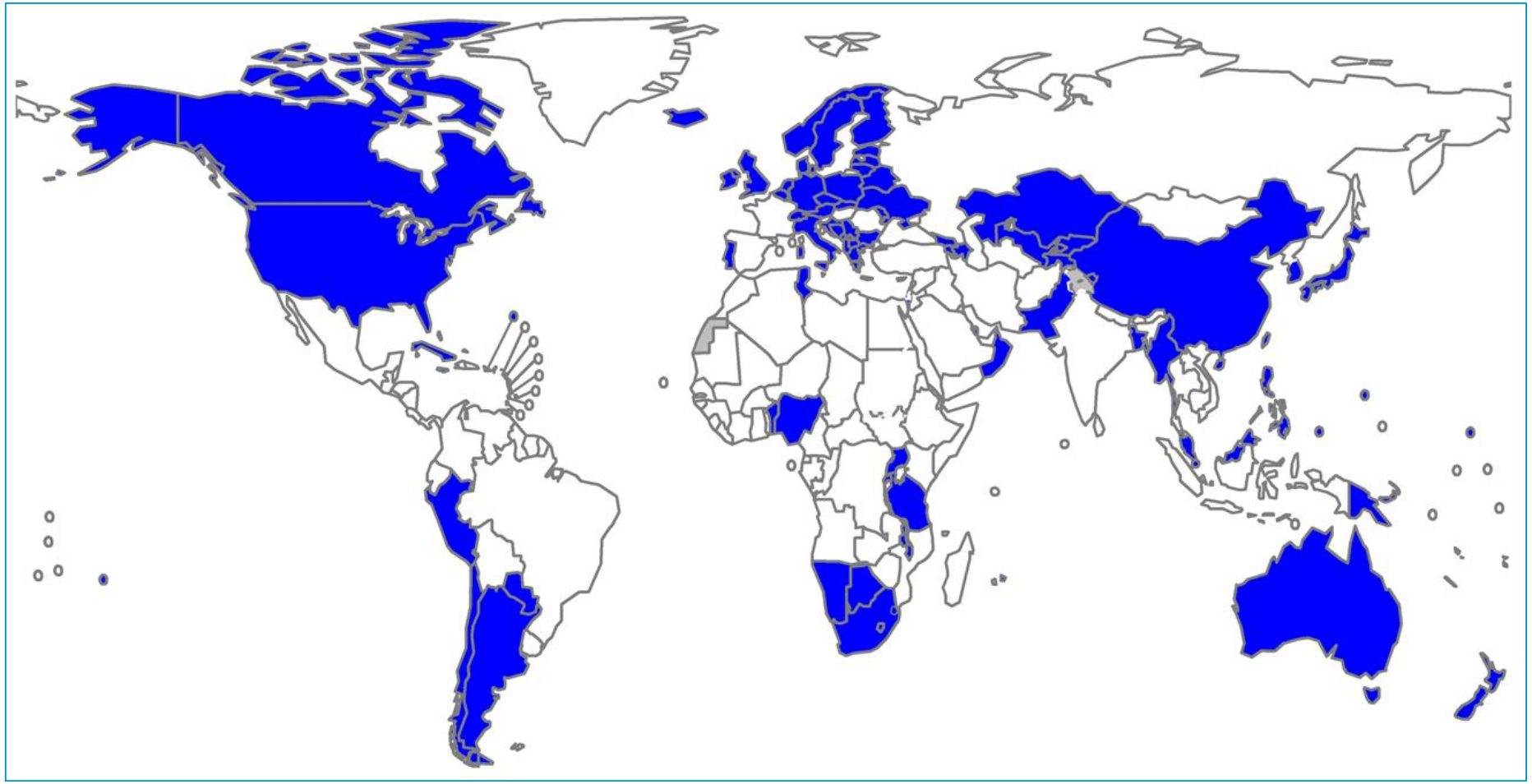
From: Geneva, World Health Organization

Challenges:

Second-line resistance among MDR-TB patients

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



Challenge: data needed for other drugs beyond rifampicin

From: Geneva, World Health Organization

Survey: transition to molecular screening

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

Challenges: limited culture capacity, logistics, reproducibility for some drugs, universal access to DST

New pulmonary TB patients | New pulmonary TB patients

Table 2 Initial rifampicin testing results by Xpert® MTB/RIF assay vs. conventional LJ culture and phenotypic DST at the NRL

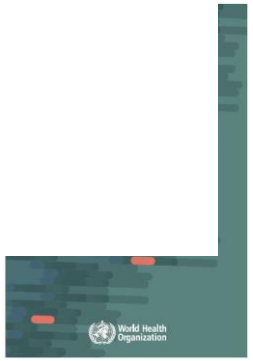
	Xpert on sputum samples						
LJ culture and phenotypic DST	MTB-positive, RIF-susceptible	MTB-positive, RIF-resistant	MTB-positive, RIF not determined	Negative	Error	Not done	Total (% of enrolled)
MTB-positive, RIF-susceptible	1416	17	10	6	23	14	1486 (75.4)
MTB-positive, RIF-resistant	15	85		1	5		106 (5.4)
NTM	2			11			13 (0.7)
Mixed infection (MTB/NTM)	25	1	1	3		3	33 (1.7)
Contaminated	138	8	1	6		1	154 (7.8)
Negative	85	3	1	36			125 (6.3)
Not done	14			1		40	55 (2.8)
Total (% of enrolled)	1695 (86.0)	114 (5.8)	13 (0.7)	64 (3.2)	28 (1.4)	58 (2.9)	1972 (100)

LJ = Lowenstein-Jensen; DST = drug susceptibility testing; NRL = National Reference Laboratory; MTB = *Mycobacterium tuberculosis*; RIF = rifampicin; NTM = non-tuberculous mycobacteria.

Tahseen S, INT J TUBERC LUNG DIS 20(4):448–455, 2016

- Valid Xpert results: 91.7% *vs* valid phenotypic DST: 80.7%
- RIF-R > Sensitivity of Xpert: 92.3% vs sensitivity of phenotypic DST: 96.2%
- Xpert specificity close to 100% (one silent mutation)
- **Discordances were resolved by sequencing**

Resistance patterns	All first-line resistance patterns including MDR-TB, as well as pre-extremely drug-resistant TB (pre-XDR-TB) and XDR-TB	Rifampicin resistance, multidrug-resistant TB (MDR-TB), pre-XDR-TB, XDR-TB



Survey: role of genome sequencing

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- Overcomes challenges of phenotypic-based surveillance by allowing testing
  - of a **greater range of drugs**
  - in **less time**
  - for **more countries**

  - **Simplified sample transport**
  - **Large high-throughput**
  - **bypass the need of laboratory capacity for culture and susceptibility testing**

- Additional information beyond prevalence of resistance
  - Other genotypic information, e.g. transmission
  - Future analyses to explore the potential of new diagnostics and new drugs

Population-based resistance of *Mycobacterium tuberculosis* isolates to pyrazinamide and fluoroquinolones: results from a multicountry surveillance project

Matteo Zignol, Anna S Dean, Natavan Alikhanova, Sönke Andres, Andrea Maurizio Cabibbe, Daniela Maria Cirillo, Andrei Dadu, Andries Dreyer, Michèle Driesen, Christopher Gilpin, Rumina Hasan, Zahra Hasan, Sven Hoffner, Ashaque Husain, Alamdar Hussain, Nazir Ismail, Mostofa Kamal, Mikael Mansjö, Lindiwe Mvusi, Stefan Niemann, Shaheed V Omar, Ejaz Qadeer, Leen Rigouts, Sabine Ruesch-Gerdes, Marco Schito, Mehriban Seyfaddinova, Alena Skrahina, Sabira Tahseen, William A Wells, Ya Diul Mukadi, Michael Kimerling, Katherine Floyd, Karin Weyer, Mario C Raviglione

Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study

Matteo Zignol, Andrea Maurizio Cabibbe*, Anna S Dean*, Philippe Glaziou, Natavan Alikhanova, Cecilia Ama, Sönke Andres, Anna Barbova, Angeli Borbe-Reyes, Daniel P Chin, Daniela Maria Cirillo, Charlotte Colvin, Andrei Dadu, Andries Dreyer, Michèle Driesen, Christopher Gilpin, Rumina Hasan, Zahra Hasan, Sven Hoffner, Alamdar Hussain, Nazir Ismail, S M Mostofa Kamal, Faisal Masood Khanzada, Michael Kimerling, Thomas Andreas Kohl, Mikael Mansjö, Paolo Miotto, Ya Diul Mukadi, Lindiwe Mvusi, Stefan Niemann, Shaheed V Omar, Leen Rigouts, Marco Schito, Ivita Sela, Mehriban Seyfaddinova, Girts Skenders, Alena Skrahina, Sabira Tahseen, William A Wells, Alexander Zhurilo, Karin Weyer, Katherine Floyd, Mario C Raviglione*

Survey: role of genome sequencing

A multi-country population-based study

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- Multi-partner project coordinated by WHO

NTPs, Supranational TB Reference Laboratories (SRLs), BMGF, USAID, TB Alliance

- Representative surveys of TB patients already conducted in seven countries

Azerbaijan, Bangladesh, Belarus, Pakistan, Philippines, South Africa, Ukraine

- Interpretation of mutations: standardised approach for grading mutations in *M tuberculosis* in terms of their association with drug resistance (Miotto et al, Eur Respir J. 2017 Dec 28;50(6))

rpoB, katG, inhA, fabG promoter, *pncA, gyrA, gyrB, rrs*, and *eis* genes

- Pooled distributions for the sensitivity and specificity of genotypic method were obtained using random effects modelling after logistic transformation and use of a restricted maximum likelihood estimator
- Prevalence of resistance estimated by sequencing obtained correlating the apparent prevalence of resistance (sequencing) and the true prevalence of resistance (bias-corrected phenotypic testing). Uncertainties about sens, spec and sampling propagated using a Bayesian model

	Loci	Rifampicin-susceptible cases		Rifampicin-resistant cases		All cases		
		Number of isolates	Sensitivity (95% CI)	Number of isolates	Sensitivity (95% CI)	Number of isolates	Sensitivity (95% CI)	
Rifampicin	*rpoB*	..	..	..	..	7010	91% (87–94)	85-95
Isoniazid	*katG*, *inhA*, and *fabG* promoter	6065	81% (66–90)	953	90% (81–95)	7018	86% (74–93)	62-97
Ofloxacin	*gyrA* and *gyrB*	4244	76% (51–90)	866	88% (83–92)	5110	85% (77–91)	75-96
Moxifloxacin	*gyrA* and *gyrB*	4010	81% (53–94)	783	91% (85–95)	4793	88% (81–92)	82-98
Pyrazinamide	*pncA*	2310	37% (22–54)	683	55% (40–70)	2993	51% (35–66)	
Pyrazinamide*	*pncA*	2310	50% (33–67)	683	54% (40–68)	2993	54% (39–68)	35-81
Kanamycin	*rrs* and *eis*	..	..	623	79% (58–91)	..	..	67-100
Amikacin	*rrs*	..	..	690	90% (82–95)	..	..	67-95
Capreomycin	*rrs*	..	..	764	81% (56–93)	..	..	33-96
Multidrug-resistant	NA	..	..	..	..	6986	85% (75–91)	
Extensively drug-resistant	NA	..	..	..	..	756	74% (53–87)	

*Adjusted with Wayne's test results.

Table: **Number of clinical *Mycobacterium tuberculosis* isolates tested and the pooled sensitivity values of genetic sequencing compared with phenotypic testing, stratified by rifampicin resistance status, for each locus or the loci conferring resistance to the indicated drug**

Prevalence of resistance estimated through sequencing compared with phenotypic testing

Emerging Bacterial Pathogens Unit
OSPEDALE SAN RAFFAELE

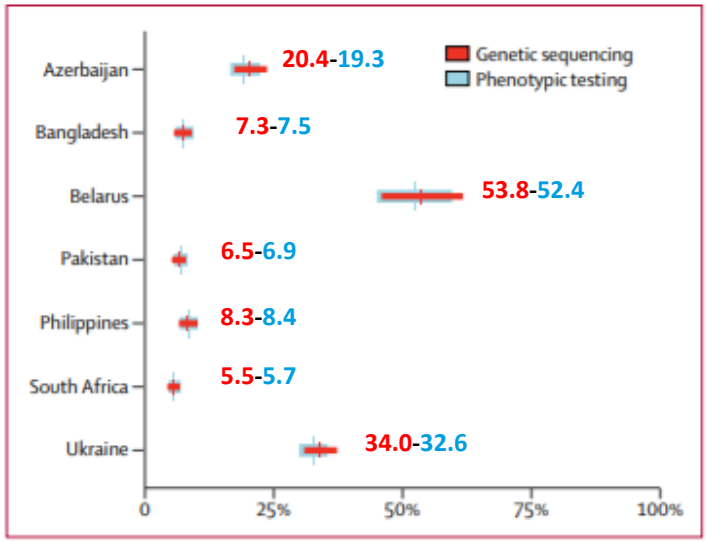


Figure 1: Prevalence of rifampicin resistance, estimated through genetic sequencing compared with phenotypic testing

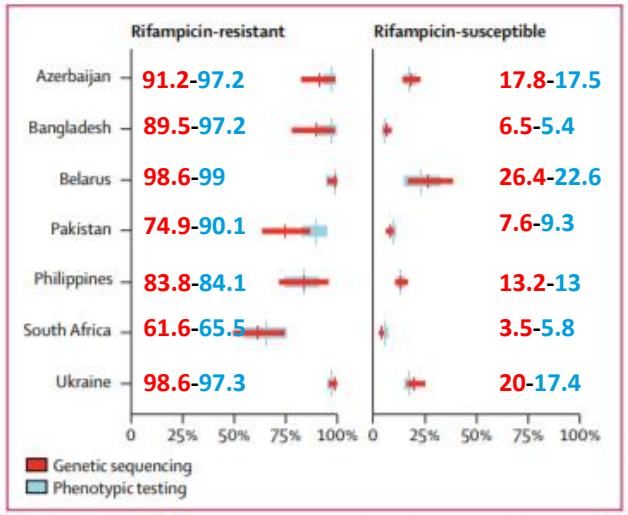


Figure 2: Prevalence of isoniazid resistance, estimated through genetic sequencing compared with phenotypic testing

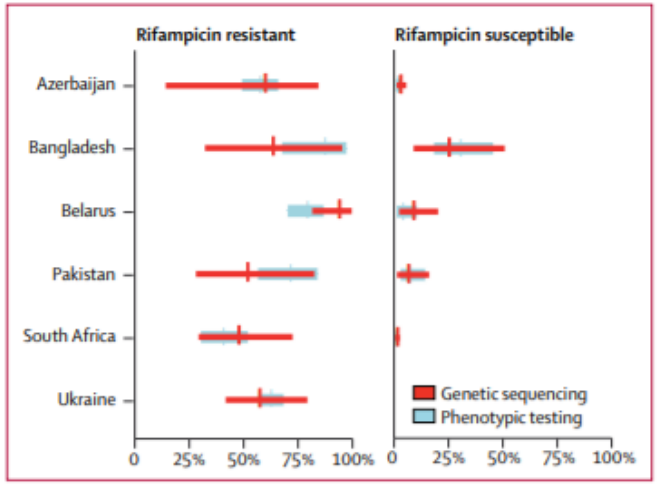


Figure 4: Prevalence of pyrazinamide resistance, estimated through genetic sequencing compared with phenotypic testing

blue as means and 95% confidence intervals
red as means and 95% credible intervals from the Bayesian model

EVIDENCE

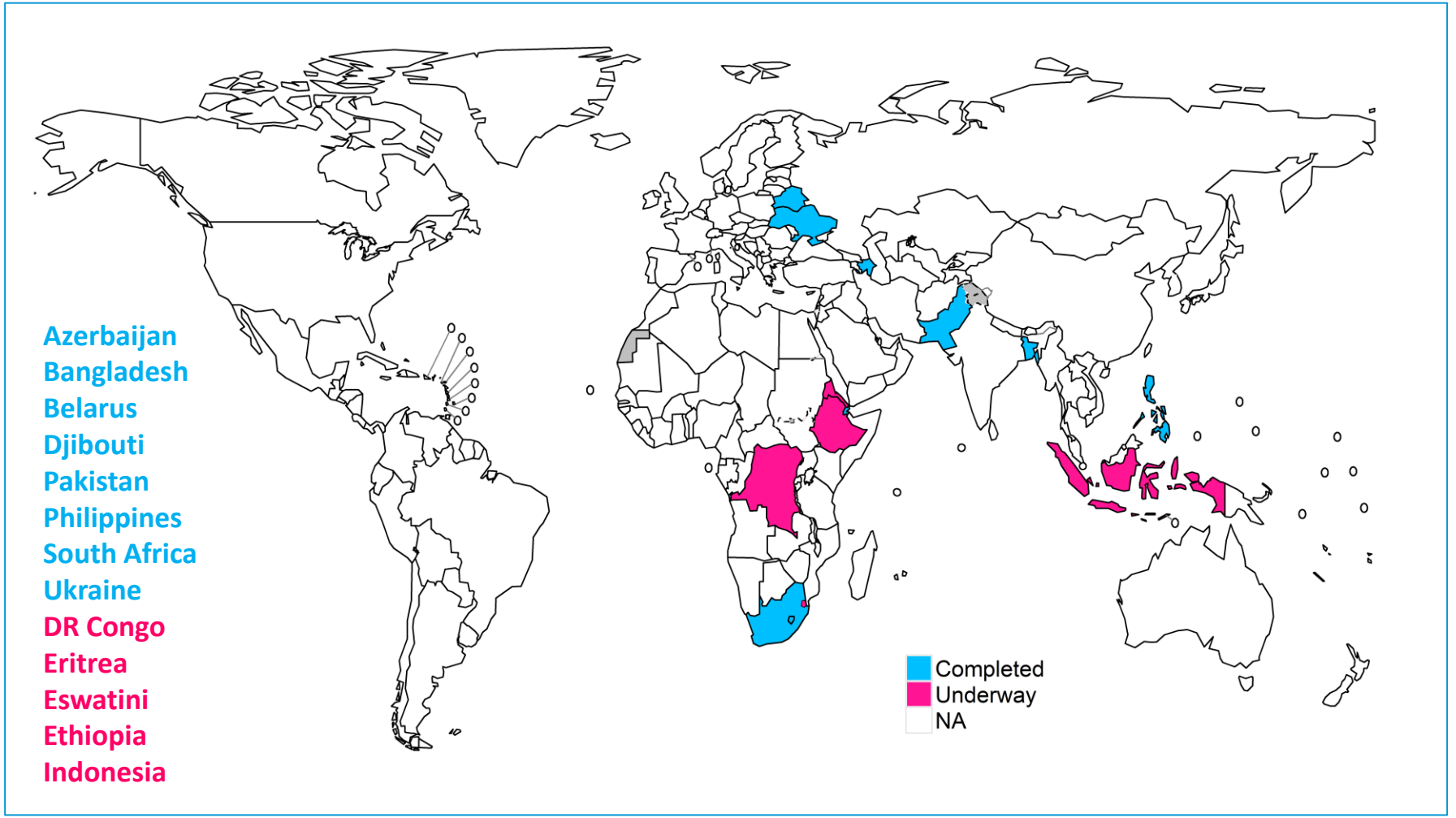
Large overlap between resistance determined by genetic sequencing, after adjustment for sensitivity, and the true prevalence of drug resistance

- ✓ Genetic sequencing can be a valuable surveillance tool to accurately predict drug resistance in low-income and middle-income countries
- ✓ Accuracy of genetic sequencing is very good at predicting phenotypic resistance to rifampicin, isoniazid, the fluoroquinolones, and (among rifampicin-resistant cases) injectable drugs
- ✓ We considered the phenotypic test to be the gold-standard test, BUT… False-negative phenotypic test results carrying graded mutations

Incorporating sequencing into surveys

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



12,000 samples from 13 countries

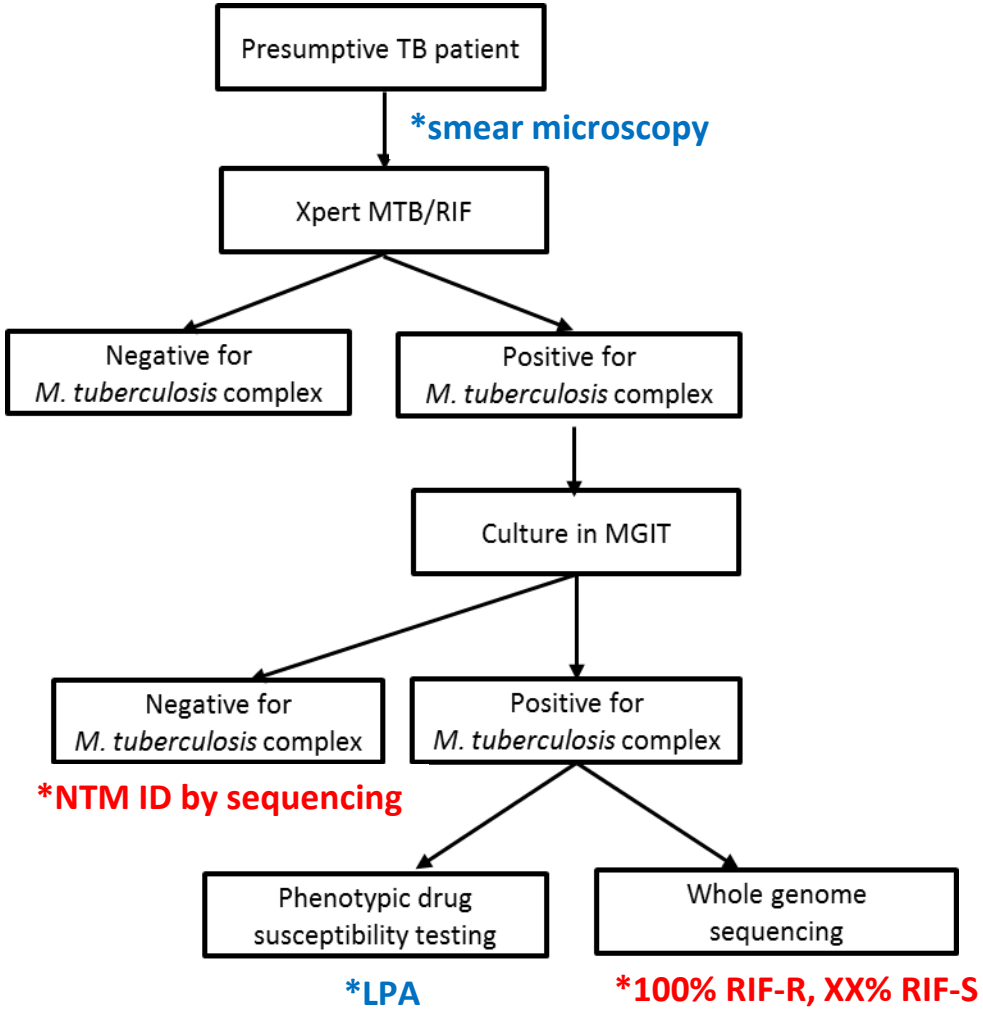
Courtesy of: Geneva, World Health Organization

DRS algorithm:

Whole genome sequencing of culture isolates

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



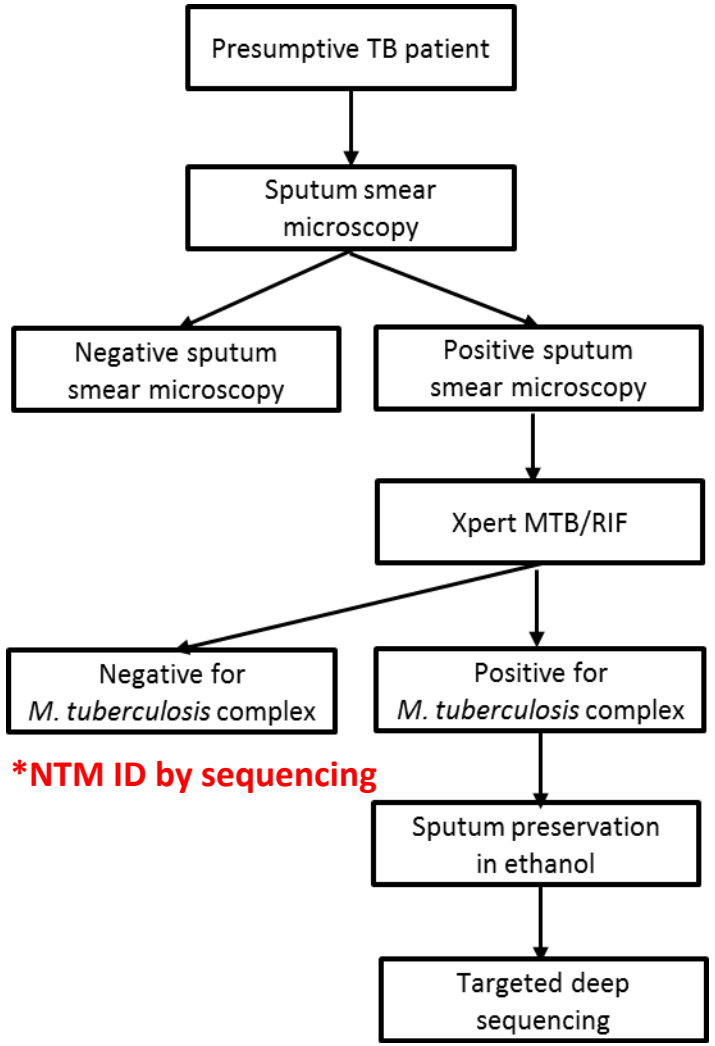
Courtesy of: Geneva, World Health Organization

DRS algorithm:

Targeted deep sequencing on sputum samples

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE



*NTM ID by sequencing

*or other transport solution

*100% RIF-R, XX% RIF-S

**Deeplex MycTB (Genoscreen)

Courtesy of: Geneva, World Health Organization

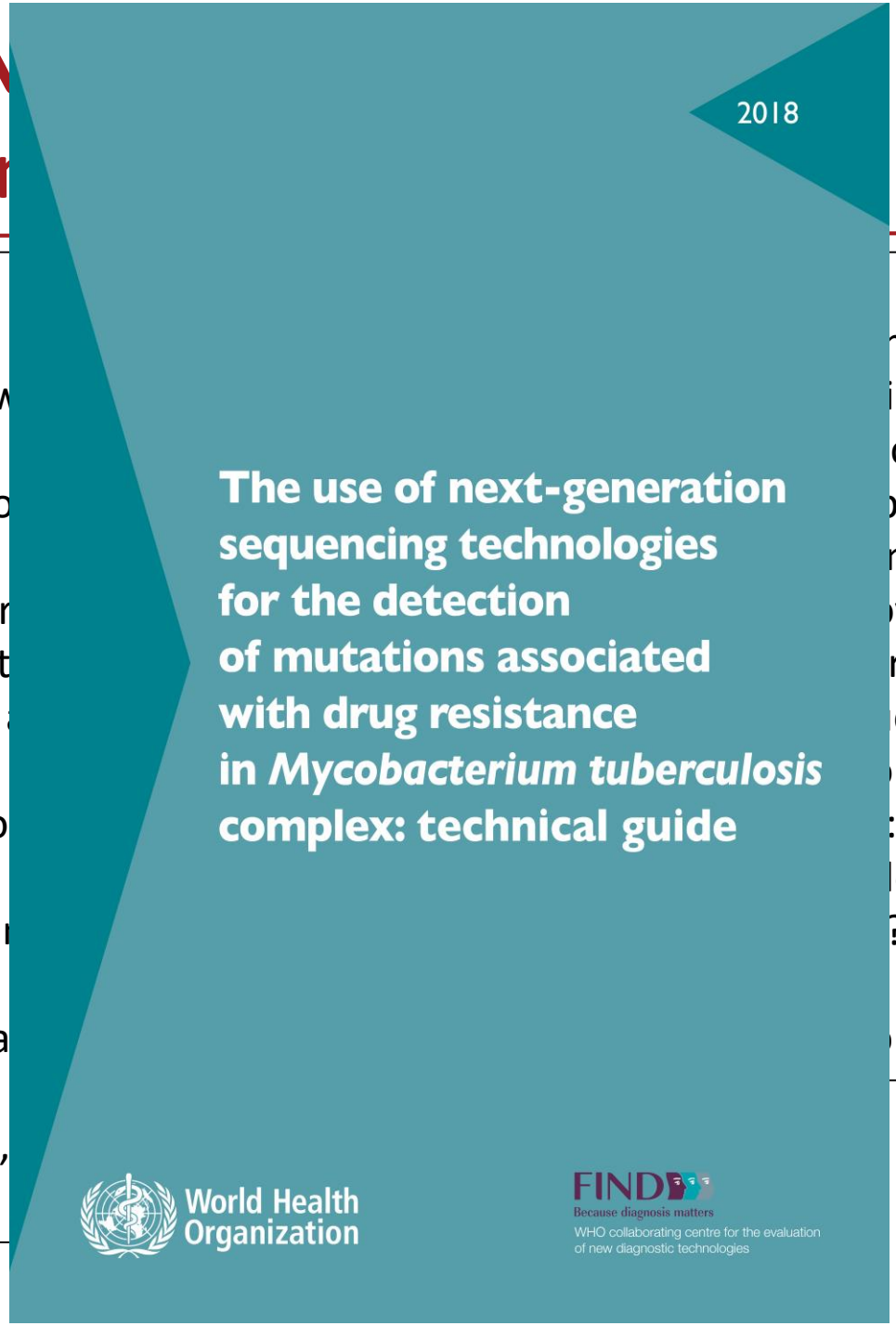
Implementation of N
Challenges and advar

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

CHALLENGES

- Capital investment
- Infrastructure: Laboratory areas, pow
  environment
- Equipment: NGS platform-specific, lo
- Procedures: Development of SOPs
- Computing: Hardware/software, stor
- Training: Laboratory and bioinformat
- Technical assistance: Manufacturers
  partners
- Quality assurance: Internal (validatio
  External (PT)
- Data analysis and interpretation: Sta
  friendly pipelines
- Nomenclature and reporting: Standa
  to clinical decisions
- Costs: Cost-benefit analysis, funding,



nostic and surveillance pros
identification, full DST, typing,
on)
proving)
m sputum specimens (tNGS
oved biosafety and logistics
retation of sequencing data (no
cture)
r epidemiological analyses
: discovery of new drug
levant mutations; studies on genetic
?)

rms

Global data platform at WHO

For surveillance of drug-resistant TB by sequencing

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- Cloud-based software at WHO (from March 2019)
- Adapted from multi-partner ReSeqTB (relational sequencing platform), hosted by Critical Path Institute
- To support countries with:
  - Analysis through standardized pipeline
  - Interpretation of mutations by expert knowledgebase
  - Linkages with patient data and phenotypic results
  - Safeguarding of data (ownership of data always remains with countries)
- A “living” platform – interpretation updated as new information become available
- Can inform future policy on sequencing and other diagnostics
- Initial focus on surveillance, to be rapidly expanded to clinical management

Courtesy of: Geneva, World Health Organization

Conclusions

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- ✓ Genome sequencing is a **valuable tool for surveillance** of drug resistance in resource-poor settings and **could potentially replace phenotypic testing** in drug resistance surveys
- Use of genome sequencing for broader surveillance of antimicrobial resistance is encouraged

- ✓ Establishment of a comprehensive **continuous surveillance system** for drug resistance, even in settings with limited laboratory capacity

- ✓ For drugs with suboptimal sensitivity of genome sequencing compared with phenotypic testing in the general patient population, the true prevalence of drug resistance can be determined using a relatively simple **statistical adjustment**
- Bridge the gaps in the determination of the whole spectrum of conferring-resistance mutations by developing **global repositories** (e.g. ReseqTB; Cryptic)

- ✓ Genetic DST can be implemented in **real-life scenarios** (population-based surveillance in low-, middle- income settings)
- Capacity building and continuous assistance

Next steps

Emerging Bacterial Pathogens Unit

OSPEDALE SAN RAFFAELE

- ✓ Development of in-country capacity for molecular biology and bioinformatics
- ✓ Development of low-cost and automated DNA extraction methods to enable direct sequencing from patient samples
- ✓ Development of kit-based and automated methodologies for sequencing
- ✓ Development of interpretation tools not requiring sophisticated infrastructure and skills
- ✓ Harmonization of quality standards and analysis pipelines
- ✓ Link to clinical decision and surveillance systems
- ✓ Design surveys to investigate proportions of resistance to PZA and SLDs (currently, insufficient power → wide CIs)
- ✓ Consider also clinical outcome data (given the suboptimal reliability of pDST)

Acknowledgements

Emerging Bacterial Pathogens Unit – TB Supranational Reference Laboratory

Daniela M Cirillo
Elisa Tagliani
Riccardo Alagna
Paolo Miotto
Emanuele Borroni

World Health Organization – Global TB Programme

Anna Dean
Matteo Zignol

All Countries: National TB Programmes, National TB Reference Laboratories
Technical partners: TB Supranational Reference Laboratories, The Union, US CDC, KNCV
Donor agencies