8th Regional TB Symposium - Tashkent, Uzbekistan

New Frontiers: Innovation and Access

The Future of DST

Next Generation Technologies

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TB Diagnostics Advisor
MSF-Access Campaign

February 28th - March 1st 2019

Ministry of Health of the Republic of Uzbekistan

MEDECINS SANS FRONTIERES

Paradigm shift

Culture Based Testing
(Phenotypic)
cDST or pDST

Measured bacterial growth in the presence at a critical concentration of an anti-TB drug which correlates with a poor clinical outcome

Solid/Liquid (MGIT) cDST and MIC plate-based technologies

Research

Molecular Based Testing
(Genotypic)
mDST or gDST

Mutations linked to resistant phenotypes.
Most knowledgeable:
INH, RIF, PZA, FQ, and SLIs

New Tools

LPA, CBNAAT, DNA Chip, and sequencing-based technologies

Features: phenotypic vs. genotypic

pDST

- Requires establishment of Critical Concentrations
- Requires BSL3 facilities
- Requires culture
- Time to results: weeks to months

gDST

- Requires knowledge of mechanisms related to DR
- Requires BSL2 facilities
- Potentially culture free
- Time to results: hours to days

Current WHO approved molecular technologies have **limited capacity,** covering only specific gene targets or regions of DNA

Pipeline molecular technologies

- Expanded use and access
- Diversified placement
- Multi-disease testing
- Remain limited in capacity

Centralized high-throughput platforms
WHO data review July 2019


Hain FluoroType


Abbott m2000


BD Max


Roche Cobas

Cepheid XDR

Gene	Encoded drug resistance
katG	Isoniazid (may also include Ethionamide)
inhA	
Ahpc-OxyR	
fabG1	
gyrA	Fluoroquinolones
gyrB	
rrs	Amikacin, Kanamycin, Capreomycin
eis promoter	

Trials start this year,
WHO review 2020


Moblio Truenat MTB/RIF

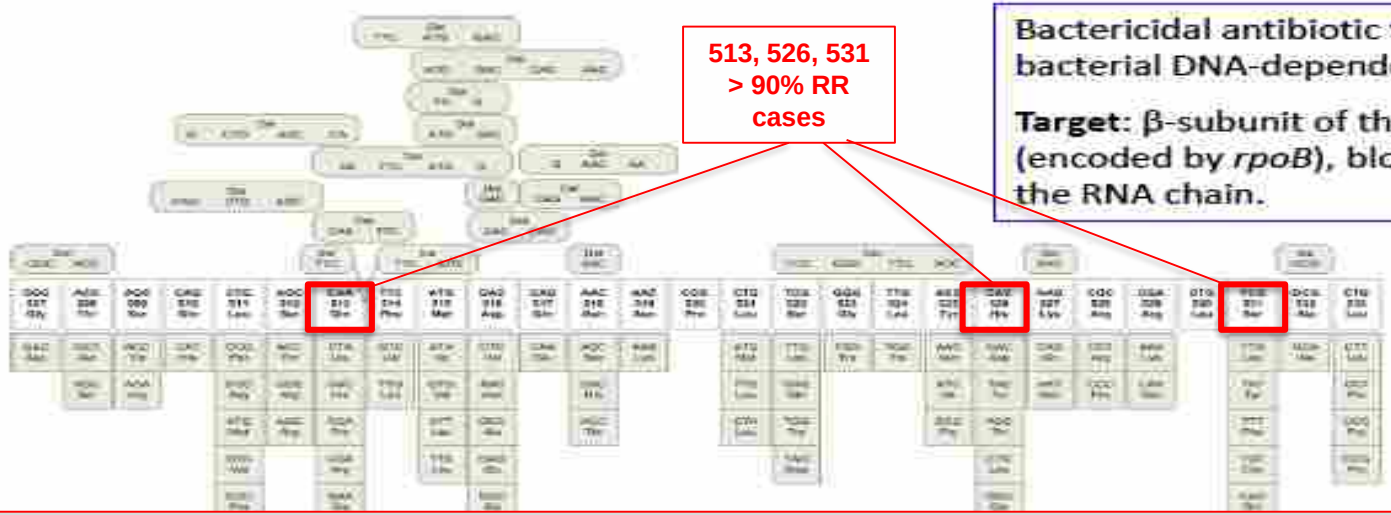
Currently Available Chip based Real Time PCR Tests

Truenat™ MTB	Truenat™ MTB-RIF Dx
Truenat™ HBV	Truenat™ H1N1
Truenat™ Dengue	Truenat™ Chikungunya
Truenat™ Dengue/Chikungunya	Truenat™ HCV
Truenat™ HIV-1	Truenat™ Rabies
Truenat™ CT	Truenat™ NG
Truenat™ CT/NG	Truenat™ Salmonella
Truenat™ Trich	Truenat™ Malaria Pf
Truenat™ Malaria Pv/Pf	Truenat™ MTB Plus
Truenat™ HPV-HR	

Trials underway,
WHO review 2020

Rifampicin resistance studies

Rifampicin (RIF)



513, 526, 531
> 90% RR
cases

Bactericidal antibiotic that inhibits the bacterial DNA-dependent RNA polymerase.

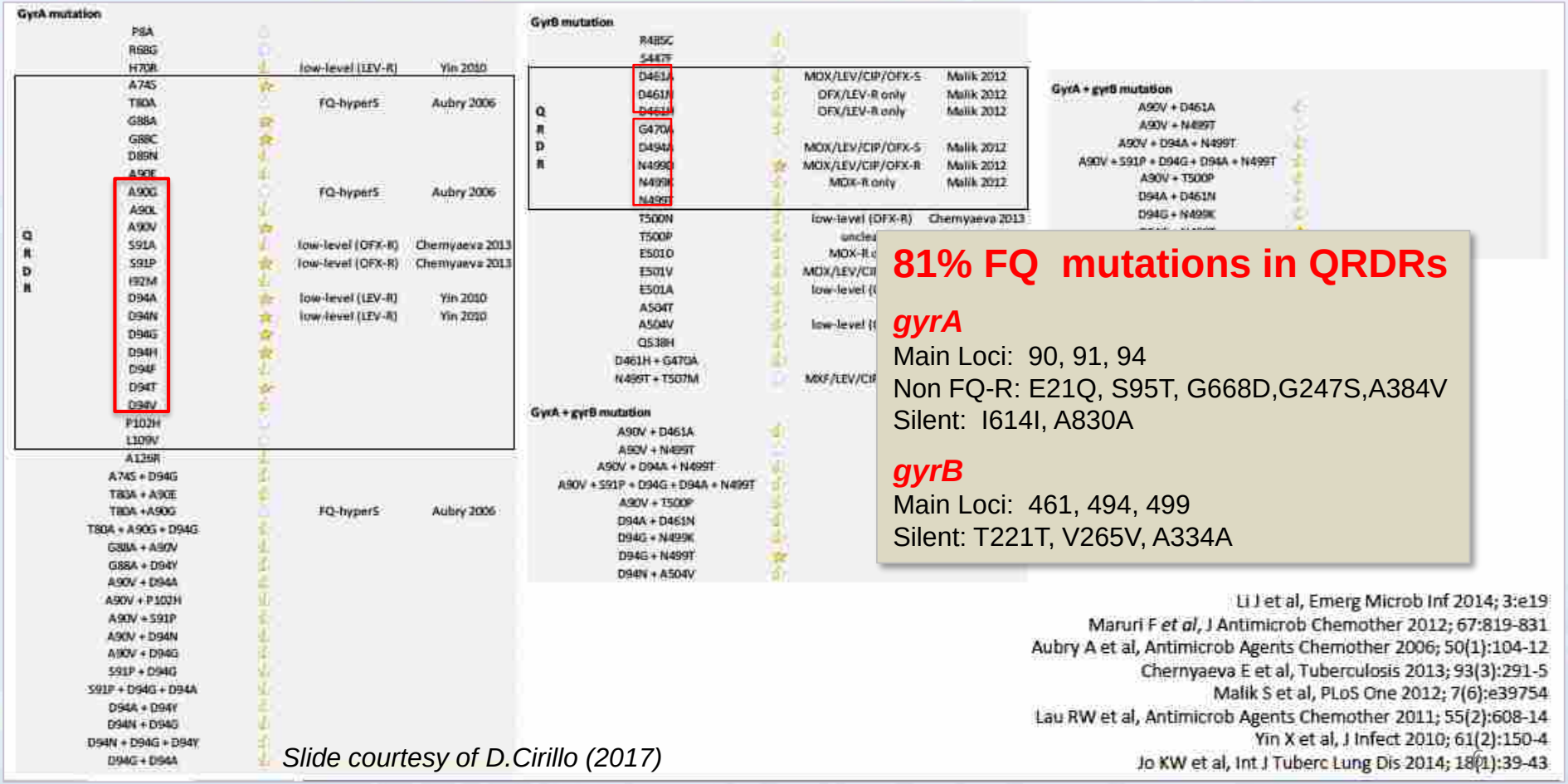
Target: β-subunit of the RNA polymerase (encoded by *rpoB*), blocking elongation of the RNA chain.

Others outside of rpoB (81bp): V146F and I572F
Silent Mutations: F506,T508,Q510,L511,Q513, F514,T525, A532, L533, P535

Mutations in a "hot-spot" region of 81 bp of *rpoB* gene (Rifampin resistance-determining region) → RIF resistance (> 95%)

Slide courtesy of P. Miotto (2016)

Fluoroquinolone resistance studies



81% FQ mutations in QRDRs

gyrA

Main Loci: 90, 91, 94

Non FQ-R: E21Q, S95T, G668D,G247S,A384V

Silent: I614I, A830A

gyrB

Main Loci: 461, 494, 499

Silent: T221T, V265V, A334A

Li J et al, Emerg Microb Inf 2014; 3:e19

Maruri F *et al*, J Antimicrob Chemother 2012; 67:819-831

Aubry A et al, Antimicrob Agents Chemother 2006; 50(1):104-12

Chernyaeva E et al, Tuberculosis 2013; 93(3):291-5

Malik S et al, PLoS One 2012; 7(6):e39754

Lau RW et al, Antimicrob Agents Chemother 2011; 55(2):608-14

Yin X et al, J Infect 2010; 61(2):150-4

Jo KW et al, Int J Tuberc Lung Dis 2014; 18(1):39-43

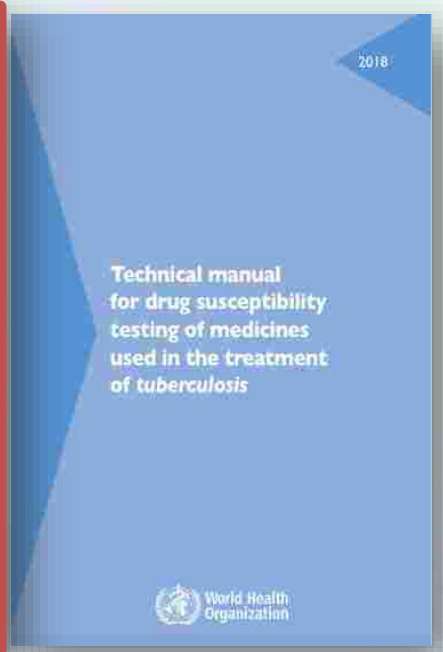
Slide courtesy of D.Cirillo (2017)

Markers of resistance to anti-TB drugs

Anti-TB Drug	Gene Targets
Isoniazid	***katG, inhA ,*** *ndh, aphC, oxyR, mshA, furA*
Rifampicin	***rpoB***
Ethambutol	***embB,*** *aftA, embA, embC, ubiA*
Pyrazinamide	***pncA****, rpsa, panD*
Streptomycin	***rpsl, rrs, gidB***
Amikacin/Kanamycin	***rrs, eis,*** *whibB*
Capreomycin	***rrs, tlyA,*** *eis, whibB*
Fluroquinolones	***gyrA, gyrB****, mfpA, pstB, lfrA, corD*
Eth/Prothionamide	***ethA, ethR, inhA****, ndh, mshA, furA*
p-aminosalycyclic acid	*thyA, dfiA, folC, ribD*
Cycloserine/Terizidone	***alr****, ald, ddl, cycA*
Linezolid	***rrl, rplC***
Clofazamine	***mmpR (Rv0678)***
Bedaquiline	***mmpR (Rv0678), atpE,*** *mmpL5, mmpS5, pepQ*
Delamanid	***ddn, fdg1, fbiA, fbiB, fbiC***

- *Know where to identify resistance conferring mutations*
- *Defined highest frequency gene targets*

New WHO guidance 2018



- **Systematic review** of pheno/genotypic data
- Defined new critical concentrations
- **Identify resistance associated mutations**

- **Critical concentrations** for reclassified drugs
- Outlined drug preparations and protocols per media
- Reliability of pDST per drug
- Recommended testing

Group	Medicine	Abbreviation	Critical concentrations (µg/ml) for DST by medium			
			Löwenstein Jensen[1]	Middlebrook 7H10[1]	Middlebrook 7H11[1]	BACTEC MGIT liquid culture[1]
Group A	Levofloxacin (CC)	LFX[2,3]	**2.0**	1.0	-	1.0
	Moxifloxacin (CC)	MFX[2,3]	**1.0**	0.5	0.5	0.25
	Moxifloxacin (CB)[4]			2.0	-	1.0
	Bedaquiline[5]	BDQ	-	-	**0.25**	**1.0**
	Linezolid[6]	LZD	-	1.0	1.0	1.0
Group B	Clofazimine	CFZ	-	-	-	**1.0**
	Cycloserine TerizidoneTerizidone	CS TZD	- -	- -	- -	- -
Group C	Ethambutol[7]	E	2.0	5.0	7.5	5.0
	Delamanid[8]	DLM	-	-	**0.016**	**0.06**
	Pyrazinamide[9]	PZA	-	-	-	100.0
	Imipenem-cilastatin Meropenem	IMP/CLN MPM	- -	- -	- -	- -
	Amikacin[10] (Or Streptomycin)	AMK (S)	30.0 4.0	2.0 2.0	- 2.0	1.0 1.0
	Ethionamide Prothionamide	ETO PTO	40.0 40.0	5.0 -	10.0 -	5.0 2.5
	Para-aminosalicylic acid	PAS	-	-	-	-

https://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/

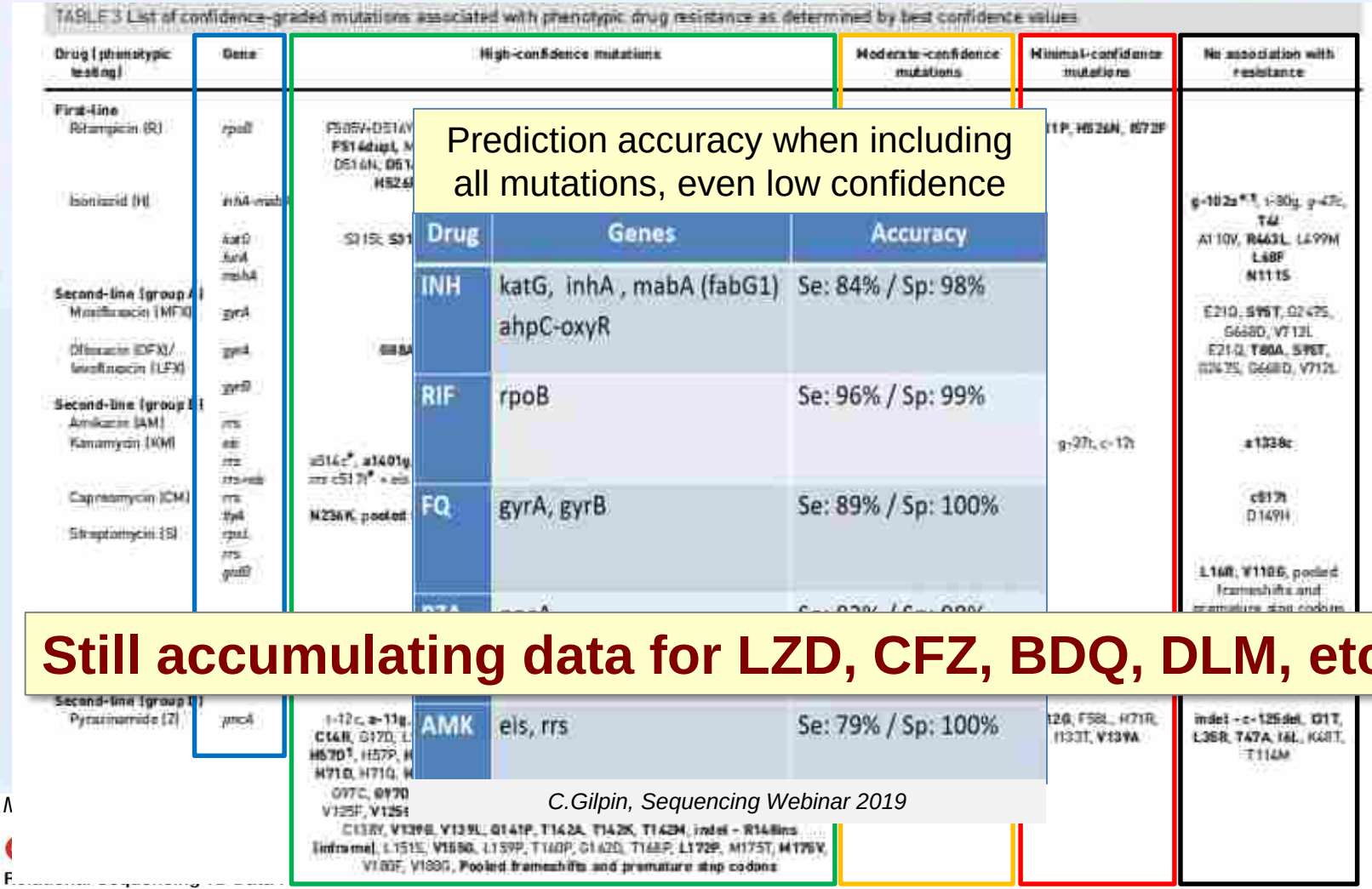
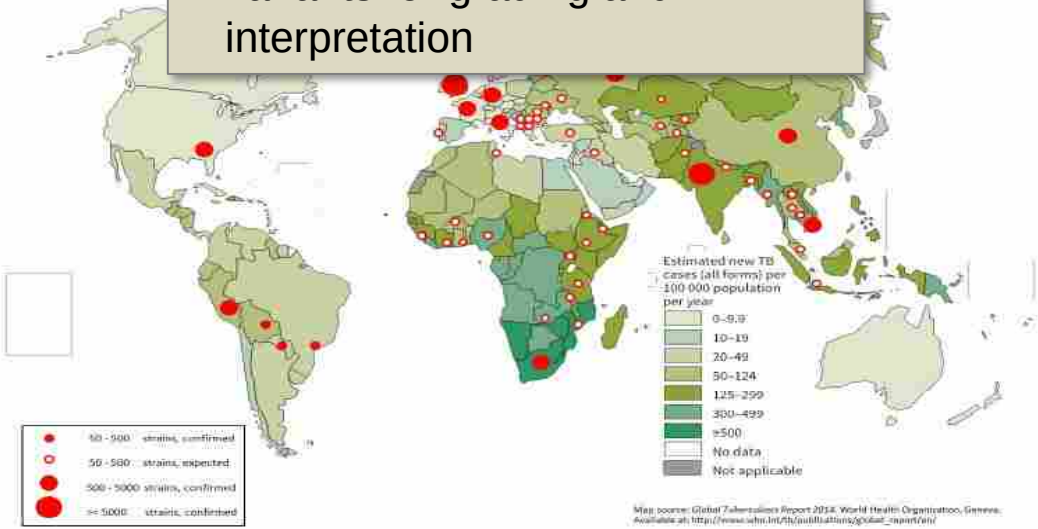


TABLE 3 List of confidence-graded mutations associated with phenotypic drug resistance as determined by best confidence values
Drug (phenotypic testing)
Gene
High-confidence mutations
Moderate-confidence mutations
Minimal-confidence mutations
No association with resistance
First-line
Rifampicin (R)
rpoB
Isoniazid (H)
Second-line (group A)
Moxifloxacin (MFX)
gyrA
Ofloxacin (OFX)/ levofloxacin (LFX)
gyrB
Second-line (group B)
Amikacin (AM)
Kanamycin (KM)
rrs
eis
Capreomycin (CM)
tlyA
Streptomycin (S)
rpsL
gidB
Second-line (group D)
Pyrazinamide (Z)
pncA
Prediction accuracy when including all mutations, even low confidence
Drug
Genes
Accuracy
INH
katG, inhA , mabA (fabG1) ahpC-oxyR
Se: 84% / Sp: 98%
RIF
rpoB
Se: 96% / Sp: 99%
FQ
gyrA, gyrB
Se: 89% / Sp: 100%
AMK
eis, rrs
Se: 79% / Sp: 100%
Still accumulating data for LZD, CFZ, BDQ, DLM, etc
C.Gilpin, Sequencing Webinar 2019
Grading:
High
Moderate
Minimal
None

Increasing knowledge to grade & interpret variants

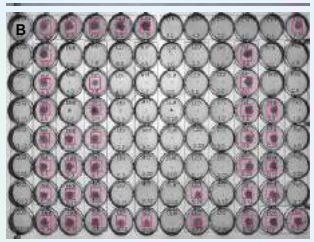
CRyPTIC
Comprehensi
Tuberculosis:

- 100,000 isolates: MDR, pre/XDR (6600 currently)
- Perform WGS / MICs
- Link data: pDST, MIC, genetic variants for grading and interpretation



http://www.crypticproject.org

- Bedaquiline
- Delamanid
- Clofazimine
- Linezolid
- Ethionamide
- PAS
- Levofloxacin
- Moxifloxacin
- Kanamycin
- Amikacin
- Capreomycin
- Pyrazinamide
- Ethambutol
- Rifabutin
- Rifampicin
- Isoniazid

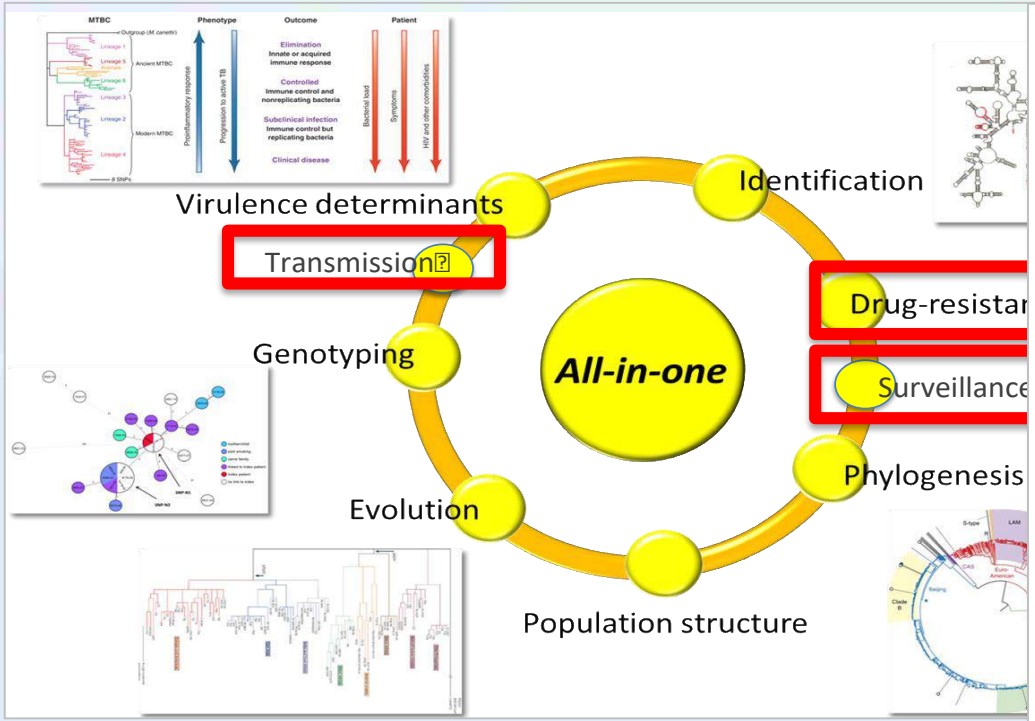


customized microtitre plates

NGS Illumina

Can we move to sequencing for DR-TB?

Next generation sequencing (NGS)



Strengths

- High accuracy for known resistance
- High throughput (200 strains/batch)
- Rapid runtime 3-4 days/batch
- Cheaper than phenotypic testing
- Systems open and adaptable as new knowledge is acquired

Limitations

- Knowledge on mutations for newer medicines is incomplete
- Contribution of hetero-resistance is not well understood
- The importance of efflux pumps and compensatory mechanisms remains unclear

Approaches for DR-TB:

Whole genome: complete DNA sequence, require

Target- based: select genes, enriched from direct

Target-based sequencing: Genoscreen Deeplex MycTB

GenoScreen

DEEPLEX®-MycTB Kit

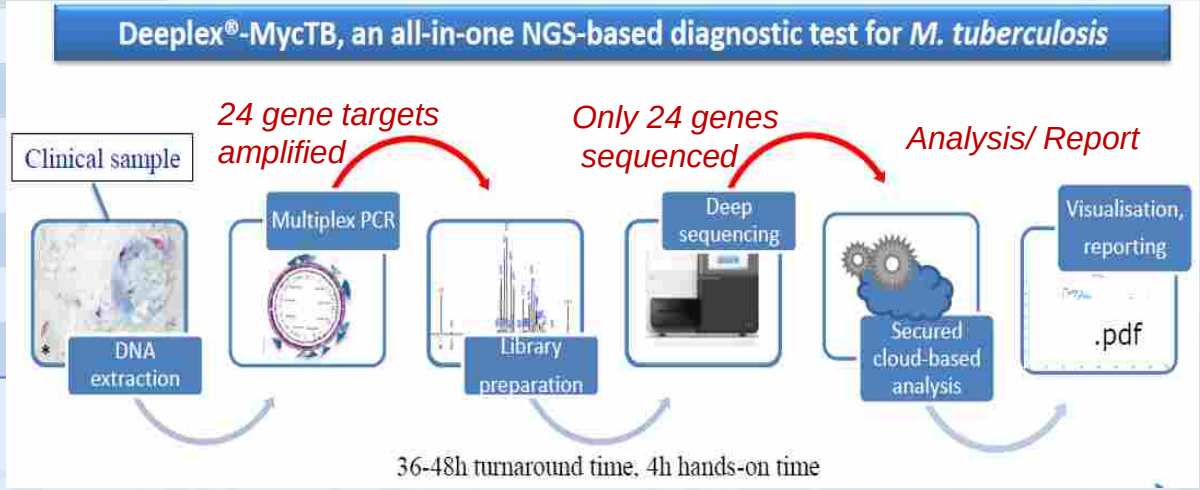
All-In-One Next Generation Sequencing-Based Diagnostics of Mycobacterium tuberculosis

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

Genotyping
Spoligotyping
CRISPR/DR
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
Fluoroquinolone	gyrA, gyrB
Linezolid	rrl, rplC
Bedaquiline	Rv0678
Clofazimine	Rv0678

- *Target amplification from direct sputum sediment = **FAST***
- *A 24-plex amplicon preparation before sequencing = **FOCUSED***
- *Identifies species, genotype, and drug resistance profiling for 18 genes targets = **FUNDAMENTAL***



WGS surveillance studies

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 Mansjo, Paolo Miotto, Ya Diul Mukadi, Lindiwe Mvusi, Stefan Niemann, Shaheed V Omar, Leen Rigouts, Marco Schito, Inta Sela, Mehriban Seyfaddinova, Girts Skenders, Alena Skrahina, Sabira Tahseen, William A Wells, Alexander Zhurilo, Karin Weyer, Katherine Floyd, Mario C Raviglione

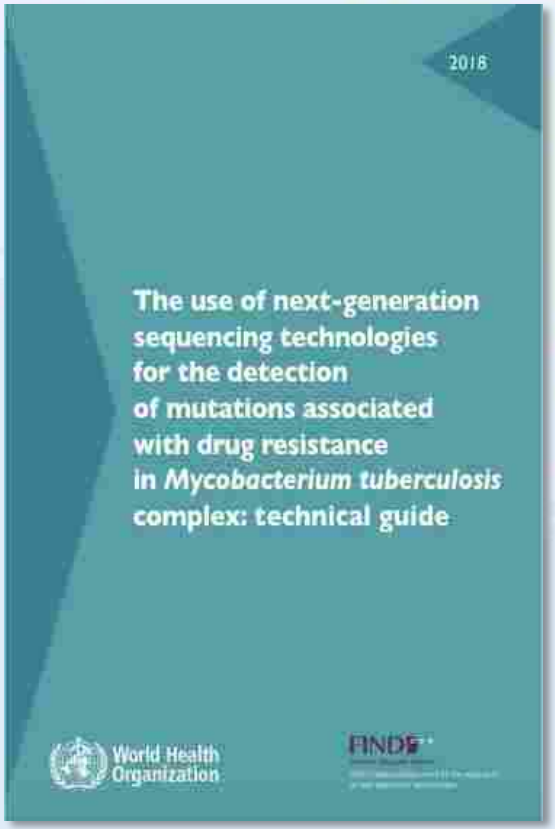
The overall pooled sensitivity for predicting resistance by WGS:

- **91%** (87–94) for *rpoB* (rifampicin)
- **86%** (74–93) for *katG, inhA*, and *fabG* promoter combined (isoniazid)
- **54%** (39–68) for *pncA* (pyrazinamide)
- **85%** (77–91) for *gyrA*/*gyrB* combined (ofloxacin/levofloxacin)
- **88%** (81–92) for *gyrA*/*gyrB* combined (moxifloxacin)

For nearly all drugs and most settings, there was a correlation in the estimated prevalence of drug resistance by sequencing and the estimated prevalence by phenotypic testing.

Zignol et al. (2018) Lancet Infect Dis.

New technical guidance



- Reviews **current NGS me**
  Mycobacterium tuberculos
- Describes the **utility and** and target-based sequenc
- Defines the principles beh conferring mutations to de
- Illustrates the **accuracy o** population-based study fo
- Provides **considerations**

https://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/

Future programmatic response

TREATMENT FOR DRUG SUSCEPTIBLE TB
(2HRZE/4HR)

STANDARDIZED SHORTER MDR-TB REGIMEN
4-6 Amk-M-Pto-Cfz-Z-Hhd-E / 5 M-Cfz-Z-E

DIAGNOSIS

TB-POSITIVE
Rif-S

RAPID DIAGNOSTIC
(XPERT MTB/RIF)

TB-POSITIVE
Rif-R

LPA-SL

CONSIDERATIONS:
pDST, preference for oral route, DR prevalence, previous treatment, tolerability, drug-drug interactions, severe forms

MDR-TB

(PRE-XDR-TB or XDR-TB)

PRECISION TREATMENT
(FOLLOWING WHO STANDARDS)

LONGER MDR-TB REGIMEN
SOCIAL SUPPORT & COUNSELLING

DIAGNOSIS
(upfront in specific settings)

TARGETED SEQUENCING
ACCESSIBLE

CENTRAL DATABASE

FEED-BACK LEARNING LOOP FOR ADJUSTMENT OF DX, RX, APPROACHES

DRTB- SURVEILLANCE

DRUG PROCUREMENT

PHARMACOVIGILANCE

Adapted from Mario C. Raviglione Global Health

Summary

- **Rapid molecular technologies remain useful**
  - Triage at near point of care (RR/FQR)
  - High-throughput testing (reference level)
  - But remain limited in capacity (targets)
- **NGS will play a significant role in the future**

  Target-based sequencing (Clinical diagnosis)
  - Rapid and accurate diagnosis using graded-mutation encyclopedia
  - Complete resistance profile in one test
  - Eliminates pDST for many drugs (INH, RIF, FQ, AMK/S)
  - pDST is unreliable for EMB, ETO/PTO, CS, PAS – rely on mutational correlates of resistance
  - Building evidence for mutation correlates of resistance for LZD, CFZ, BDQ, DLM

  Whole genome sequencing (Surveillance/Transmission)
  - Identify new mutations as strains evolve
  - Study the role of efflux pumps
  - Understand compensatory mechanisms underpinning resistance
  - Evaluate transmission events
- **Future convention will rely on gDST over pDST as more knowledge is gained.**

Rahmat
Спасибо
THANK YOU

Ministry of Health of the Republic of Uzbekistan

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