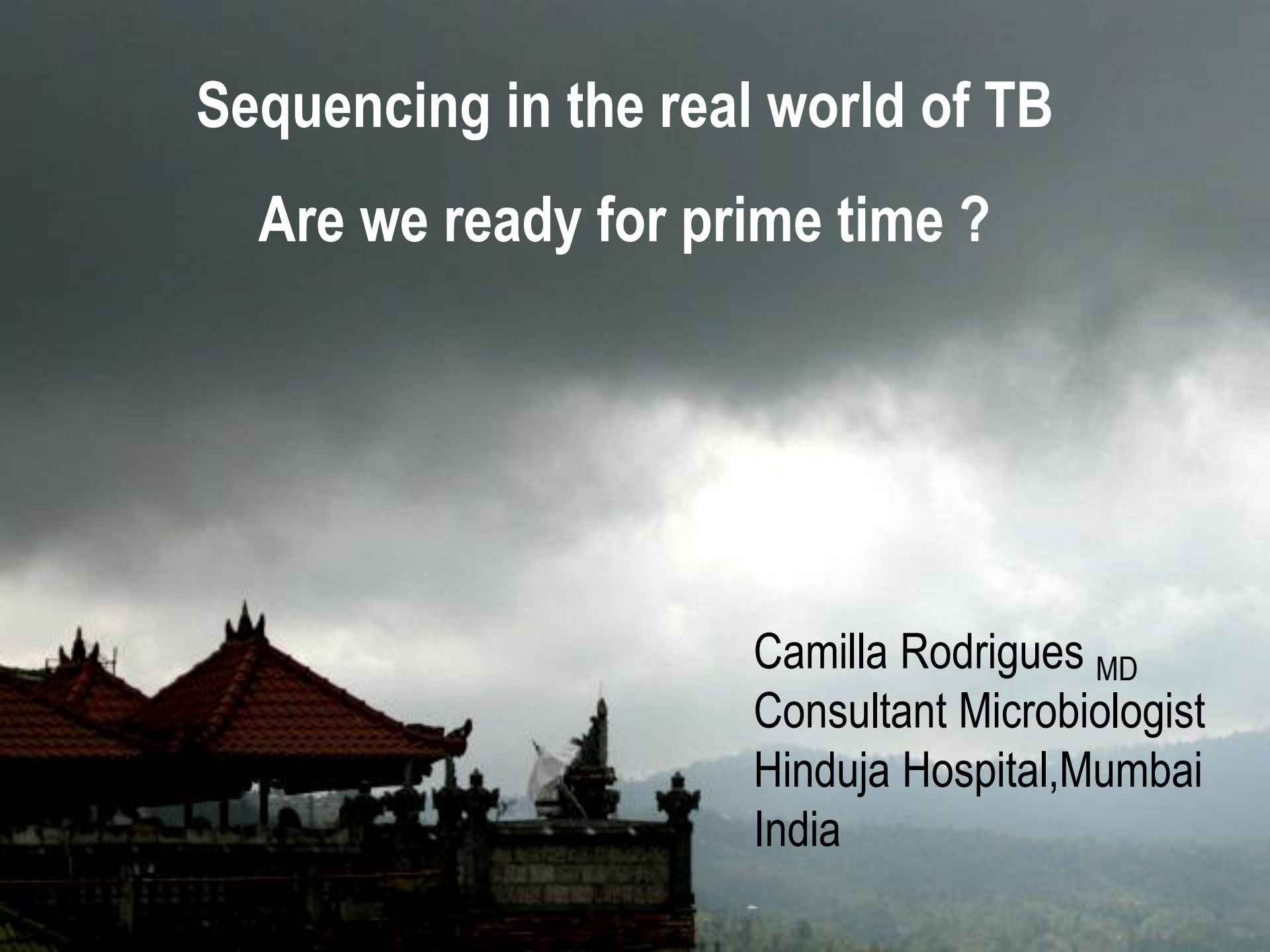
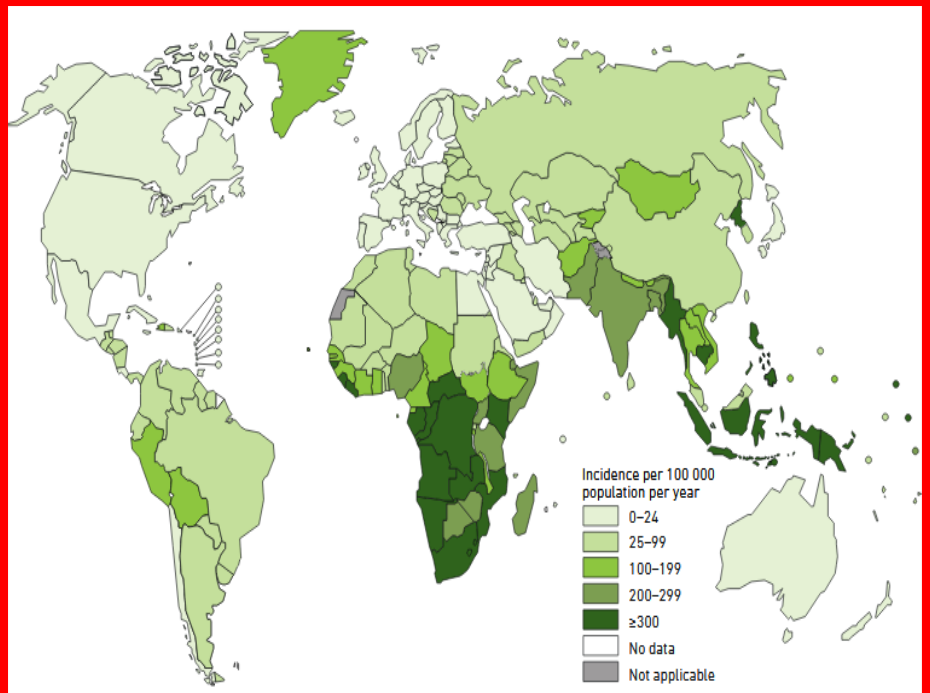
Sequencing in the real world of TB

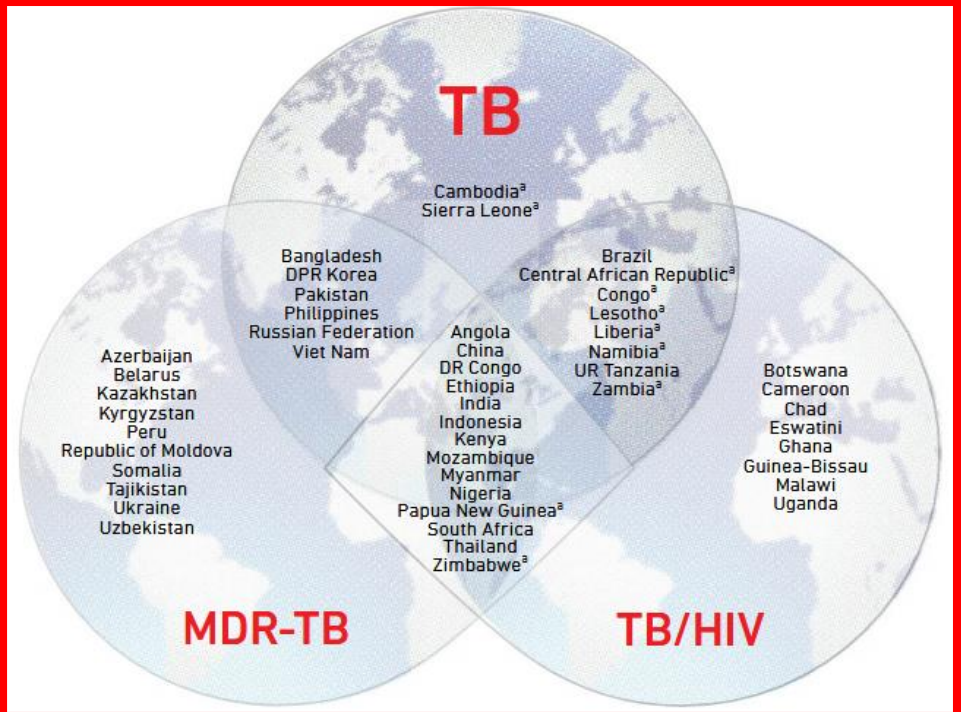
Are we ready for prime time ?



Camilla Rodrigues MD
Consultant Microbiologist
Hinduja Hospital,Mumbai
India

The real world of tuberculosis





Case

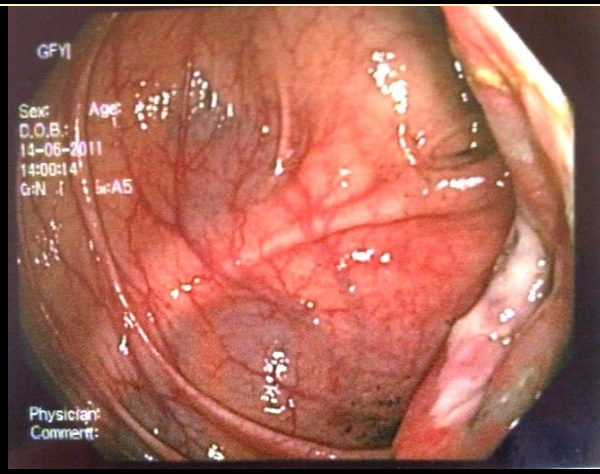
SK 41 M

8 Nov 2010 : LRKT, immuno suppresives

11 June 2011 : Fever, bleeding PR,

Cecal ulcer biopsy : nonspecific colitis

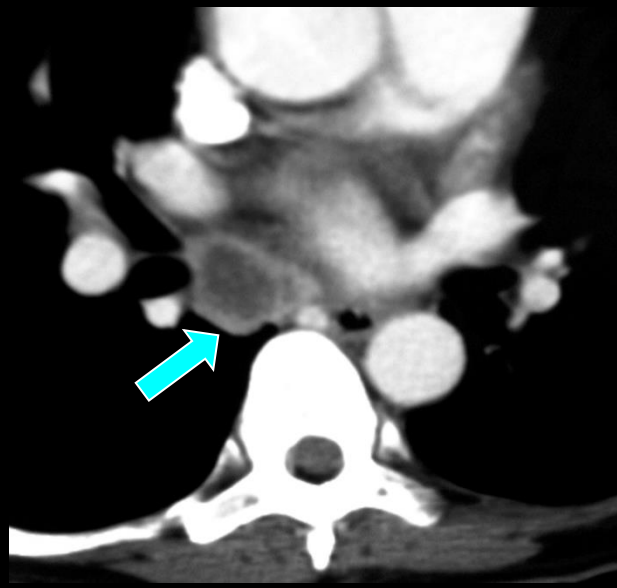
Bone marrow biopsy : normal



Fever continued

22 June 2011 :

CT chest : necrotic sub carinal LN mass



Patient refused a CT guided biopsy

Empirically started on INH,EMB,PZA,LVF

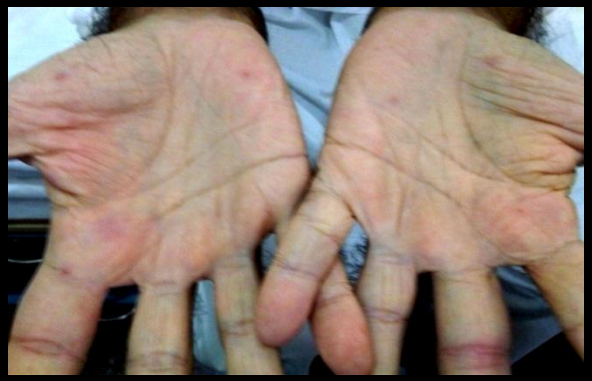
11 July 2011: readmitted with pancytopenia

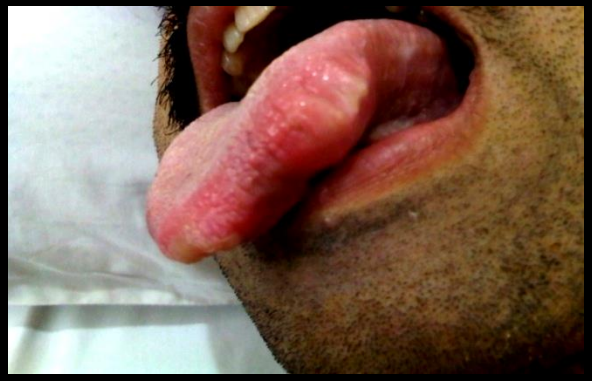
Skin biopsy septic vasculitis

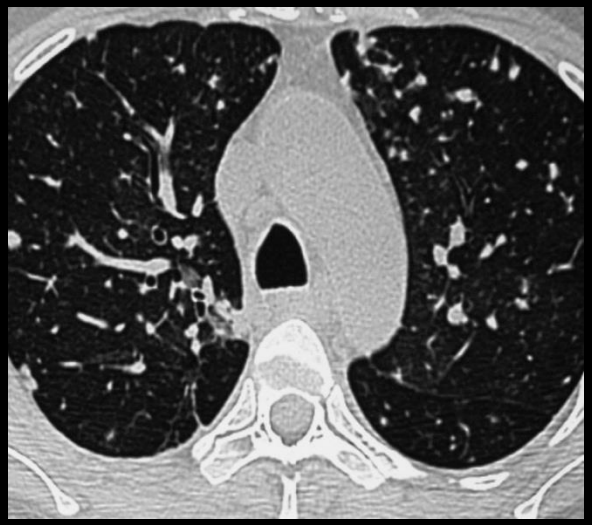
Tuberculosis cutis orificialis

13 July 2011: miliary TB
Sepsis Tuberculosa Acutissima
hematogenous, disseminated TB & shock

16 July 2011: Hypotension, acidosis, massive GI bleed & **Expired**





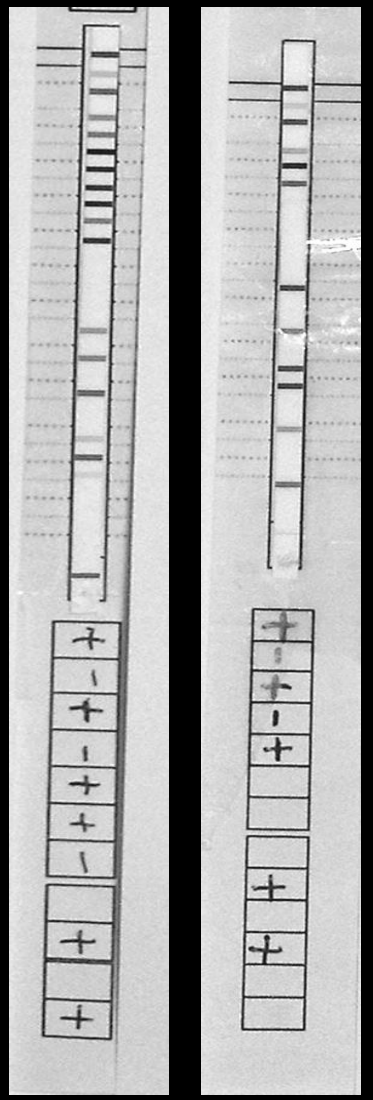


…Too little ..too late

16 July 2011 : Bone marrow culture(17 June) : MTB
LPA GenoType MTBDR *plus* & *sl* *:* XDR
Confirmed by MGIT DST

10 August 2011 : Blood culture (11 July) : XDR TB

MTBDR *plus* MTBDR *sl*

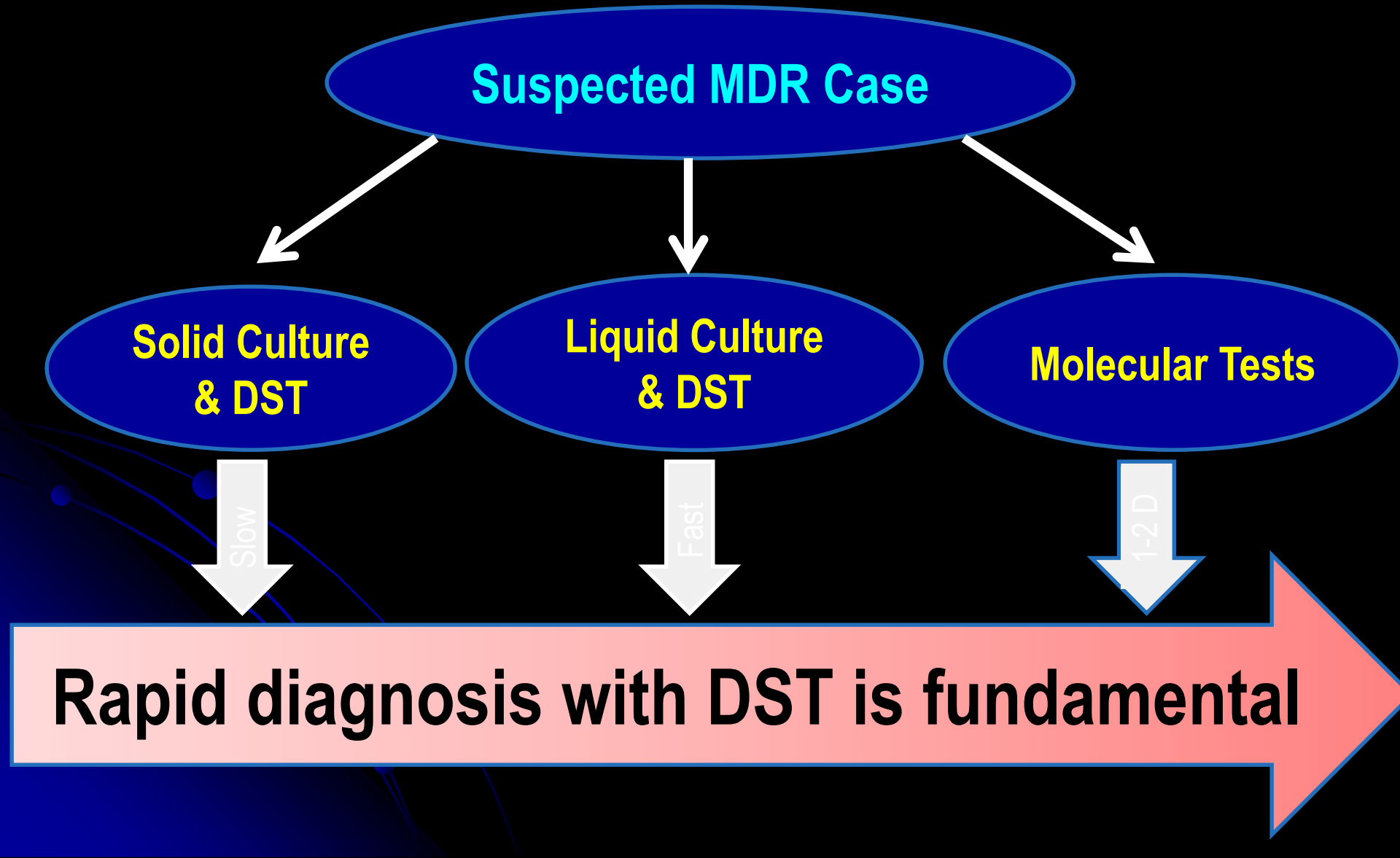


What did this case teach us ?

In the Immune compromised, in high DR endemic regions
(poly R, MDR , pre XDR, XDR)

- Simply cannot treat TB empirically
- Need to get DST results **fast**

With increasing drug resistance, DST is vital



Outline

- **Empiric treatment with local lab epidemiology**
- Pyrosequencing applications
- Expanding the mutation knowledge base
- Sequencing directly from clinical samples

Regional prevalence specifics for DR conferring mutations

Drug	Reference Genes	Mutations	% global R	% Mumbai	% Moldova	% South Africa
INH	*katG* *inhA-fabG1*	**S315T,** S315N, S315I **- c15t,-17t**	78.5	**89** **18**	**92** **46**	**44** **47**
RIF	*rpoB*	**S450L,** **D435V,** **H445Y, H 445D** H445R,S450W,H445L , D435G,H445C, H445G Q432K, Q432P, D435F, Q432L,F433,S450F,L452P	83.1	**83** **2** **3**	**84** **2** **2**	**17** **57** **low**
MXF	*gyrA*	**D94G,** **A90V,** **D94A, D94N/Y**	82.8	**46** **26** **8/8**	**6** **19** **19**	**46** **9** **9**
KAN	*rrs* *eis*	**A1401g**, c1402t, **g1484t** **-c14t**, -c12t -g10a	81.7	**85-95** **4**	**7** **53**	**85-95** **4**
AMK	*rrs*	**A1401g, g1484t**	78.6	**85-95**	**33**	**85-95**
CAP	*rrs* *tylA*	**A1401g**, c1402t, **g1484t** N236K	77	**85-95**	**40**	**85-95**

Paolo M Eur Respir J 2017;50:1701354
Georghiou SB. AMAC 2016; doi:10.1128/AAC.00222

Use of Xpert MTB/RIF in Decentralized Public Health Settings and Its Effect on Pulmonary TB and DR-TB Case Finding in India

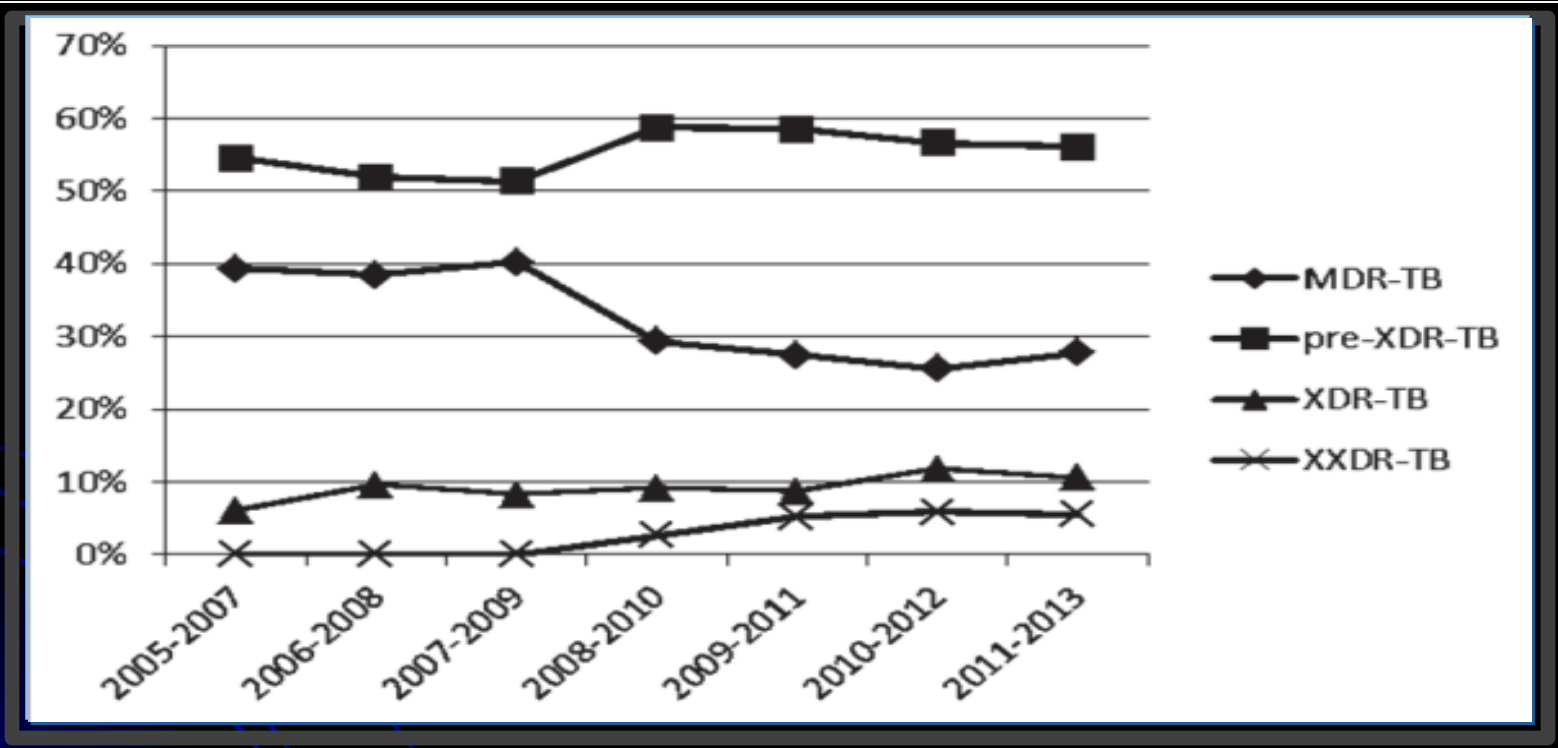
Kuldeep Singh Sachdeva[1], Neeraj Raizada[2]*, Achuthan Sreenivas[3], Anna H. van't Hoog[4], Susan van den Hof[4,5], Puneet K. Dewan[3¤], Rahul Thakur[2], R. S. Gupta[1], Shubhangi Kulsange[2], Bhavin Vadera[2], Ameet Babre[2], Christen Gray[2], Malik Parmar[3], Mayank Ghedia[1], Ranjani Ramachandran[3], Umesh Alavadi[2], Nimalan Arinaminpathy[6], Claudia Denkinger[2], Catharina Boehme[2], C. N. Paramasivan[2]

Implementation of Xpert MTB / RIF for suspected PTB at the district level, **increased case notification by 39% & rifampicin resistance notification by five fold**

PLoS ONE 10(5):e0126065. doi:10.1371/journal.pone.0126065

Resistance Patterns among Multidrug-Resistant Tuberculosis Patients in Greater Metropolitan Mumbai: Trends over Time

Alpa Dalal[1], Akshay Pawaskar[2], Mrinalini Das[3], Ranjan Desai[4], Pralhad Prabhudesai[5], Prashant Chhajed[6], Sujeet Rajan[7], Deepesh Reddy[8], Sajit Babu[9], Jayalakshmi T. K.[10], Peter Saranchuk[3], Camilla Rodrigues[11], Petros Isaakidis[3]*



Higher rates of Pre XDR TB than MDR TB (56.8% vs 29.4%)

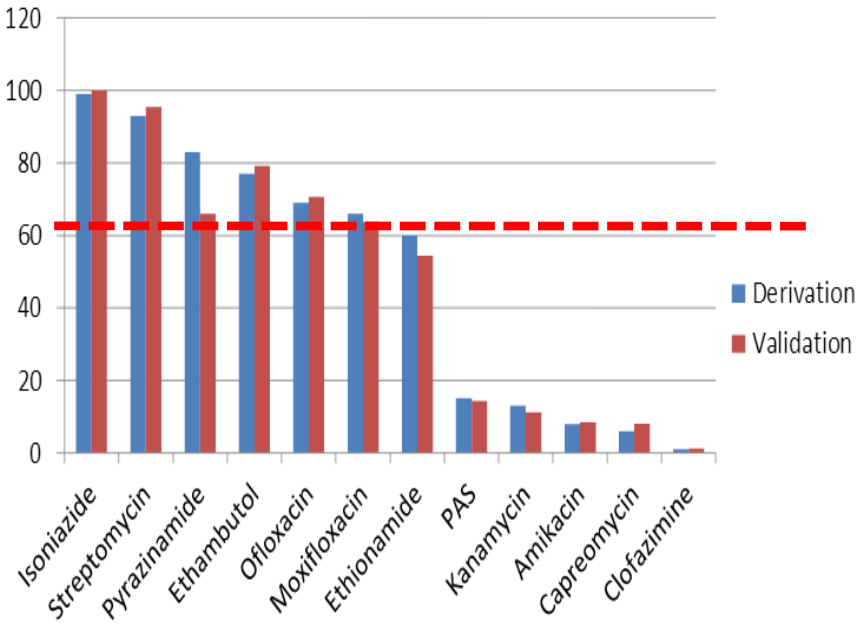
PLoS One 2015 :10(1):e0116798

AGORA
RESEARCH LETTER

Rifampicin-resistant tuberculosis: what is the best initial empiric regimen in Mumbai, India?

TABLE 1 Positive predictive values of rifampicin resistance in predicting resistance to individual drugs in the derivation and validation cohorts

Drug	Positive predictive value of rifampicin resistance in predicting resistance (derivation cohort)	Positive predictive value of rifampicin resistance in predicting resistance (validation)
Isoniazid	99 (97–100)	100 (98.6–100)
Streptomycin	93 (89–96)	95.4 (92–97.6)
Pyrazinamide	83 (78–88)	66 (59.9–71.8)
Ethambutol	77 (72–83)	79.2 (73.7–83.9)
Ofloxacin	69 (63–75)	70.7 (64.7–76.1)
Moxifloxacin	66 (60–72)	63.7 (57.5–69.6)
Ethionamide	60 (54–66)	54.4 (48.2–60.6)
PAS	15 (10–20)	14.3 (10.3–19.1)
Kanamycin	13 (9–18)	11.2 (7.6–15.7)
Amikacin	8 (5–12)	8.5 (5.4–12.6)
Capreomycin	6 (4–10)	8.1 (5.1–12.1)
Clofazimine	1 (0–3)	1.2 (0.2–3.3)



J Mullerpattan et al doi.org.10.1183/13993003.02182.2016

At HNH, in Rif R
the best derived initial empiric regimen

SLI (KAN, CAP, AMK)

Clofazimine

Linezolid

Cycloserine

Ethionamide

PAS

Moxifloxacin

J Mullerpattan et al doi.org.10.1183/13993003.02182.2016

Stratifying FQ R mutations that maybe overcome with HD MOX

Molecular tests may better predict treatment outcomes

gyrA A90V & *gyrA* D94A maybe treated with HD MOX

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Moxifloxacin Improves Treatment Ou
Ofloxacin-Resistant Multidru

Jung-Yien Chien, [a,b,d] Shun-

Fluoroquinolone Resistance Mutation Detection Is Equivalent to Culture-Based Drug Sensitivit
Predicting Multidrug-Resistant Tu
Outcome: A Retrospecti

Maha R. Farhat,[1,2] Karen R. Jacobson [3]

[1]Department of Biomedical In
University Schoo

DRUG DISCOVERY AND RESISTANCE

Correlating Minimum Inhibitory Concentrations of ofloxacin and moxifloxacin with gyrA mutations using the genotype MTBDRsl assay

Priti Kambli [a], Kanchan Ajbani [a], Meeta Sadani [a], Chaitali Nikam [a], Anjali Shetty [a], Zarir Udwadia [b], Timothy C. Rodwell [c], Antonino Catanzaro [c], Camilla Rodrigues [a,*]

LPA DST : clinical implications with positive mutant bands

Mutation	Isoniazid	Ethionamide
katG S315T MUT 1	X	√
inhA MUT1 C15T MUT 2 A16G MUT3 A / B 8A / C	HD	X
katG S315T *plus* *inhA*	X	X

Mutation	Rifampicin	Rifabutin
rpoB S531L MUT3 H526Y/D MUT 2A/2B	X	X
rpoB D516V MUT 1	X	Check DST

Mutation	Moxiflox	Levoflox	Ofloxacin
gyrA D94 / (N /Y) /G / MUT 3B / 3C	X Check DST at 2	X	X
gyrA A90V MUT 1	√ HD	X	X
gyrA D94A MUT3A	√ HD	X	X

Mutation	Kanamycin	Amikacin	Capreo
rrs A1401G MUT 1	X	X	X check DST
rrs G1484C MUT 2	X	X	X
eis promoter C14T MUT 1	X	√	√

Sequencing in the DR TB world

- Sanger Sequencing
- Pyrosequencing (PSQ)
- NGS (Targeted & WGS)

JCM
Journals.ASM.org

Predicting Extensively Drug-Resistant *Mycobacterium tuberculosis* Phenotypes with Genetic Mutations

Timothy C. Rodwell,[a] Faramarz Valafar,[b] James Douglas,[c] Lishi Qian,[c] Richard S. Garfein,[a] Ashu Chawla,[b] Jessica Torres,[b] Victoria Zadorozhny,[b] Min Soo Kim,[b] Matt Hoshide,[c] Donald Catanzaro,[b] Lynn Jackson,[a] Grace Lin,[d] Edward Desmond,[d] Camilla Rodrigues,[e] Kathy Eisenach,[f] Thomas C. Victor,[g] Nazir Ismail,[h] Valeru Crudu,[i] Maria Tarcela Gler,[j] Antonino Catanzaro[a]

Sensitivity and Specificity of Mutations for Predicting M/XDR-TB Phenotypes in India, Moldova, Philippines and South Africa

Phenotype	Primary Gene/s Associated with Resistance	No. of unique SNPs	Sensitivity	Specificity
INH^R	*katG, inhA* prom.	6	±95% (n=894)	98-100%
RIF^R	*rpoB*	15	±97% (n=777)	97-100%
MOX/OFX^R	*gyrA*	8	±92% (n=562)	99-100%
AMK^R	*rrs*	1	±86% (n=293)	99-100%
KAN^R	*rrs, eis* promoter	6	±88% (n=404)	96-100%
CAP^R	*rrs*	1	±87% (n=279)	98-100%

< 30 SNPs in 6 genes could predict XDR TB with 90 - 98% sens

J Clin Microbiol 2014 ;52(3):781-789 doi 10.1128/JCM.02701-13.

Outline

- Empiric treatment with local lab epidemiology

- **Pyrosequencing applications**
- Expanding the mutation knowledge base
- Sequencing directly from clinical samples

Pyrosequencing (PSQ) to detect XDR TB

- **Rapid, accurate, robust, high throughput tech**

  delivers targeted sequencing

- **Clinically relevant results in < 6 hrs directly from samples**

- **Flexible :**

  adaptable to regional prevalence specifics & new mutations

PSQ : detecting > 64 mutations in < 6 hrs

	Target	Location	Mutations	Length
M tuberculosis complex	IS6110		-	24
Isoniazid	*katG*	312 – 316	5	13
	inhA promoter	- 4 to -20	7	17
	ahpC- oxyR	- 4 to -23	5	24
Rifampicin	*rpoB*	507-521 522-533	32	45 35
Fluoroquinolone	*gyrA*	88-96	13	25
SLI KAN,AMK,CAP	*rrs*	1397-1406	2	10
KAN	*eis*	-6 to -47	5	42

Pyrosequencing (PSQ) applications

What do we use PSQ for ?

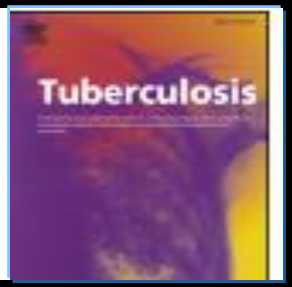
(i) Smear Positive Samples with LPA any WT band absent but with no corresponding mutant band

(ii) Resolving discordant Xpert & MGIT DST

(iii) Smear negative TB

Evaluation of pyrosequencing for extensive drug resistance-defining anti-tuberculosis drugs for use in public healthcare

Remya Nambiar[a,**], Daksha Shah[b], Kanchan Ajbani[a], Mubin Kazi[a], Meeta Sadani[a], Anjali Shetty[a], Padmaja Keskar[b], Sanjeev Kamble[b], Alex van Belkum[c], Camilla Rodrigues[a,*]



Comparison of PSQ, GenotypeMTBDR & MGIT DST

Drug	Molecular locus	Number of isolates corresponding to the locus	Mutation(s) detected by PSQ (n = number of isolates)	Point mutations included in Genotype MTBDRplus/sl (theoretical assumption)	Incremental value of PSQ over Genotype MTBDRplus/sl	Phenotypic MGIT DST pattern (total number of isolates)		
						Drug	Resistant	Sensitive
INH	*katG*	71	S315T (n = 69) S315G (n = 1) S315T (n = 1)	*katG*MUT1 – *katG*MUT2	1.41%	INH	100	0
	inhA	1 4	T−8C (n = 1) C−15T (n = 4)	*inhA*MUT3A *inhA*MUT1	0.00%			
	katG & *inhA*	19	S315T + C−15T (n = 18) S315T + −17T (n = 1)	*katG*MUT1 + *inhA*MUT1 *katG*MUT1	0.00%			
	No mutations detected by PSQ	5						
	Genotypic resistance + Phenotypic susceptibility	0						
RMP	*rpoB*	1	L511P (n = 1)	–	13.00%	RMP	99	1
		2	Q513K (n = 1)	–				
			Q513K (n = 1)	–				
		2	D516Y (n = 1)	–				
			D516Y + S339GAG (n = 1)	–				
		6	H526Q (n = 1)	–				
			H526D (n = 3)	*rpoB*MUT2B				
			H526C (n = 1)	–				
			H526L (n = 1)	–				
		85	S531Q (n = 1)	–				
			S531L (n = 84)	*rpoB*MUT3				
		4	L533P (n = 4)	–				
	Genotypic resistance + Phenotypic susceptibility	1						
KAN	*eis*	1	10C (n = 1)	–	100% (sample size = 4)	KAN	17	83
		3	C12T (n = 3)	–				
		2	NI (n = 2)	–				
	No mutations detected by PSQ	94						
	Genotypic resistance + Phenotypic susceptibility	3						
Injectable drugs	*rrs*	9	A1401G (n = 9)	*rrs*MUT1	0% (sample size = 9)	KAN CAP AMK	17 10 12	83 90 88
	No mutations detected by PSQ	91						
	Genotypic resistance + Phenotypic susceptibility	KAN = 1 CAP = 1 AMK = 0						
FQs	*gyrA*	1	88TGC + S95T (n = 1)	–	8.20%	MXF	58	42
		9	A90V + S95T (n = 9)	*gyrA*MUT1				
		1	A90V + S91P + S95T (n = 1)	*gyrA*MUT1 + *gyrA*MUT2				
		4	D94Y (n = 4)	*gyrA*MUT3B				
		1	D94G (n = 1)	*gyrA*MUT3C				
		29	D94G + S95T (n = 29)	*gyrA*MUT3C				
		3	D94N + S95T (n = 3)	*gyrA*MUT3B				
		3	D94H + S95T (n = 3)	*gyrA*MUT3D		OFX	76	24
		4	D94A + 95T (n = 4)	*gyrA*MUT3A				
		4	94GTC + S95T (n = 4)	–				
		2	D94Y + S95T (n = 2)	*gyrA*MUT3B				
		6	NI (n = 6)	–				
		31	S95T (n = 31)					
	No mutations detected by PSQ	2						
	Genotypic resistance + Phenotypic susceptibility	MXF = 9 OFX = 2						

Incremental increase in SNP detection over LPA

INH : 1.3%

RIF : 13%

FQL : 8.2%

Tuberculosis 2018 ;110:86-90

Pyrosequencing (PSQ) applications

What do we use PSQ for ?

(i) Smear Positive Samples with a LPA WT band present but with no corresponding mutant band

(ii) Resolving discordant Xpert & MGIT DST

(iii) Smear negative TB

Pyrosequencing to resolve discrepant Xpert MTB/RIF and Mycobacterial Growth Indicator Tube 960

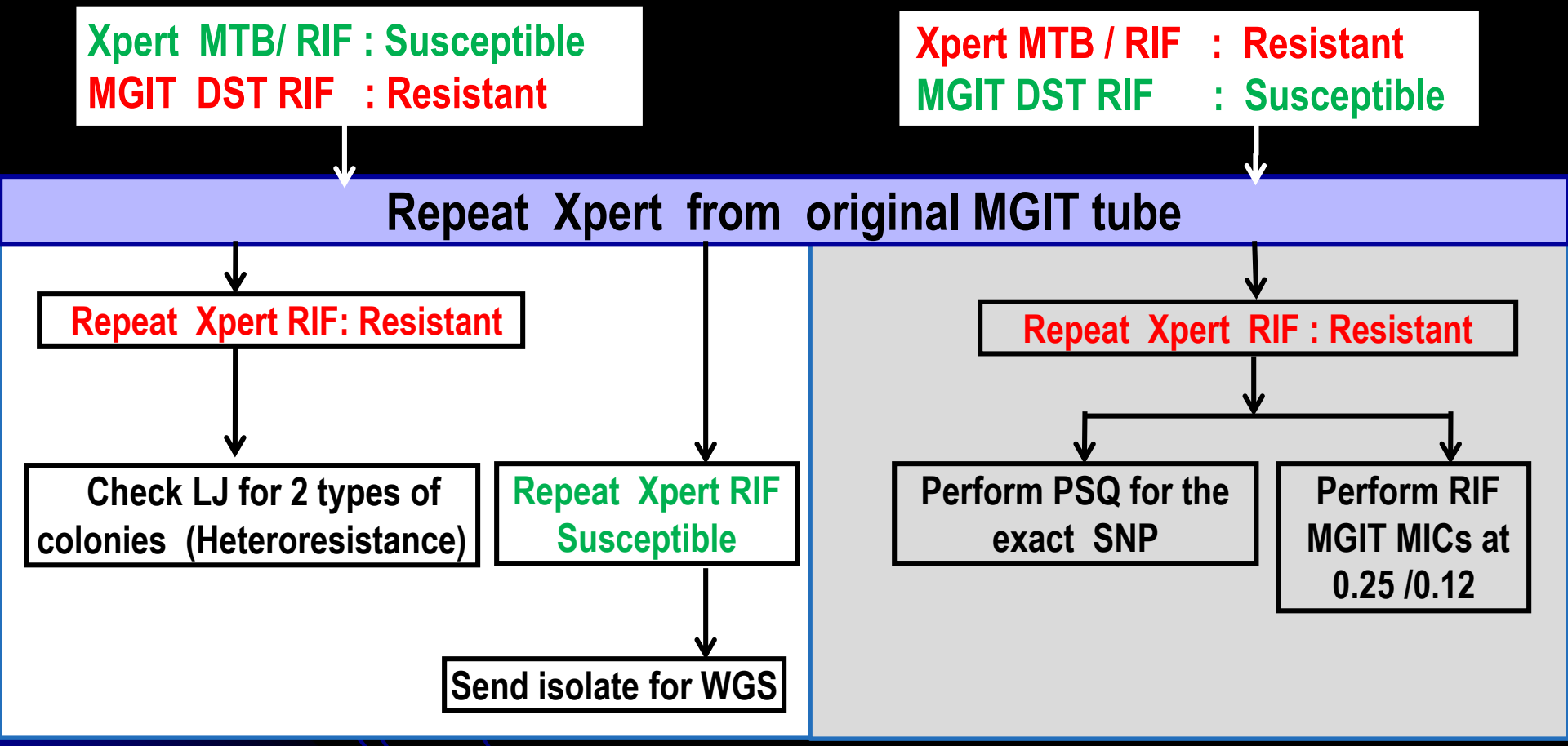
Kanchan Ajbani, Mubin Kazi, Jeffrey Tornheim[1], Swapna Naik, Rajeev Soman[2], Anjali Shetty, Camilla Rodrigues

Departments of Microbiology and [2]Medicine, P. D. Hinduja Hospital and Medical Research Centre, Mumbai, Maharashtra, India, [1]Johns Hopkins University School of Medicine, Division of Infectious Diseases, Center for Clinical Global Health Education, Baltimore, MD USA

- Operator related
- Protocol related
- Heteroresistance
- Inadequate knowledge about mutations

Lung India 2018;35(2):168-170

Discrepant samples : Algorithm for Xpert MTB / RIF & MGIT DST



Lung India 2018;35(2):168-170

Pyrosequencing (PSQ) applications

What do we use PSQ for ?

(i) Smear Positive Samples with a LPA WT band present but with no corresponding mutant band

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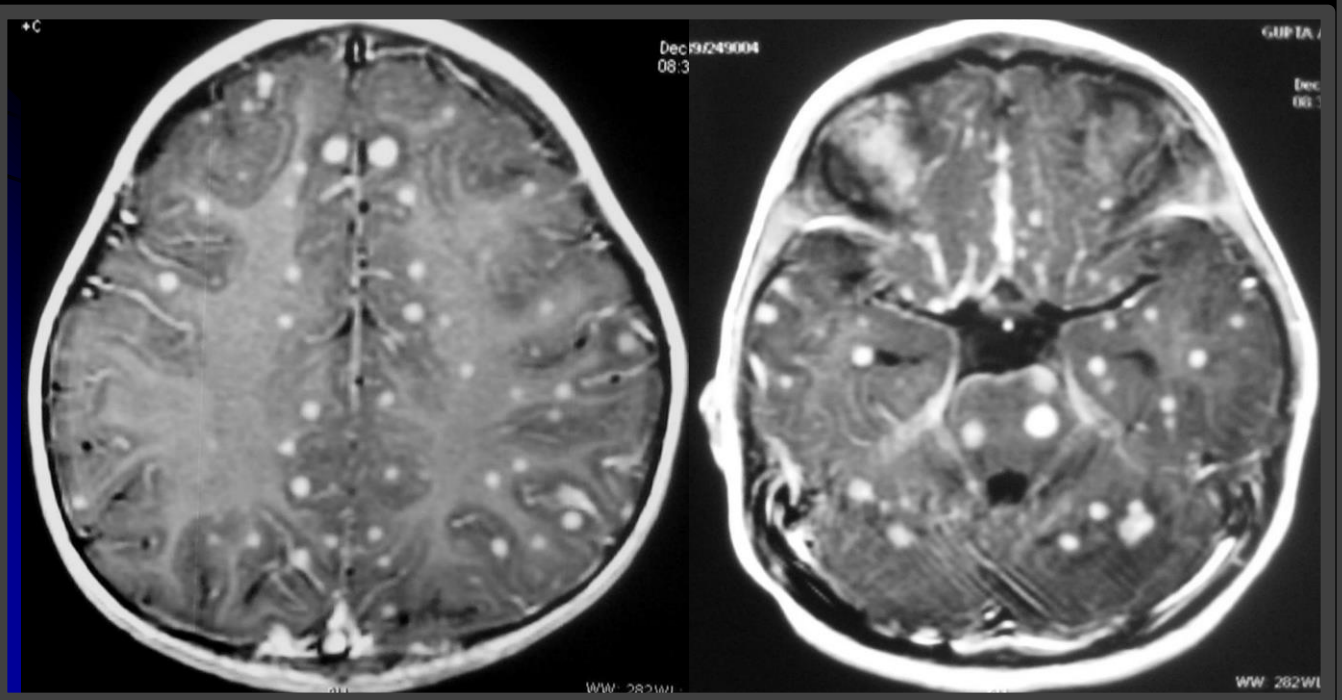
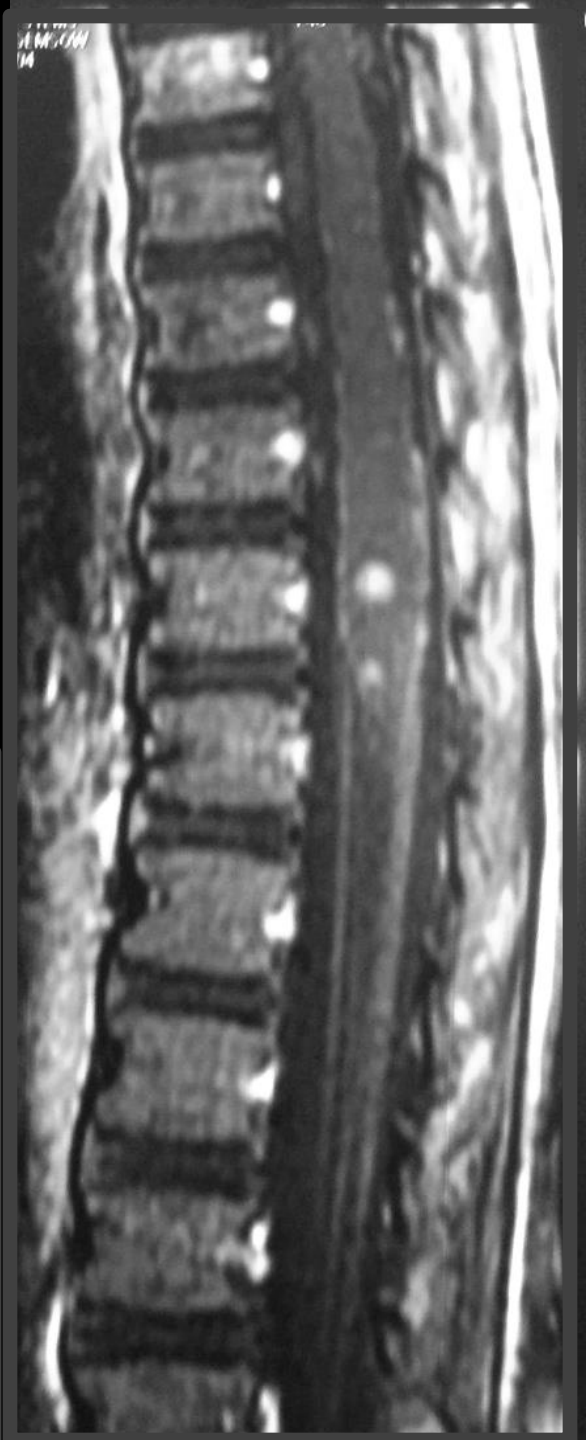
(iii) Smear negative TB

AG 2 year old F treated elsewhere in October for fever,chest infiltrate started HREZ

In December, presented to HNH drowsiness, seizures, ptosis miliary TB & tuberculomas

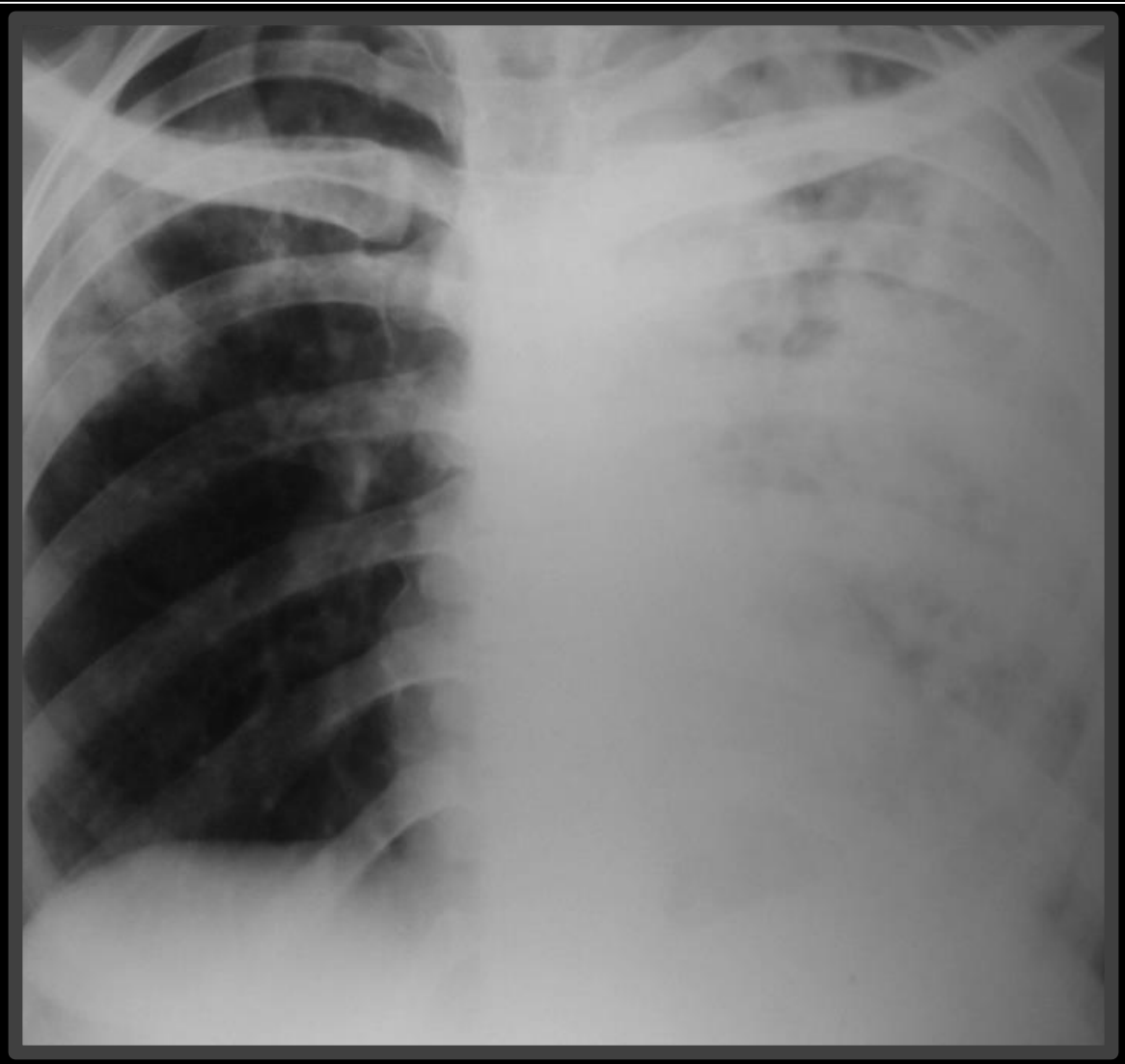




Case contd…..

Childs's mother
On HRZE for TB
for 5 months but
not improving

Mother's sputum &
Child's gastric
aspirate sent for culture
& PSQ done

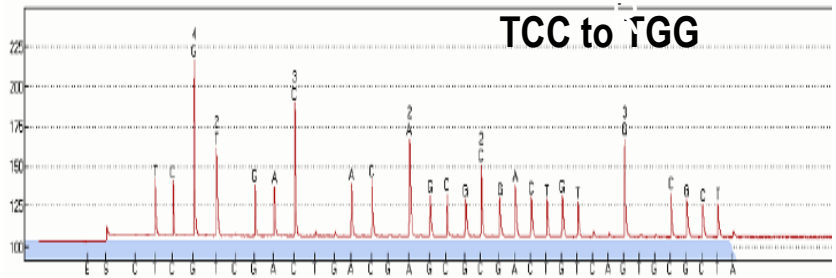


Pyrosequencing: XDR TB detected in 6 hrs

Well: H12
PSQ run: 09_26_full plate
Entry ID: rB-S2A-522-533-090611
Sequence library: 8 target-rBMB [507-521]-rBSW (522-533] library-07-20-11 (2011-09-14, 8:18:06 PM)
Query sequence: TCGGGGTTGACCCACAAGCGCCGACTGTGGGCGCT

Result: rpoB-S2A mutation 531 tcg/tgg-(RIF-R)
Quality: Good

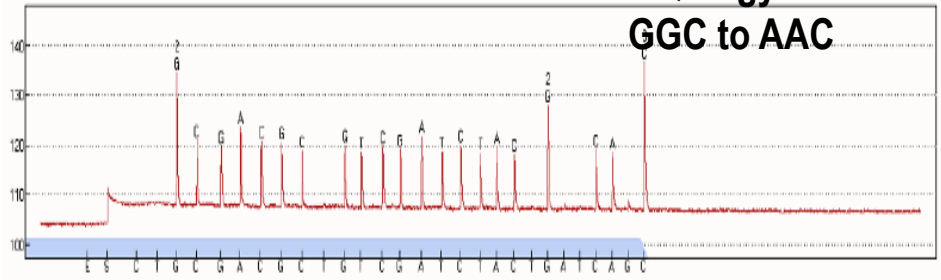
Rif R rpo531
TCC to TGG



Well: E5
PSQ run: 09_26_full plate
Entry ID: gA-S2-090611
Sequence library: 8 target-rBMB [507-521]-rBSW (522-533] library-07-20-11 (2011-09-14, 8:18:06 PM)
Query sequence: GGCGACGCGTCGATCTACGGCACCC

Result: gyrA mutations 94GGC & 95ACC (fQ-R) **Score:** 100
Quality: Good

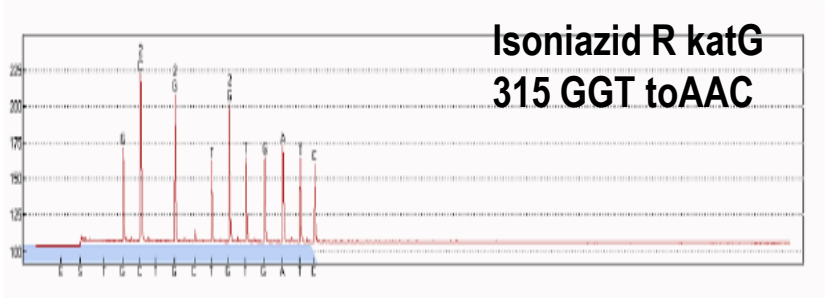
FQ R gyrA 94
GGC to AAC



Well: B11
PSQ run: 09_26_full plate
Entry ID: kG-S2-090611
Sequence library: 8 target-rBMB [507-521]-rBSW (522-533] library-07-20-11 (2011-09-14, 8:18:06 PM)
Query sequence: GCCGGTGGTGATC

Result: katG mutation 315ggt(acc)-(INH-R), katG mutation 315ggt(acc) & 314 ggc(gcc)-(INH-R) **Score:** 100
Quality: Good
Information: Top result contains multiple hits (2).

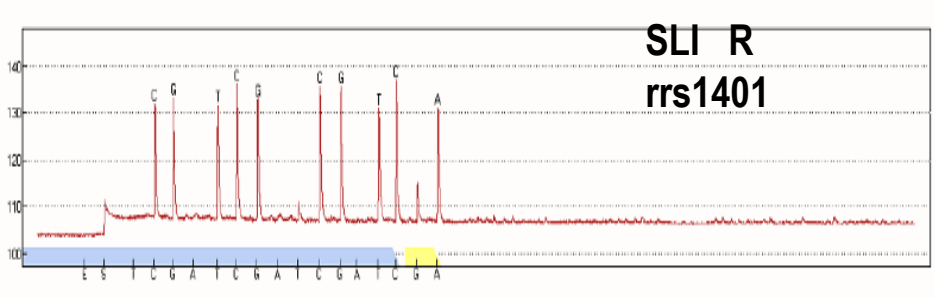
Isoniazid R katG
315 GGT toAAC



Well: F6
PSQ run: 09_26_full plate
Entry ID: rrs01-S4-090611
Sequence library: 8 target-rBMB [507-521]-rBSW (522-533] library-07-20-11 (2011-09-14, 8:18:06 PM)
Query sequence: CGTCGCGTCA

Result: rrs-1401 mutation A/G (aminoglycoside-R) **Score:** 100
Quality: Good

SLI R
rrs1401



Global Consortium for Drug resistant TB Diagnostics (GCDD) funded by NIH, USA : Grant #5U01AI082229

Case contd…..

- The child was on IV dexamethasone & anti epileptics
- Salvage Treatment

  clofazimine (1 mg/kg)

  linezolid (10 mg/kg/day)

  PAS (150 mg/kg)

  amox clav (875/125 mg/kg)

Referred to ID for optimizing treatment

- cycloserine + ethionamide added

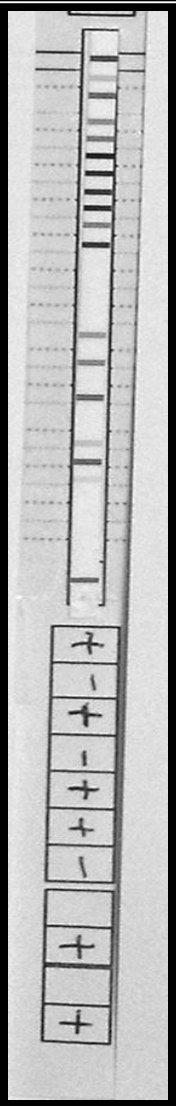
Case contd…..

Sputum of mother
LPA GenoType MTBDR *plus* & *sl* :

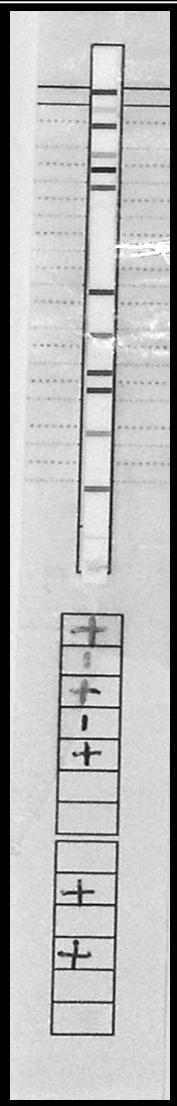
XDR
Subsequently confirmed by
MGIT DST in both mother & child

Needless delay of 4-6 weeks avoided….

Both child & mother well at 2 year FU



MTBDR *plus*



MTBDR *sl*

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com

Utility of pyrosequencing for rapid detection of tubercular meningitis (TBM) and associated susceptibility directly from CSF specimens

Kanchan Ajbani[a], Mubin Kazi[a], Swapna Naik[a], Rajeev Soman[b], Anjali Shetty[a], Camilla Rodrigues[a, *]

Sr. No	Age	Sex	Phenotypic		Genotypic			
			Culture	Culture-DST	Xpert		Pyrosequencing	
					MTB	RIF	MTB	Mutations
1	22	Female	MTB	MDR	POS	RES	POS	531 TTG rpoB, katG 315 ACC
2	17	Male	N.G.	N.A.	POS	RES	POS	531 TTG rpoB, katG 315 ACC, gyrA 94 GGC, rrs 1401G
3	25	Female	N.G.	N.A.	NEG	N.A.	**POS**	**rpoB 516 GTC, katG 315 ACC, inhA -15T**
4	64	Male	N.G.	N.A.	NEG	N.A.	**POS**	**rpoB 531 TTG, katG 315 ACC**
5	13	Male	N.G.	N.A.	NEG	N.A.	NEG	N.A.
6	34	Male	N.G.	N.A.	POS	SUS	POS	wild type
7	57	Female	MTB	SUS	NEG	N.A.	POS	wild type
8	28	Female	N.G.	N.A.	NEG	N.A.	**POS**	**katG 315 ACC, rrs 1401G, rpoB NI, gyrA NI**
9	28	Female	N.G.	N.A.	NEG	N.A.	**POS**	**rpoB 531 TTG, katG 315 ACC, inhA -15T, gyrA 94 GGC**
10	28	Male	N.G.	N.A.	NEG	N.A.	**POS**	**kat G 315 ACC**
11	20	Male	N.G.	N.A.	NEG	N.A.	NEG	N.A.
12	53	Male	N.G.	N.A.	NEG	N.A.	POS	wild type
13	32	Female	N.G.	N.A.	POS	RES	POS	rpoB 521 TTG, kat G 315 ACC

TBM : CSF PSQ

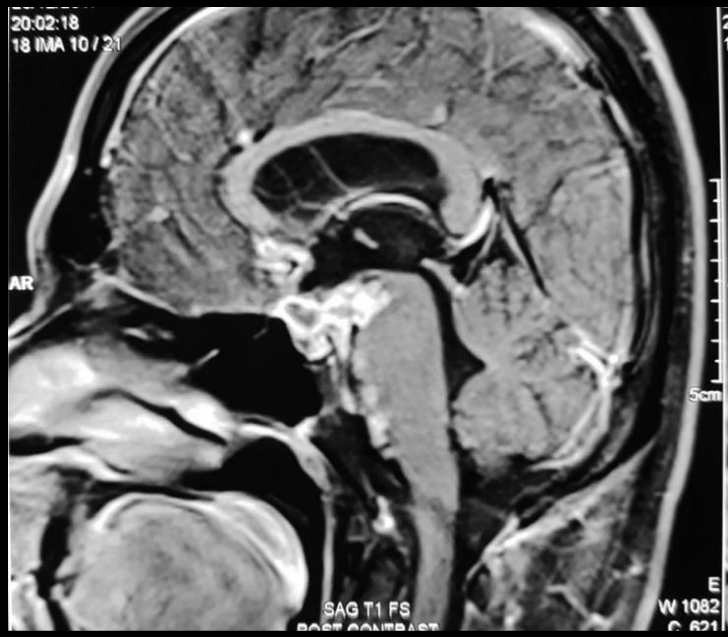
Sr. No	Age	Sex	PHENOTYPIC		GENOTYPIC				
			Culture	Cullture-DST	Xpert		Pyrosequencing		
					MTB	RIF	MTB	Mutations	MDR/ XDR
14	65	Male	NG	NA	Neg	NA	Pos	katG 315 ACC	INH monoresistance
15	33	Male	Pos	MDR	Pos	Res	Pos	526 AAC rpoB, katG 315 ACC	MDR
16	55	Female	Pos	MDR + FQ	Pos	Res	Pos	rpoB 531 TTG, katG 315 ACC, gyrA 94 GGC	MDR + FQ
17	19	Male	Pos	Sus	Pos	Sus	Pos	wild type	Pan S
18	28	Female	Pos	Sus	Pos	Sus	Pos	wild type	Pan S
19	53	Male	Pos	Sus	Pos	Sus	Pos	wild type	Pan S
20	25	Male	NG	NA	Pos	Sus	Pos	wild type	Pan S
21	37	Male	NG	NA	Neg	NA	Pos	NA	NA
22	50	Male	NG	NA	Neg	NA	Pos	wild type	Pan S
23	38	Male	NG	NA	Neg	NA	Pos	wild type	Pan S
24	87	Female	NG	NA	Neg	NA	Neg	NA	NA
25	71	Female	NG	NA	Neg	NA	Neg	NA	NA
26	55	Female	NG	NA	Neg	NA	Neg	NA	NA
27	39	Male	NG	NA	Neg	NA	Neg	NA	NA
28	55	Female	NG	NA	Neg	NA	Neg	NA	NA
29	58	Female	Pos	Sus	Pos	Sus	Pos	wild type	Pan S
30	31	Male	Pos	Sus	Pos	Sus	Pos	wild type	Pan S
31	21	Female	Pos	MDR	Pos	Res	Pos	531 TTG rpoB, katG 315 ACC	MDR
32	68	Female	NG	NA	Neg	NA	Pos	wild type	Pan S
33	31	Female	NG	NA	Neg	NA	Pos	wild type	Pan S
34	53	Male	NG	NA	Pos	MDR	Pos	531 TTG rpoB, katG 315 ACC	MDR
35	38	Male	NG	NA	Neg	NA	Pos	wild type	Pan S
36	35	Female	NG	NA	Neg	NA	Pos	wild type	Pan S
37	18	Male	NG	NA	Neg	NA	Pos	wild type	Pan S
38	6	Male	NG	NA	Neg	NA	Pos	wild type	Pan S
39	32	Female	NG	NA	Neg	NA	Pos	531 TTG rpoB, katG 315 ACC	MDR
40	10	Female	NG	NA	Neg	NA	Neg	NA	NA
41	64	Female	NG	NA	Neg	NA	Pos	rpoB 531 TTG, katG 315 ACC, gyrA NI	MDR
42	47	Male	NG	NA	Neg	NA	Neg	NA	NA
43	43	Female	NG	NA	Neg	NA	Pos	wild type	Pan S
44	16	Female	NG	NA	Neg	NA	Neg	NA	NA
45	34	Female	NG	NA	Neg	NA	Neg	NA	NA
46	27	Female	NG	NA	Neg	NA	Neg	NA	NA
47	64	Female	NG	NA	Neg	NA	Neg	NA	NA

Of 47 TBM suspects, 34 PSQ +ve with with 10 MGIT positive & 14 Xpert positive

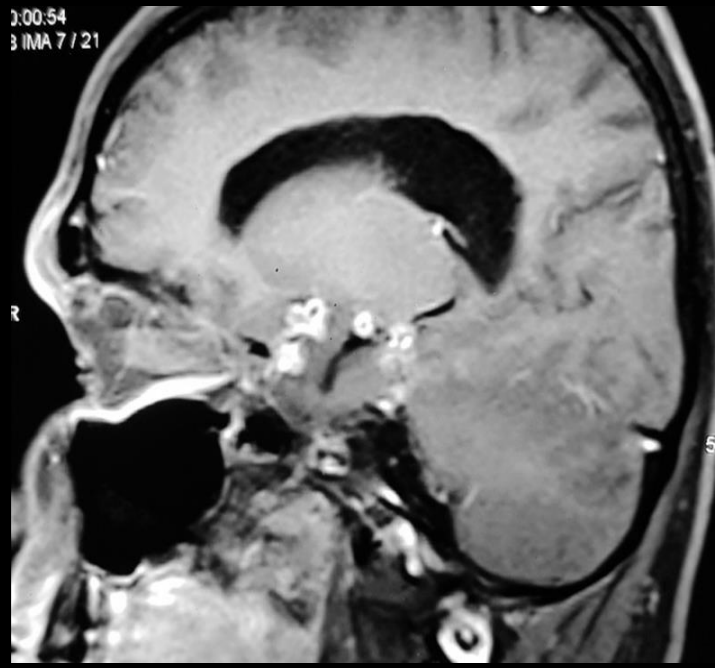
Dilemmas in management of TBM / tuberculomas

- **Drug resistance**
- **Paradoxical response**
- **Adequate drug penetration**
- Vasculitis / infarcts
- Hyponatremia
- Brain edema
- Hydrocephalus
- Seizures
- Mixed infections (HIV)

AK 16 years
TBM
Started on HRZE & steroids
partial improvement



After tapering of steroids,
c/o severe headache
Intercisternal tuberculomas with exudates
Referred to ID



? Drug Resistant TB or paradoxical response

Xpert : MTB not detected

PSQ:Mono R to INH *katG* 315ACC

- INH replaced with ETH
  (better CSF penetration & EBA)
- Re started steroids

Patient improved on FU

Sample Type: CSF

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF Assay G4	5	In Vitro Diagnostic

Test Result: MTB NOT DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0.0	-3	NEG	PASS
Probe C	0.0	8	NEG	PASS
Probe E	0.0	-2	NEG	PASS
Probe B	0.0	0	NEG	PASS
SPC	25.6	291	PASS	PASS
Probe A	0.0	1	NEG	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

PYROSEQUENCING FOR XDR

Sample- CSF

Drug	*Target*	*Mutation*	*Interpretation*
	MTBC (IS6110)	POSITIVE	*Mycobacterium tuberculosis* detected
Isoniazid	katG	315 ACC	Resistant
	inhA promoter	No mutation	
Rifampin	rpoB	No mutation	Susceptible
Kanamycin	eis	No Mutation	Susceptible
Amikacin, Kanamycin, Capreomycin	rrs	No Mutation	Susceptible
Fluoroquinolones i.e. Ofloxacin, Moxifloxacin	gyrA	No Mutation	Susceptible

PSQ is a rapid screening technique for molecular detection of drug resistance. For the confirmation of the PSQ results, culture-based drug susceptibility testing should be performed. A negative result (e.g. no mutation) does not rule out contributory mutations present elsewhere in the genome. The test results should not be used as the sole criterion but can be used in conjunction with other clinical data for the diagnosis of drug resistant tuberculosis.

Sequencing in the DR TB world

- Sanger Sequencing
- Pyrosequencing (PSQ)
- **NGS (Targeted & WGS)**

Current TAT with DST methods

MGIT, Genotype MTB DR *plus* & MTB DR *sl*, PSQ, NGS (Targeted & WGS)

Culture MGIT DST	Genotype MTB DR *plus* MTB DR *sl*	Pyrosequencing	Targeted NGS	WGS from culture
4 -8 weeks Isolation 4-6 weeks + DST 2 weeks	**2 days**	**< 6 hrs**	**37 hrs** Enrichment 4 hrs Prep 5 hrs Sequencing MiSeq 28 hrs Batch 48 samples	**9 -12 days** Enrichment 7-10 days Prep 5 hrs Sequencing MiSeq 28 hrs Batch 9 samples

HNH 2017 : DR conferring mutations detected by MTBDR*plus* & MTBDR*sl*

Resistant Phenotype	Reference Genes	DR conferring mutations	No(%) Detected	ONLY WT absent	% Only WT absent
INH^R	***kat G***	**S315T1/ S315T2**	**1728 (87%)**	katG 315	49(2.23%)
	inhA	**C15T**	**202 (9.2%)**	inhA -15	-
		A16G		-16	-
		T 8C	**27 (1.23%)**	-8	
RIF^R	***rpob***	**D516V**	**32 (1.4%)**	WT 1 : 505-509	-
		H526Y	**6 (0.2%)**	WT 2 : 510-513	12(0.5%)
		H526D	**4 (0.18%)**	WT 2/3 : 510-517	8 (0.3%)
				WT 3/4 : 513-519	13 (0.5%)
				Wt 4/5 : 516-522	6 (0.27%)
				WT 5/6 : 518-525	-
		S531L	**1717 (93%)**	WT 7 : 526-529	52 (2.3%)
				WT 8 : 530-533	21 (0.95%)
OFX^R	***gyrA***	**A90V**	**318 (26%)**	gyrA	-
		S91P	14 (1.1%)	WT1 : 85-90	-
		D94A	30 (2.4%)	WT2 : 89-93	48 (3.96%)
		D94N/Y	21 (1.73%)	WT3: 92-97	
		D94G	**423 (64%)**		
	gyrB	**D94H**	12 (1%)		
		N538D/ E540G	2 (0.1%)	gyrB WT1 : 538-540	4 (0.3%)
KAN^R	***eis***	**C14T**	**4 (0.33%)**	eis	5 (0.4%)
				WT1: G37T,	44 (3.63%)
				WT2: -10-14 : WT3 : -2	
KAN^R /AMK^R / CAP^R	***rrs***	**A1401G**	**106 (94%)**		
		G1484T		rrs WT 1: 1401-1402	9 (0.8%)
				WT2 1484	

WGS for DR conferring mutations : Incremental value over current LPA / PSQ ?

XDR drugs : <10% (LPA WT absent with no mutant)

Oral FLD : Ethambutol , PZA

Oral SLD : Ethionamide, PAS, cycloserine

Re purposed drugs : CFZ, LZD

New Drugs : BDG, DLD

Outline

- Empiric treatment with local lab epidemiology
- Pyrosequencing applications

- **Expanding the DR mutation knowledge base**

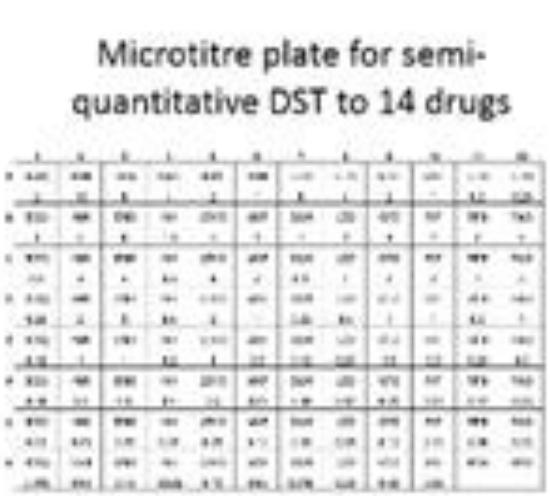
  WGS for identification of more genomic variants

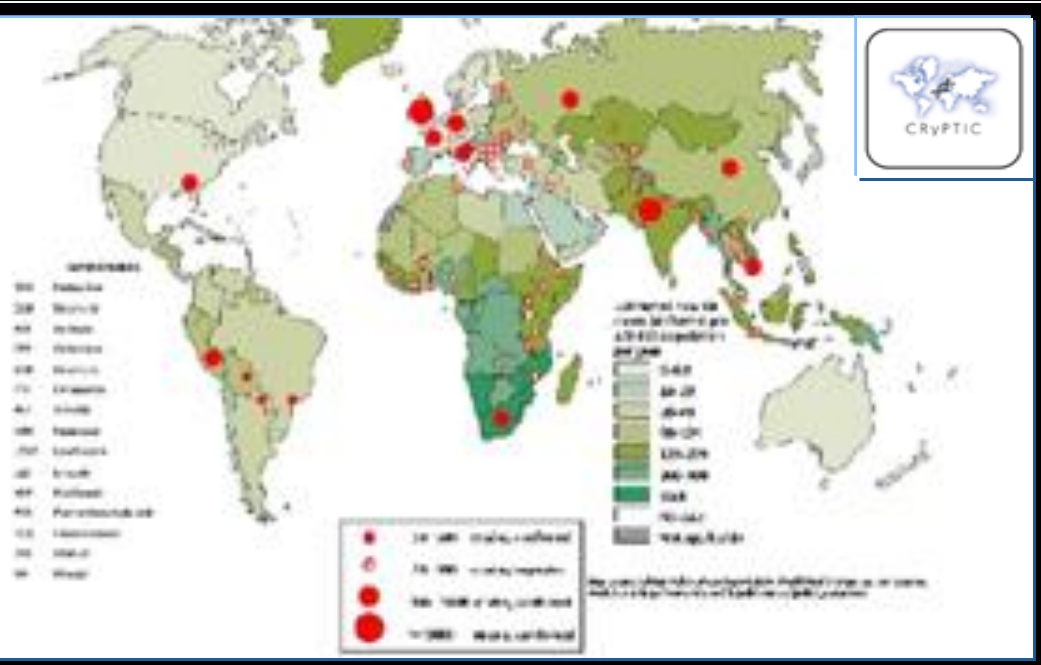
  & detection of associations of genetic variants with quantitative DST

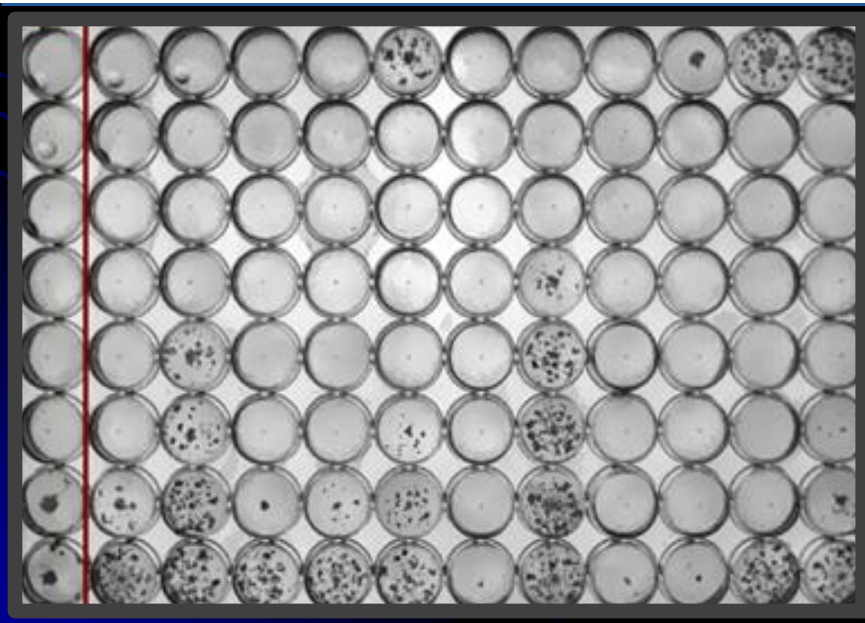
- Sequencing directly from clinical samples

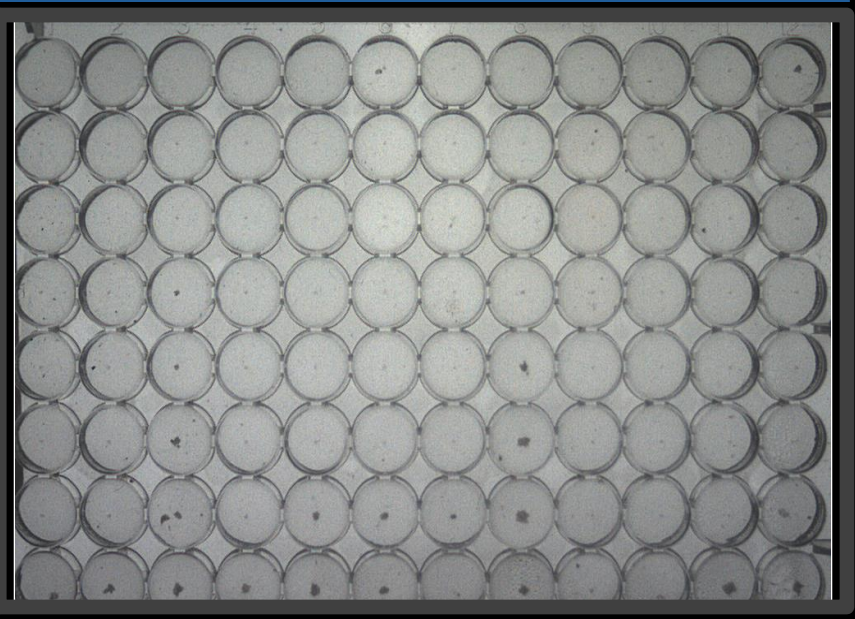
CRyPTIC : Microtitre phenotypic MICs vs Whole Genome Sequencing

Microtitre plate for semi-quantitative DST to 14 drugs







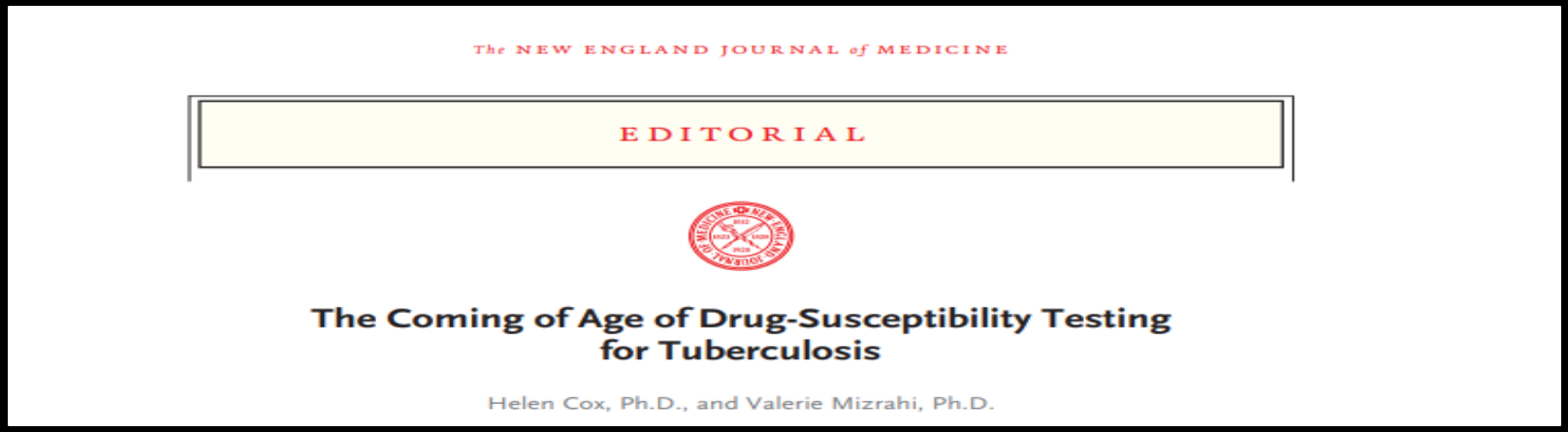


A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*

ReSeqTB
Relational Sequencing TB Data Platform

Drug (phenotypic testing)	Gene	High-confidence mutations	Moderate-confidence mutations	Minimal-confidence mutations	No association with resistance
First-line					
Rifampicin (R)	*rpoB*	F505V+D516Y, S512T, Q513H+L533P, Q513-F514ins, **Q513K**, **Q513L**, **Q513P**, **F514dupL**, M515I+D516Y, **D516A**, **D516F**, **D516G**, D516G+L533P, D516ins, D516N, **D516V**, Del N518, **S522Q**, **H526C**, **H526D**, H526F, **H526G**, **H526L**, **H526R**, **H526Y S531F**, **S531L**, S531Q, **S531W**, S531Y, D626E	**D516Y**, **S522L**, H526P, **L533P**	**L511P**, **H526N**, **I572F**	
Isoniazid (H)	*inhA-mabA*	g-102a[#,¶]	**c-15t**		**g-102a**[#,¶], t-80g, *g-47c*, **T4I**
	katG	S315I, **S315N**, **S315T**, pooled frameshifts and premature stop codons			A110V, **R463L**, L499M
	furA				**L68F**
	mshA		A187V[#,¶]		**N111S**
Second-line (group A)					
Moxifloxacin (MFX)	*gyrA*	G88C, **A90V**, **S91P**, **D94A**, **D94G**, **D94N**, **D94Y**			E21Q, **S95T**, G247S, G668D, V712L
Ofloxacin (OFX)/ levofloxacin (LFX)	*gyrA*	**G88A**, **G88C**, **S91P**, **A90V**, **D94A**, **D94G**, **D94H**, **D94N**, **D94Y**	D89N		E21Q, **T80A**, **S95T**, G247S, G668D, V712L
	gyrB	E459K, **A504V**			
Second-line (group B)					
Amikacin (AM)	*rrs*	**a1401g**, **g1484t**			
Kanamycin (KM)	*eis*	**c-14t**, **g-10a**		g-37t, c-12t	**a1338c**
	rrs	a514c[#], **a1401g**, c1402t, g1484t			
	rrs+eis	*rrs* c517t[#] + *eis* g-37t			
Capreomycin (CM)	*rrs*	**a1401g**, **c1402t**, **g1484t**			**c517t**
	tlyA	**N236K**, **pooled frameshifts and premature stop codons**			D149H
Streptomycin (S)	*rpsL*	**K43R**, K43T, **K88Q**, **K88R**, T40I			
	rrs	a1401g[#], **a514c**, a514t, c462t, c513t, **c517t**			
	gidB		**E92D**[#,¶]		**L16R**, **V110G**, pooled frameshifts and premature stop codons
Second-line (group C)					
Ethionamide and prothionamide (ETO/PTO)	*inhA*	c-15t+I194T, c-15t+S49A	**c-15t**		
	ethA				Q347Stop
Second-line (group D)					
Pyrazinamide (Z)	*pncA*	t-12c, **a-11g**, t-7c, **A3E**, L4S, **I6T**, V7G, D8E, **D8G**, D8N, **Q10P**, **D12A**, **D12N**, **C14R**, G17D, L19P, G24D, **Y34D**, **A46V**, **K48T**, **D49G**, D49N, **H51Q**, H51R, P54S, **H57D**[¶], H57P, **H57R**, **H57Y**, S59P, P62L, P62Q, **D63G**, S66P, **S67P**, W68C, **W68R**, **H71D**, H71Q, **H71Y**, **C72R**, **T76P**, **H82R**, **L85P**, **L85R**, **F94L**, **F94S**, K96N, K96R, G97C, **G97D**, **G97S**, **Y103H**, **S104R**, **G108R**, L116P, **L116R**, **L120P**, R123P, V125F, **V125G**, **V128G**, G132A, G132D, G132S, A134V, **T135N**, **T135P**, H137P, C138Y, **V139G**, **V139L**, **Q141P**, **T142A**, **T142K**, **T142M**, **indel - R148ins (inframe)**, L151S, **V155G**, L159P, T160P, G162D, T168P, **L172P**, M175T, **M175V**, V180F, V180G, **Pooled frameshifts and premature stop codons**	**V7G**, Q10R, **P54L**, **W68G**, K96E, K96T, A171E, M175I	**D12G**, F58L, H71R, I133T, **V139A**	**indel - c-125del**, **I31T**, **L35R**, **T47A**, **I6L**, K48T, T114M

Eur Respir J 2017;50:1701354



The NEW ENGLAND JOURNAL of MEDICINE

EDITORIAL

The Coming of Age of Drug-Susceptibility Testing for Tuberculosis

Helen Cox, Ph.D., and Valerie Mizrahi, Ph.D.

1. Database validation **for all drugs** with High, moderate & minimal confidence resistance conferring mutations

2. Development of standardized analytical pipelines to differentiate DR mutations from phylogenetic markers & synonymous mutations

Outline

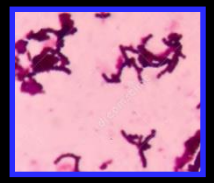
- Empiric treatment with local lab epidemiology
- Pyrosequencing applications
- Expanding the mutation knowledge base

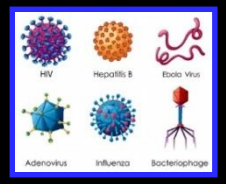
- **Sequencing directly from clinical samples**

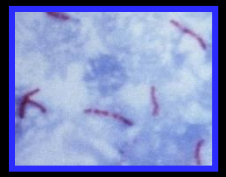
Sequencing directly from sputum

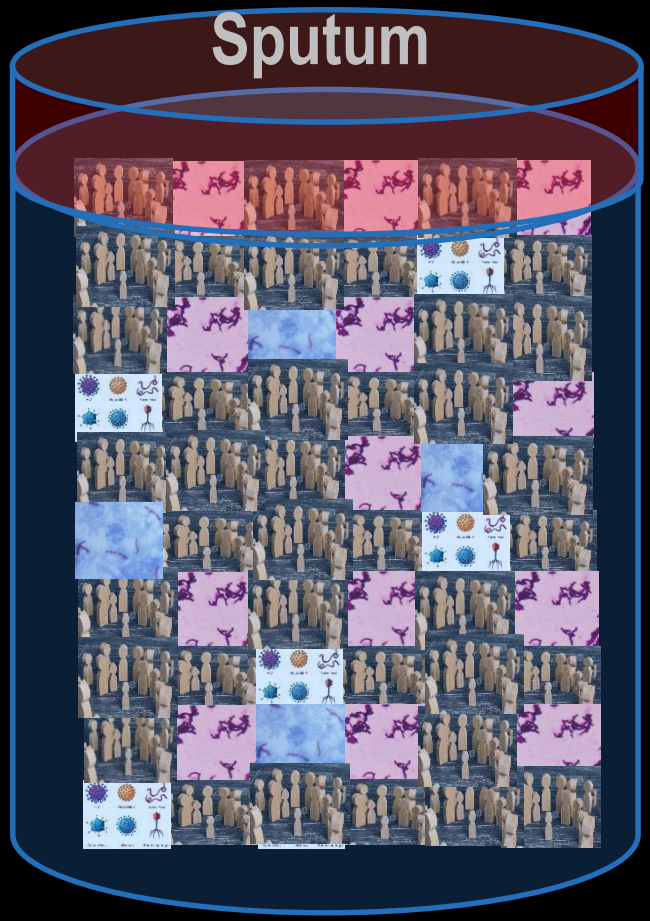
Sputum contains a mix of DNA

- Mostly human
- Bacteria / NTM
- Viruses
- TB

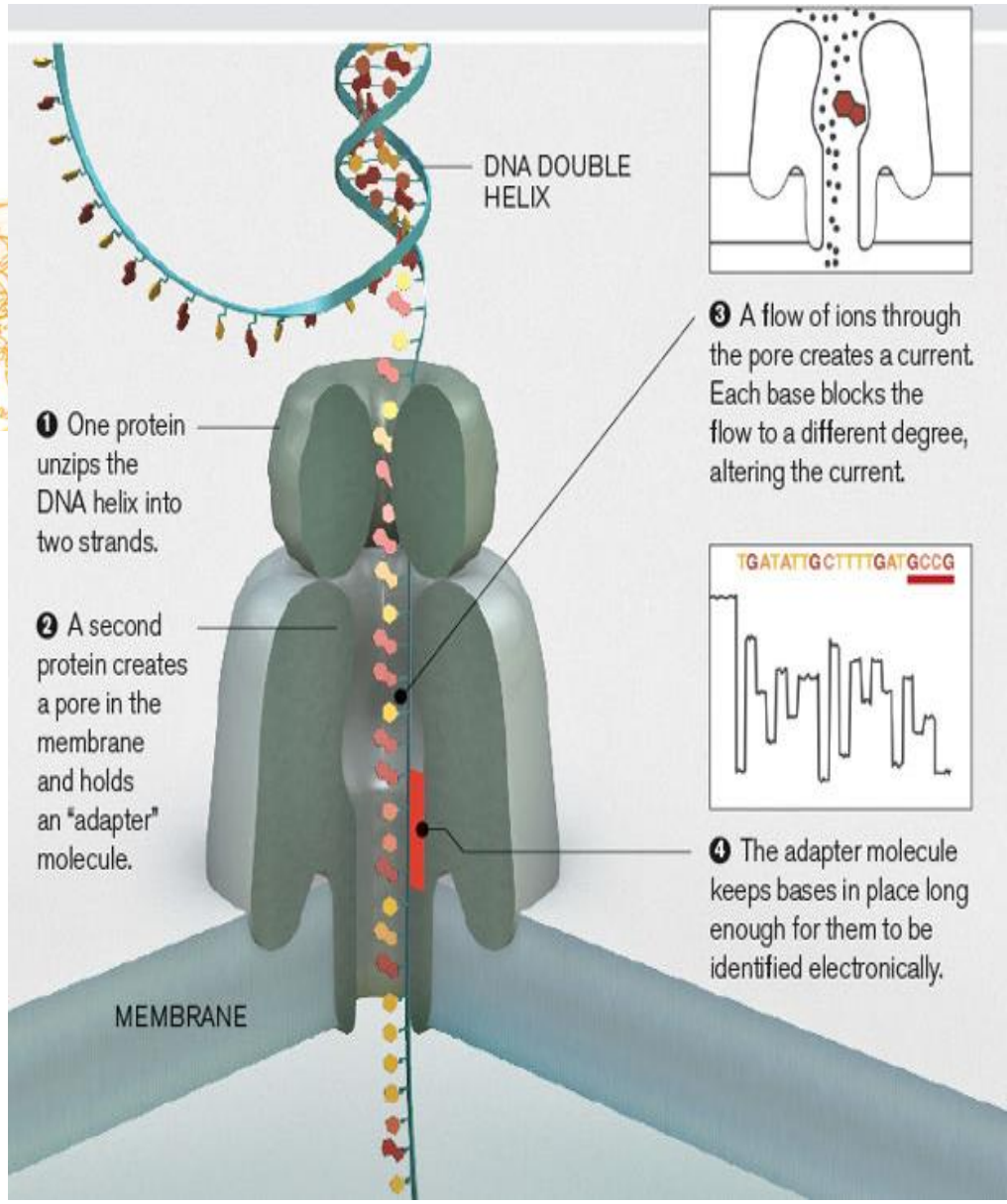








Oxford Nanopore MinION Sequencing

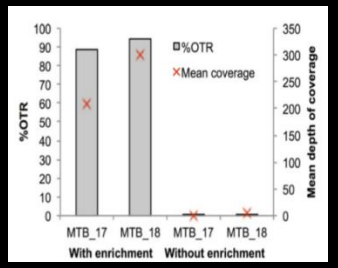


http://www.technologyreview.com/sites/default/files/legacy/nanopore_x616[1].jpg

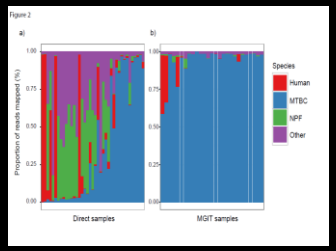
Sputum : How do you enrich the TB DNA signal ?

1. Sequence all DNA present at high depth (MTB coverage 0.002 – 0.7x)

2. Enrich with Baits (83% samples achieved >20x depth)



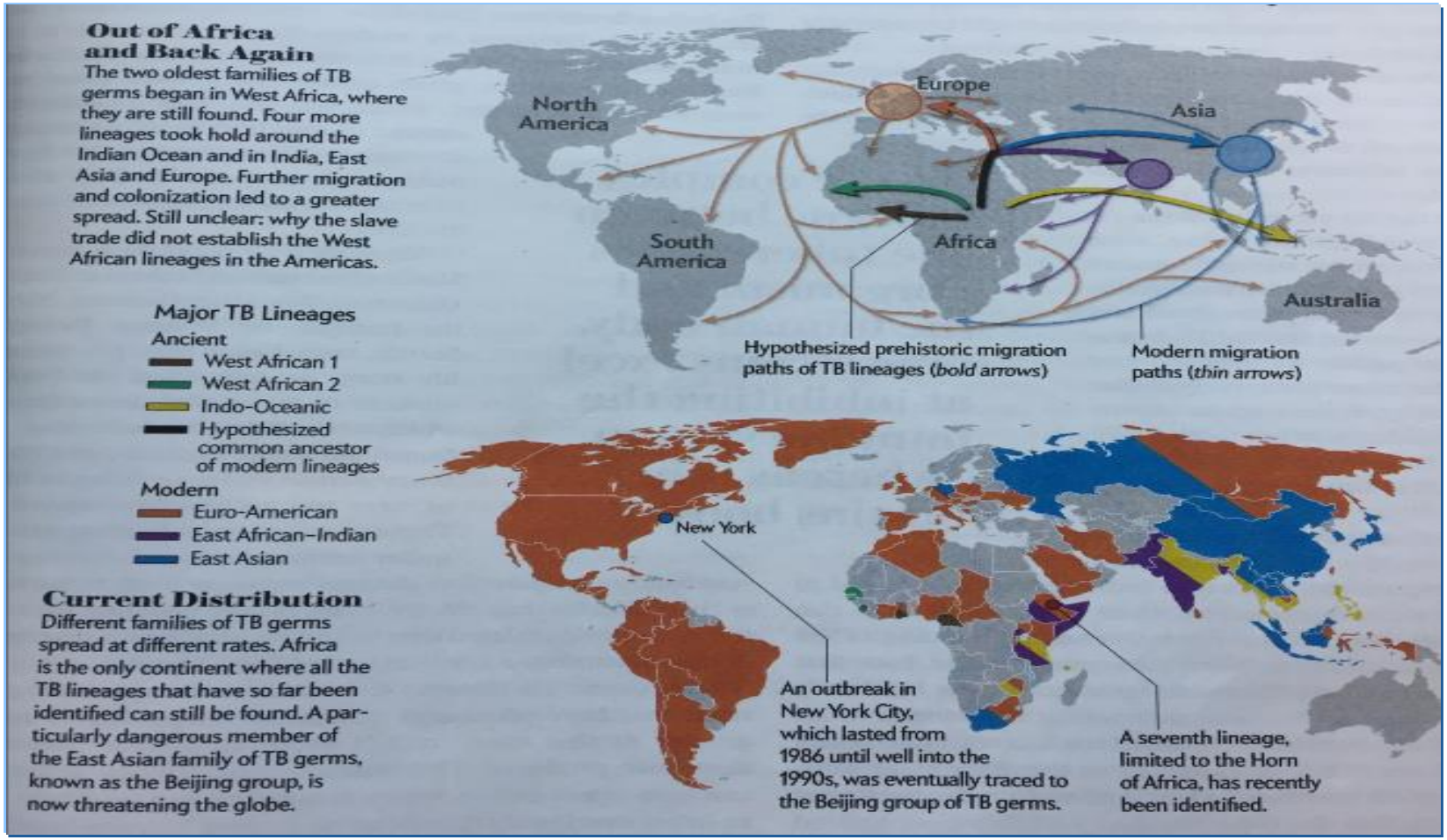
3. Lysis to remove target DNA (65% > 3X depth)



4. Early MGIT culture (1-5 days)

Doughty, et al. Peer J 2014
Brown, et al. J Clin Micro 2015
Doyle RM J Clin Micro 2018
Votintseva, et al. J Clin Micro 2017

How TB continues to conquer the world



Evolved into 7 lineages

When does bacterial resistance become global ?

- When the strain is easily transmissible (high density settings)
- When resistance imposes little fitness burden

Global implementation of NGS
are we ready for prime time ?

- Direct WGS still not standard
- Not near enough to the patient
- Current pipelines focus XDR defining drugs
- For oral SLD / repurposed drugs not all mutations are known
- Expertise for analysis with complex workflows
- Cost

- ..we certainly need to get on with WGS for surveillance

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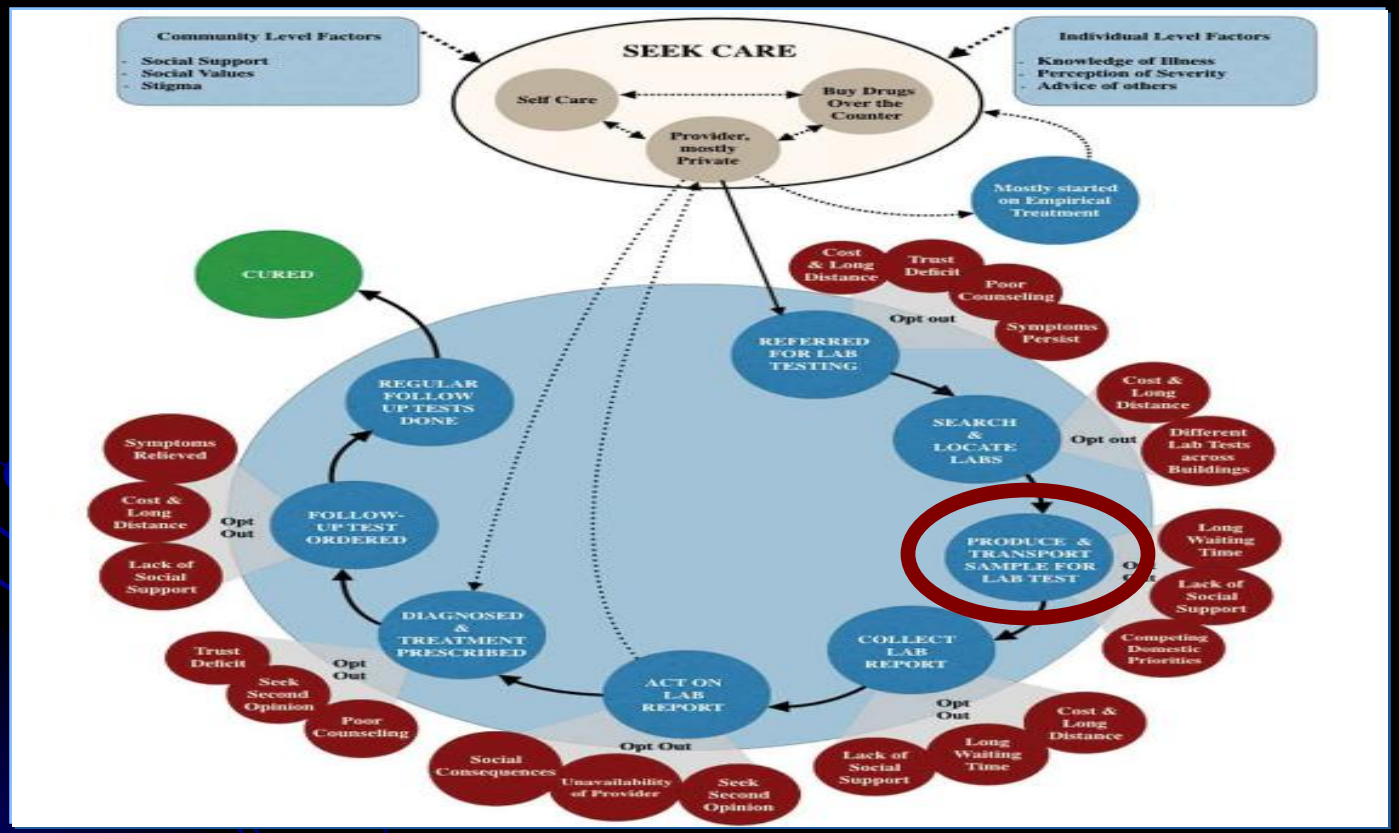
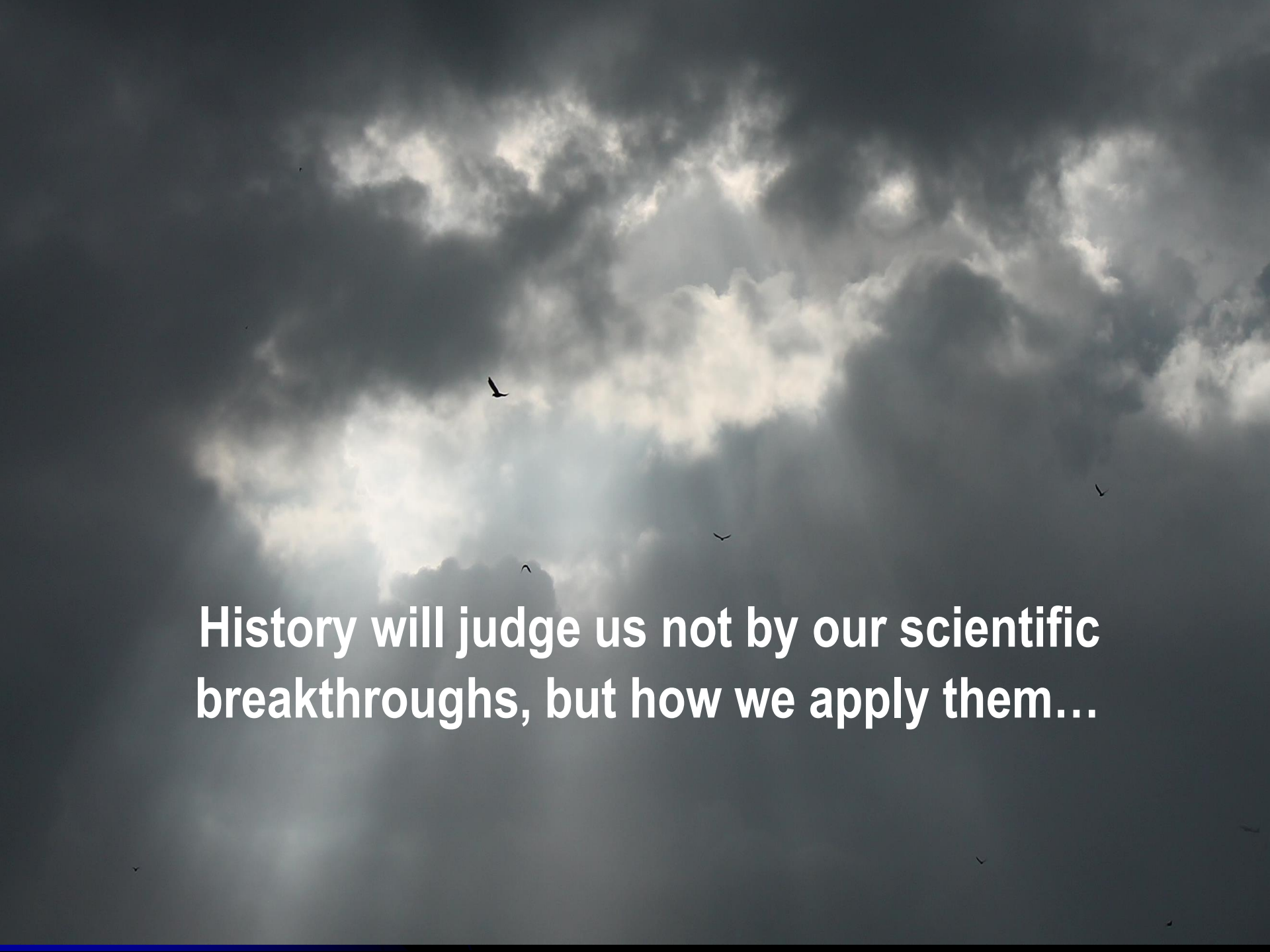
Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project



M Pai et al. BMJ Global Health doi:10.1136/bmjgh-2018-000755


History will judge us not by our scientific breakthroughs, but how we apply them…