Stop TB Partnership

New Diagnostics Working Group
Annual Meeting 2013

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Palais des Congrès, Paris

Moving to Point of Care Diagnostics

Role of next generation whole genome sequencing and clinical relevance

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11.15 – 11.30

WGS - Clinical Impact

Whole genome sequencing (WGS) and resistance genotyping

Predictive information to manage MDR/XDR-TB treatment

- 9 – 18 months
- Requires Good clinical utility
  - give best possible evidence-based advice to clinicians
  - likely resistance/sensitivity
  - likely MICs
  - likely cross-resistance between different members of drug families

Needs good predictive genotype-phenotype correlation data

Evidence base – tbdreamdb, literature, in house 2nd line DST + WGS

Genetic Determinants of Resistance

Drug	Gene	Gene product/ function
Isoniazid	*katG, inhA,* *ahpC,* *kasA*	catalase peroxidase, enoyl acp reductase alkyl hydroperoxide reductase β-ketoacyl-ACP synthase
Rifampicin	*rpoB*	RNA polymerase β subunit
Pyrazinamide	*pncA*	pyrazinamidase
Ethambutol	*embA, embB, embC*	arabinosyl transferase
Streptomycin Kanamycin, AMK,CAP	*rrs, whiB7* *rpsL, tlyA* *gidB, eis* promoter	16S ribosomal RNA, promotor eis and tap ribosomal subunit 12, methyltransferase 16Smethyltransferase, acetyltransferase
Quinolones PAS Cycloserine Prothionamide	*gyrA, gyrB* *thyA,dfrA,folC,* *nhoA.acc* *alr, ddlA, cycA* *inhA, eta*	DNA gyrase Thymidylate synthase, folate reductase/ synthase, acetyltransferease D-ala ligase, racemase, transporter InhA, activator monoxygenase

Genotypic tests depend on strength of associations

High confidence calls

e.g.	INH^R	*katG* or *inhA* SNPs
	$Rifampicin^R$	*rpoB* SNPs in RRDR – codons 507-533
	$Quinolone^R$	*gyrA* SNPs in QRDR

More Complex Associations - hard to make predictions

e.g.

KAN^R	rrs	A1401G & C517T plus eis promoter G-10A or C-14
CAP^R	rrs	A1401G, C1402T, G1158T + possible *eis* C-12T
AMK R	rrs	A1401G + eis promoter

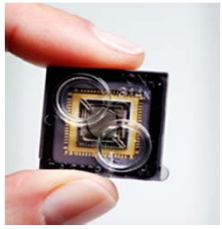
Difficulties:

Cross resistance
no DST data for new SNPs
DST and MICs
strain variation
compensatory and secondary mutations

WGS – Clinical use at St George's ?

XDR-TB patients
MGIT positive cultures

TTP = 12 days
3 mL = 150ng DNA yield



- 20-100ng DNA
- 1.8million reads
- mean 128 bp
- M.tb genome = 4.4Mb
- > 35x coverage (perfect reads)

WGS IonTorrent
clinically timely turnaround time – 24 hours
high cost but high value test - ~£180 per genome

Informs treatment options

DST PRIOR to Tx	- weeks
Clinical perspective	- often too little, too late

XDR-TB clinical case

Genotype Results - PCR for *gyrA*, *gyrB* and *pncA*
reference strain H37Rv

• SNPs in *gyrA*

*269nt GCG→GTG codon 90, Alanine to Valine

Evidence to call

gyrA A90V mutation

- significant associated with MIC of 1- 2 µg/ml for moxifloxacin (*www.tbdreamdb.com*) multiple publications with frequency data. Wilby et al 2012 i.e. predictive evidence

•WT *gyrB*

Treatment informed:

- Moxifloxacin dose increased to 600 mg
- Theoretically achieves in vivo MIC sufficient for treatment ~ 2ug/ml

Feasey et al (2011) IJTLD 15(3):417-20.

XDR-TB clinical case

- ***pncA*** AAT→ATT nt 335 = codon 112 = stop codon at 118.

Treatment informed:

- Pryazinamide – stopped -
- Weight of evidence of SNP and DST for PZA is unreliable

Some SNPs found in XDR-TB clinical case

Gene	Resistance	H37Rv Position	Mutation
gyrA	FLQ	7,582	Asp94Gly
gyrA	FLQ	7,585	Ser95Thr
rpoB	RIF	761,155	Ser450Leu*
rpsL	SM	781,822	Lys88Arg
rrs	AMI;SM	1,473,246	A1400G
fabG1	ETH;INH	1,673,425	-15 C/T*
katG	INH	2,154,724	Arg463Leu
katG	INH	2,155,168	Ser315Thr*
pncA	PZA	2,288,847	Gly132Asp
accD6	INH	2,521,428	Asp229Gly
embA	EMB	4,243,221	-12 C/T
embB	EMB;INH;RIF	4,247,513	Tyr334His

Genotype matched the XDR phenotype

*** Matched Hain test**

Rif^R SNPs – cross-resistance

- **1 high level high confidence Rif^R mutation present in rpoB gene:**
  - 761155CT S450L = **S531L** (ec nomenclature)
  - reported phenotype: MICs:
    - >8mg/ml rifampicin
    - 4->8mg/ml rifabutin
    - >8mg/ml rifapentin

Report for clinicians: need for assessment of evidence

Results	Good evidence of resistance
	• **Fluoroquinolone resistance:**
	  ○ **1 high level high confidence FLQ^R mutation present in gyrA:**
	  ▪ 7582AG = Asp94Gly (D94G)
	  ▪ >8ug/ml ciprofloxacin; gatofloxacin 1-4, moxafloxin 1-8, levofloxacin 2-8, ofloxacin 16-16
	  ○ 3 other gyrA mutations, for which there is evidence they do <u>not</u> affect function. No SNPs in gyrB
	• **Isoniazid resistance:**
	  ○ **1 high level high confidence INH^R mutation present in katG gene:**
	  ▪ KatG mutation S315T (Ser315Thr)

XDR-TB clinical cases

- Early prediction of TB drug resistance would:
  - allow faster and more effective treatment
  - improve patient outcomes
  - reduce onwards transmission - ↓time of infectivity
  - reduce the chance of further resistance developing
  - cost-effective compared to total care package ~£1000/day
  - Genotype matched the XDR phenotype.

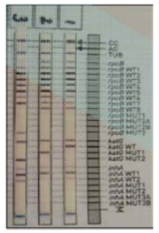
Genomics

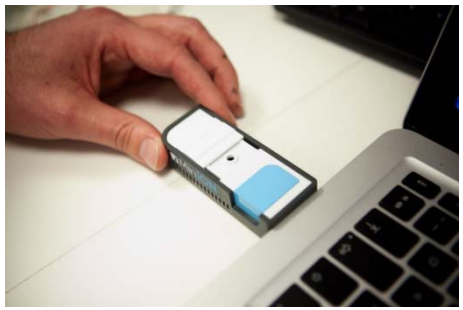
Direct impact on treatment selection

Role in clinical management and public health

WGS Point of Care- Are we there yet?

PoC - possibly in 3 – 5 years
- need to prepare for technology advances
- clinically timely – hours (PoC)
– days (WGS)
– weeks (DST)
- multiplex R+ gene PCR sequencing or hybridisation
viz Hain or GeneXpert

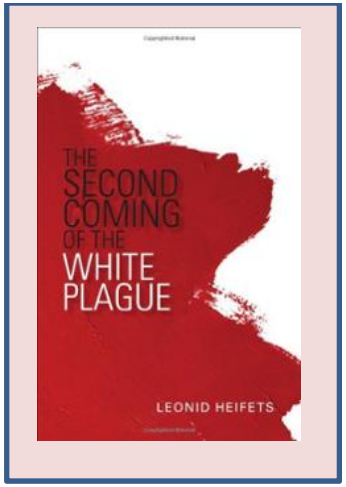




MinIon
Oxford Nanopore Technologies Ltd

QuantuMDx Q-POC or Q-SEQ
nanowires

Where Next?



- Need phenotypic DSTs – maintain capacity

  Leonid Heifets 2010 The second coming of the white plague.

- Rapid sequencing direct from specimens

- Database for routine clinical use

  e.g. TBDReaMDB, *WIKI &* reportable front end

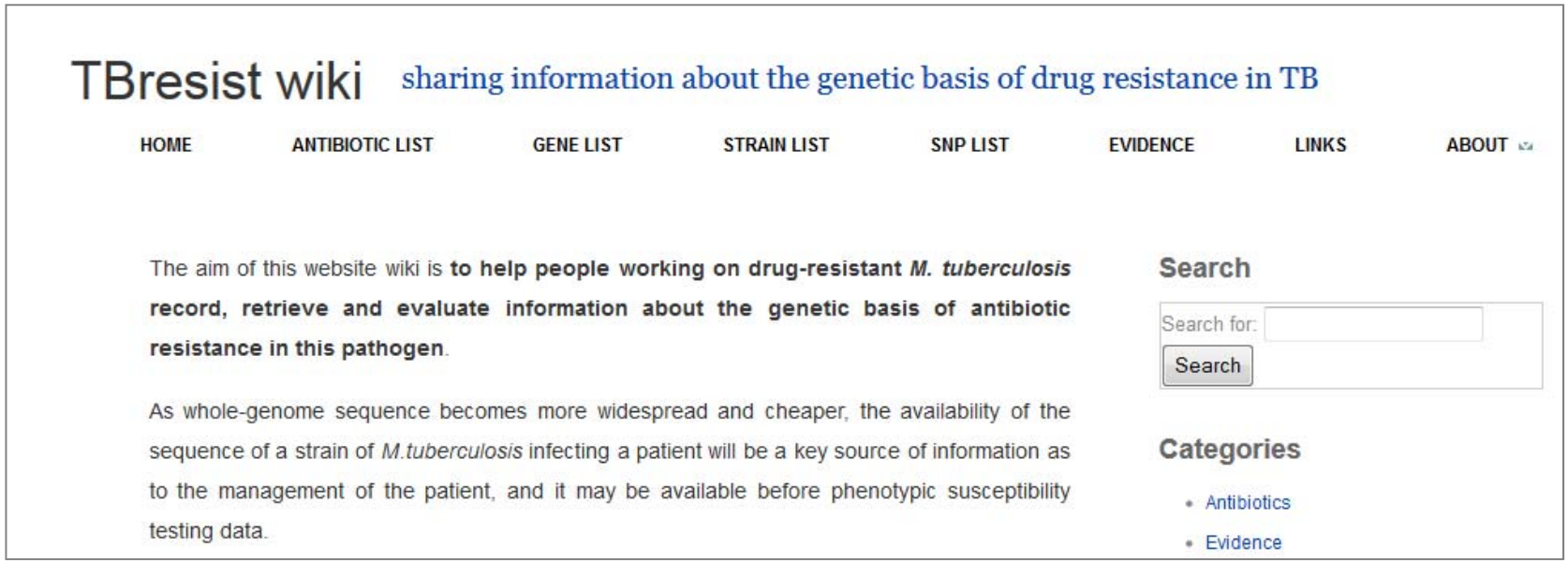
- Regional and global frequencies for accurate prediction

  - large scale WGS

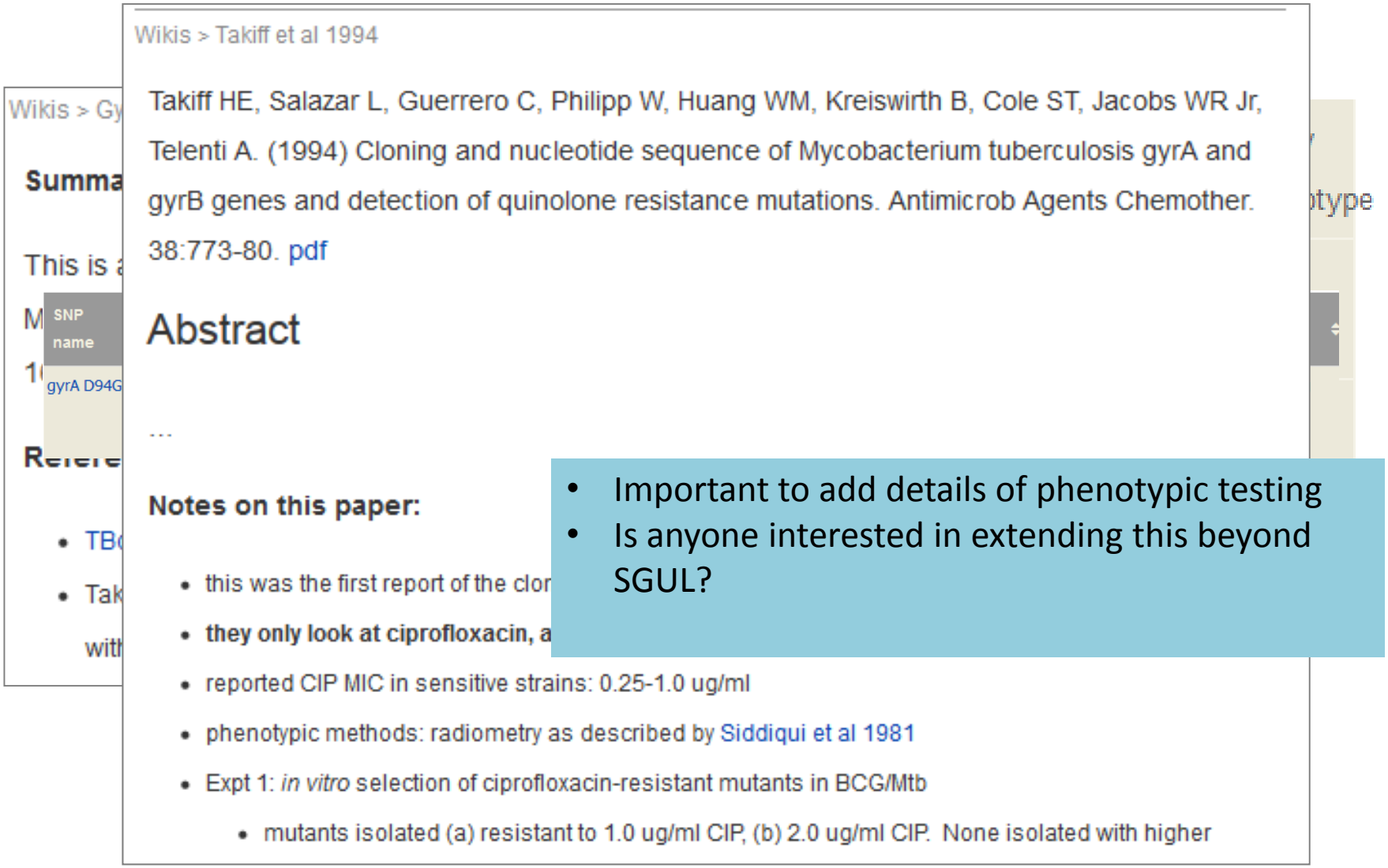
Proposal – a wiki for sharing evidence

(stoker.neil@gmail.com)

- **tbresist.org –** may change address
- Sharing of expertise
  - SNP evidence
  - DST details – methods, MICs, interpretation
- **Evidence base of phenotype- genotype correlations**
- permanent / temporary (data can be moved)



Strain – SNP list → wiki SNP list → individual SNP evidence summary → summary and review of individual paper



New database for *Mtb* genomes?

- Initiative by people with multiple genomes planned

  (at this meeting)

- combine with DST Ab^R data → powerful tool for predicting SNP function

- **meeting this lunchtime here to discuss**

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