

CNR-MyRMA
Centre National de
Référence des
Mycobactéries
et de la Résistance
des Mycobactéries
aux Antituberculeux

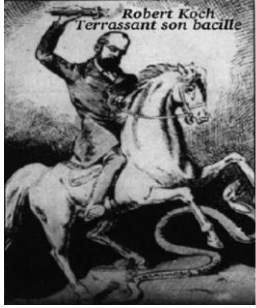


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**First evaluation in routine use of the combination of GeneLEAD VIII
to extract and detect *Mycobacterium tuberculosis* DNA
and Deeplex-MycTB to predict drug resistance and TB transmission in
less than 7 days from clinical samples**

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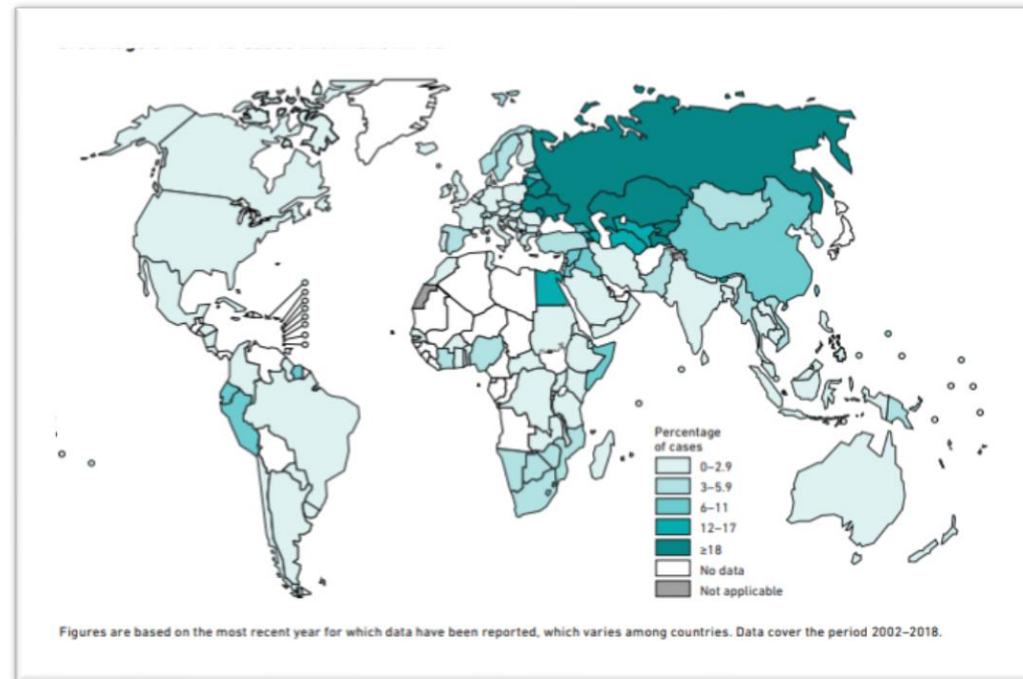
Conflicts of interest

Nothing to declare

Background

	Incidence	% of deaths
All form of TB	10 ⁷	13%
MDR-TB	550.000	42%

WHO, Global Tuberculosis Report 2018



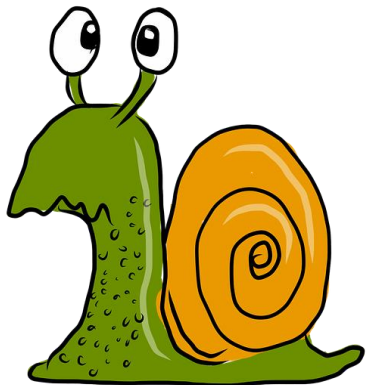
New TB cases with MDR/RR-TB

Drug Susceptibility Testing (DST)

Phenotypic DST	Genotypic DST	
	Routine methods	Recent technology
Reference method	DNA strip assay	WGS
	Sanger DNA sequencing	



Long delay



DNA strip assay:

- limited to 2 to 5 antiTB drugs

Sanger sequencing for 12 drugs:

- long delay
- technically time-consuming
- expensive



Long delay

- from cultures

Complexity of interpretation

Difficult to standardize

Innovative approach: GeneLEAD VIII/Deeplex-MycTB

1. GeneLEAD VIII (Diagenode, France)

- all-in-one system = extraction and amplification
- real-time PCR of *IS6110* (id of *M. tuberculosis* complex)

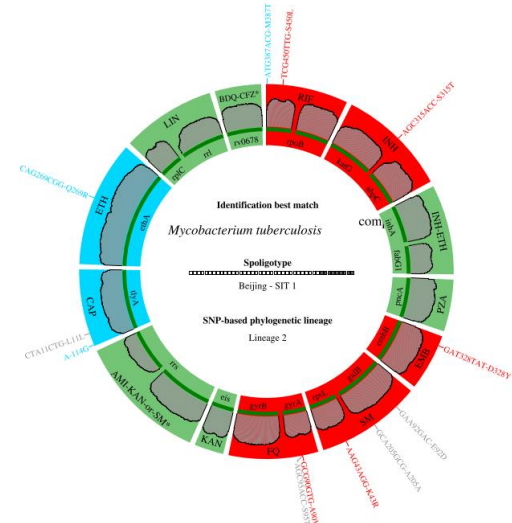


GeneLEAD POSITIVE (in 3h)

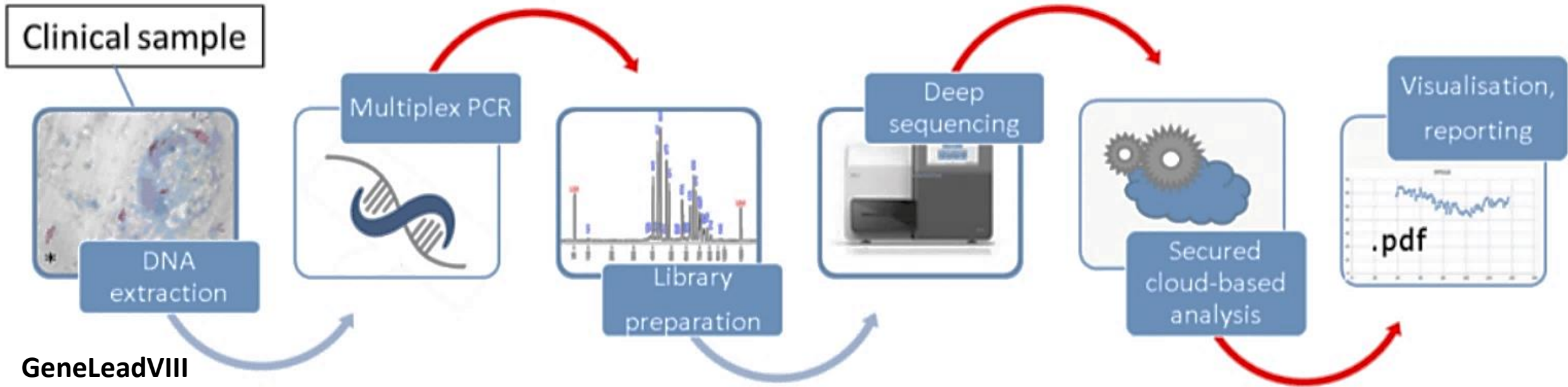


2. Deeplex-MycTB (GenoScreen, Lille) from DNA extracted by GeneLead

- NGS-based assay
- prediction of resistance to 13 antituberculous drugs
- genotyping and spoligotyping of MTBC strains
- highly sensitive (identify minority populations)



Deeplex-MycTB



- Libraries preparation: Nextera XT kit
- Sequencing platform: MiSeq/NextSeq Illumina
- NGS-based targeted deep sequencing of 18 target genes for resistance
- Sequencing data uploaded via a web app

Deeplex-MycTB

<i>pncA</i>	Pyrazinamide	<i>eis</i>	Kanamycin
<i>embB</i>	Ethambutol	<i>rrs</i>	Amikacin
<i>rpoB</i>	Rifampicin	<i>tlyA</i>	Capreomycin
<i>ahpC, fabG1, katG, inhA</i>	Isoniazid	<i>gyrA, gyrB</i>	Fluoroquinolones
		<i>gidB, rpsl</i>	Streptomycin
<i>hsp65</i>	Species ID	<i>ethA, inhA, fabG1</i>	Ethionamide
CRISPR/DR	Spoligotyping	<i>Rv0678</i>	Bedaquiline, Clofazimine
phyloSNPs	Genotyping	<i>rrl, rplC</i>	Linezolid



Simultaneous prediction of mycobacterial identification, resistance to 13 antiTB drugs, and MTBC genotyping

Deeplex-MycTB: example of report for resistance

- Resistance prediction based on the detection of SNVs and interrogation of a database
- Resistance-associated variants and uncharacterized variants are listed
- Heterogeneous mutations can be detected (down to 2% of mutant bacteria)

Drug resistance associated variants³

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Confidence	PMID
<i>embB</i>	4247429	ATG306GTG	76.0	2018.00	M306V	EMB	n/a	Campbell PJ et al., 2011
<i>embB</i>	4247431	ATG306ATC	20.8	718.50	M306I	EMB	n/a	Campbell PJ et al., 2011
<i>embB</i>	4248002	CAG497AAG	23.5	1638.00	Q497K	EMB	n/a	Plinke C et al., 2010
<i>katG</i>	2155168	AGC315ACC	100	557.25	S315T	INH	High	Marttila HJ et al., 1996
<i>pncA</i>	2288719	ATG175GTG	47.5	948.75	M175V	PZA	High	n/a
<i>rpoB1</i>	761155	TCG450TTG	18.2	1634.25	S450L	RIF	High	Campbell PJ et al., 2011
<i>rpsL</i>	781687	AAG43AGG	83.8	13069.50	K43R	SM	High	Feuerriegel S et al., 2012

No drug resistance associated variants detected in the other gene targets.

Uncharacterized variants³

Uncharacterized variants designate sequence variants of as yet unknown association with drug sensitivity or resistance.

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*
<i>gidB</i>	4407797	TCA136CCA	31.0	1415.25	S136P	SM
<i>rpoB1</i>	761277	ATC491CTC	83.6	14776.50	I491L	RIF
<i>tlyA</i>	1917972	CTA11CTG	99.7	591.75	L11L	CAP

Deeplex-MycTB: example of report for resistance

- Resistance prediction based on
- Resistance-associated variants
- Heterogeneous mutations

and interrogation of a database
Antibiotic resistance variants are listed
(Number of mutant bacteria)

Drug resistance associated variants

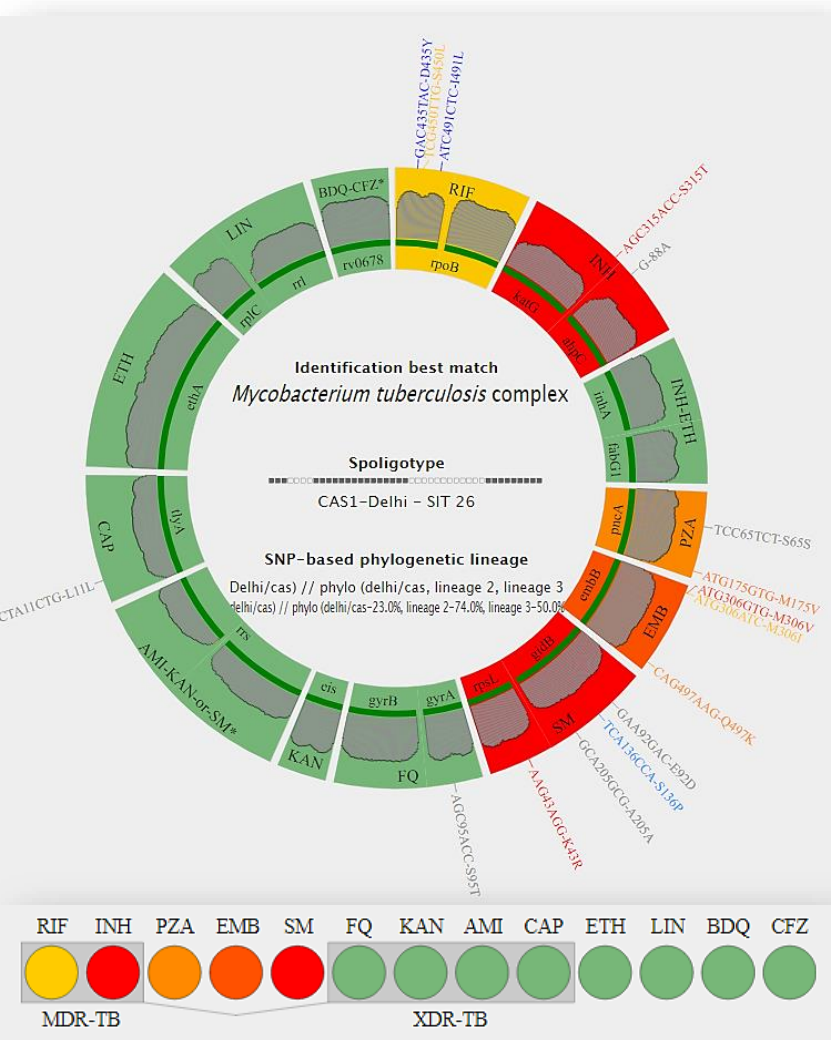
Gene	Genomic position	Codon change
embB	4247429	ATG>G
embB	4247431	ATG>G
embB	4248002	CAG>G
katG	2155168	AGC>G
pncA	2288719	ATG>G
rpoB1	761155	TCG>G
rpsL	781687	AAG>G

No drug resistance associated variants

Uncharacterized variants

Uncharacterized variants detected

Gene	Genomic position
gidB	4407797
rpoB1	761277
tlyA	1917972



Prediction of resistance to 13 antiTB drugs

gidB	4407797	TCA136CCA	31.0	1415.25
rpoB1	761277	ATC491CTC	83.6	14776.50
tlyA	1917972	CTA11CTG	99.7	591.75

Antibiotic resistance associated variants

Antibiotic resistance	PMID
n/a	Campbell PJ et al., 2011
n/a	Campbell PJ et al., 2011
n/a	Plinke C et al., 2010
high	Marttila HJ et al., 1996
high	n/a
high	Campbell PJ et al., 2011
high	Feuerriegel S et al., 2012

Uncharacterized variants

Uncharacterized variants detected

AA change	Drug*
S136P	SM
I491L	RIF
L11L	CAP

Deeplex-MycTB: example of report for genotyping

- Species identification based on *hsp65* analysis: MTBC, 143 NTM
- Spoligotyping (SITVITWEB database)
- SNP-based lineage identification

hsp65-based species identification

Av coverage depth (x)	Consensus length	% Identity	E-value	Best match
51265.8	401.0	100	0.0	<i>Mycobacterium tuberculosis</i> complex

Spoligotype

Octal code/ Binary code	Av. coverage depth (x)	SIT	SITVIT occurrence	Clade
703777740003771	223.3	26	896	CAS1-Delhi
1110000111111111111110000000000011111111				

SNP-based phylogenetic lineage

Delhi/cas) // phylo (delhi/cas, lineage 2, lineage 3 delhi/cas) // phylo (delhi/cas-23.0%, lineage 2-74.0%, lineage 3-50.0%

GeneLEAD – Deeplex: STUDY DESIGN

All consecutively clinical samples and positive cultures submitted to the French National Reference Centre

* in case of suspicion of resistant TB and susceptible control strains

* between May 2018 and June 2019

GeneLEAD/DEEPLEX

Genotypic routine procedures

DNA strip assay

Sanger sequencing of PCR

Solid proportion method

(ongoing)

GeneLEAD – Deeplex: MATERIALS AND METHODS

Nature of clinical samples (% MDR or XDR)		Strains (% MDR or XDR)	
Respiratory sample	35 (57)	Liquid medium	24 (67)
Lung biopsy	2 (50)	Solid medium	45 (42)
Pleural fluid	2 (0)	<i>Total</i>	69 (51%)
Lymph node	8 (50)		
Spine	4 (75)		
Abscess iliac fossa	2 (0)		
<i>Total</i>	53 (53%)		

GeneLEAD – Deeplex: RESULTS

Deeplex result	Clinical samples (%)					Strains	
	>100/field	10-99 /field	1-9/field	<1/field >10/slide	Negative	Liquid medium	Solid medium
Successful	5 (83)	12 (92)	8 (89)	2 (25)	4 (24)	24 (100)	42 (93)
Failed*	1 (17)	1 (7)	1 (11)	6 (75)	13 (76)	0	3 (7)
Total	6	13	9	8	17	24	45

* GeneLead+ Deeplex-



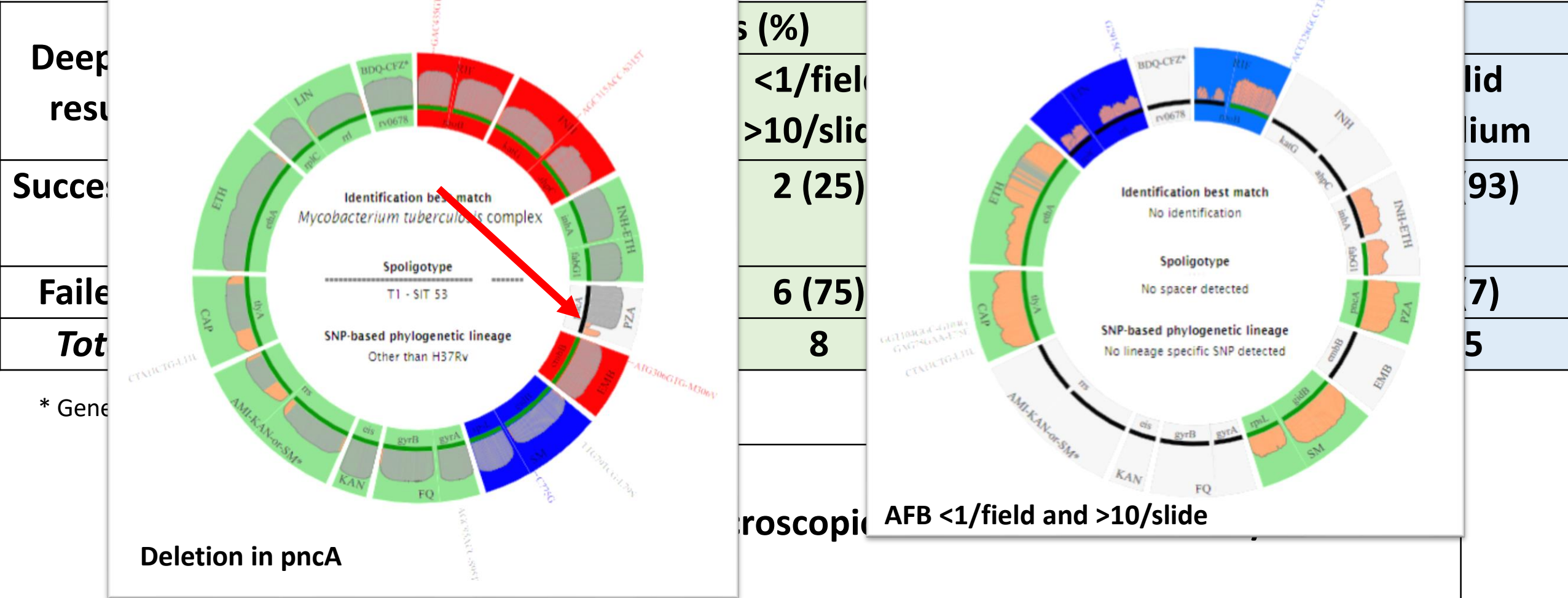
Deeplex works:

- for clinical samples if the microscopic examination is > 1 AFB/field
- for strains

Deeplex is less efficient:

- for clinical samples when the microscopic examination is < 1 AFB/field

GeneLEAD – Deeplex: RESULTS



Deeplex is less efficient:

- for clinical samples when the microscopic examination is < 1 AFB/field

GeneLEAD – Deeplex: UNCHARACTERIZED VARIANTS

Gene	N uncharacterized variants	N strains (%) <i>Total</i> = 80
<i>rpoB</i>	4	9 (11)
<i>katG</i>	7	6 (8)
<i>fabG</i>	1	1 (1)
<i>inhA</i>	1	1 (1)
<i>pncA</i>	10	10 (13)
<i>embB</i>	6	10 (13)
<i>gyrA</i>	3	4 (5)
<i>gyrB</i>	6	9 (11)
<i>eis</i>	2	2 (3)
<i>rrs</i>	3	3 (4)
<i>ethA</i>	29	30 (38)
<i>rplc</i>	2	2 (3)
<i>rri</i>	0	0
<i>Rv0678</i>	7	5 (6)

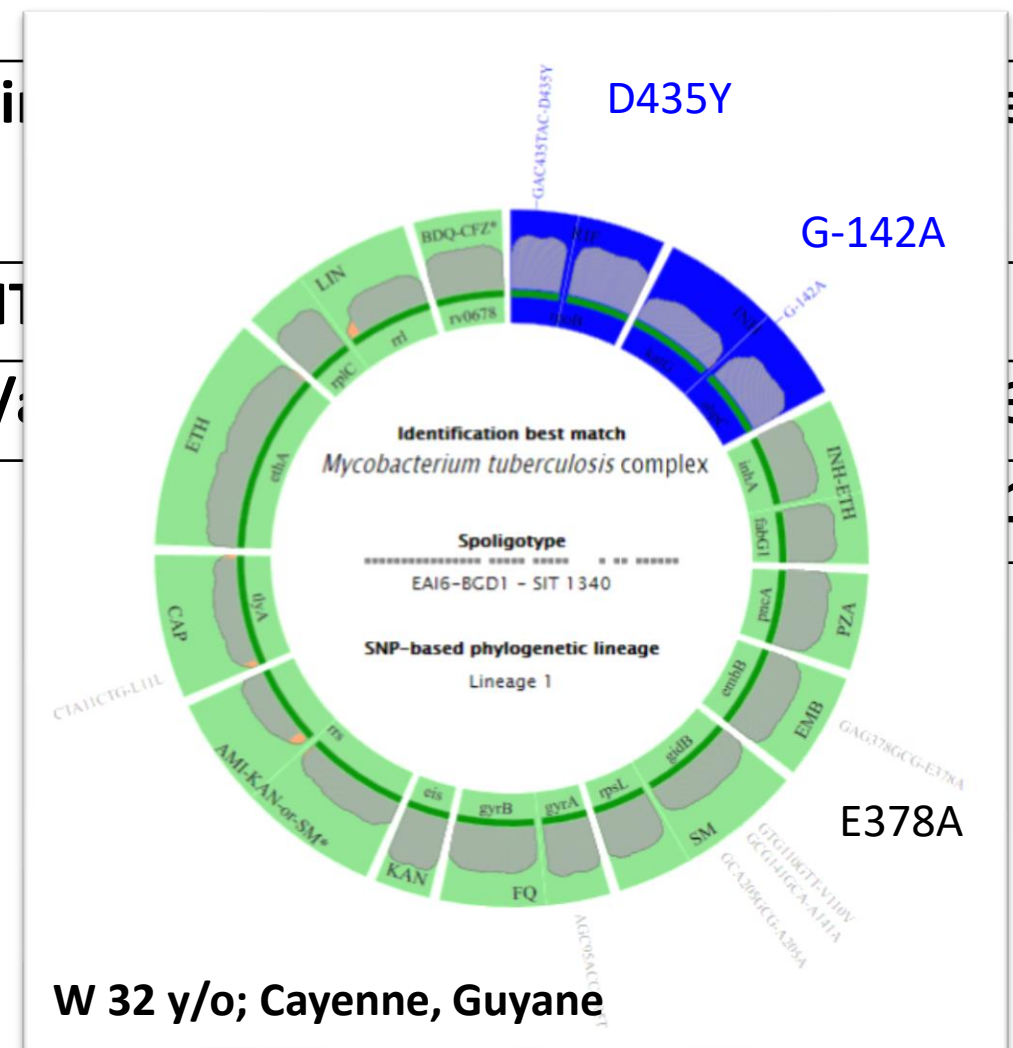
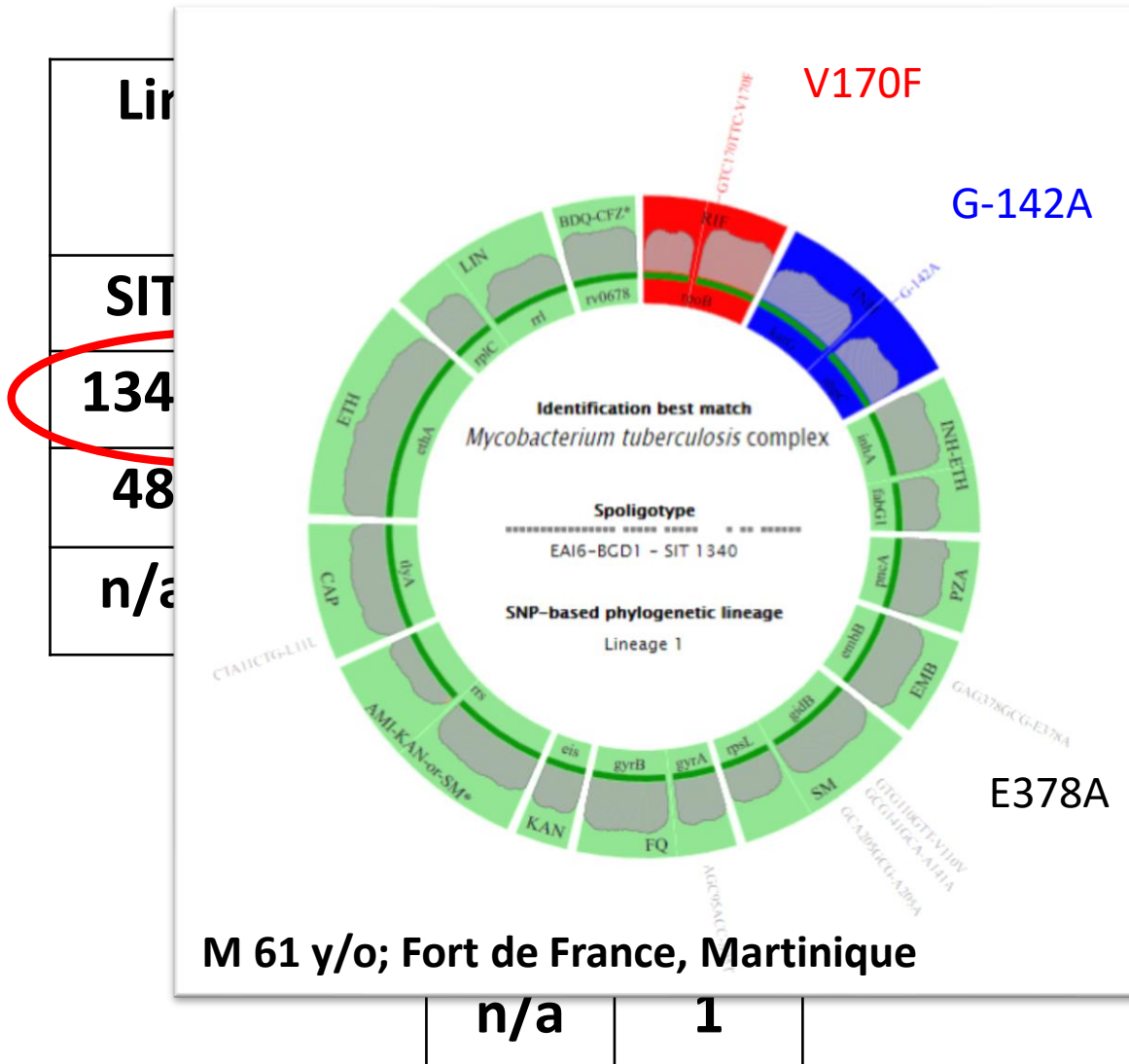


<i>rpoB</i> (n)	<i>Rv0678</i> (n)
M387T (7)	779181 inser (1)
Q429R (1)	779454 inser (1)
I491L (2)	193 del (1)
S450S (1)	N4N (1)
	R89P (1)
	I67T (1)
	Y92 stop (1)

GeneLEAD – Deeplex: PHYLOGENY

Lineage 1		Lineage 2		Lineage 4.3		Lineage 5		Lineage 6		No lineage specific	
SIT	n	SIT	n	SIT	n	SIT	n	SIT	n	SIT	n
1340	2	1	18	20	2	n/a	1	2669	1	N = 21 ≠	32
48	1	190	2	42	2			n/a	2	n/a	11
n/a	1	260	1	1070	1						
		265	2	1176	1						
		632	1	2669	1						
		2669	1	n/a	4						
		n/a	1								

GeneLEAD – Deeplex: PHYLOGENY



A link between both strains cannot be excluded

- Identification + full resistance genotyping + spoligotyping in 7.8 (5-14) days
- Performance
 - 89% of clinical samples containing >1 AFB/field
 - very good performance on solid/liquid cultures
- Digital spoligotyping: identification of potential patient-to-patient transmission events
- To be improved
 - sensitivity (<1 AFB/field)
 - « uncharacterized mutations »
 - delamanide
 - identification of clades

Strain sent to the Reference National Centre on 2/11/18

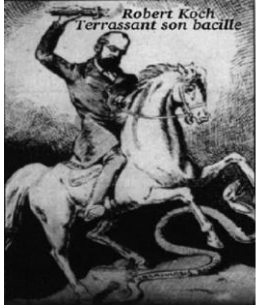
- for a resistance to rifampicine without mutation according to GeneXpert

- Rifampicine resistance verified by phenotypic method

- *rpoB* - MTBDR+ = WT

- *rpoB* – Deeplex = V170F

- mutation conferring resistance to rifampicine (Heep M *et al.*, 2001)



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