Missed opportunities for detecting MDR-TB in South-Africa

Increasing epidemiological importance of a rifampicin resistance mutation undetected by commercial molecular assays

Ndivhuho Makhado, Léonie Goeminne, Edith Matabane, Fairouz Boutachkourt, Rosine Gros, Maphoshane Nchabeleng, Bouke de Jong, Emmanuel André

Contact: emmanuel.andre@uclouvain.be

Conflicts of interest : None

Introduction

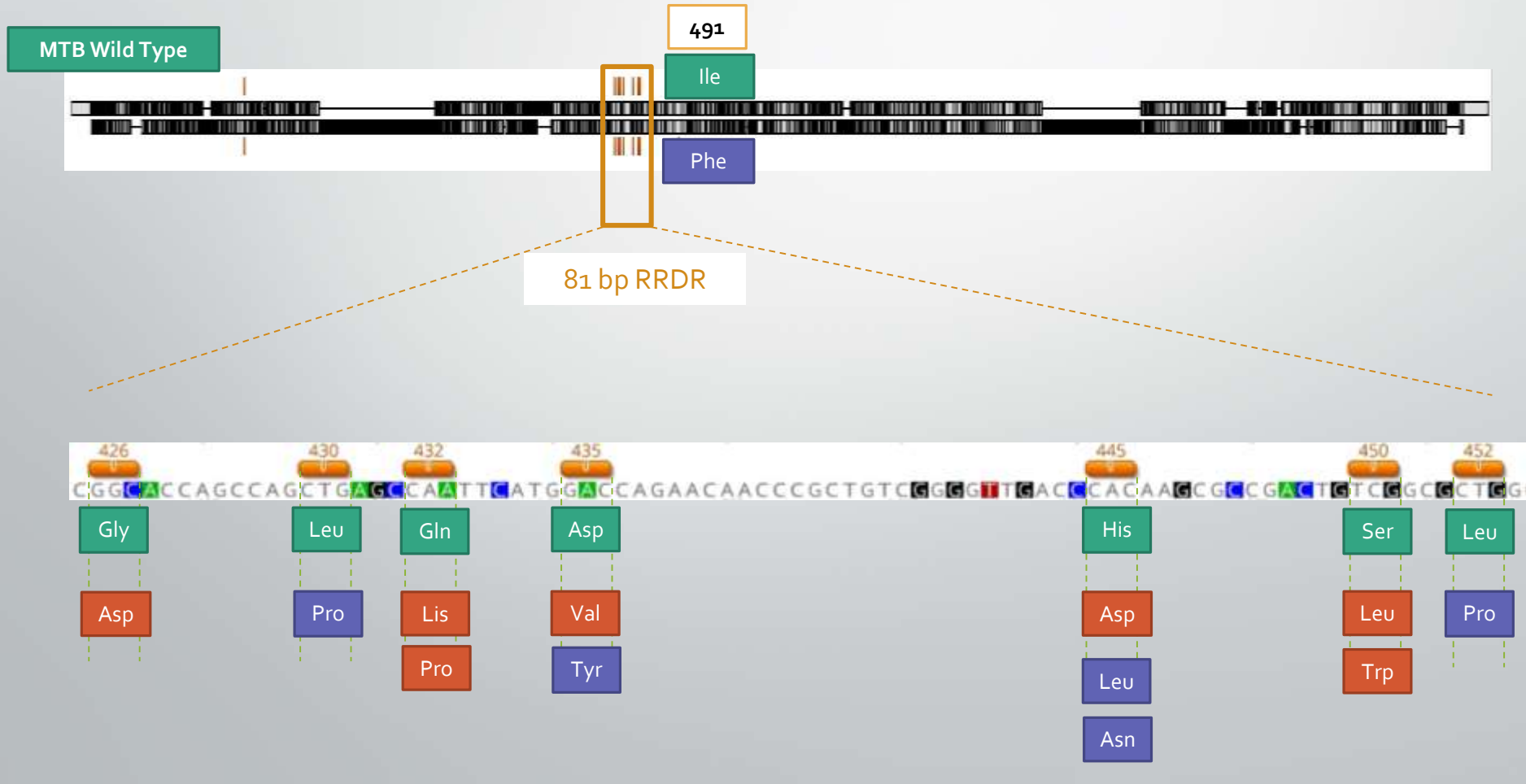
How good are laboratory methods for detecting Rifampicin resistance?

Liquid culture-based DST(Rigouts, et al. 2013)

Generally detected

Regularly missed

- Represent up to 13% of RIF-resistant strains (Van Deun, et al. 2013)
- Are associated with poor clinical outcome (Van Deun, et al. 2015) (Shah, et al. 2016)



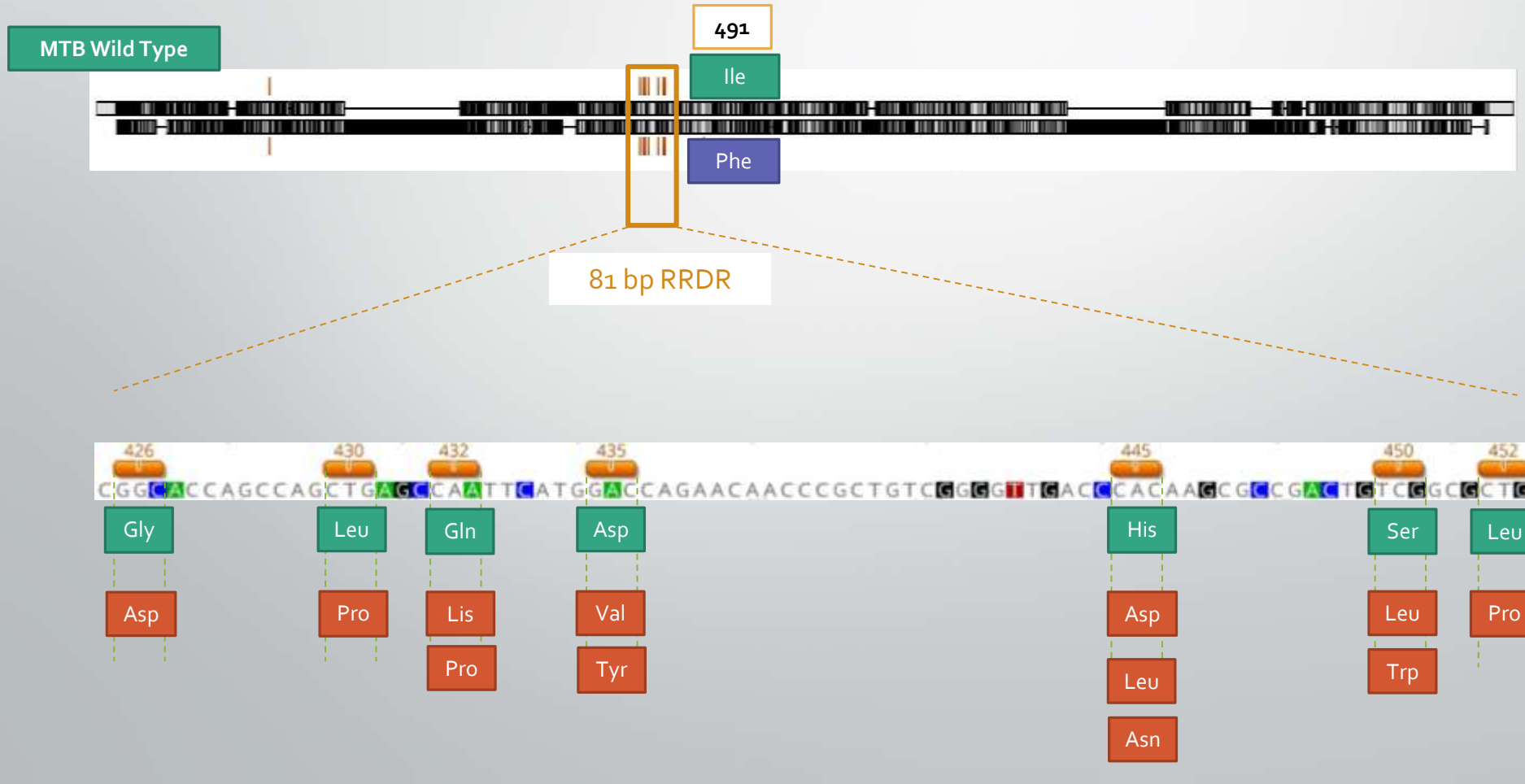
Commercial PCR assays : Xpert MTB/RIF and LPA

(Van Deun, et al. 2013)(Andre, et al. 2017)

Generally detected

Always missed

- Frequent : ?



The Ile491Phe rpoB mutation

Detection methods

- Liquid-based DST 👎
- Commercial molecular assays 👎
- *rpoB* sequencing 👍

First alarming report from Swaziland (Sanchez-Padilla, et al. 2015)

- 30% of MDR-TB strains harbour the 491 mutation
- These strains are clustered
- (…) More studies are needed to assess the prevalence of similar mutations in neighboring countries.

« To-do list »

1. Data mining in Public WGS repositories
2. Develop a rapid screening method
3. Screen in neighboring countries

Detection of Drug-Resistant Tuberculosis by Xpert MTB/RIF in Swaziland

TO THE EDITOR: Tuberculosis is a major global health problem that has worsened with the increasing emergence of *Mycobacterium tuberculosis* (MTB) complex strains that are resistant to rifampin (RIF) and isoniazid. As recommended by the World Health Organization (WHO), the timely detection of drug resistance with the use of rapid molecular diagnostic tests, such as the Xpert MTB/RIF assay (Cepheid), is essential for appropriate treatment of patients with tuberculosis and for limiting the further spread of multidrug-resistant disease.[1,2]

We used 24-loci mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) analysis and spoligotyping to perform classic genotypic analysis of MTB complex strains from the most recent (2009) national survey of tuberculosis-drug resistance in Swaziland, a country with a high prevalence of tuberculosis (945 cases per 100,000 persons, or approximately 1%).[3] We found that 38 of 125 multidrug-resistant strains (30%) that were isolated during the survey carried the *rpoB* I491F mutation, which confers resistance to rifampin (Table 1; and the Supplementary Appendix, available with the full text of this letter at NEJM .org). This mutation, which was previously reported with low frequency in clinical isolates from Hong Kong and Australia,[4] is not detected by the Xpert MTB/RIF assay.

Xpert MTB/RIF, a cartridge-based point-of-

mutation substantially reduces the sensitivity of Xpert MTB/RIF–based diagnosis in Swaziland and presumably results in underdiagnosis and potentially inadequate treatment. This is problematic in a country where an estimated 26% of adults are infected with the human immunodeficiency virus (HIV) and 80% of patients with tuberculosis are coinfected with HIV. In addition, coinfected patients are more likely than

Table 1. Mutations in *rpoB* in 125 Multidrug-Resistant Strains from the 2009 Survey Regarding Tuberculosis-Drug Resistance in Swaziland.*

Mutation	Strains with Mutation	Mutation in *rpoB* Hot-Spot Region†
	no. (%)	
D435F	1 (0.8)	Yes
D435F, N437D	3 (2.4)	D435F, yes; N437D, yes
D435V	1 (0.8)	Yes
G442R‡, I491F	1 (0.8)	G442R, yes; I491F, no
H445D	7 (5.6)	Yes
H445L	6 (4.8)	Yes
H445Y	6 (4.8)	Yes
I491F, R552C	1 (0.8)	I491F, no; R552C, no
I491F	38 (30.4)	No
QF432–433del	1 (0.8)	Yes
S450L	58 (46.4)	Yes
S450W	1 (0.8)	Yes
Unmutated	1 (0.8)	No

NB: Clusterered!!

1. Data mining in public WGS repositories

NB: WGS selection bias

Most are from pre-diagnosed MDR-TB strains (exception : UK)

Most are from low TB burden countries

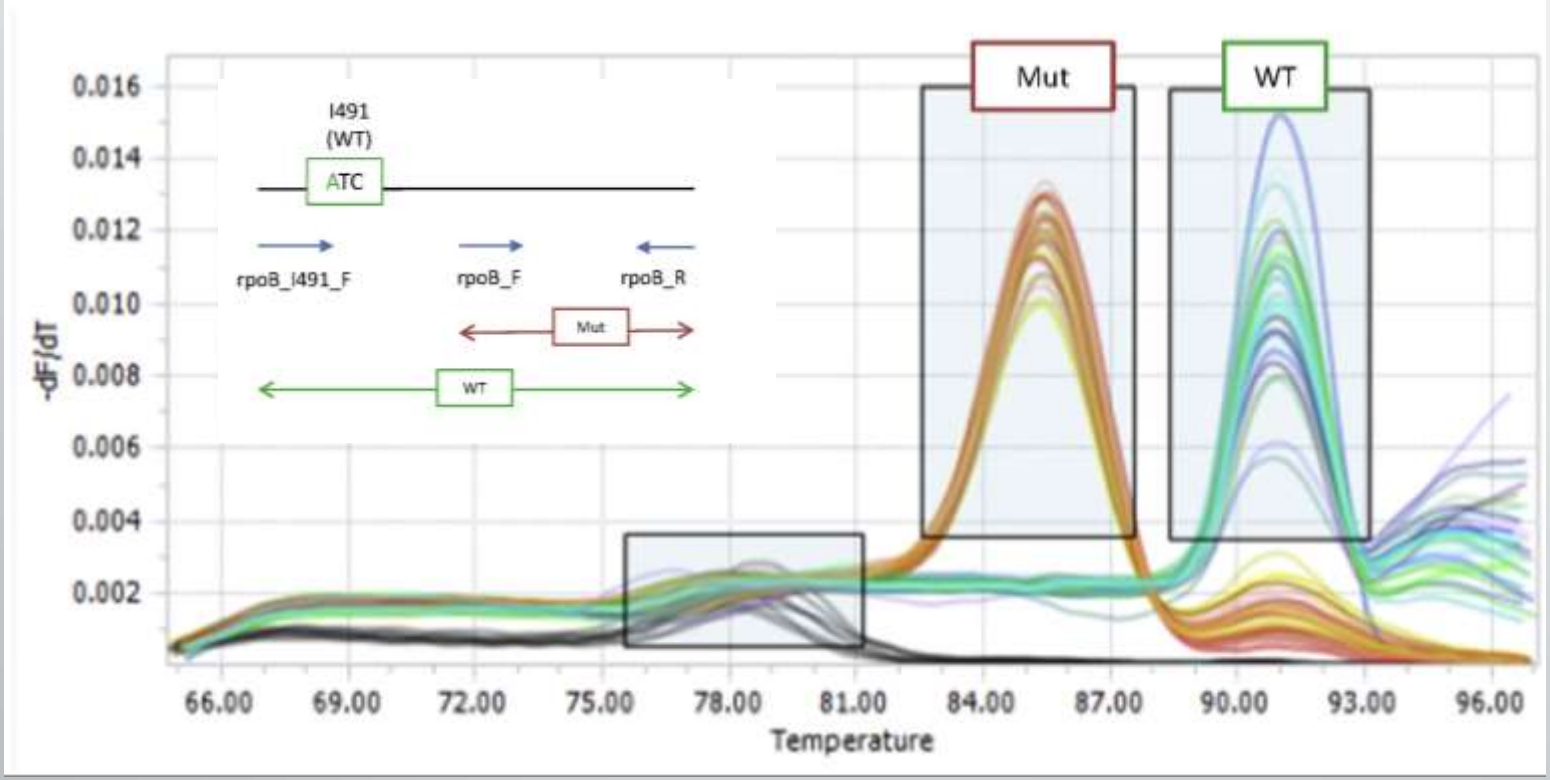
Public WGS data, April 2016

ReSeqTB

- 2051 *rpoB* sequences (unmutated > mutated)
- 26 (1,2%) harbour the Ile491Phe resistance
- **Origin**: UK, South-Africa (3), Vietnam, USA, …

2. Rapid detection of the Ile491Phe mutation

Cfr publication (Andre, et al. 2017) and Poster #1661



Design of the multiplex allele-specific PCR for the detection of the Ile491Phe rpoB rifampicin-resistance mutation. The rpoB_I491_F primer is specific to the wild-type profile, whereas the rpoB_F and rpoB_R primers are universal.

Results: the method allowed the correct identification of the rpoB Ile491Phe mutation among the 39/78 rifampicin-resistant strains.

3A. Screening of the Ile491Phe RIF resistance mutation in South-Africa

Why?

- Small distance from Swaziland, **important cross-border migrations** associated with the mining sector
- Rapid molecular tests and liquid-based drug susceptibility testing performed in programmatic conditions => **possible amplification of this mutation**
- High number of TB patients, so higher probability to find this mutation even if it was infrequent

Epidemiological background

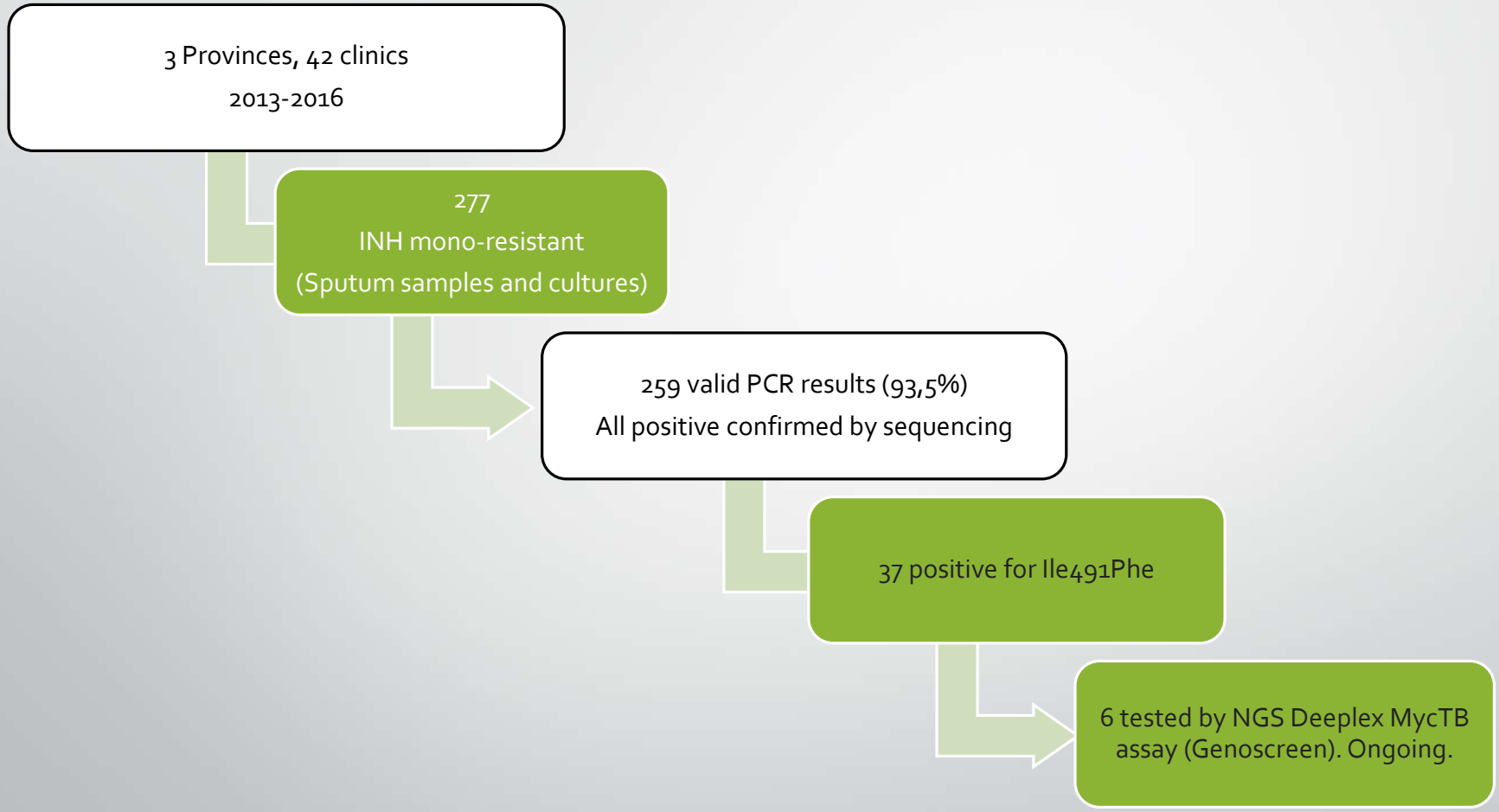
National Drug Resistance Survey
South-Africa, 2012

2012-14	New (%, 95%CI)
MDR	2.1 (1.5-2.7)
Any rifampicin	3.4 (2.5-4.3)*
Rifampicin mono[†]	1.4 (0.9-1.8)
Rifampicin mono (strict)[1]	0.9 (0.5-1.3)*
Rifampicin mono (other)[2]	0.4 (0.1-0.7)*
Any isoniazid[††]	7.6 (6.4-8.7)
Isoniazid mono	5.5 (4.6-6.5)
Isoniazid mono (strict)[3]	4.5 (3.6-5.3)*
Isoniazid mono (other)[2]	1.1 (0.3-1.8)
Ethambutol	2.0 (1.2-2.8)*
Streptomycin	3.9 (2.8-5.1)
Pyrazinamide	2.9 (2.2-3.6)

Province	New Cases %	95% CI
Eastern Cape	1.7	0.8 - 2.6
Free State	1.8	0.8 - 2.8
Gauteng	2.7	1.3 - 4.1
KwaZulu-Natal	1.8	0.6 - 3
Limpopo	1.4	0.4 - 2.4
Mpumalanga	4.2	2.8 - 5.6
North West	1.9	0.8 - 3.1
Northern Cape	1.3	0.4 - 2.1
Western Cape	2	0.7 - 3.2

Province	New Cases %	95% CI
Eastern Cape	5.4	3.3 - 7.5
Free State	7	4.9 - 9.1
Gauteng	4.8	3.3 - 6.3
KwaZulu-Natal	4.8	2.1 - 7.4
Limpopo	5.1	3.8 - 6.5
Mpumalanga	6.3	4 - 8.7
North West	5.8	4.3 - 7.2
Northern Cape	7.3	5.4 - 9.2
Western Cape	6.9	5.1 - 8.7

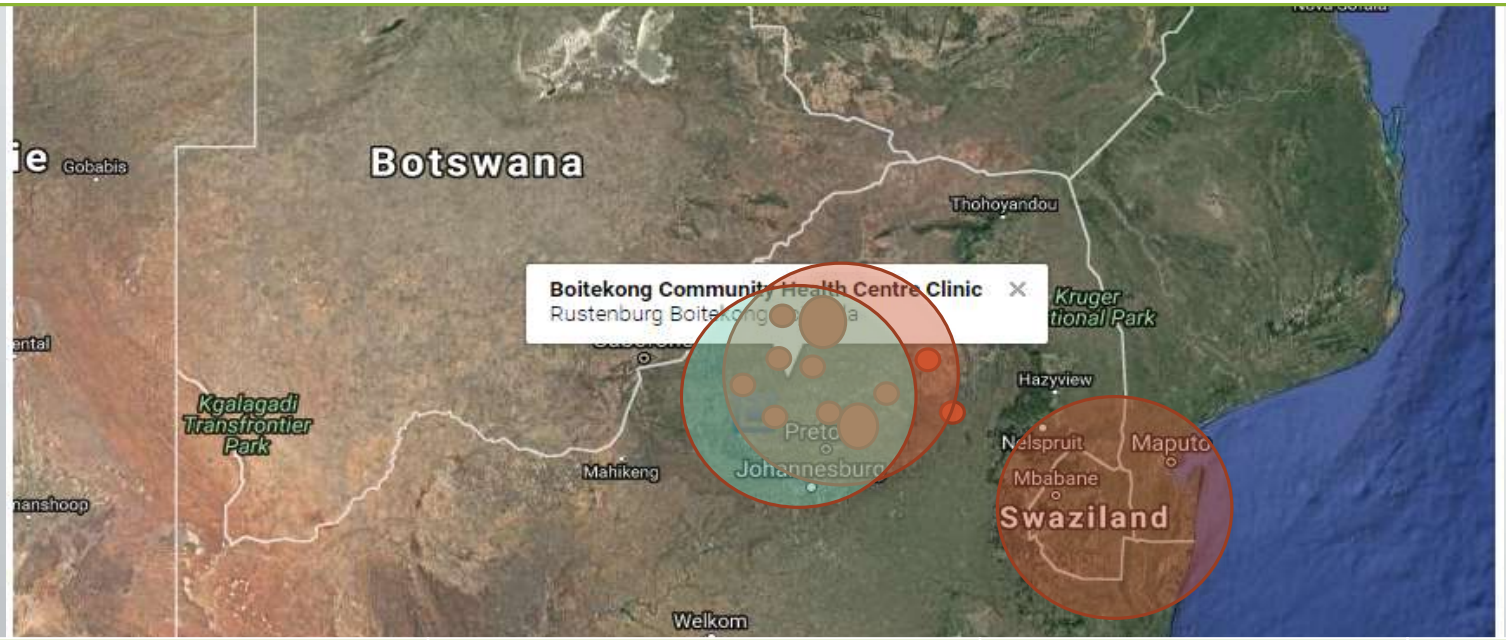
Material, methods and results



14,3% of INH –R, « RIF-S » strains are actually RIF-R
… and therefore undetected MDR-TB

Origin of patients presenting the mutation

We found at least two clusters based on spoligotyping and resistance profiles, including one similar to the Swaziland report (oingoing analysis)



	« Undetected MDR-TB » rpoB 491 mutated	« Real INH mono-R » rpob 491 wild-type	
Number of clinics reporting cases	15/42 (36%)	32/42 (76%)	P=0,0002
Number of cases reported	[1-8], mean: 2,4		

Number of patients in the Rutsenburg area

Results : patients characteristics (preliminary analysis)

	« Undetected MDR-TB » (*rpoB* 491 mutated)	« Real INH mono-R » (*rpob* 491 wild-type)	
High-level INH resistance (*katG* S315T mutation)	18/18 (100%)	8/20 (40%)	P = 0.0001
Resistant to R, H, E, SM	4/6 (ongoing testing)		
Resistant to R, H, E, SM and PZA	2/6 (ongoing testing)		
HIV infection	25/28 (89,3%)	40/41 (97,6%)	P=0,11

3B. Screening of the Ile491Phe RIF resistance mutation in the rest of the world

Conclusion

- Current algorithms implemented in South-Africa and elsewhere miss-diagnose patients with this mutation
- MDR-TB strains harbouring the Ile491Phe *rpoB* mutation are not limited to Swaziland
- At least 2 different clusters circulate in South-Africa
- These strains harbour resistance mutations to other first-line TB drugs
- Highly-mobile mining workforce may play an important role in spreading this hard-to-detect MDR-TB strain
- Mass-screening for this mutation in Southern-African countries is urgently needed. It may be justified in other coutries.

Acknowledgments

Léonie Goeminne
Fairouz Boutachkourt
Dr Rosine Gros
Alexandre Colmant
Prof. M. Delmée

Prof. B de Jong

Dr Ndivhuho Makhado
Edith Matabane
Maphoshane Nchabeleng

Marco Schito

Contact: emmanuel.andre@uclouvain.be