

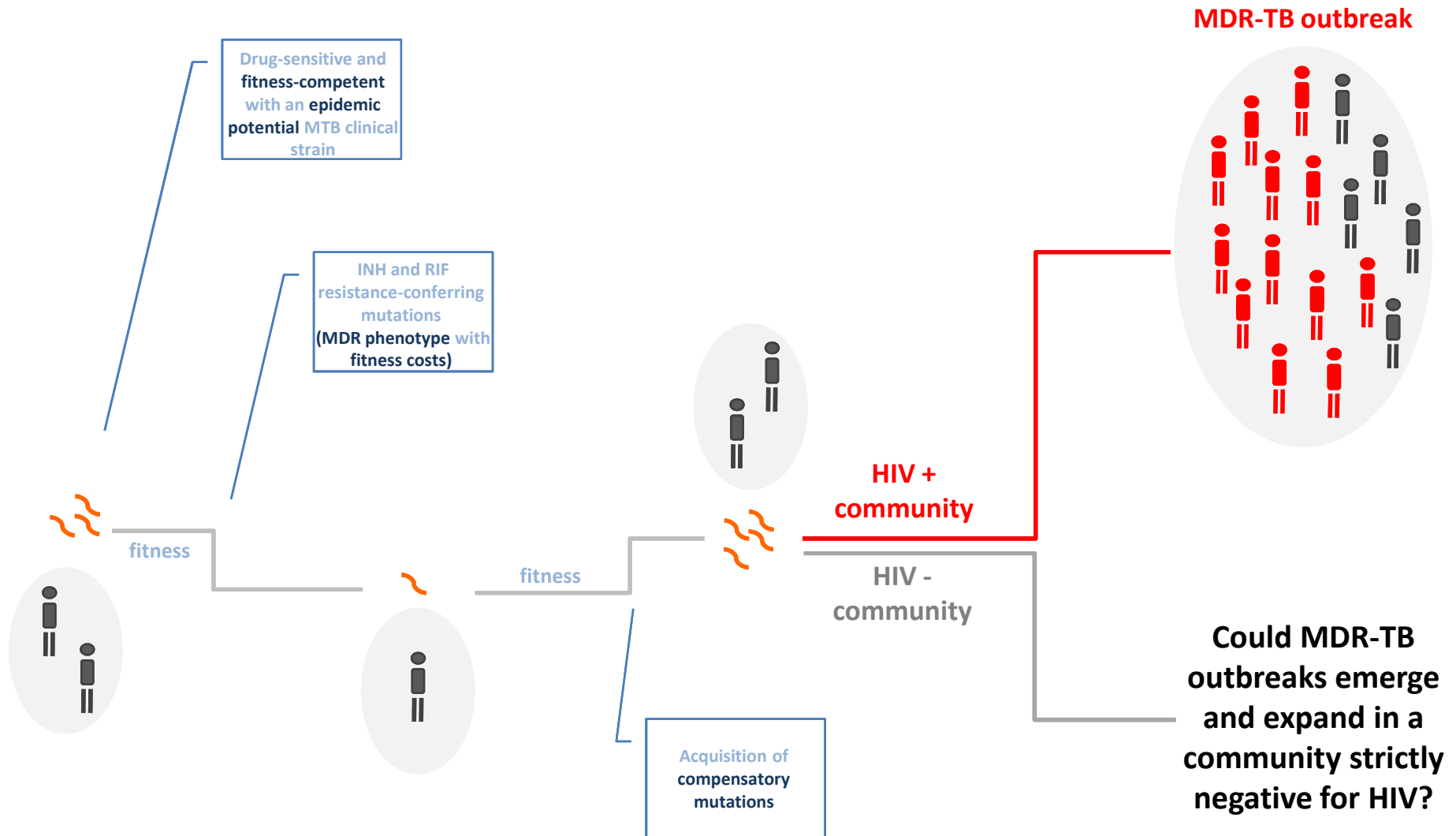
# A ten-year evolution of a multidrug-resistant tuberculosis (MDR-TB) outbreak in an HIV-negative context, Tunisia (2001-2011)

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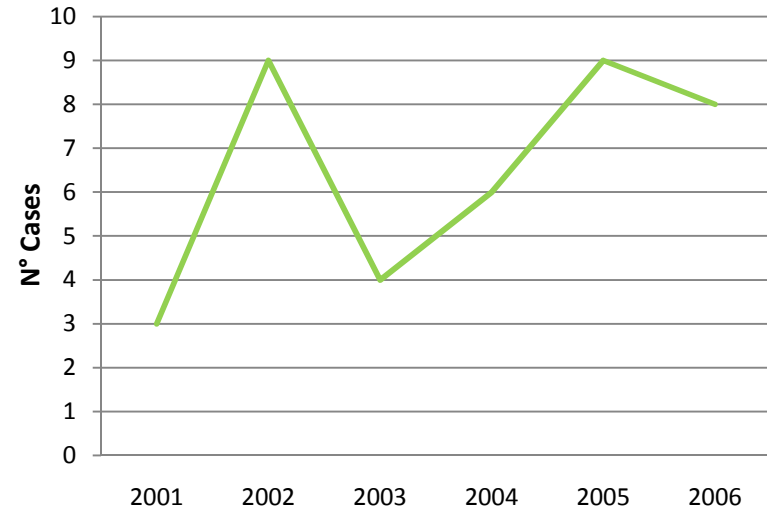
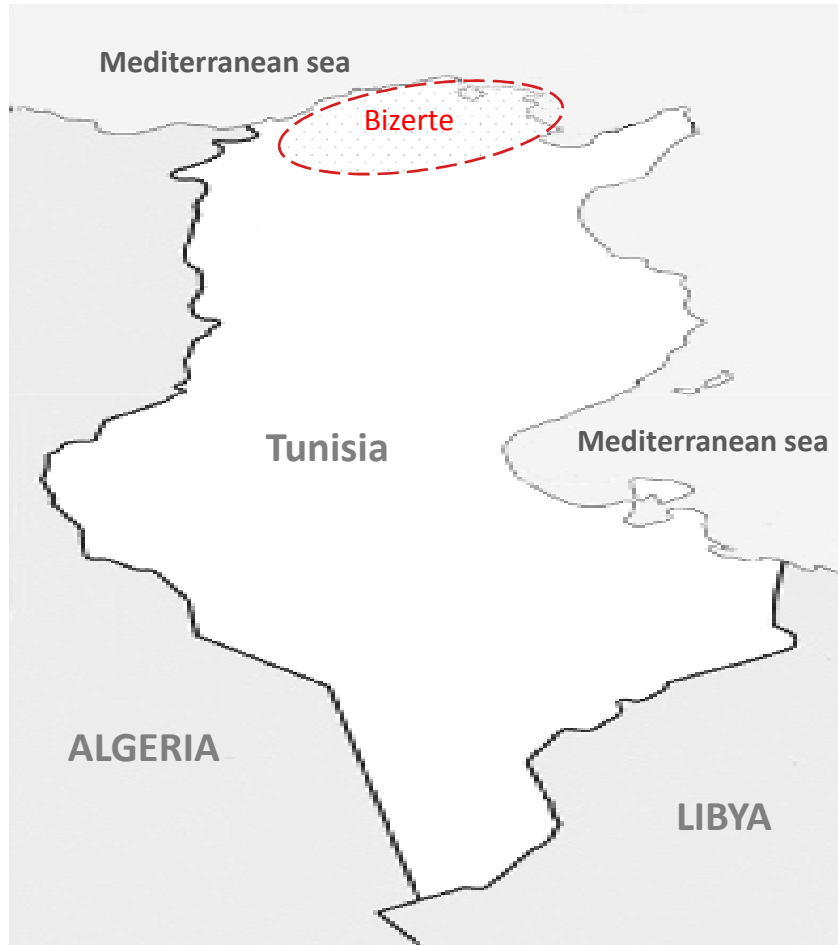
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# MDR-TB outbreaks have been mainly described in HIV-positive institutionalized individuals



# An MDR-TB outbreak emerged in Tunisia and expanded in an HIV-negative context

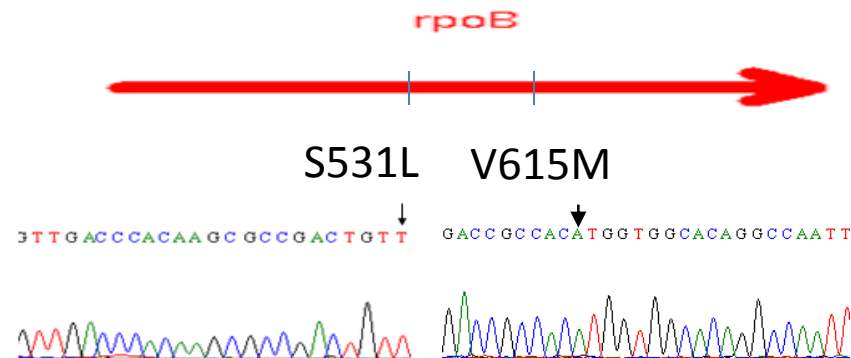
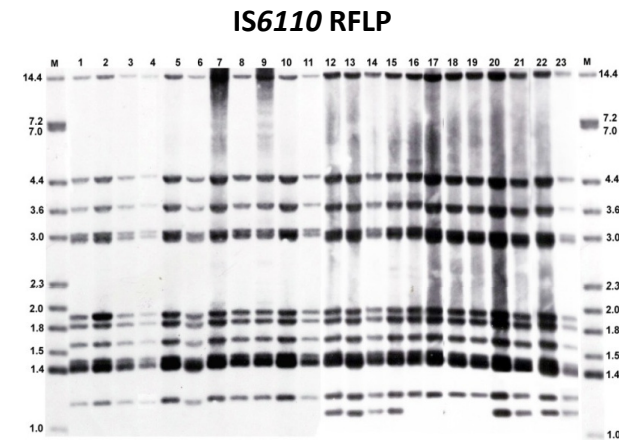
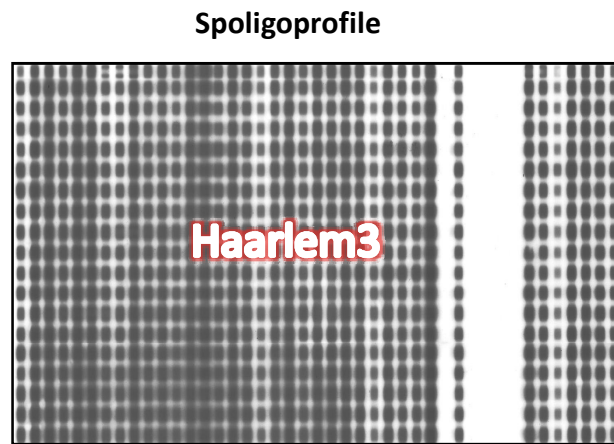


## The Tunisian MDR-TB outbreak major characteristics

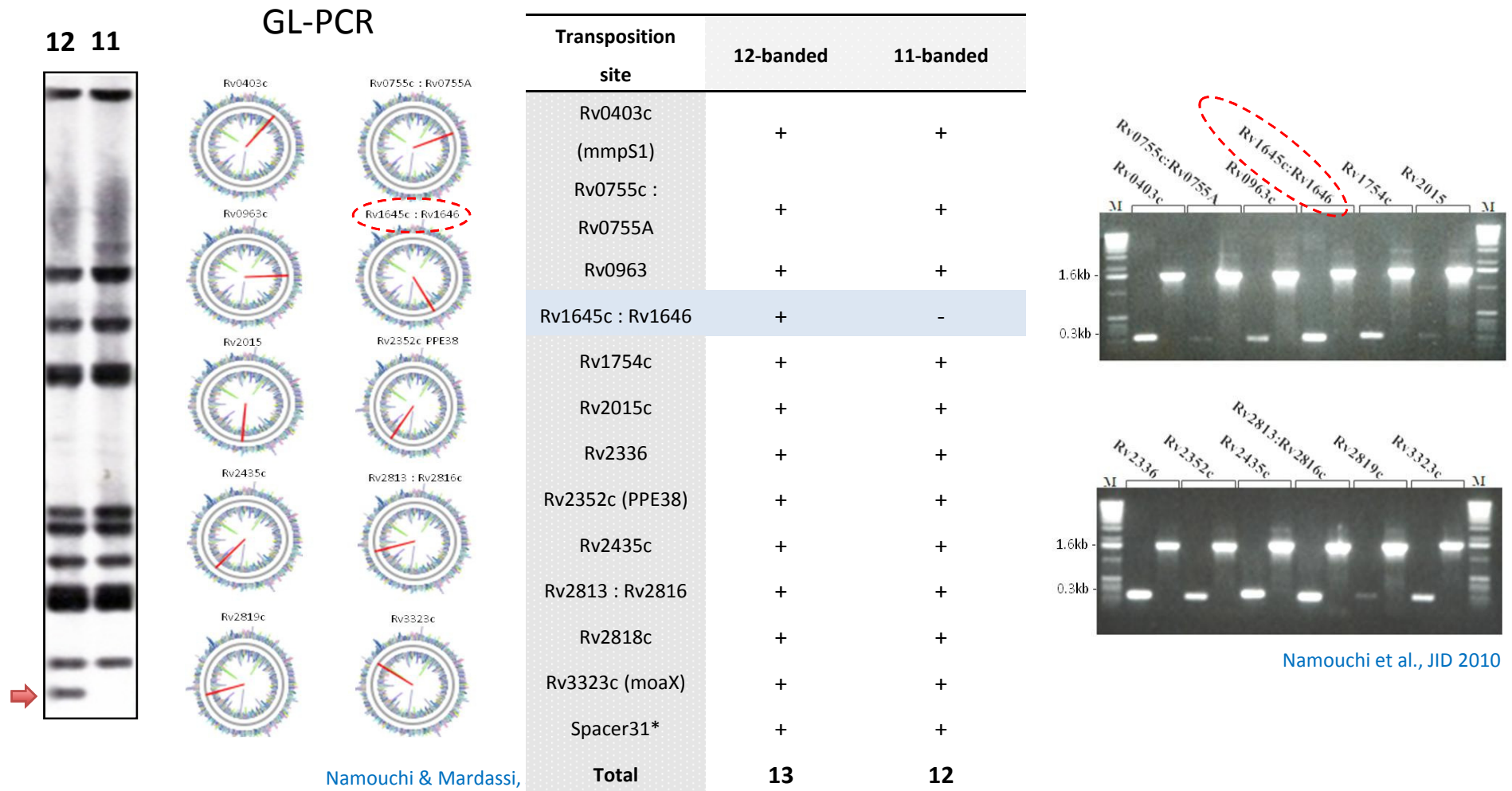
- ☀ The outbreak expanded in the general community (non institutionalized)
- ☀ Affected young (mean age ~27 yrs), immuno-competent and HIV-negative individuals
- ☀ The involved strains grow profisciently « *in vitro* »

(Mardassi et al., EID 2005)

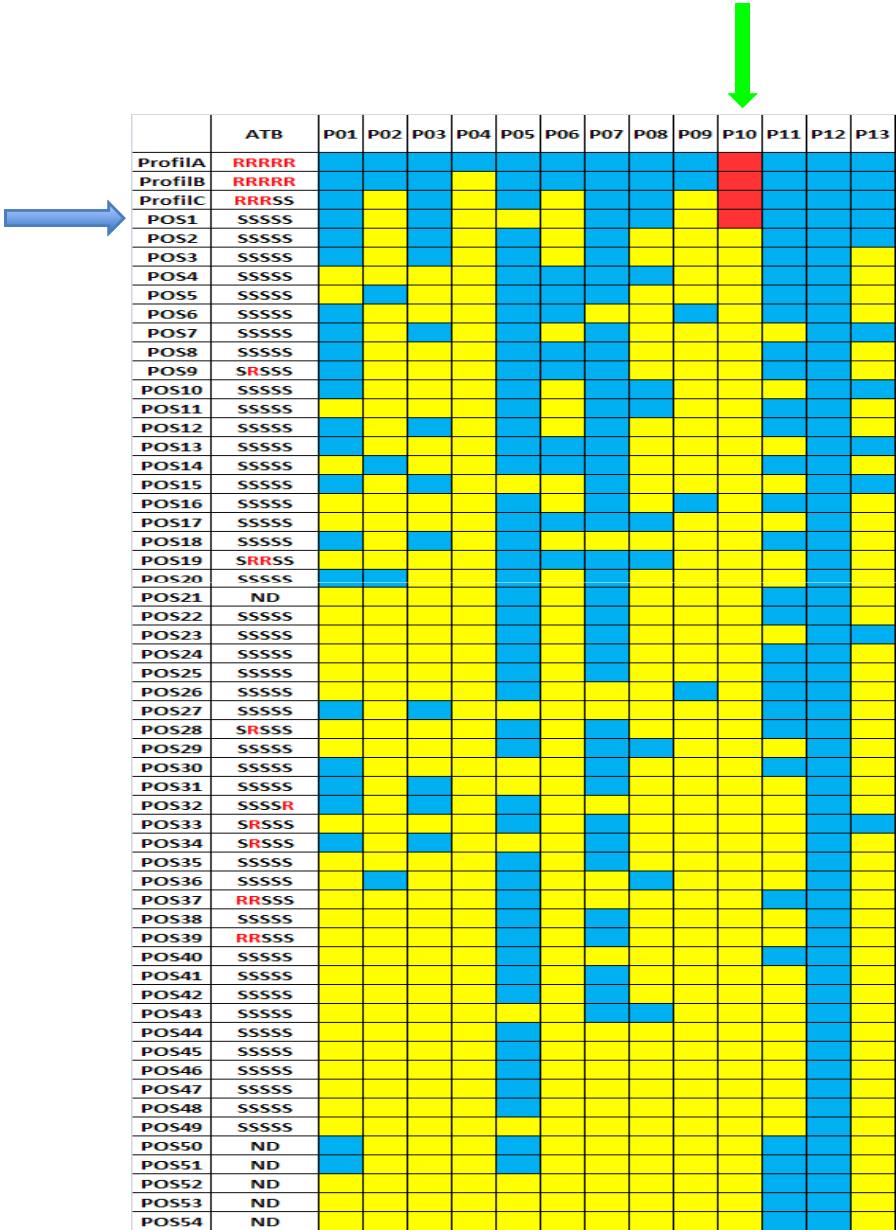
# The Tunisian MDR-TB outbreak involved a Haarlem3 genotype clone harboring a rare *rpoB* secondary site mutation, V615M



# Development of a PCR-based test for the differentiation of the 12- and 11-banded profiles



# Identification of the closest drug-sensitive pre-outbreak strain



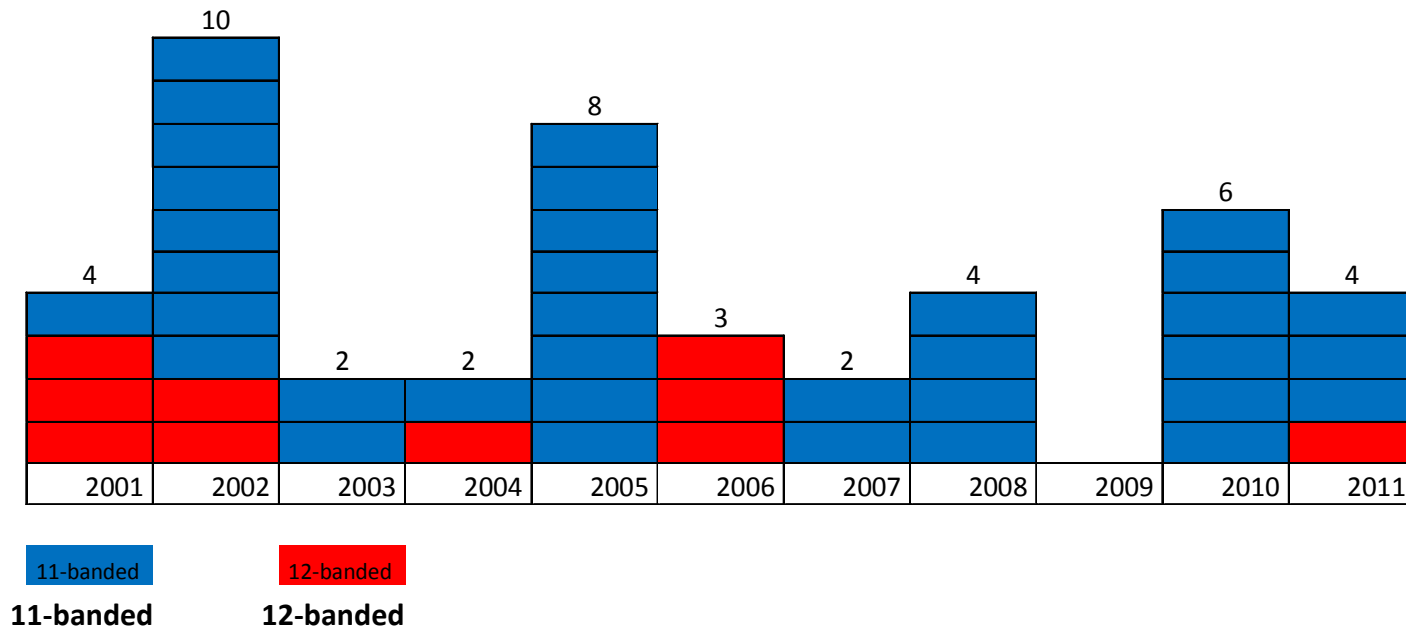
# Objectives



- ✓ Appreciate over a 10-year period (2001-2011) the clinical characteristics of the MDR-TB outbreak and the treatment outcome
- ✓ Carry out a comparative genomics analysis to better understand the molecular basis underlying the epidemic phenotype
- ✓ Carry out an in-depth, 10-year spanning, genotypic analysis of the strains circulating in the epidemic region



The MDR-TB outbreak was more frequently associated with the 11-banded IS6110 RFLP profile



## Aside from smear positivity, the 11- and 12-banded outbreak strains behave similarly

| Characteristics                      | All outcomes<br>N=45 (%) | 11-banded<br>IS6110 RFLP<br>N=35 | 12-banded<br>IS6110 RFLP<br>N=10 | Matched OR<br>(95% CI) | P value<br>(Pearson's Chi-square test) |
|--------------------------------------|--------------------------|----------------------------------|----------------------------------|------------------------|--|
| Age (years,means)                    | 29,72                    | 29,46                            | 30,63                            |                        | 0,774                                  |
| <20 (                                | 4 (8,89%)                | 3                                | 1                                | 1,19 (0,107-13,3)      | 0,887                                  |
| 20-40                                | 27 (60%)                 | 21                               | 6                                | 1 (0,163-6,138)        | 1                                      |
| >40                                  | 5 (11,11%)               | 4                                | 1                                | 0,857 (0,082-8,965)    | 0,898                                  |
| Male                                 | 40 (88,89)               | 31                               | 9                                | 0,861 (0,085-8,706)    | 0,899                                  |
| Smear-positive                       | 15 (33,33%)              | 9 (25,71%)                       | 6 (60%)                          | 4,333 (0,992-18,938)   | 0,043*                                 |
| epidemiological link                 | 10 (22,22%)              | 8                                | 2                                | 0,633 (0,107-3,733)    | 0,612                                  |
| Duration of treatment (months,means) | 30,4 (67,56%)            | 29,83                            | 32,4                             |                        | 0,81                                   |
| Outcome category:                    |                          |                                  |                                  |                        |  |
| Cure                                 | 17 (37,78%)              | 14                               | 3                                | 0,6 (0,120-3,007)      | 0,532                                  |
| Failure                              | 6 (13,33%)               | 5                                | 1                                | 0,657 (0,065-6,605)    | 0,72                                   |
| Relapse                              | 4 (8,89%)                | 4                                | 0                                | 0,750 (0,614-0,916)    | 0,257                                  |
| Death                                | 9 (20%)                  | 5                                | 4                                | 4,6 (0,849-24,929)     | 0,064                                  |

# Treatment outcome: The outbreak proved difficult to treat

|     | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 |
|-----|------|------|------|------|------|------|------|------|------|------|------|
| P1  | ●    | ●    |      | ●    | ●    | ●    | ●    | ●●   |      | ●    |      |
| P2  |      | ●    |      |      |      | ●    |      |      |      |      |      |
| P3  |      | ●    |      |      |      |      | ●    |      |      |      |      |
| P4  |      | ●    | ●    | ●    |      |      |      |      |      |      |      |
| P5  |      | ●●   | ●    |      |      |      |      |      |      |      |      |
| P6  |      | ●    | ●●   |      | ●    |      |      |      |      |      |      |
| P7  | ●    |      | ●    | ●    |      |      |      |      |      |      |      |
| P8  |      |      |      |      | ●●●  | ●    | ●●   | ●    | ●●   | ●●   |      |
| P9  |      |      |      |      | ●●   | ●●   |      |      |      |      |      |
| P10 |      |      | ●    |      | ●    | ●    |      | ●    | ●●   | ●    | ●●●  |
| P11 |      |      |      | ●    | ●●●  | ●●●  | ●●●  | ●●●  |      |      |      |
| P12 |      |      |      |      | ●    | ●    | ●●●  | ●●   | ●    | ●●   |      |
| P13 |      |      |      |      |      | ●●●  | ●●●  | ●●●  |      |      |      |
| P14 |      |      |      |      | ●    | ●●●  | ●●   | ●●●● | ●●   | ●●●● | ●    |
| P15 |      | ●●   |      |      | ●    | ●    | ●    | ●    |      |      |      |
| P16 |      |      |      |      | ●    |      |      |      | ●    |      |      |

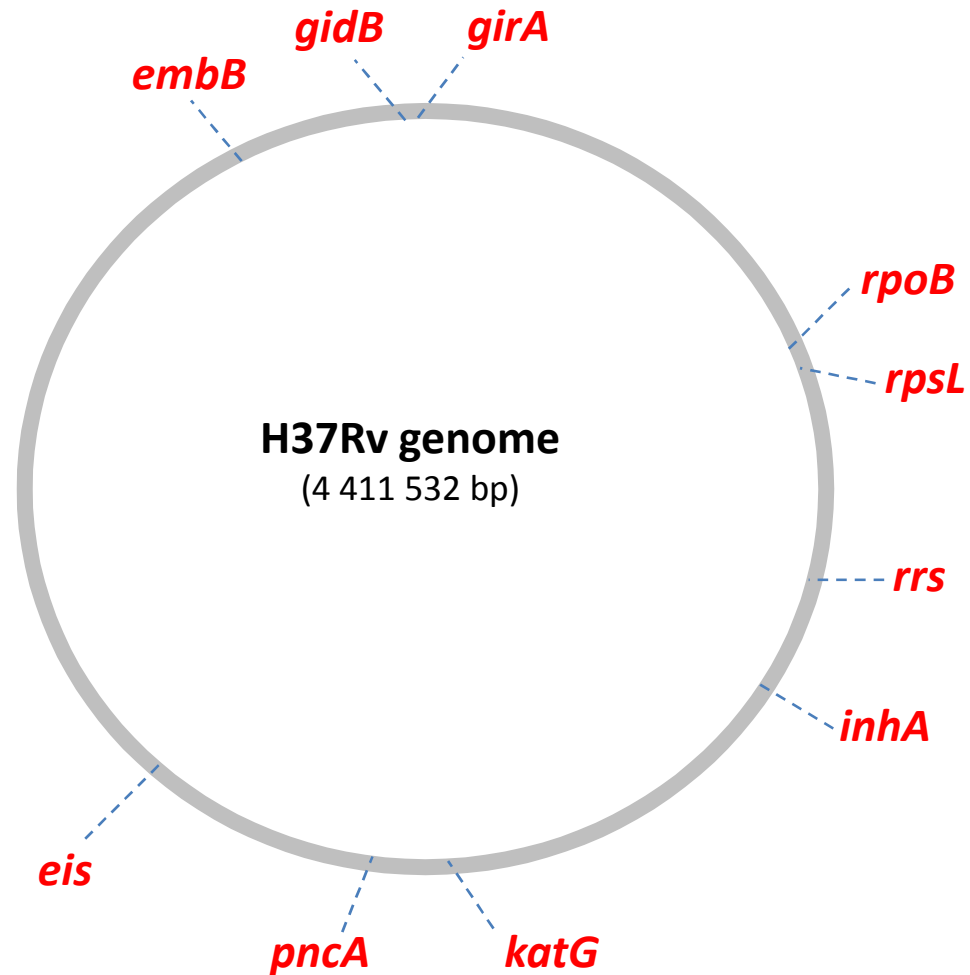
● 12-banded

● 11-banded

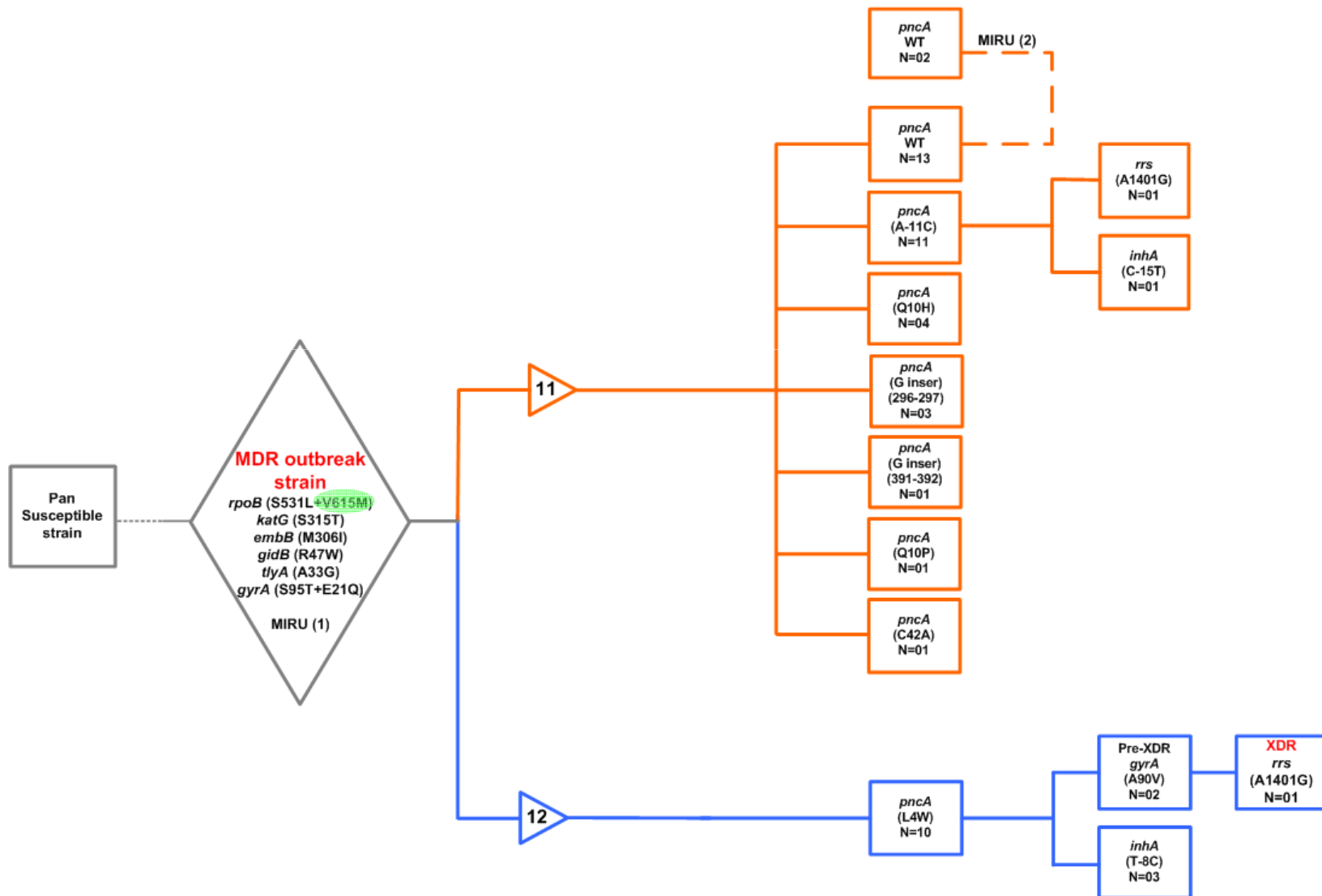
## Death was significantly associated with relapse and chronic cases

|             | Age, mean   | Smear positivity                     | Relapse                                      |
|-------------|-------------|--------------------------------------|--|
| Death (N=9) | $P = 0,082$ | $P = 0,197$                          | $P = 0,002$                                  |
|             |             | Matched OR (95% CI)<br>4 (0,431-3,7) | Matched OR (95% CI)<br>0,052 (0,00562-0,492) |
| %           |             | 11,11%                               | 88,88%                                       |

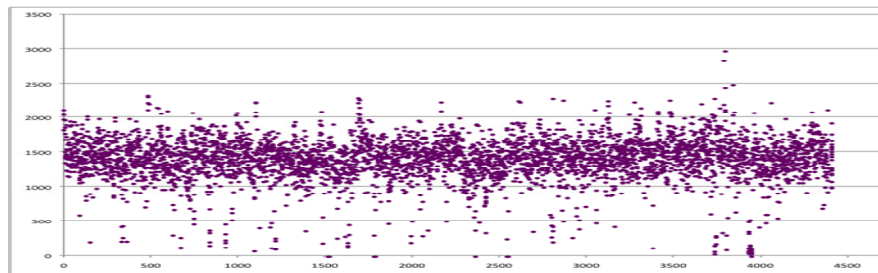
# Mutational analysis of drug resistance genes



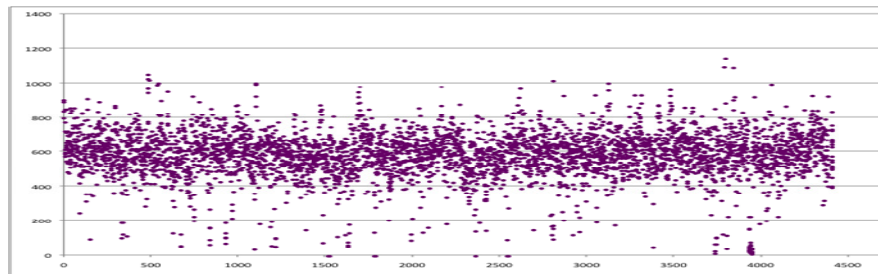
# Evolution of the MDR-TB outbreak based on mutations in drug resistance genes



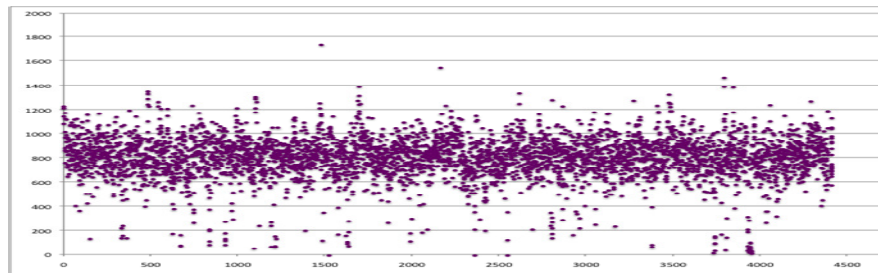
# Genome sequencing using the Illumina platform



11-banded MDR H3 outbreak strain 2078



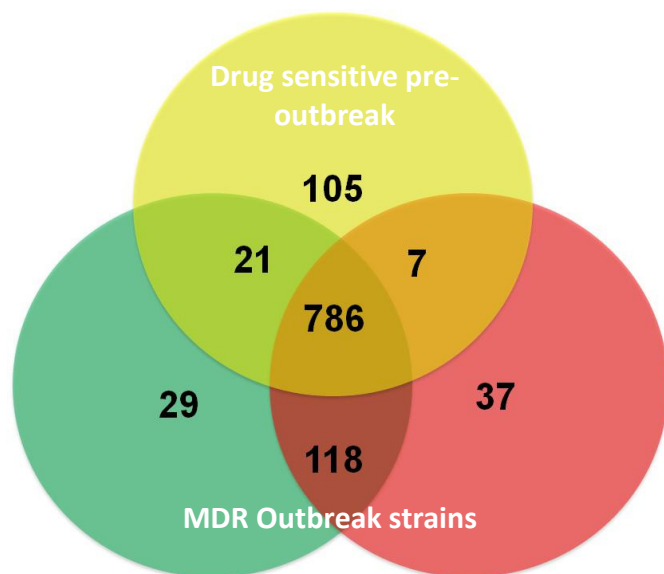
12-banded MDR H3 outbreak strain 1183



Susceptible genetically related H3 strain 233

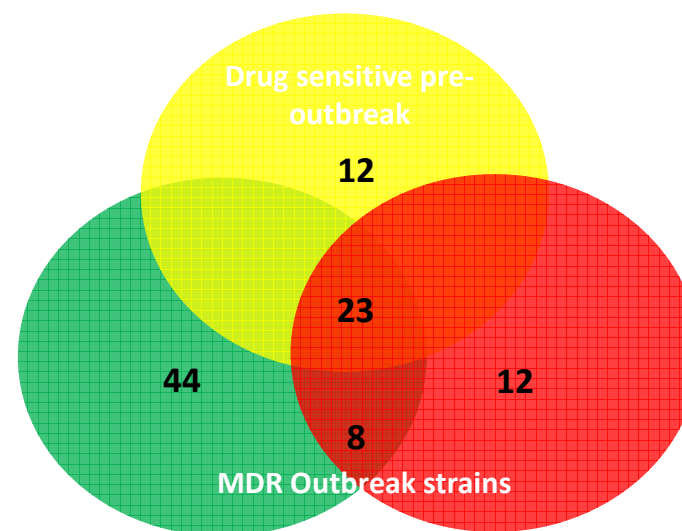
# Comparative genomics: Statistics

Whole genome **SNPs**-based Venn diagram



SNP: Single Nucleotide Polymorphism

Whole genome **LSPs**-based Venn diagram



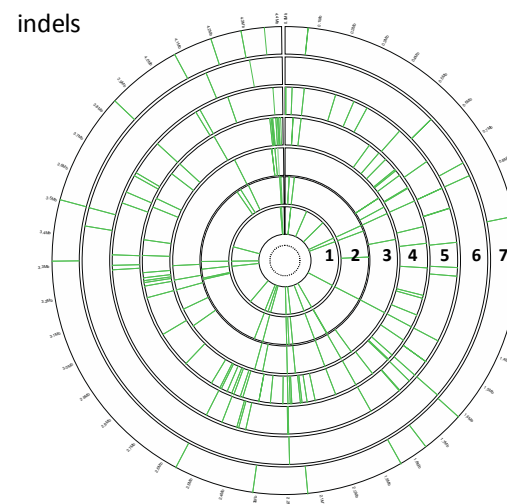
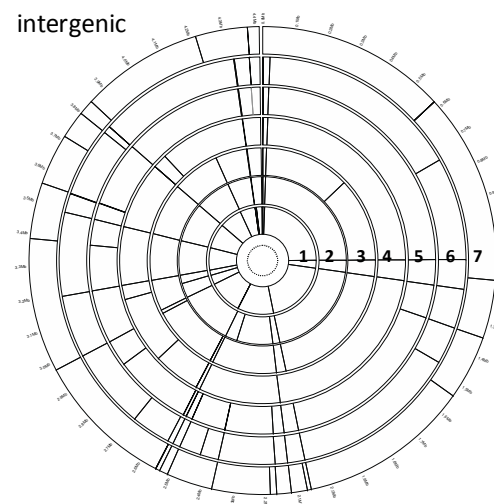
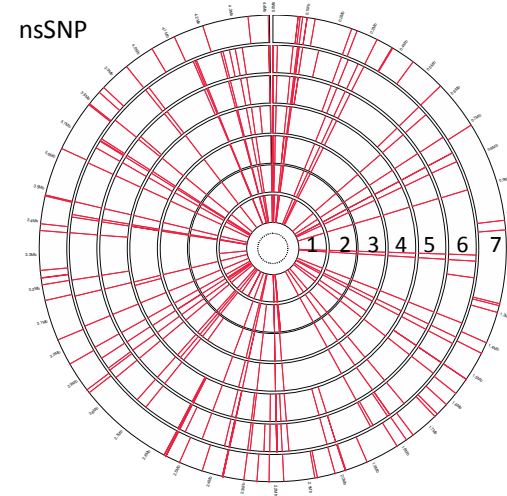
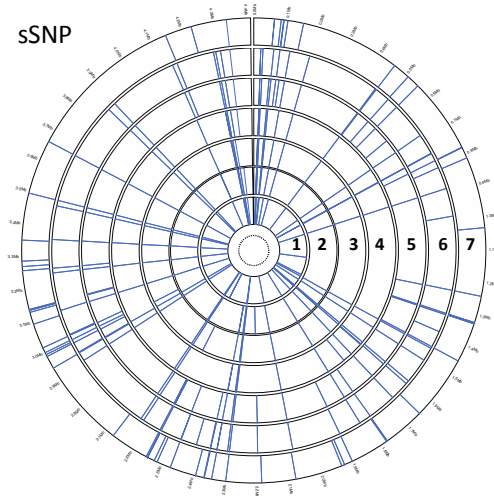
LSP: Large Sequence Polymorphism ( $\geq 10$  bp)



## Few Indel events differentiate other outbreak strains described worldwide

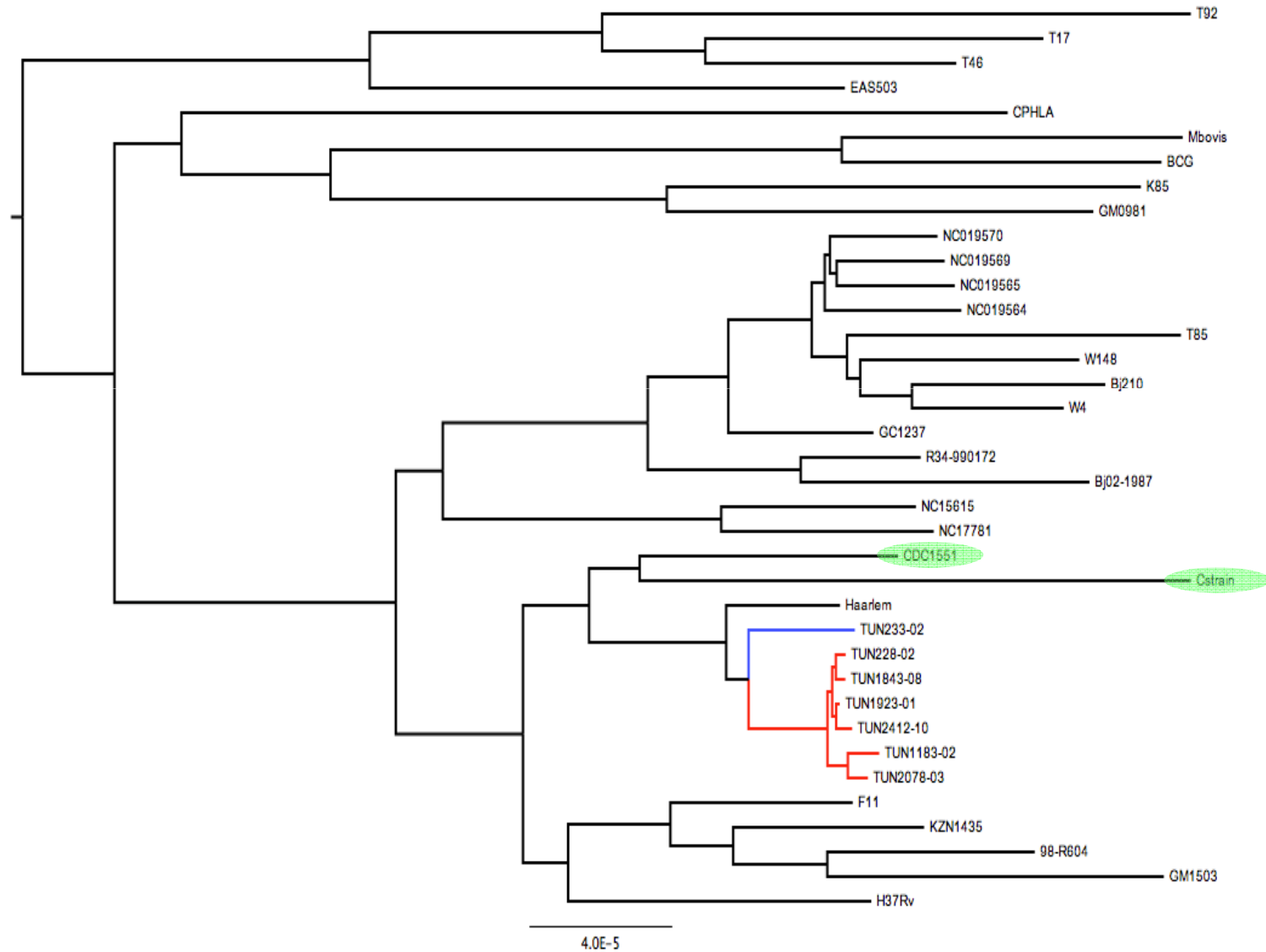
| Haarlem3<br>(Hamburg)<br>N= 89 | Outbreak<br>(San Francisco)<br>N=09 | Beijing<br>(Uzbekistan)<br>N=02 |
|--------------------------------|-------------------------------------|---------------------------------|
| 5 SNPs + 5 short deletions     | 7 SNPs+ 0 indels                    | 130 SNPs+ 1 large deletion      |
| (Andreas Roetzer,2013)         | Midori Kato-Maeda, 2013)            | (Niemann S, 2009)               |

# Indels contributed significantly to the clonal diversification of the MDR-TB outbreak-associated strains



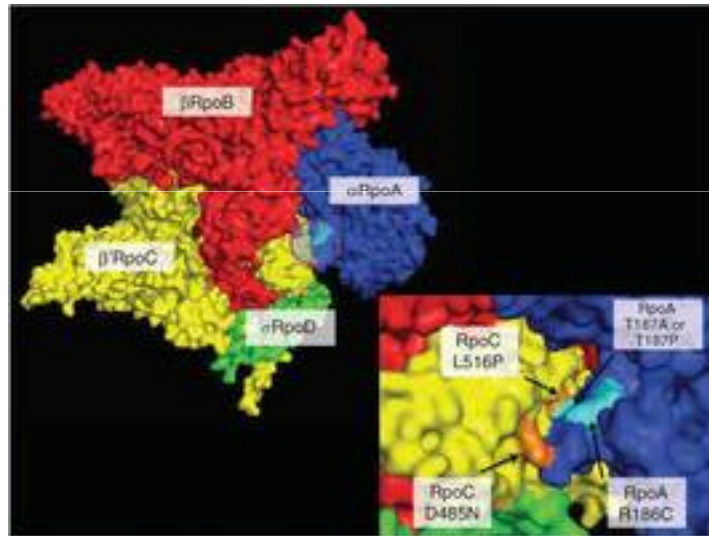
- 1: TUN2412-10
- 2: TUN1923-01
- 3: TUN1843-08
- 4: TUN228-02
- 5: TUN2078-03
- 6: TUN1183-02
- 7: TUN233-02

# Genome-wide-based Maximum Likelihood phylogenetic tree

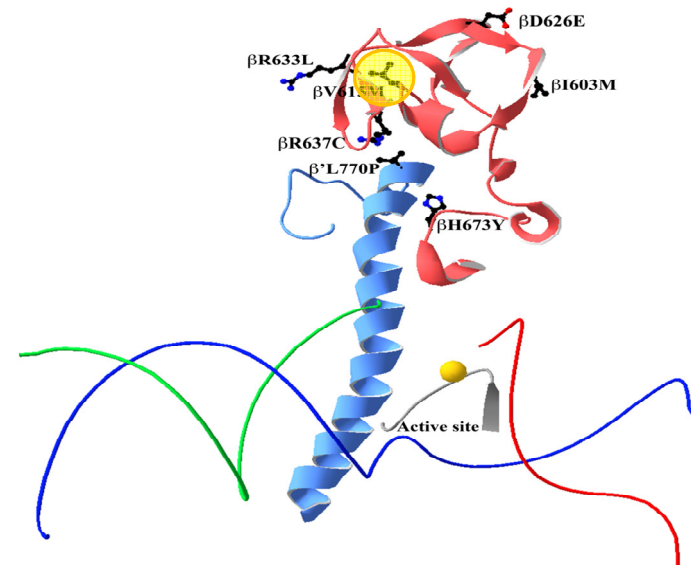


What have we learned from  
microgenomics on the biology of  
the MDR-TB outbreak?

Comparative genomics coupled to structural analysis disclosed the possible role of the *rpoB* secondary mutation, V615M, in fitness cost compensation



No putative compensatory mutations either in *rpoC* or in *rpoA* could be identified

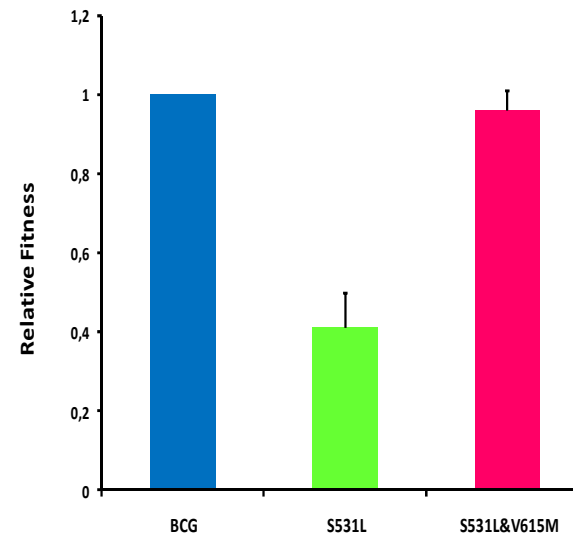


V615M maps to the flexible bridge helix structure which interact with DNA

# The outbreak-restricted secondary site *rpoB* mutation, V615M, did indeed restore the fitness costs of S531L

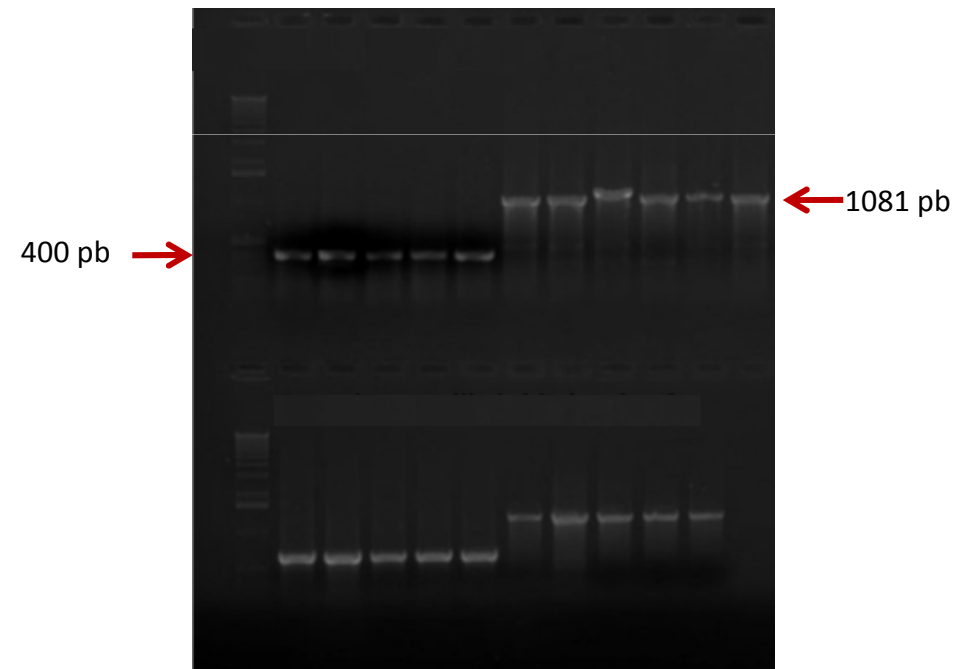
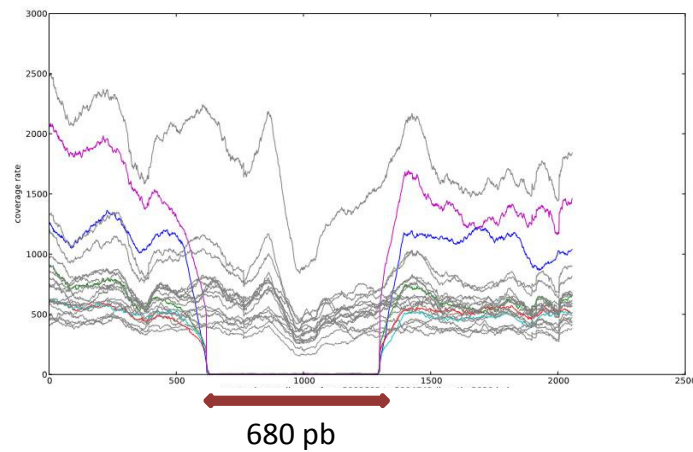
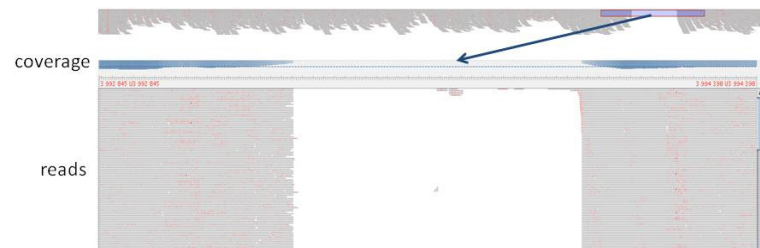


Engineered mutant BCG harboring V615M +S531L grows as efficiently as WT-BCG

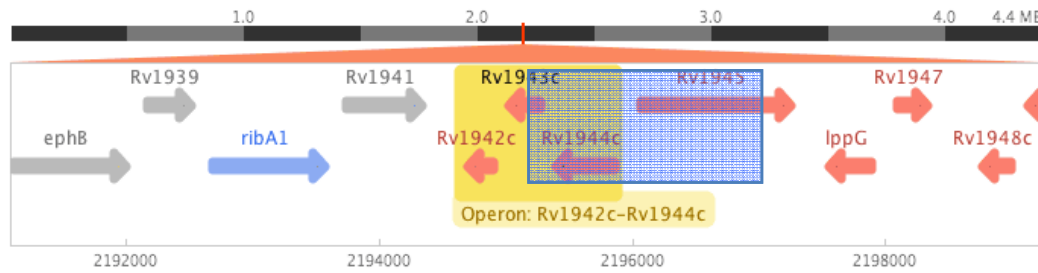


Engineered mutant BCG harboring V615M +S531L and WT-BCG display comparable fitness

# An in-frame deletion in the ferredoxin gene is likely to be critical to the epidemic potential of the Tunisian MDR outbreak strain



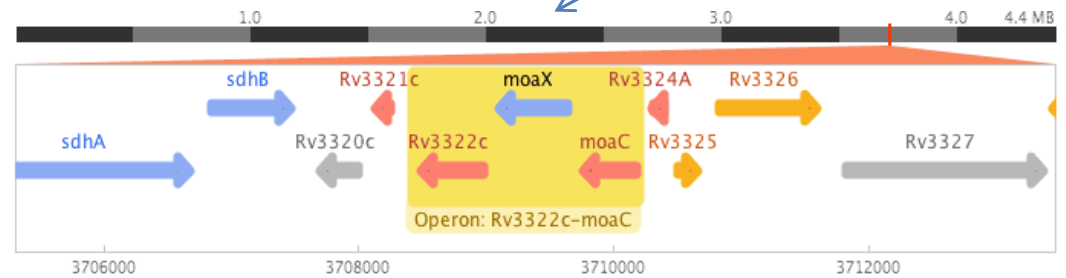
# Shared, outbreak-restricted, deletions to be further explored by functional genetics



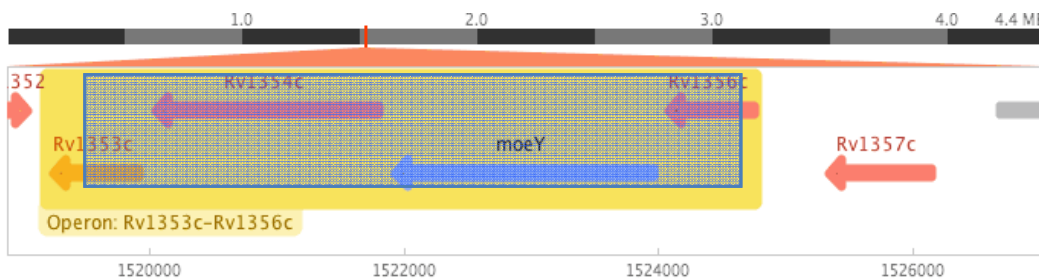
1.9 kb



1 kb



4.7 kb





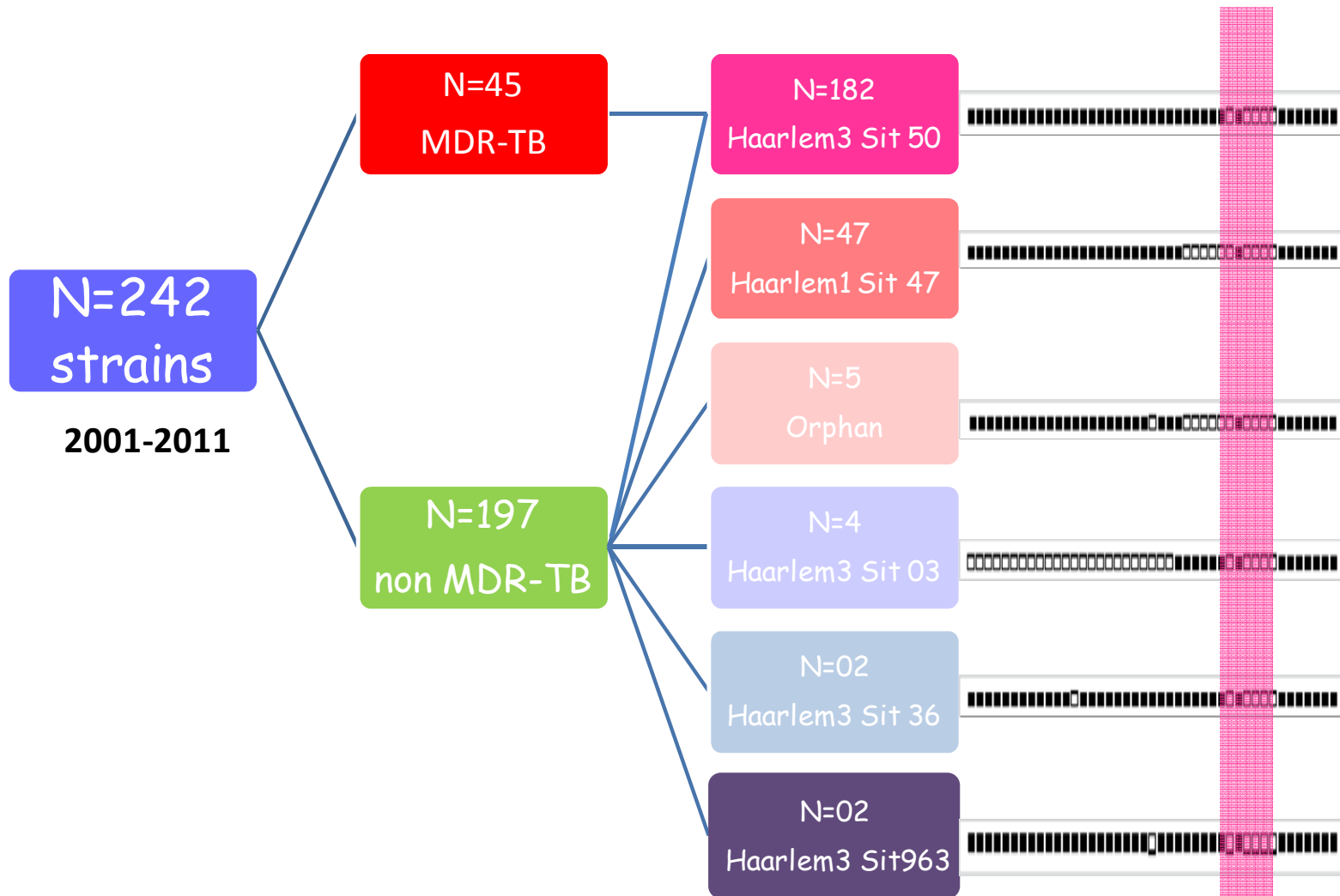
## Main outputs from comparative genomics

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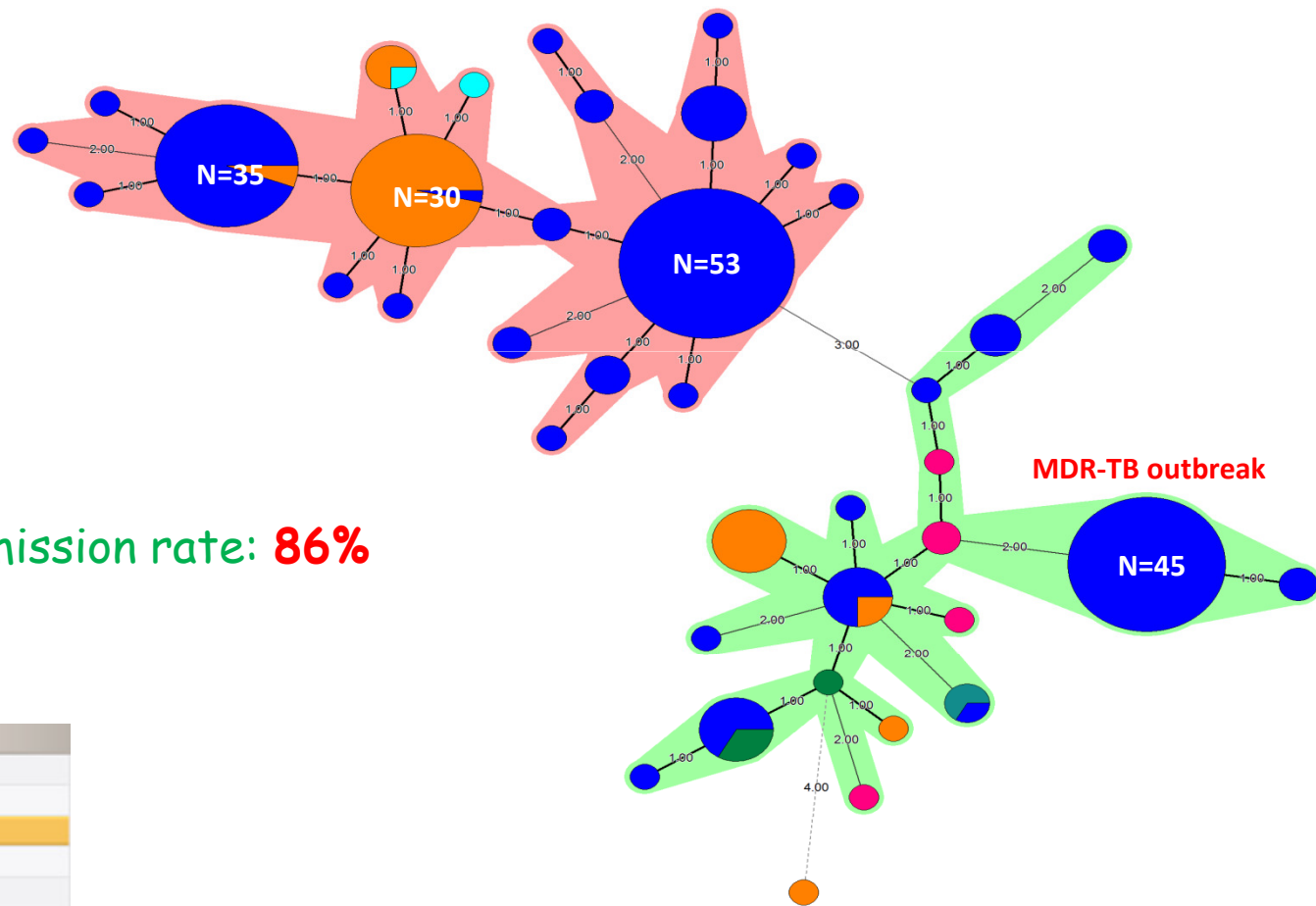
- Phylogenomics confirm the relatedness of the Tunisian MDR-TB outbreak strain with the epidemic CDC1551 and C strains
- The genome of the MDR-TB outbreak strain appears to evolve rapidly, mainly through frequent indel events
- Rationale comparative genomics identified key deletion events which could have contributed to the epidemic phenotype of the Tunisian MDR outbreak strain

An in-depth snapshot of the molecular epidemiology of the *M. tuberculosis* Haarlem genotype in the epidemic region

All Haarlem strains and variants co-evolving with the MDR-TB outbreak (2001-2011) were included in a MIRU-VNTR24 typing analyses



# The MTB Haarlem strain family, northern Tunisia, is likely to be intrinsically epidemic and genetically unstable



| Size | Spoligotype |
|------|-------------|
| 182  | H3 SIT50    |
| 47   | H1 SIT47    |
| 5    | Orphan      |
| 4    | H3 SIT03    |
| 2    | H3 SIT36    |
| 2    | H3 SIT963   |

# Conclusions

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- MDR-TB outbreaks can emerge and successfully expand in a HIV-negative context
- Evolution to the XDR phenotype is likely to be associated with the genetic trait of the involved strain rather than treatment default
- The Tunisian MDR-TB outbreak benefited of an intrinsic epidemic potential coupled to a rapid genomic evolution, mainly through indels

THANKS

A 3D rendered scene featuring the word "THANKS" in large, colorful, block letters. Each letter is held by a small, white, cartoonish character with a single eye and thin limbs. The characters are standing on a light gray reflective surface. The letters are colored as follows: 'T' is red, 'H' is orange, 'A' is yellow, 'N' is light green, 'K' is dark green, and 'S' is blue. The background is a plain, light gray gradient.



