

*Robert Koch
Terrassant son bacille*



March 24th 1882 : announcement of
Discovery of TB bacillus

Multi Drug Resistant tuberculosis (MDR-TB)

Vincent Jarlier
Pitié-Salpêtrière hospital
Paris, France

**National reference
center for
mycobacteria**

MDR-TB definition (WHO)

2 major antituberculous drugs :

Isoniazid (INH)

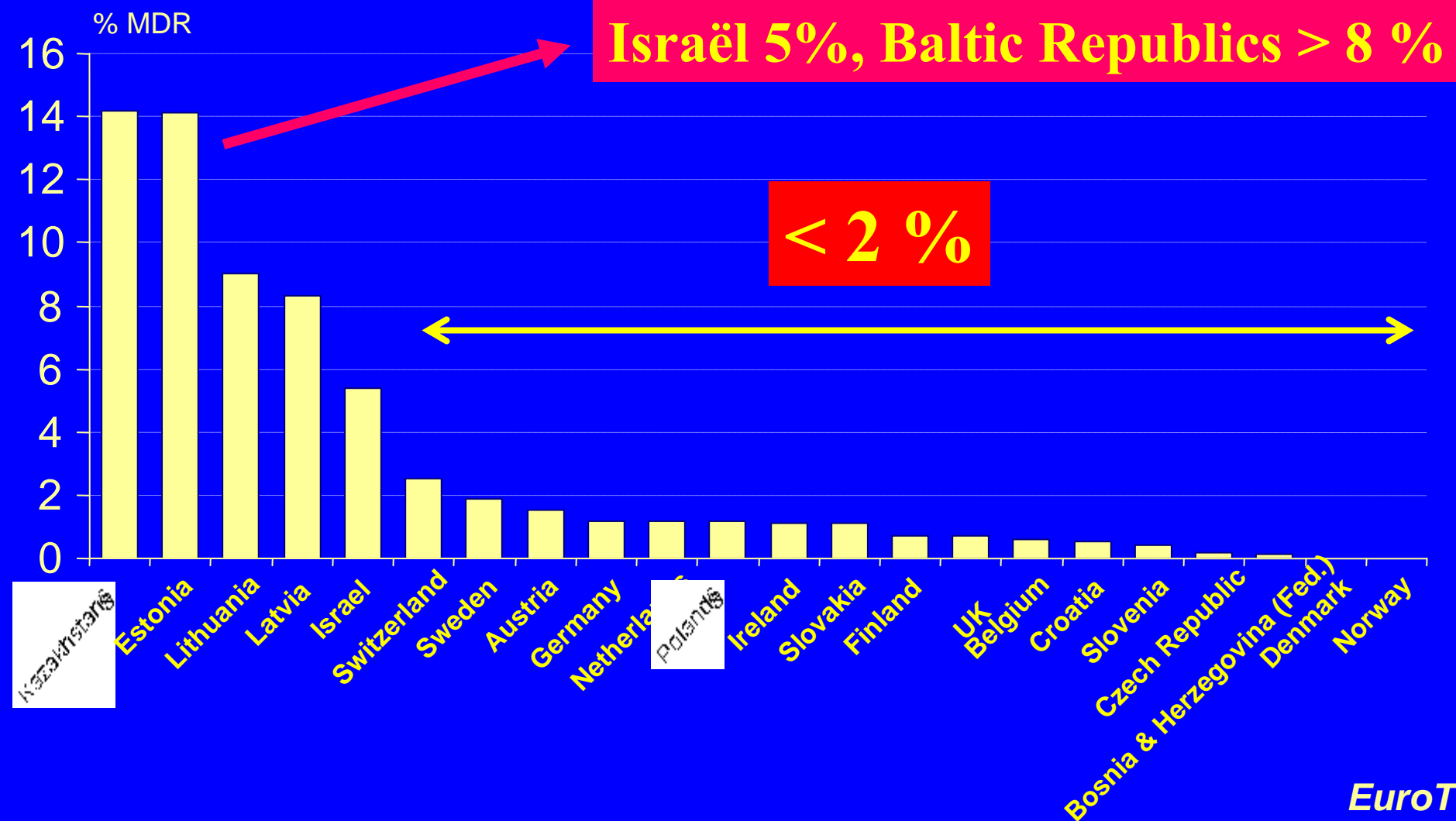
Rifampicine (RIF)

MDR =

resistance to INH and RIF

% MDR in new TB cases in Europe

EuroTB 2003



MDR-TB in the world

WHO 3rd report 2004

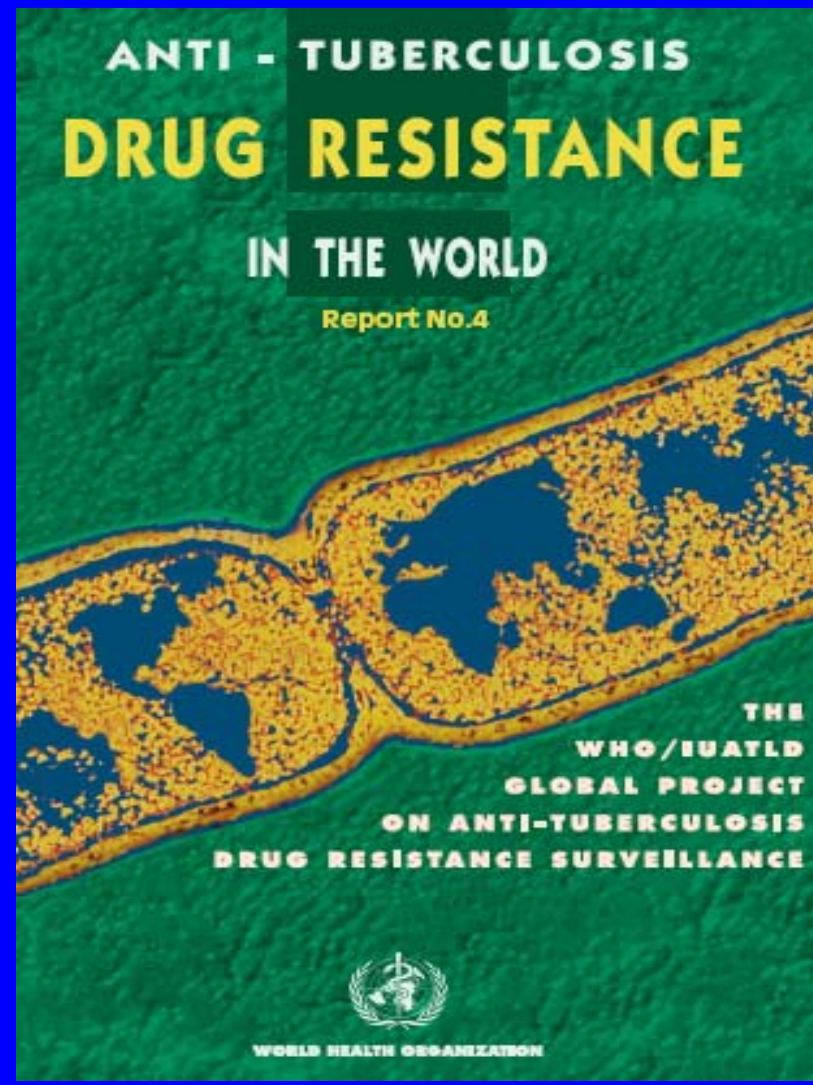
- Estimation : 424 000 cases/year
- (~ 4% of ~ 10 millions cases)
- **Primary** : 243 000 (**3%** of the **new cases**)
- **Secondary** : 181 000 (**19%** of the **prev. Tt cases**)
- **China, India, Russia** : 261 000 (= **62%** of total **MDR cases**)
- **> 10% MDR** : Estonia (17%), Georgia (16%), Azerbaijan (15%), Moldavia (15%), Kazakhstan (14%), Ouzbekistan (13%)

TB drug resistance in the world

WHO 4th report 2008

- 138 countries/regions
- data on drug susceptibility tests for **91,577 patients** in 81 countries, years **2002-06**

n.b. previous reports 1997,
2000, 2004



MDR TB

key findings 4th report 2008 (global)

- Estimated MDR cases in 2006 :

489,139

(95% CLs, 455,093 to 614,215)

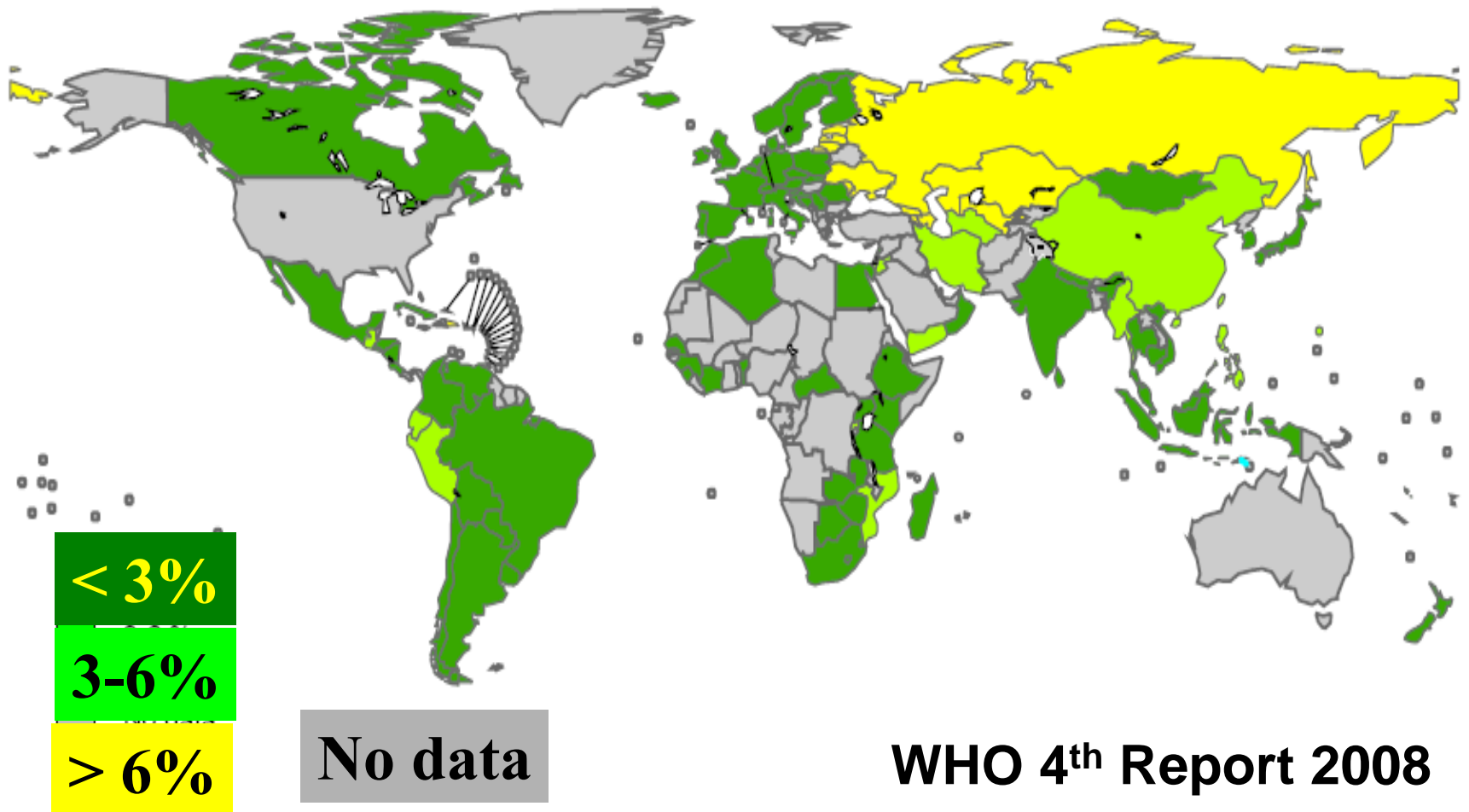
- Global proportion of MDR among all cases :

4.8 %

(95% CLs, 4.6 to 6.0)

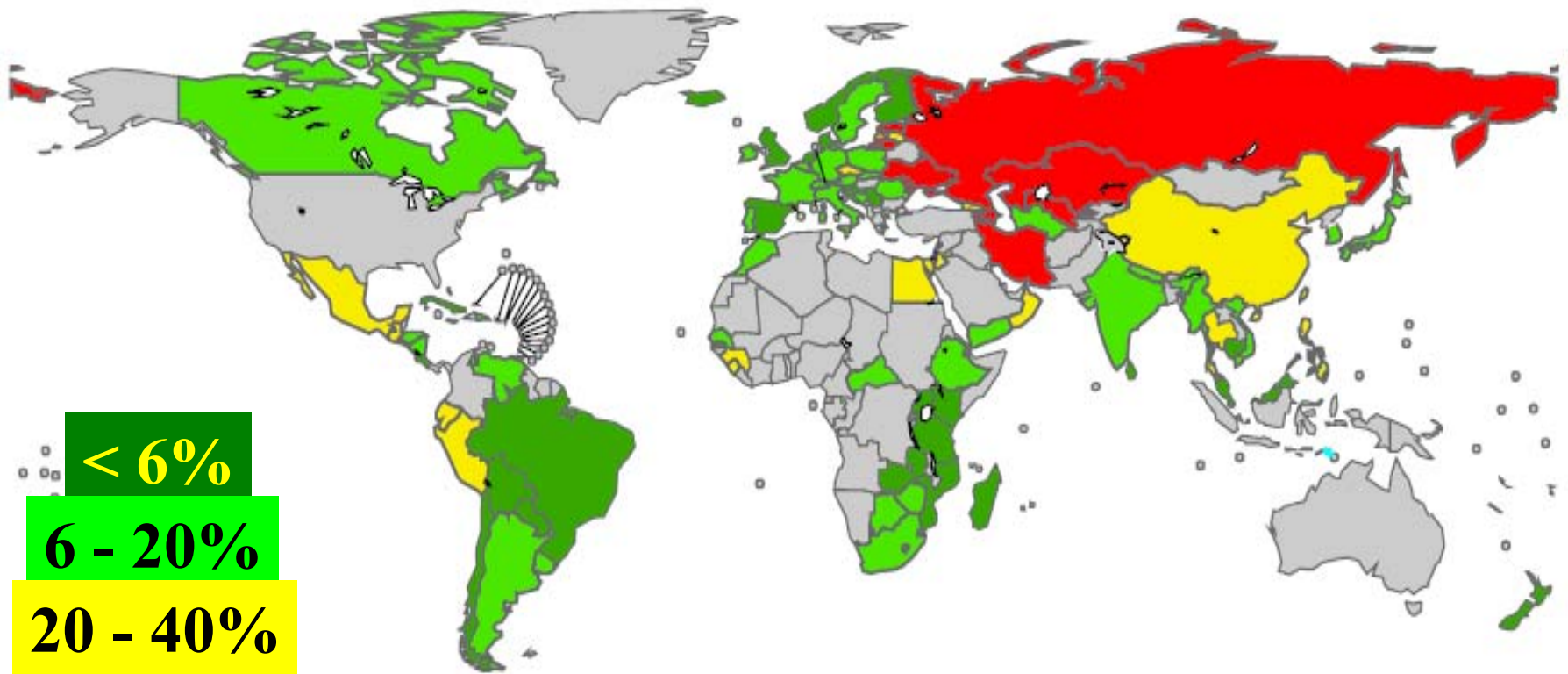
MDR-TB in new cases 1994-2007 (in %)

* Sub-national coverage in India, China, Russia, Indonesia.



MDR-TB in previously treated cases 1994-2007 (in %)

* Sub-national coverage in India, China, Russia, Indonesia.



No data

WHO 4th Report

Main antituberculous drugs for MDR

Aminoglycosides

Fluoroquinolones

Ethionamide

Ethambutol

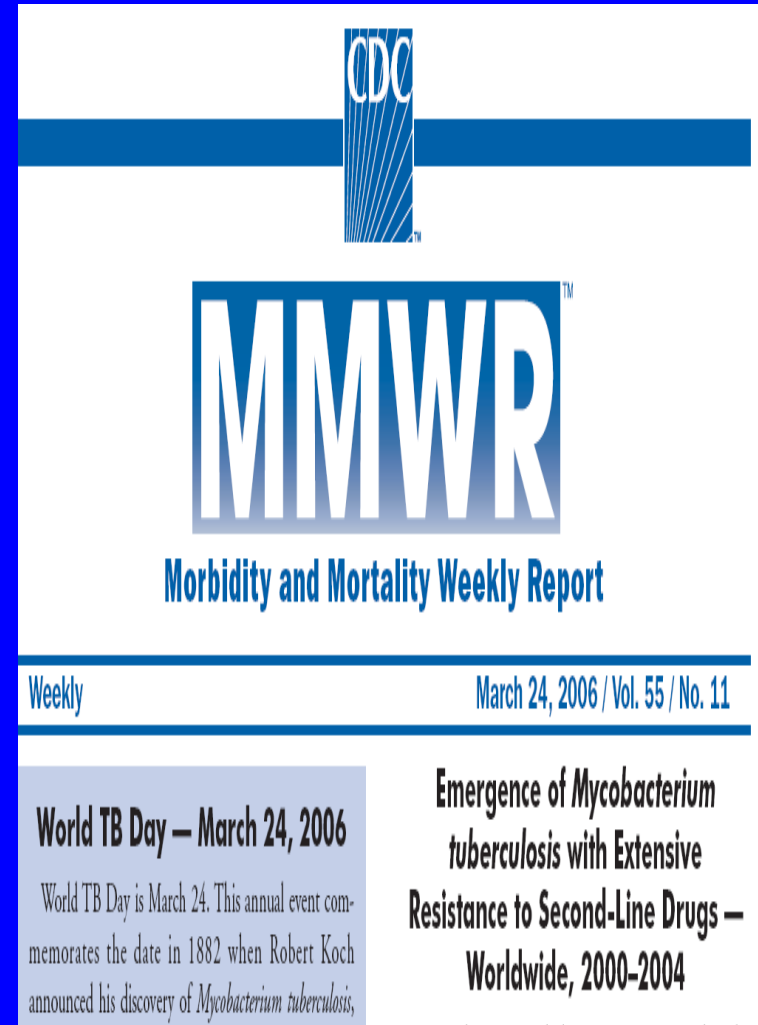
Pyrazinamide

PAS

Cycloserine

TB Extensive Resistance to 2nd line Drugs (CDC, MMWR March 2006)

XDR = resistance to :
INH and **RIF**
(MDR)
and at least to
3 of the 6 main classes
of second line drugs
(“old definition”)



Revised Definition XDR-TB

October 2006

XDR = resistance to :

INH and RIF (MDR-TB)

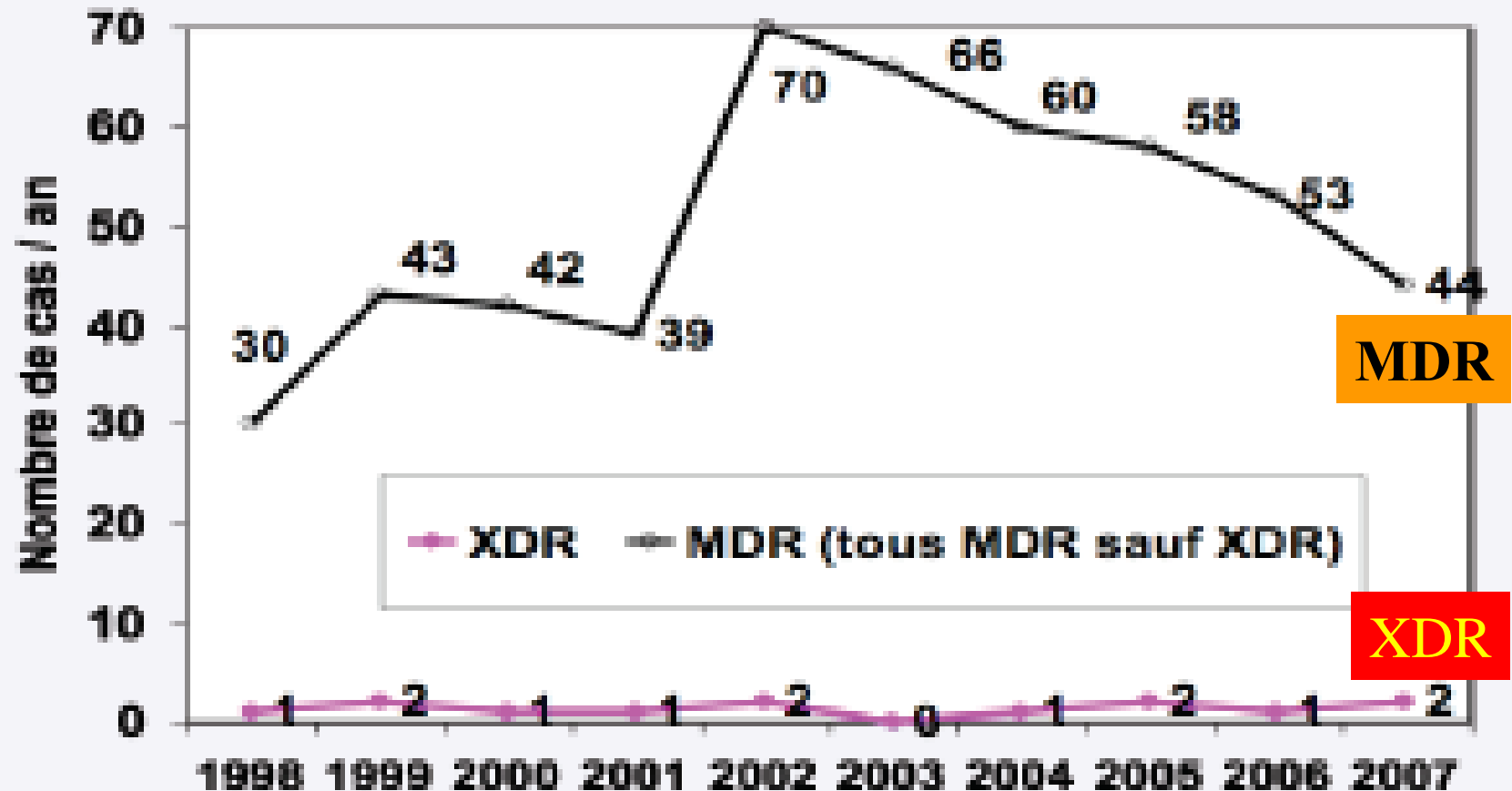
and

amikacin, kanamycin or capreomycin
(injectable agents other than streptomycin)

and

fluroquinolones

MDR-TB and XDR-TB in France 1998 - 2007

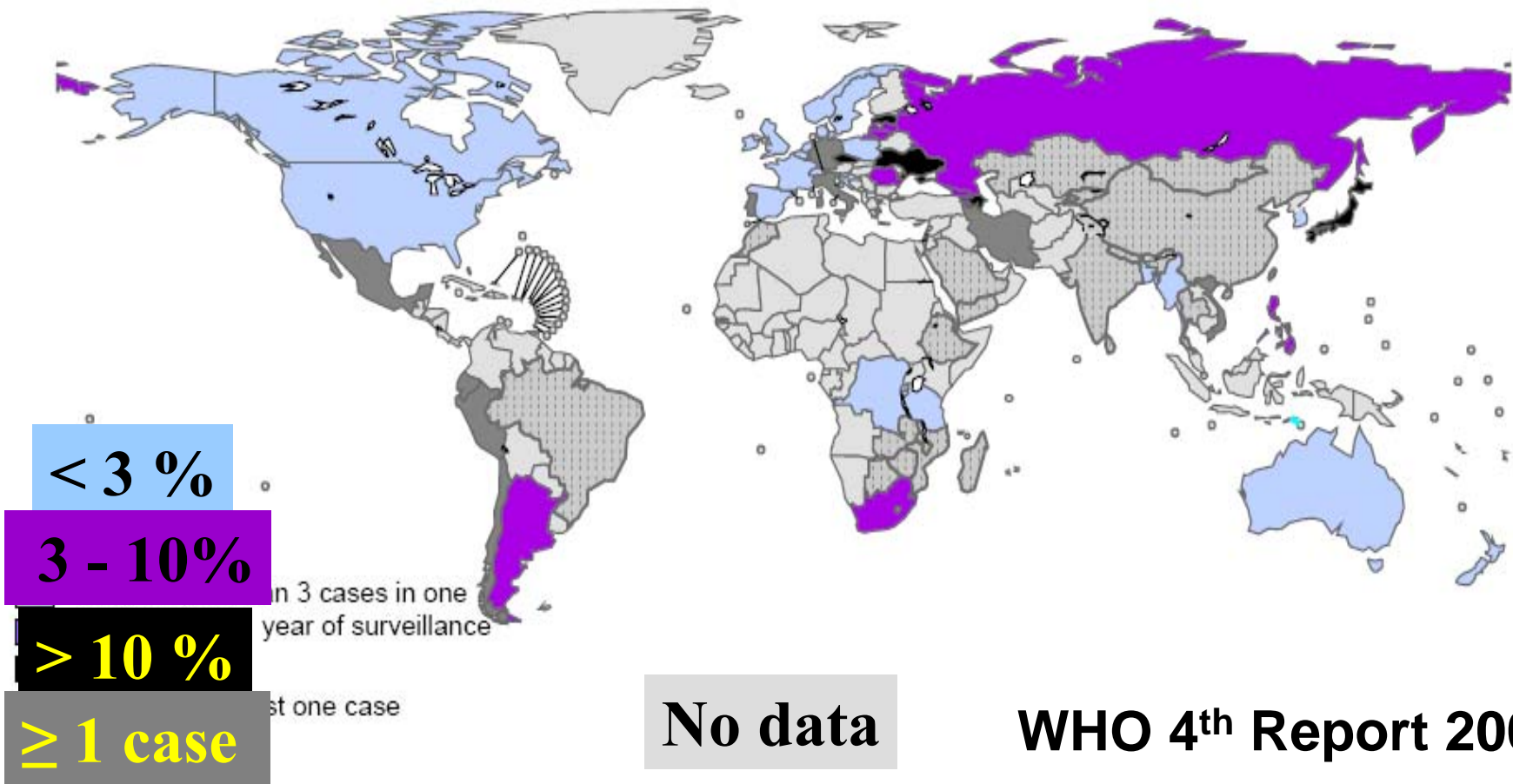


In 10 years :
517 MDR and 13 XDR (1 to 2 XDR per year)

Kanitz 2008
submitted

XDR-TB (new definition) in % of MDR cases

* Sub-national averages applied to Russia



WHO 4th Report 2008

XDR-TB : key findings 4th report 2008

- Representative data for 39 countries or regions (24 in Europe)
- >> data for ~ 5.000 MDR TB cases 2002-07

% XDR TB among MDR TB :

- **0 - 1% in 14/39** (e.g. Canada, UK, France, Denmark)
- **2 - 10% in 15/39** (e.g. USA, Australia, Netherlands Sweden, Latvia, Romania, Moldova, Armenia, Georgia)
- **> 10% in 10/39** (33% Ireland, 33% Slovenia, 31 % Japan, 24% Estonia, 20% Czech, 15% H.Kong, 15% Ukraine, 14% Lithuania, 13% Azerbaijan)

XDR-TB : key findings 4th report 2008

- Representative data for 39 countries or regions (24 in Europe) for ~ 5.000 MDR TB cases 2002-07

Only 4 countries with 10-20 cases/year :

Japan, Estonia, Latvia, Azerbaijan

- Non representative data for **South Africa** 2004-07
200 cases / year

Challenges of MDR TB

2 main endpoints :

- MDR patient outcome
(management of the cases)

- Number of MDR cases
(genesis of cases)

Task 1

Optimize the management
of MDR cases

Outcome of MDR cases background

Cure : 40 - 75 %

Failure : 10 - 30 %

Death : 5 - 20 %

Death in HIV + : 80 %

Lost to follow-up : ≥ 10 %

Suarez 2002, Tahaoglu 2001, Goble 1993,
Frieden 1996, Chan 2004, Mitnick 2003

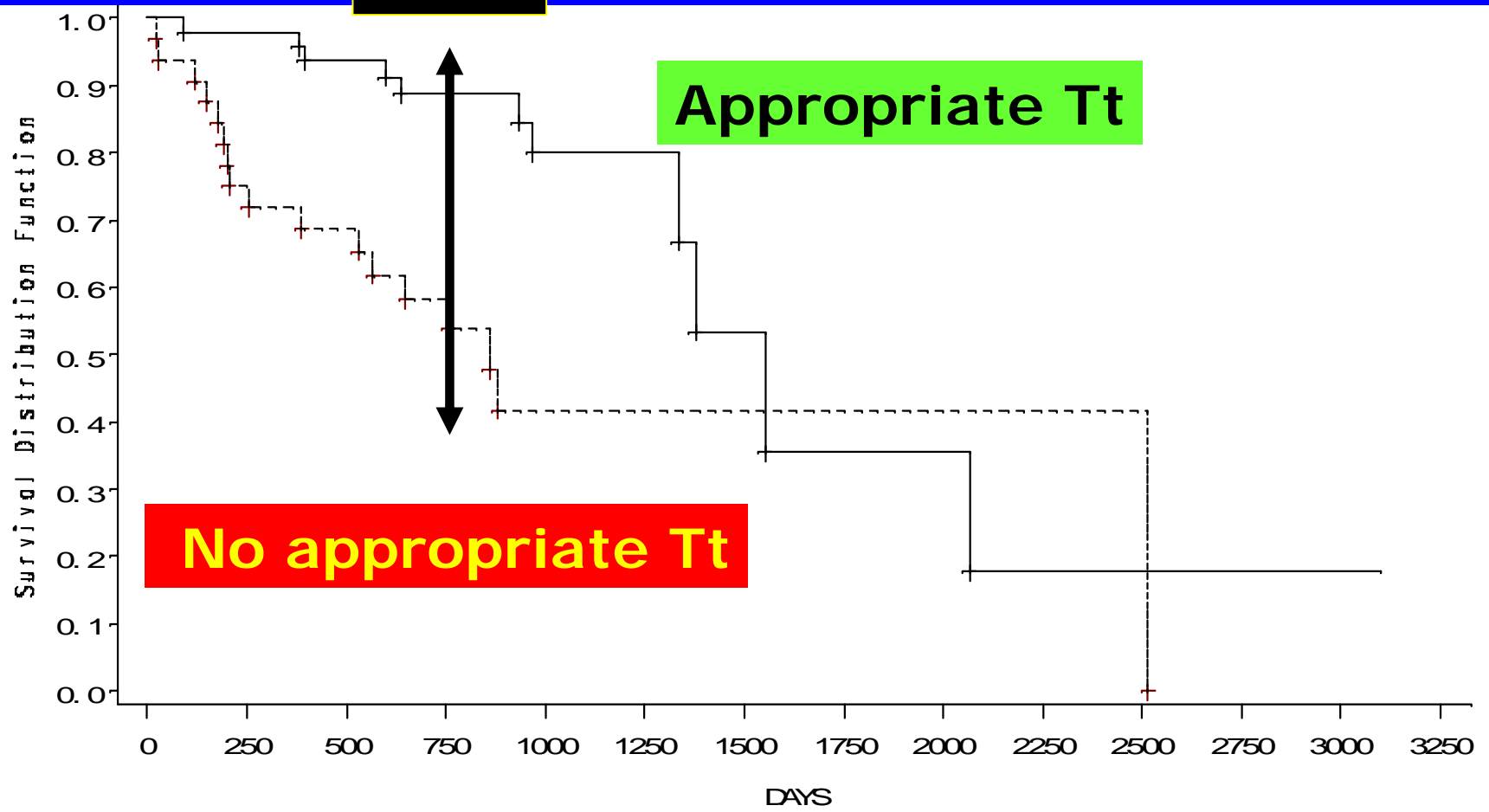
Survival of MD-RTB in the 1990s

Year 2

UK

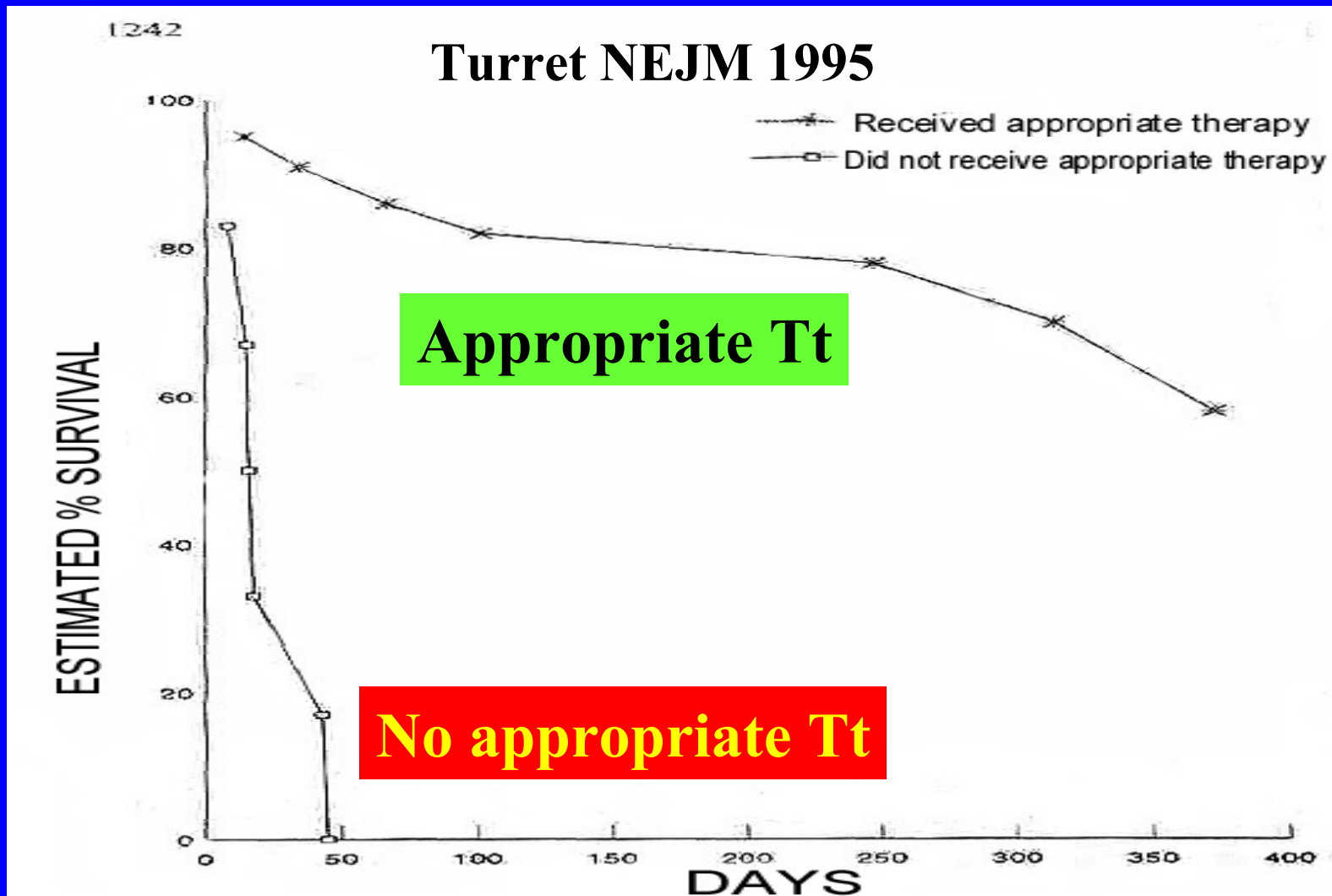
Appropriate Tt

No appropriate Tt



Drobniewski, Thorax 2002, 90 MDRTB patients

Survival of HIV-associated MDR-TB in the 1990s New York city, USA



Outcome of MDR-TB France

| | 1994 (n=51)* | 1999 (n= 45)** | 2006 (n= 53)*** |
|---|-----------------|-------------------|----------------------|
| No of tested drugs (including STR, EMB) | 5 | 8 | 11 |
| Treatment with > 3 active drugs | 47% | 84% | 85% |
| Succes | 41% | 67% | evaluated in 2008 |

* Saillour Am Resp Crit Care Med 1999 : non specialized teams

** Uffredi Inter J Antibiot 2006 : specialized team (lab/physicians)

*** Veziris 2008 : specialized team (idem but systematic)

Outcome (%) of MDR et XDR Lithuania (old and new definitions)

| | cure* | failure |
|-------------------------|-------|---------|
| MDR | 67 | 13 |
| XDR (old definition**) | 58 | 30 |
| XDR (new definition***) | 28 | 55 |

- completed treatment

** R to 3 2nd line drugs

*** R to FQs and 1 injectable

Management of MDR cases

Speeding up identification
and susceptibility tests
(directly on sputum)

Recommended standards for modern TB laboratory services

Laboratories should aim to identify TB and rifampicin resistance in over 90% of cases **directly from smear + sputum** where resources are available for this...

... rapidly within 1-2 days

Eur Respir J 2006; 28: 1-7
DOI: 10.1183/09031936.06.00084906
Copyright ©ERS Journals Ltd 2006



Recommended standards for modern tuberculosis laboratory services in Europe

F.A. Drobniowski*, **S. Hoffner[#]**, **S. Rusch-Gerdes[†]**, **G. Skenders⁺**,
V. Thomsen⁵ and the **WHO European Laboratory Strengthening Task Force**

Performances of genomic identification tests (classical PCR) in smear + sputum (meta-analysis by Sarmiento, JCM 2003)

- **Sensitivity** : 95-100 %
- **Specificity** : 95-98%
- **PPV** : very high due to :
 - good specificity
 - high prevalence of TB in smear + patients (> 90%)

« **Typing PCR** »

(ATS 1997, AJRCCM 155:1804-14)

Identification of *M.tuberculosis* in smear + sputum by using marketed DNA amplification and strip assay

- **INNO-LIPA mycobacteria (Innogenetics) :**
spacer 16s-23s : 16 species (including Mtb complex)
- **Genotype mycobacterium (Hain Lifescience) :**
23s gene : 13 species (including Mtb complex)
- **Genotype MTBC (Hain Lifescience) :**
23s, RD1, *gyrB* : species within Mtb complex

Resistance detection in *M.tuberculosis*

- Rifampicin (surrogate for MDR)
- (Isoniazid)

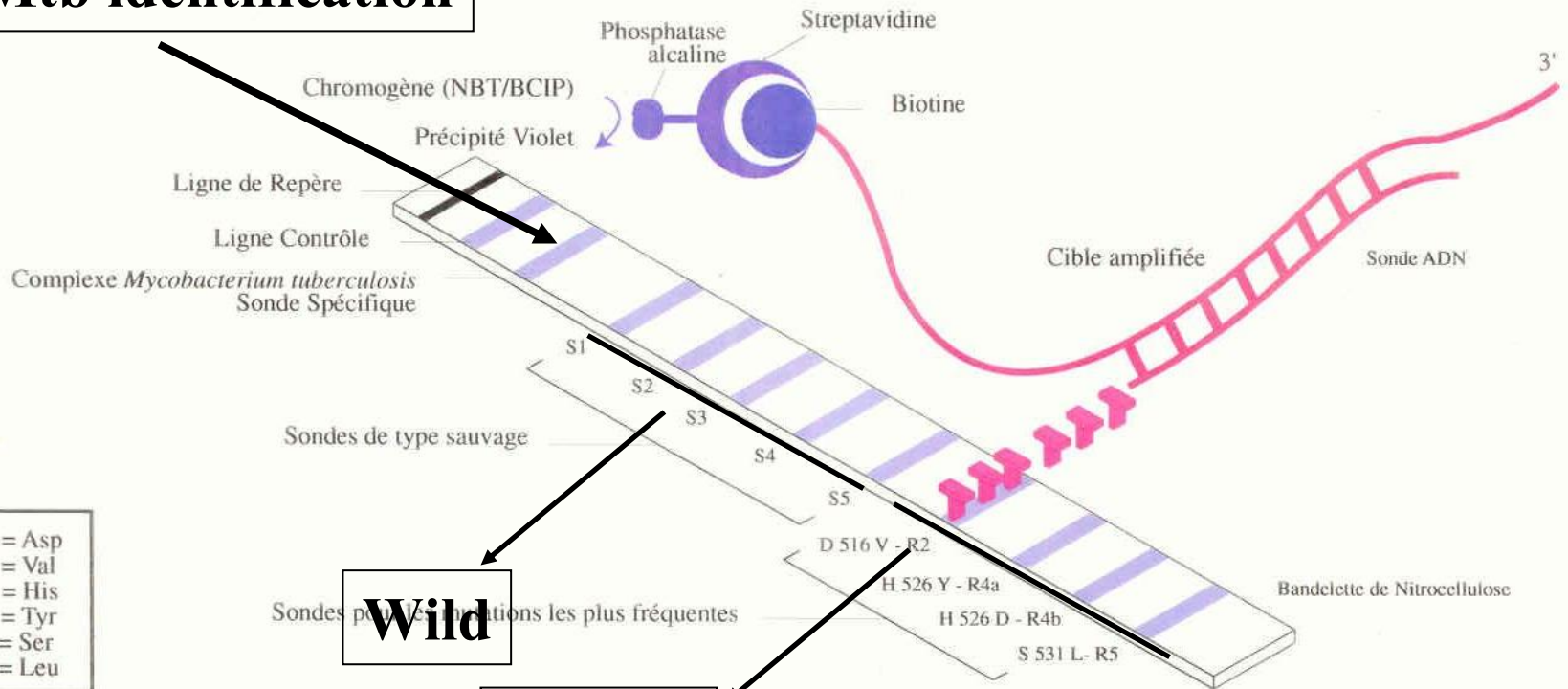
Genomic resistance detection
in *M. tuberculosis*
directly in Smear positive specimens

- DNA source : smear + specimen
- Sometimes requires additional specimen
- Often requires double PCR (nested)
- Technologies : hybridation strips, chips, others...

INNO-LiPA - Rif-TB

Sondes pour les mutations les plus fréquentes

Mtb identification



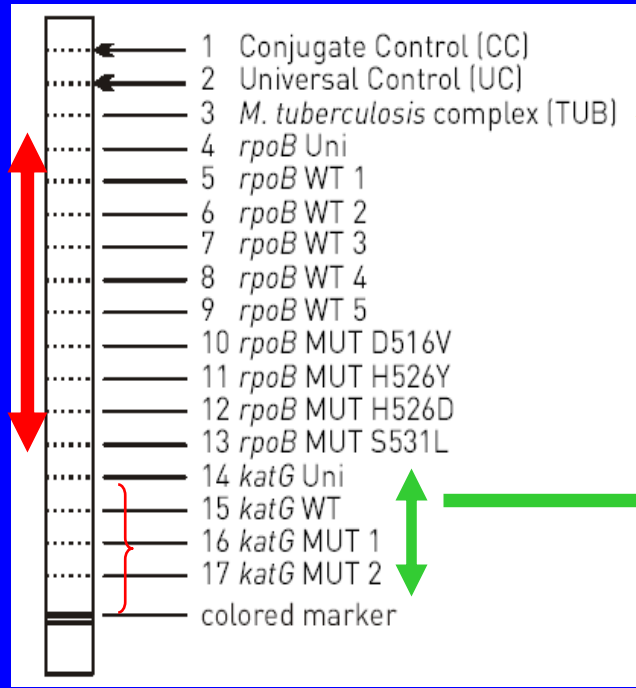
Performances of InnoLiPA RIF. TB® (Innogenetics)

**95-100 % of detection
in RIF-R strains**

Rossau 1997, Cooksey 1997, Marttila 1898,
Matsiota 1998, Watterson 1998, Gamboa 1998,
Kiepela 1998, Sintchenko 1998, Gonzalez 1999,
Hirano 1999, Traore 2000.....

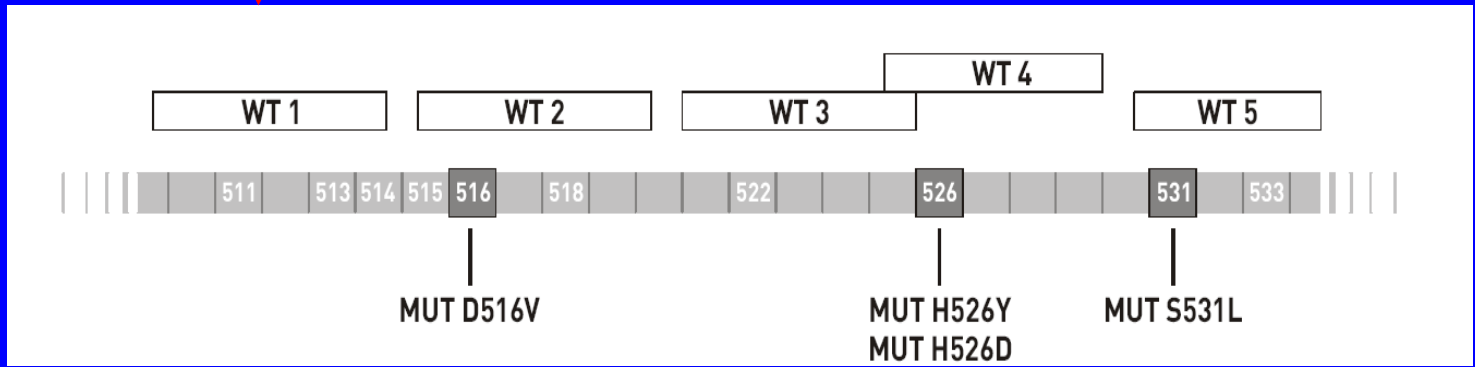
DNA strip assay MTBDR[®]

Rifampicine
rpoB



Mtb identification

INH
katG 315



From D. Hilleman et coll., J Clin Microbiol, 2005.

Strip DNA assay MTBDR[®] for detecting rifampicine resistance (77 R strains, France)

| Région étudiée | Mutations MTBDR | | Séquençage | N souches (%) |
|----------------|---------------------|------------------|-------------------|---------------|
| | MUT WT ^a | MUT ^b | | |
| 511-513 | MUT WT1 | | Q513K | 1 |
| | | | Q513P | 1 |
| 514-516 | MUT WT2 | | D516Y | 3 |
| | | | M515I+D516Y | 2 |
| | | | MUT1:D516V | 4 (5%) |
| 522 | MUT WT3 | | S522L | 2 |
| 526 | MUT WT4 | | H526A | 1 |
| | | | H526L | 3 |
| | | | H526R | 3 |
| | | | MUT2B:H526D | 8 (10%) |
| | | | MUT2A:H526Y | 6 (8%) |
| 531-533 | MUT WT5 | MUT3 :S531L | S531L | 37 (48%) |
| | | | S531W | 2 |
| | | | S531T | 1 |
| | | | L533P | 2 |
| MUT WT5+WT1 | | | S531C+L511P+F505L | 1 |

**100% detection
of rifampicine-R**

Brossier 2006 JCM

Brossier Int J Tub Lung Dis 2008

Strip DNA assay MTBDR® for detecting INH resistance (96 R strains, France)

| | | % mutation among Inh ^R | Inh ^R high level | Inh ^R low level |
|----------------------------------|-------------------|-----------------------------------|-----------------------------|----------------------------|
| <i>katG</i> : | S315 | 68 % | 60 | 5 |
| <i>inhA</i> promotor : | -15c->t | 19 % | 3 | 15 |
| Other <i>inhA</i> or <i>katG</i> | | 13 % | 4 | 9 |

**With KatG 315 only
only 68 % detection of INH-R**

**Brossier
2006 JCM**

Strip DNA assay MTBDR[®] plus for detecting INH resistance (96 R strains, France)

| | | % mutation among Inh ^R | Inh ^R High level | Inh ^R Low level |
|----------------------------------|-------------------|---|-----------------------------------|----------------------------------|
| <i>katG</i> : | S315 | 68 % | 60 | 5 |
| <i>inhA</i> promotor : | -15c->t | 19 % | 3 | 15 |
| Other <i>inhA</i> or <i>katG</i> | | 13 % | 4 | 9 |

**KatG 315 and InhA promotor
87 % detection of INH-R
(NPV still too low if high prevalence)**

**Brossier
Int J Tub Lung Dis
2008**

Strip DNA assay MTBDR® plus directly in Smear + sputum (MDR context, South Africa)

- 536 consecutive specimens
- 97 % of results interpretable in 2 days
- Rifampicine :
 - **Sensitivity 99 %**
 - **Specificity 99 %**
- Isoniazid :
 - Sensitivity 94 % (but many clonal strains !)
 - Specificity 99 %

Phenotypic susceptibility test directly from Smear + specimen

- Possible when > 1 afb/microscopic field
- Source of bacilli : specimen itself (homogeneized and decontaminated)
- Dilutions according to afb count
- Requires technical training
- Results obtained at the same time as primo-cultures

Particularly important for 2nd line drugs (can be done as soon as genotypic test proves RMP-R)

Management of MDR cases

Design and organize
treatment

Actions and recommendations

- **Implement WHO *Guidelines for the programmatic management of drug-resistant tuberculosis***
- **Green Light Committee (GLCs) to facilitate access to high-quality second-line drugs**



WHO ranked classification of second line antituberculosis drugs according to their effectiveness (1997)

| rank | | activity |
|----------|------------------------|------------------------|
| 1 | AMINOGLYCOSIDES | Bactericidal |
| a | Streptomycine | |
| b | Kanamycine/ Amikacine | |
| c | Capreomycine | |
| 2 | ETHIONAMIDE | Bactericidal |
| 3 | PYRAZINAMIDE | Bactericidal acidic pH |
| 4 | OFLOXACINE | Bactericidal |
| 5 | ETHAMBUTOL | Bacteriostatic |
| 6 | CYCLOSERINE | Bacteriostatic |
| 7 | P.A.S. | Bacteriostatic |

« Simple » MDR cases (susceptible to all second line drugs)

At least 3 months of initial intensive phase :

Amikacin or kanamycin

Moxifloxacin

Ethionamide

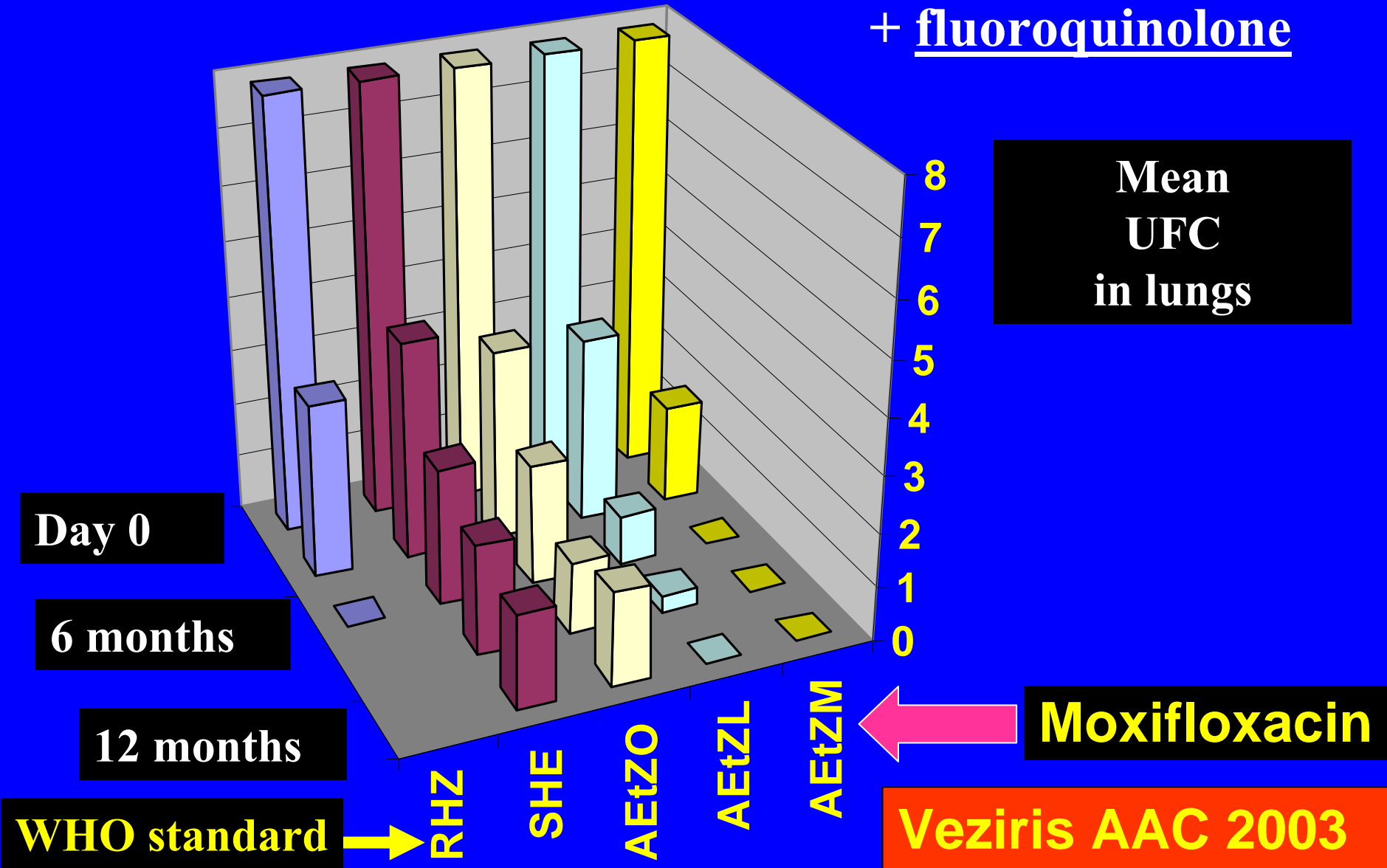
Pyrazinamide

+/- Ethambutol

At least 12 months of continuation phase (stop aminoglycoside after culture negativation)

Moxifloxacin in MDR TB treatment in the mice

amikacin + ethionamide + pyrazinamide (AEtZ)
+ fluoroquinolone



Sterilizing activity in MDR TB (mice) : relapse 3 months after end of treatment

Veziris ICAAC 2008

% relapses

6 months : 58%
Not long enough !

9-12 months required

gold standard
suscept TB

11%

2RHZ
+ 4RH

2JRZ+2JR

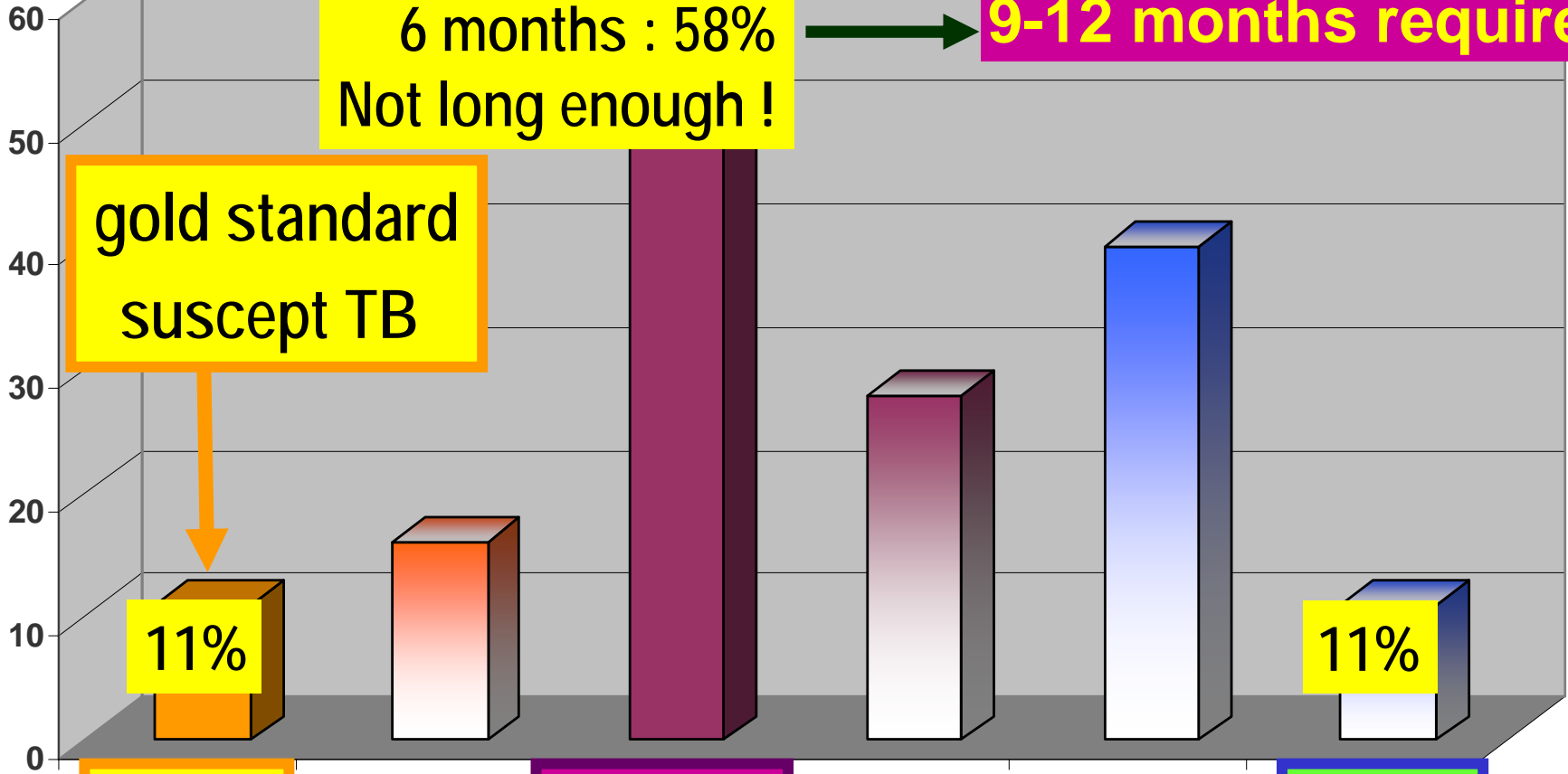
2AEtMZ
+ 4EtM

2JAEtMZ+4JEtM

2JMZ+2JM

11%

2JMZ
+ 4JM



« Complicate » MDR cases
(e.g. R to ethionamide, pyrazinamide..)
and XDR cases

- « Hand tailored » treatment designed by an expert team
 - Last chance for patient survival

Management of MDR cases

Discover and evaluate
new antituberculous
agents

Discover and evaluate new antituberculous agents

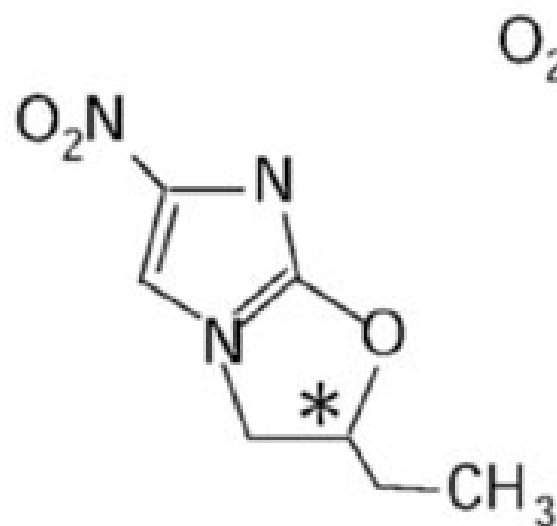
- Screening of drug libraries
- (design based on new targets identified by genomics or proteomics)
- In vitro testing
- Target identification
- Identification of acquired mechanisms of resistance
- In vivo testing : animal model (mice)
- Trials in human

Nitro-imidazoles : PA-824

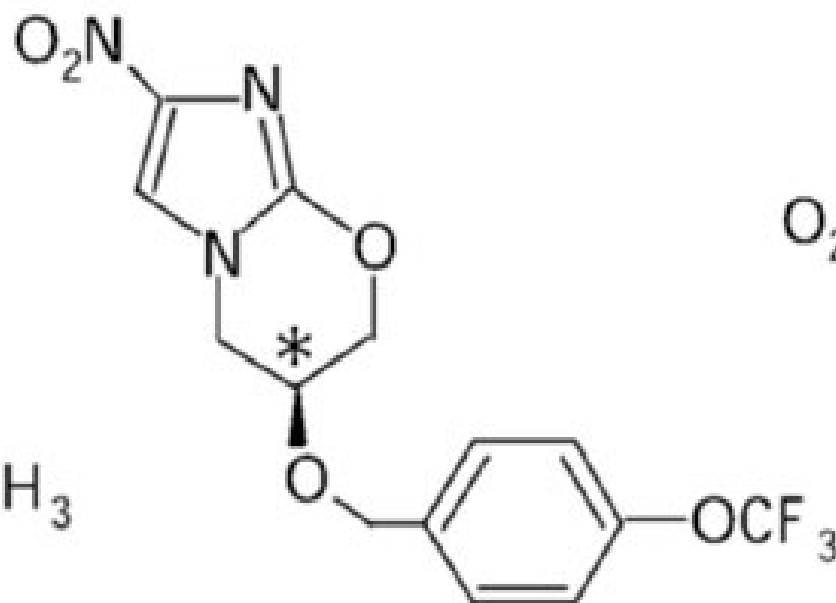
(PathoGenesis Corp.

>> global Alliance against TB)

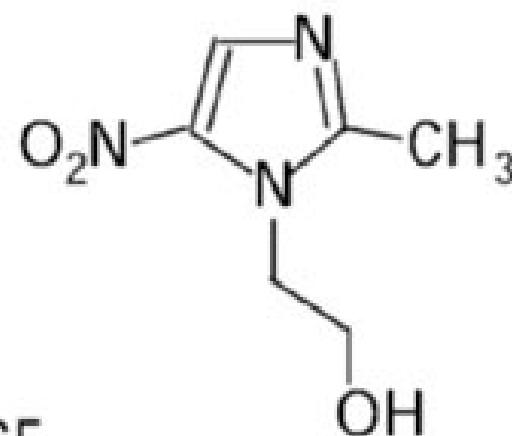
MIC on *M.tuberculosis* : 0.06 - 0.1 mg/L



CGI-17341



PA-824



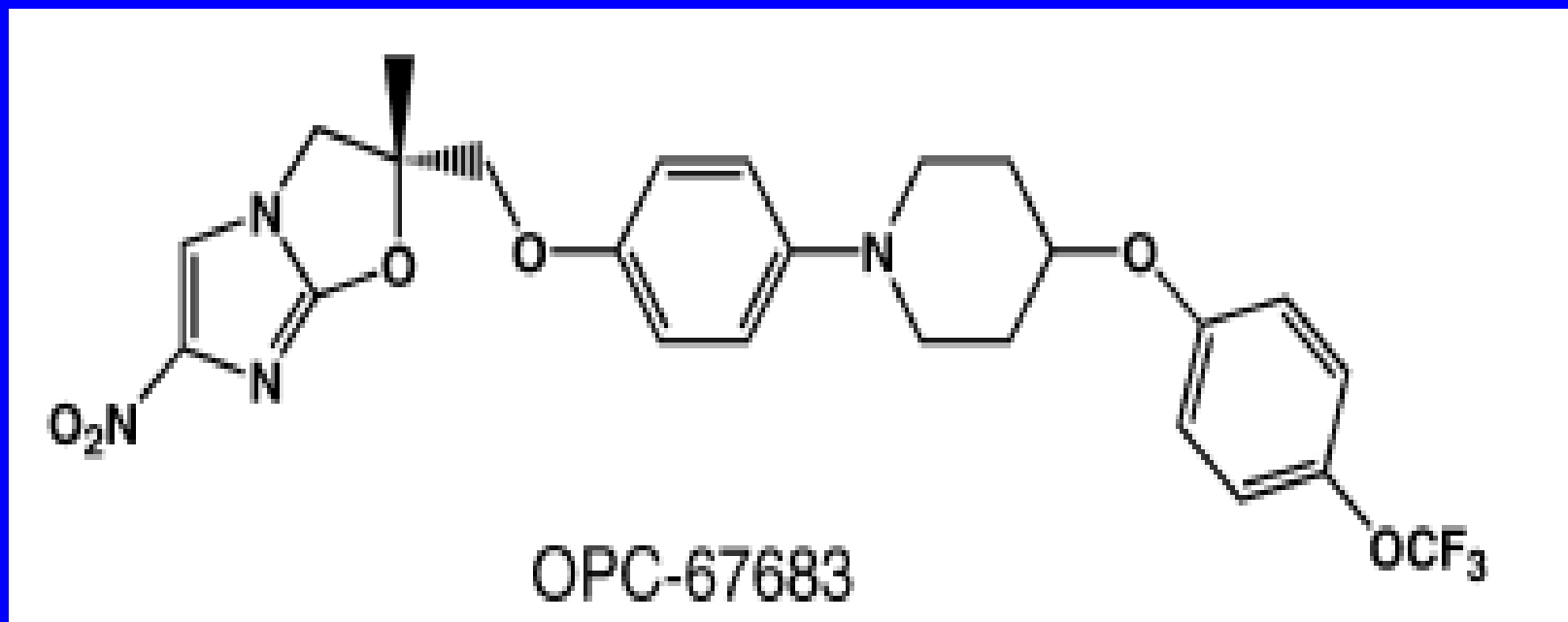
Metronidazole

Target unclear

Nitro-imidazoles : OPC-67683

(Otsuka Pharmaceutical, Tokushima)

MIC on *M.tuberculosis* : 0.01 mg/L



Target unclear

Diarylquinoline : R207910 (TMC207)

Inhibition of
ATP synthase

H⁺

H⁺

H⁺

F₀

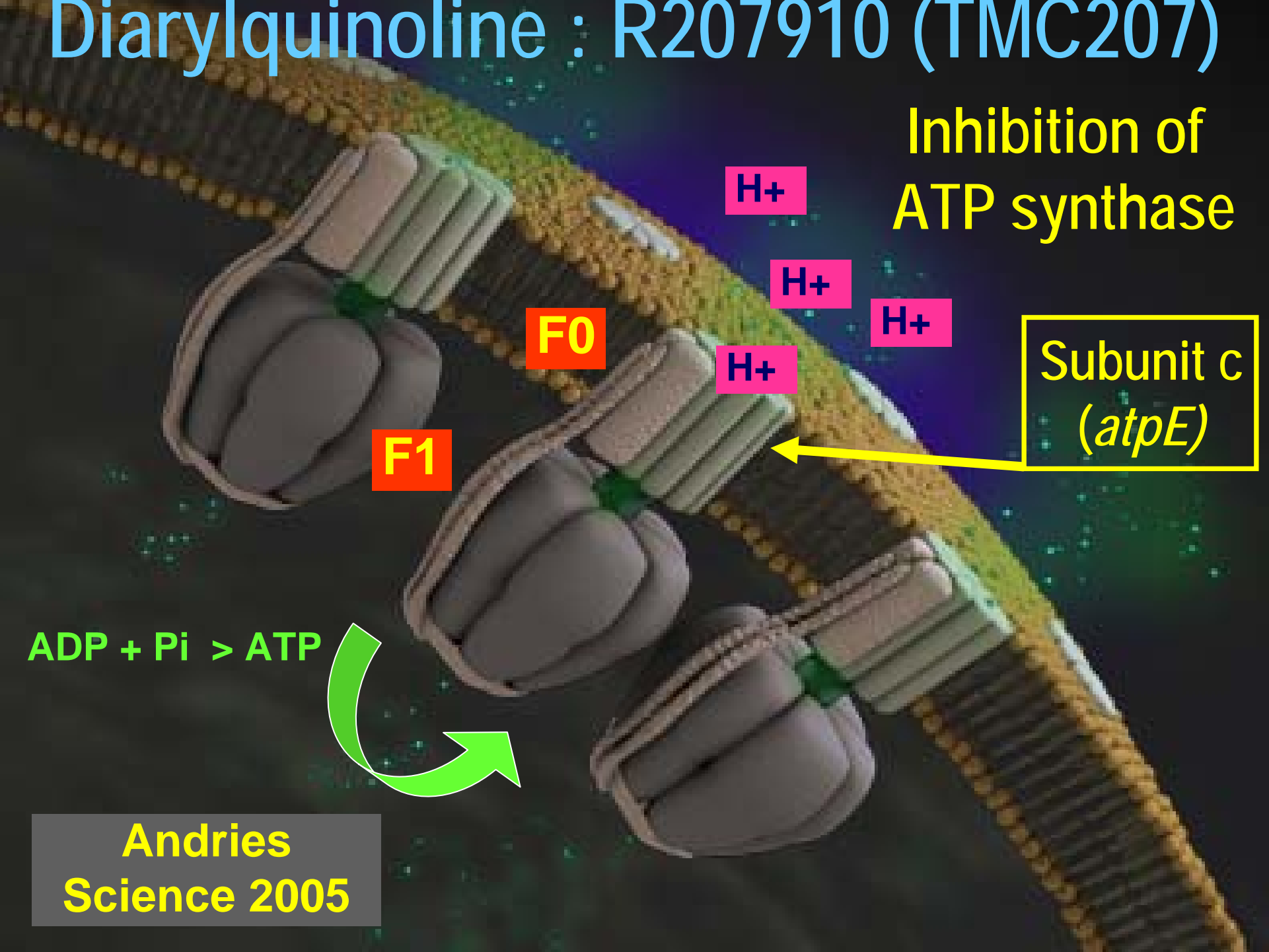
F₁

Subunit c
(*atpE*)

ADP + Pi > ATP



Andries
Science 2005



Task 2

Decrease the number of
MDR cases :

Curb the genesis of new
cases

Curb the genesis of new
cases

Prevent cross transmission

South Africa (Tugela Ferry) XDRTB and HIV



Fears of 'extreme' TB strain

New drug-resistant infection is 'nightmare' say health experts

by Robin McKie
Science Editor

HEALTH EXPERTS are to hold an emergency meeting in Johannesburg this week, following the discovery of a deadly new strain of tuberculosis.

The strain – known as extreme drug-resistant TB – has horrified World Health Organisation doctors. In one outbreak in South Africa, 52 of 53 patients died within weeks of becoming infected.

'This new strain leaves us facing a nightmare,' said Paul Nunn, coordinator of the WHO's drug-resistance unit. 'It is resistant to nearly every drug in our arsenal. We are now on the threshold of the appearance of a strain of TB that is resistant to every medicine known to science.'

The strain was originally discovered by scientists earlier this year. They looked at cases of multiple drug-resistant TB – which has developed over the past decade in many parts of the world – and discovered that among these a worrying new 'extreme' strain had evolved.

'Mainstream drugs are ineffective against multiple drug-resistant TB,' said Nunn. 'However, there are half a dozen second-line medicines that can be used to tackle it. Now this new extreme resistant strain has appeared. It is not only resistant to our principal anti-TB drugs, but to many of our second-line defences. In short, we are now on the last line of our defences against tuberculosis.'

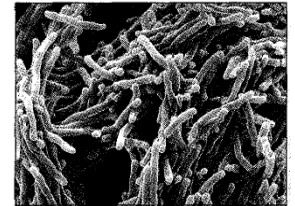
Among the areas found to have been affected by extreme drug-resistant TB are Latvia and South Africa. Scientists discovered the strain last month among HIV-infected patients in the KwaZulu-Natal region. 'Fifty two of the 53 infected people are already dead, and the last may well have died by now,' added Nunn.

An estimated 4.5 million people in South Africa have HIV. Extreme drug-resistant TB could devastate the population. 'If countries don't have the diagnostic capacity to find these patients, they will die without proper treatment,' said Nunn.

As a result, WHO is to hold its emergency meeting in Johannesburg to help establish measures that will lead to the rapid diagnosis of the new strain.

'It appears to kill within a few weeks and that does not give us a lot of time to spot it and treat it with the right drugs,' added Nunn. The few classes of drugs that are still effective against this strain of TB are expensive and can be toxic.

The meeting will be attended by officials from WHO and its partners, including the South African Medical Research Council and the US Centers for Disease Control and Prevention.



A new super-TB is threatening Latvia.

Gandhi Lancet 2006

South Africa XDR-TB 2006

- Majority : no previous treatment
- Suggests newly infected with XDR
- 26 of 30 (87%) XDR isolates = **genetically similar**
- **Nosocomial transmission in hospitals** likely
- **Transmission in community** possible for 36% of the patients who had no prior hospitalizations
- 52 of 53 patients died
- All patients tested for HIV (n=44) : HIV +

Gandhi Lancet 2006

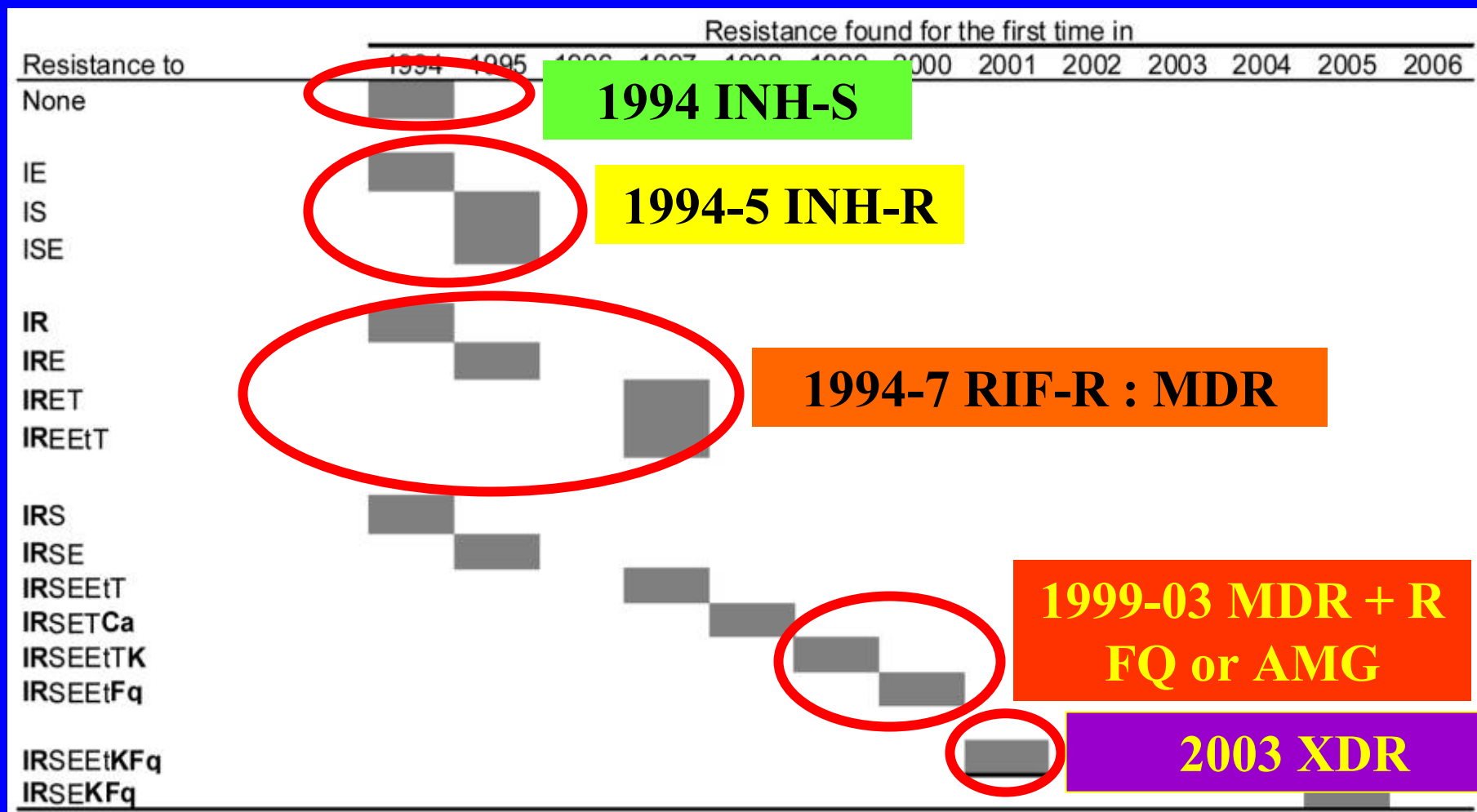
Prevent cross transmission of MDR-TB

- Out-patients dispensaries
- Hospital
- Prisons
-

Curb the genesis of new
cases

Prevent transformation of
MDS-TB in MDR-TB
(and of MDR-TB in XDR-TB)

Stepwise resistance in strain F15/LAM4/KZN in South Africa



Prevent transformation of MDS-TB in MDR-TB

- TB program
- Resources and expertise
- Standardized protocols (DOT, **combined drugs**)
- Organization
- Training
- Evaluation (indicators : % completed treatment, % cases with previous treatment, resistance rates...)

**Commitment : health authorities,
medical community**

Conclusion

When your boat is sinking...

Drain the water
(diagnose and
treat MDR)



....but most crucial

Seale the hole
(do not generate
new MDR cases)

