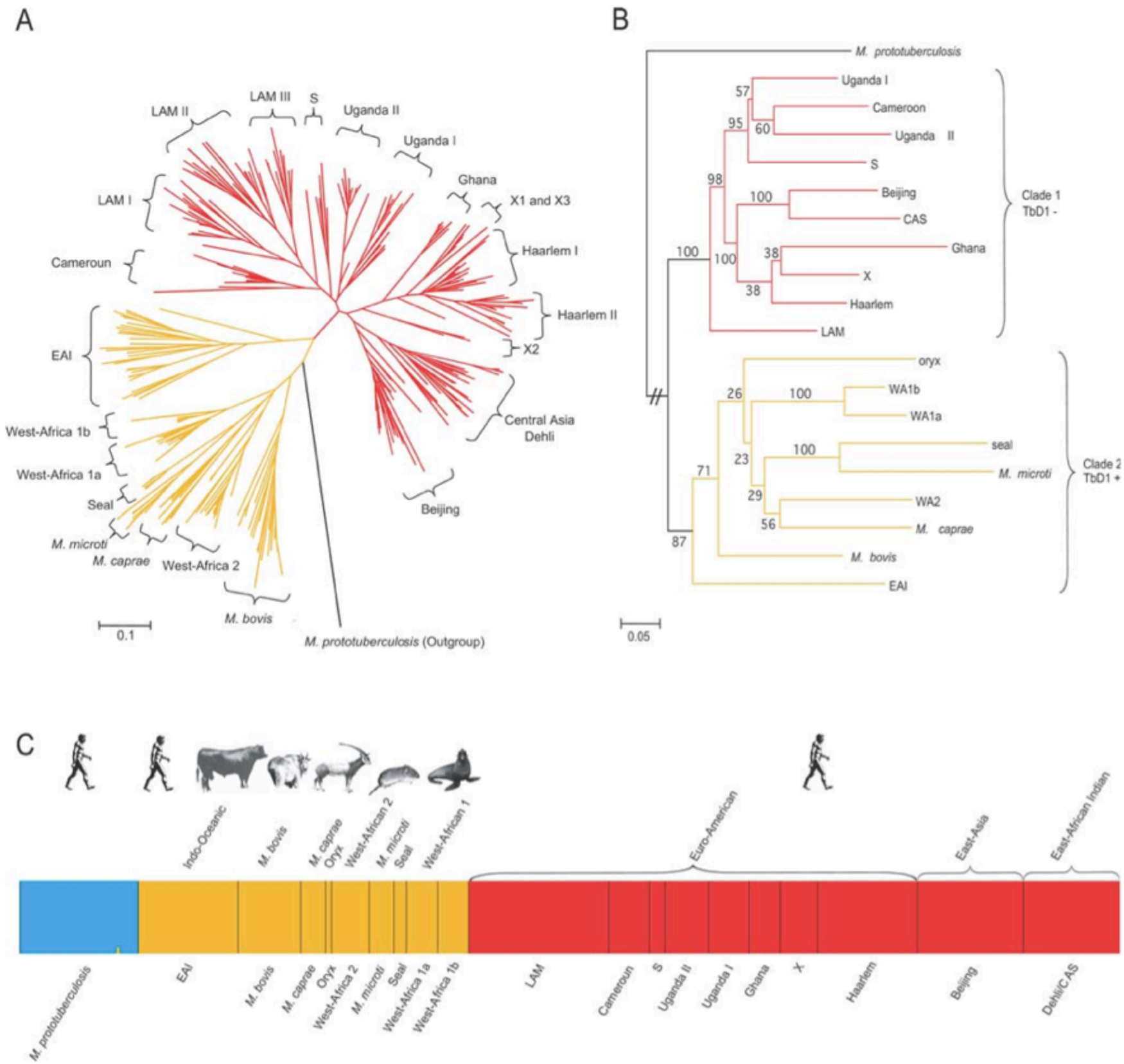
# Understanding Drug-Resistant Tuberculosis

Dean Tsukayama




Wirth et al. PLOS 4:e1000160, 2008

# KwaZulu Natal

Gandhi et al. Lancet 368:1575, 2006

- 53 cases of extensively drug-resistant tuberculosis reported from a hospital in South Africa
- Represented 6% of all patients with culture-confirmed tuberculosis
- All 44 patients tested for HIV were positive
- 52 patients died
- The mean time to death was 16 days from the time of diagnosis
- 55% had no prior TB treatment
- 67% had a recent hospital admission

Andrew Speaker is currently in isolation at National Jewish hospital in Denver. National Jewish Medical and Research Center

**TB Patient Ordered into Isolation**

# TB Drugs

**FIRST LINE**

isoniazid

rifampin

pyrazinamide

ethambutol

**SECOND LINE**

injectable agent*

quinolone**

ethionamide

cycloserine

PAS

* amikacin, kanamycin, capreomycin, streptomycin

** levofloxacin, moxifloxacin

# Definitions

| Drug Resistance | |
|---|---|
| **Single** | ethambutol |
| | isoniazid |
| | pyrazinamide |
| | rifampin |
| **Poly** | two or more first-line drugs, but not MDR |
| **Multi (MDR)** | isoniazid and rifampin |
| **Extensive (XDR)** | isoniazid, rifampin, quinolone, injectable agent |

# TDR?

Report from Iran in 2009 and India in 2012 of patients with tuberculosis resistant to all first-line and second-line drugs

Velayati et al. Chest 136:420, 2009
Udwadia et al. Clin Infect Dis 54:579, 2012

# Rifampin Resistance

- Resistance extends treatment duration to a minimum of one year
- Rarely see single drug resistance. Usually a surrogate for MDR.
- Rifampin dependent strains have been reported

# Drug-resistant Tuberculosis
## Consequences

- longer and more expensive treatment
- less effective and more toxic medication
- less likely to be cured
- epidemic of drug-resistant tuberculosis
- specter of untreatable tuberculosis

Bull World Health Organ 2012, 90:111

# Drug-resistant tuberculosis
## Outcome of treatment

| Resistance | Failure or relapse (%) |
|---|---|
| Pansensitive | 0.8 |
| Single Drug | 6 |
| Polydrug | 14 |

# MDR Tuberculosis

## Outcome of treatment

| | |
|---|---|
| Successful | 62 |
| Failure | 0.8 |
| Died | 6 |
| Defaulted | 14 |
| Transferred | 2 |

# Treatment Outcome of XDR Tuberculosis

- Individualized treatment of in Peru had a cured/completed treatment rate of 60% versus 66% with MDR [1]
- In the US, 83 cases from 1993-2007[2]
  - sputum culture conversion to negative in 66%
  - completed treatment, 44%
  - mortality (44%) higher in HIV (70%) than non-HIV (9%)
  - mortality compared to MDR (2x) and drug-susceptible (6x)


1. Mitnick. NEJM 359:563, 2008
2. Shah. JAMA 300.2153, 2008

# Economic cost of DRTB

Cost of treating XDR is $483,000, about twice the cost of treating MDR

**Plan to Combat Extensively Drug-Resistant Tuberculosis**
**Recommendations of the Federal Tuberculosis Task Force**
**MMWR Recommendations and Reports** February 13, 2009 / Vol. 58 / No. RR-3

Estimated cost treatment of pan-sensitive pulmonary tuberculosis is approximately $10,000

# MDR Tuberculosis
## Epidemiology

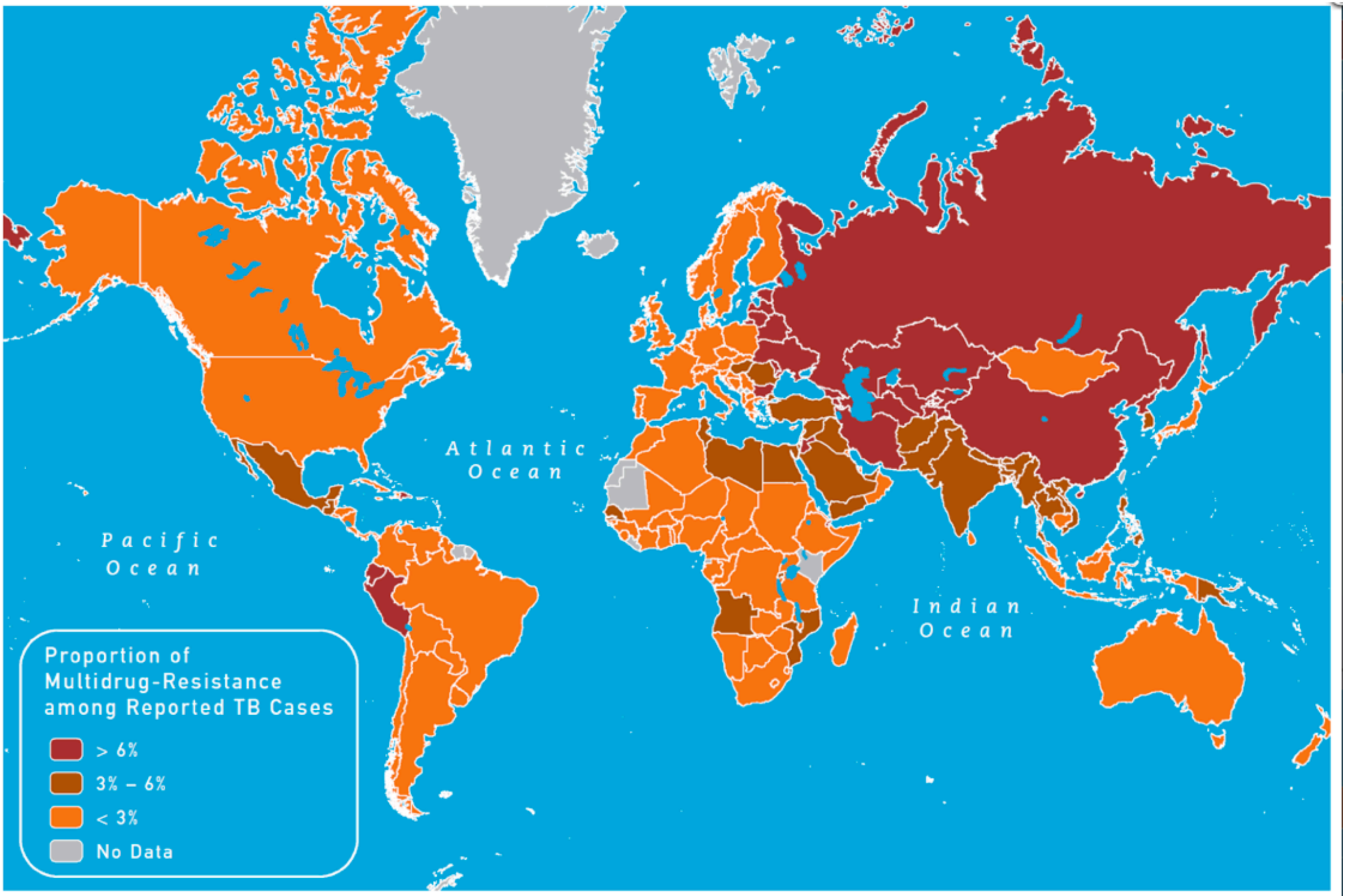
- The global proportions of TB cases showing MDR were:
  - 3.4% in newly diagnosed cases
  - 19.8% in previously treated cases
- The highest rate in new cases was 28.9% in Murmansk, Russia
- The highest rate in previously treated cases was 65.1% in the Republic of Muldova
- Approximately 500,000 new cases annually in the world

Bull World Health Organ 90:111, 2012

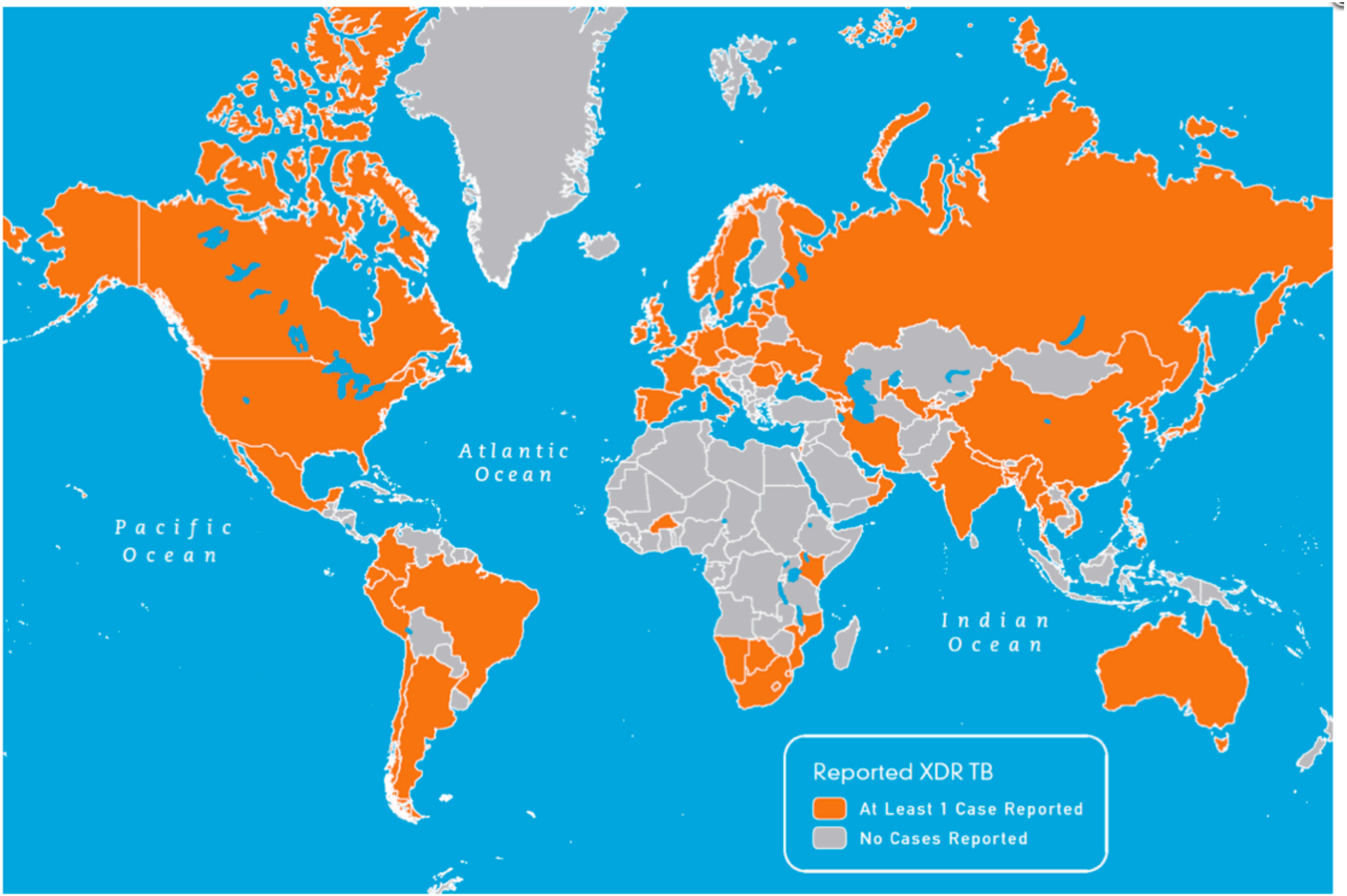
# MDR Tuberculosis
## Epidemiology

- The global proportion of MDR-TB that was XDR was 9.8%
- In 2010 only 16% of the TB patients estimated to have MDR-TB were diagnosed and given appropriate treatment
- There is a lack of data on drug resistance in Africa which has 80% of the cases of tuberculosis occurring in HIV-infected persons.

Bull World Health Organ 90:111, 2012




Map 3-16 Proportion of MDR TB among new TB cases, 2009




Map 3-17 Distribution of countries and territories reporting at least one case of XDR TB as of 2010

# Risk factors for acquiring drug-resistant tuberculosis

- Previously treated tuberculosis
- Inadequate therapy
  - unrecognized initial drug resistance
  - poor adherence to therapy
  - inadequate dosing of medication
  - needed medications are unavailable
- Exposure to drug-resistant case

# Components of TB Control

- Identification and treatment of active tuberculosis cases
- Drug susceptibility testing
- Screening and treatment of latent tuberculosis
- Contact investigation
- Respiratory isolation

# Management Errors Leading to Resistance

- Ineffective Drugs
- Adding single drug to a failing regimen
- Inconsistent compliance with medication
- Inadequate isolation of contagious patients

# Lack of resources

- Most cases of pulmonary cases in the world are diagnosed by sputum smear or clinically
- Less than 20% of estimated MDR cases are diagnosed and treated appropriately
- Very little treatment of latent tuberculosis
- Limited ability to do contact investigation
- Lack of effective respiratory isolation in hospitals

# Poverty and TB

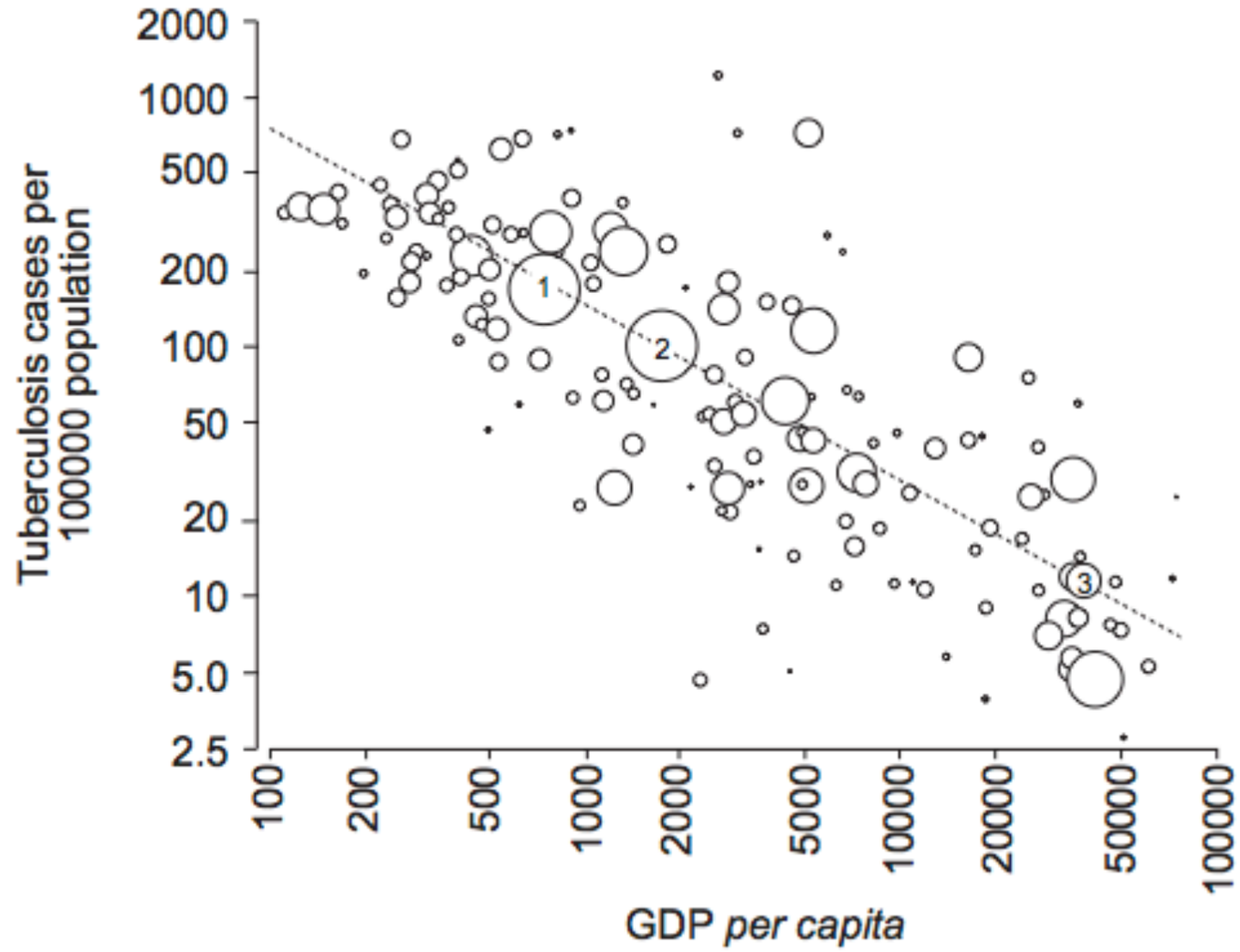



**FIGURE 1.** Relationship between *per capita* gross domestic product (GDP; Wold Bank data, 2005) and incidence of tuberculosis per 100,000 population. For graphical presentation, the third root of the population (millions) divided by 10 was used to determine the size of the symbols. This arbitrary choice provides a visual appreciation that large sized populations do not bear an excessive weight at the extremes of the axes, but are rather distributed across the entire scale. Both abscissa and ordinate were drawn logarithmically. 1; India; 2: China; 3: UK.

**Eur Resp J 32:1415, 2008**

# AFB Smear

- False-positive result from nontuberculous mycobacteria
- False negative in up to 50% of culture-positive cases
- Only test available for diagnosing TB in many parts of the world

http://www.google.com/imgres?imgurl=http://www.cosmosbiomedical...HWDQ&esq=4&page=4&ndsp=14&ved=1t:429,r:1,s:43

# Evolution of F15/LAM4/KZN Strain of *Mycobacterium tuberculosis* in KwaZulu-Natal

| Resistance first noted in: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| INH | RIF | STR | ETB | THI | ETH | CAP | KAN | FQN |
| 1994 | 1994 | 1994 | 1994 | 1997 | 1997 | 1998 | 1998 | 1999 |

INH- isoniazid
RIF- rifampin
STR- streptomycin
ETB- ethambutol
THI- thiacetazone
ETH- ethionamide
CAP- capreomycin
KAN- kanamycin
FQN- fluoroquinolone

MDR found in 1994
Progressively increasing poly-drug resistance with time
XDR found in 2001
XDR outbreak in 2005

# Hospital-acquired XDR in Tugela Ferry, KZN

- 1428 patients presented with signs and symptoms of tuberculosis
- 475 had culture-confirmed tuberculosis
- 185 (39%) had XDR
- 53 (6%) had XDR
- 55% had never had a diagnosis of tuberculosis in the past
- 30% had a history of successful treatment of tuberculosis
- 67% had history of hospital admission for any cause within 2 years
- 2 XDR patients were hospital workers
- 39 of 46 genotyped isolates of XDR had similar strains

# HIV Infection and Multidrug-Resistant Tuberculosis—The Perfect Storm

Charles D. Wells, J. Peter Cegielski, Lisa J. Nelson, Kayla F. Laserson, Timothy H. Holtz, Alyssa Finlay, Kenneth G. Castro, and Karin Weyer
JID 2007:196 (Suppl 1): S86

- HIV is the strongest risk factor for developing active TB. TB notification rates in countries in sub-Saharan Africa, with HIV infection prevalences of >10% in the general population, have increased 5%–10% annually over the past decade, including in countries with well-established TB control programs

- TB is also the leading cause of death among HIV-infected persons and may accelerate the course of HIV infection, increasing the HIV load in some patients

- HIV infection has been associated with acquired rifamycin resistance among patients with TB in controlled clinical trials and other studies

- Institutional outbreaks of MDR-TB have primarily affected HIV-infected persons.

- Delayed diagnosis, inadequate initial treatment, and prolonged infectiousness led to extraordinary attack rates and case-fatality rates among HIV-infected persons.

# Biology of Drug Resistance

- Genetic resistance to an anti-tuberculous drug is due to spontaneous chromosomal mutation occurring at a frequency of $10^{-6}$ to $10^{-8}$ mycobacterial replications.
- No resistance due to plasmids or transposons

# Drug Resistance in M. tuberculosis

**Table 3 Mutant genes responsible for resistance to antituberculosis drugs**

| Drug | Mutant resistant gene(s) (protein product it encodes) | Mechanism of resistance |
|---|---|---|
| Rifampin | *rpoB* (RpoB = β chain of RNA polymerase) | Mutation of RpoB reduces the ability of rifampin to inhibit it. |
| Isoniazid | *katG* (KatG = catalase-peroxidase) | Mutation of KatG reduces its ability to activate prodrug INH to a range of highly reactive bactericidal species. |
| | *inhA* (InhA = NADH-dependent enoyl ACP reductase) | InhA is involved in mycobacterial mycolic acid synthesis. Mutation of InhA results in reduced binding of NADH to InhA → reduces vulnerability of InhA to attack by INH |
| | *ahpc* (AhpC = alkyl hydroperoxide reductase) | Mutation in the promoter of the *ahpc* gene results in the overexpression of the antioxidant enzyme AhpC, which removes peroxide needed for INH activation, resulting in less INH activation. |
| | *kasA* (KasA = β-ketoacyl ACP synthase) | Overexpression of KasA, an enzyme involved in fatty acid and mycolic acid synthesis, leads to low levels of INH resistance. Higher resistance seen with comutation of InhA. |
| | *ndh* (Ndh = NADH dehydrogenase) | Mutation of Ndh increases the NADH/NAD+ ratio → NADH competes with INH-NAD adduct to the active site of InhA. |
| Pyrazinamide | *pncA* (PncA = pyrazinamidase) | Mutation of PncA results in lack of conversion of PZA to pyrazinoic acid |
| Ethambutol | *embB* (EmbB = a mycobacterial membrane protein) | Mutation of EmbB results in decreased binding of ethambutol to it. |
| Streptomycin | *rpsL* (RpsL = S12 ribosomal protein) | Mutation of RpsL leads to decreased binding of streptomycin to the S12 ribosomal protein. |
| | *rrs* (Rss = 16S ribosomal RNA) | Mutation of Rss leads to decreased binding of aminoglycosides to 16S rRNA. |
| Amikacin/ kanamycin | *rrs* (see above) | See above |
| Quinolones | *gyrA* (GyrA = bacterial DNA gyrase) | Various mutations of GyrA result in the inability of FQNs to inactivate it. |
| Ethionamide | *inhA* (see above) | See above |

ACP, acyl carrier protein; INH, isoniazid; NADH, nicotinamide adenine dinucleotide; PZA, pyrazinamide. Adapted from [66].

Edward D. Chan and Michael D. Iseman

Current Opinion in Infectious Diseases 2008, 21:587–595

# Genetic mutations account for drug resistance

- Rifampin- 95% (rpoB)
- Isoniazid- 80% (katG and inhA)
- Pyrazinamide- 70% (pncA)
- Ethambutol- 70% (embB)
- Streptomycin- 70% (16rRNA and rpsL)

# Resistance- clinical settings

- Primary- transmission of drug-resistant strain to an uninfected person
- Acquired- conversion of a pan-sensitive strain to a drug-resistant strain during therapy
- Amplified- progressive acquisition of more drug resistance during repeated treatment episodes

Sally M Blower & Tom Chou. Nature Medicine 10:1111, 2004

# Traditional Drug Susceptibility Testing

- Liquid and solid media
- 1% proportional growth is considered resistance
- Requires growth of bacteria
- Significant technical expertise, infrastructure required

# TREATING MDR-TB

## First Line

isoniazid

rifampin

pyrazinamide

ethambutol

streptomycin

## Second Line

injectable agent*

quinolone**

ethionamide

cycloserine

PAS

* amikacin, kanamycin, capreomycin
** levofloxacin, moxifloxacin

1) Use available first line agents
2) Add quinolone
3) Add injectable agent
4) Add other agents to total 4-6 drugs

# Strategies for combating DRTB

## We have the tools we need

- Diagnostic Laboratory
  - AFB culture
  - Drug susceptibility testing for 1st and 2nd line drugs
  - Rapid tests for diagnosis and susceptibility
- Surveillance, epidemiology, and contact/outbreak investigation
- Infection control
- Best practices in clinical care
- Adequate resources to ensure appropriate management

# New Diagnostics

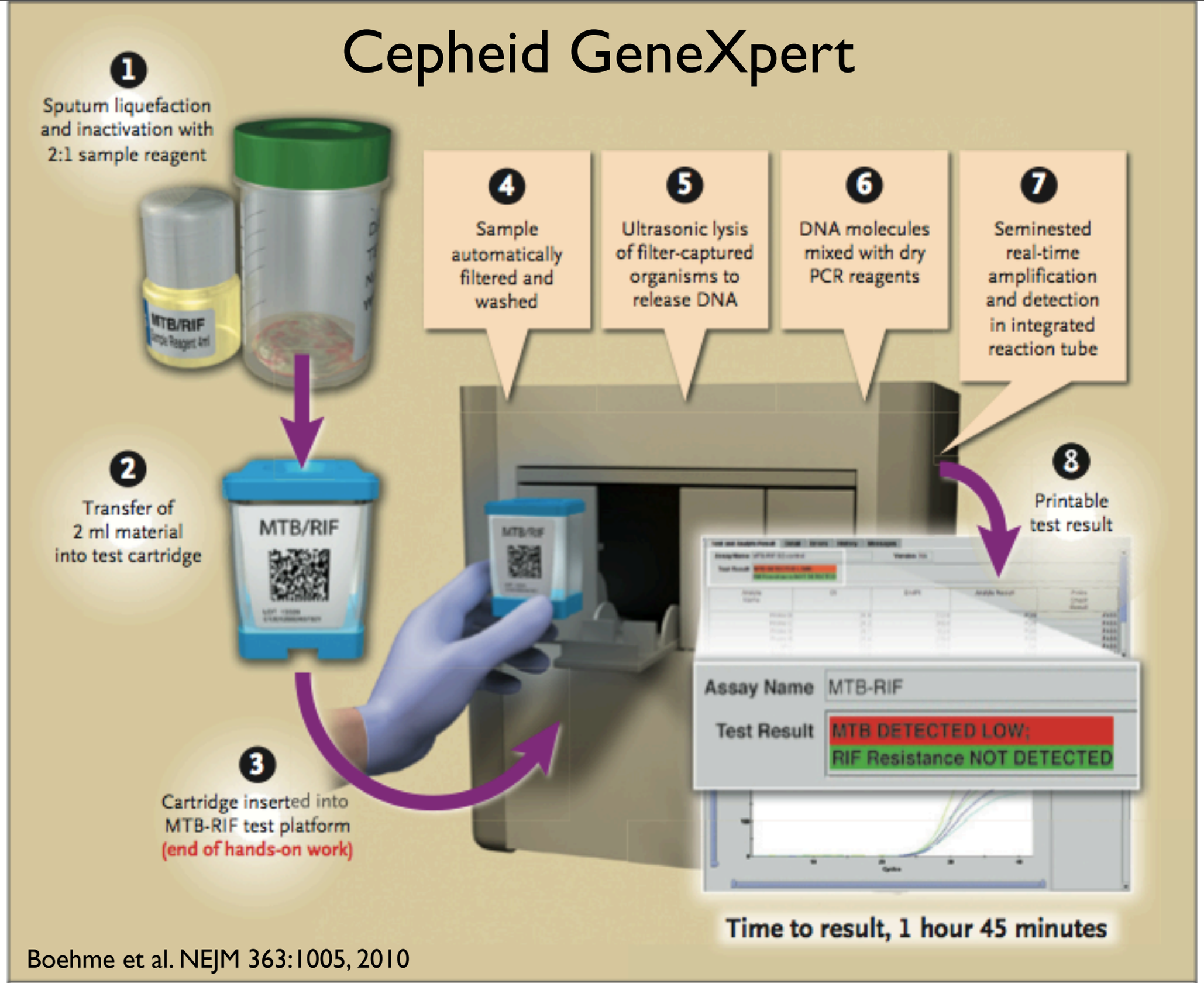
# Nucleic acid amplification

- identifies unique sequence for MTB from ribosomal RNA/DNA by sequencing or probe
- M. tuberculosis can be identified in one day
- More sensitive on smear-positive specimens
- Also available for avium-intracellulare, kansasii, gordonae

# Molecular Detection of MTB

- Extract MTB DNA/RNA from clinical specimens or from culture
- Increase amount of DNA/RNA by nucleic acid amplification
- Identify MTB by:
  - Sequencing DNA
  - Hybridization to specific probes

# Cepheid GeneXpert




Boehme et al. NEJM 363:1005, 2010

**Figure 2. Assay Procedure for the MTB/RIF Test.**

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.

# Limitations of molecular diagnosis

- Insufficient DNA or contamination
- Mixed susceptible and resistant populations
- Neutral polymorphisms
- Silent mutations (falsely interpreted as resistant)
- Not all mutations associated with resistance are known

# **M**icroscopic **O**bservation **D**rug **S**usceptibility

Figure 8. Microscopic observation drug-susceptibility assay. Characteristic tangling appearance of cords of *Mycobacterium tuberculosis* as visualized at ×20 magnification under an inverted light microscope. Image courtesy of David AJ Moore and Luz Caviedes and reproduced with permission.

- Sputum sample is directly inoculated into liquid medium
- Growth is recognized by characteristic cord formation
- Can inoculate into medium with antibiotic to test for drug resistance.
- Excellent sensitivity and specificity

# Dog-sized rats sniff out TB in patients

***Chris Tachibana writes:*** Could a giant, spit-sniffing rat save your life? Maybe — they can be better than humans at diagnosing tuberculosis.

Eric Nathan

Yes, it's nearly as big as a chihuahua but don't be afraid. Rodents of unusual size, such as this one, could save your hide. A pilot program in Tanzania has trained them to detect land mines and also sniff out tuberculosis.

A pilot program in Tanzania is using trained rats to smell TB in sputum samples. Up to 1,000 samples a week are collected from local hospitals by APOPO, a nonprofit that also trains rats to sniff out landmines. Although the TB samples have already been checked by a human under the microscope, the rat pack's sniff tests have improved disease detection by 44 percent because the clever rodents often find TB that was missed.

While the World Health Organization estimates that 2 billion people around the world are infected with TB, it can be hard to detect under a microscope, particularly in those who are also HIV positive. That's where the giant rats can help. They might turn out to be just as accurate at finding TB. They're low-tech and could screen for TB in resource-limited countries. They're also faster than the standard microscope test, says Bart Weetjens, APOPO founder. A human with a microscope can process 40 samples a day. A rat can do 40 in seven minutes, he says.

# New Treatment

# TB Drugs

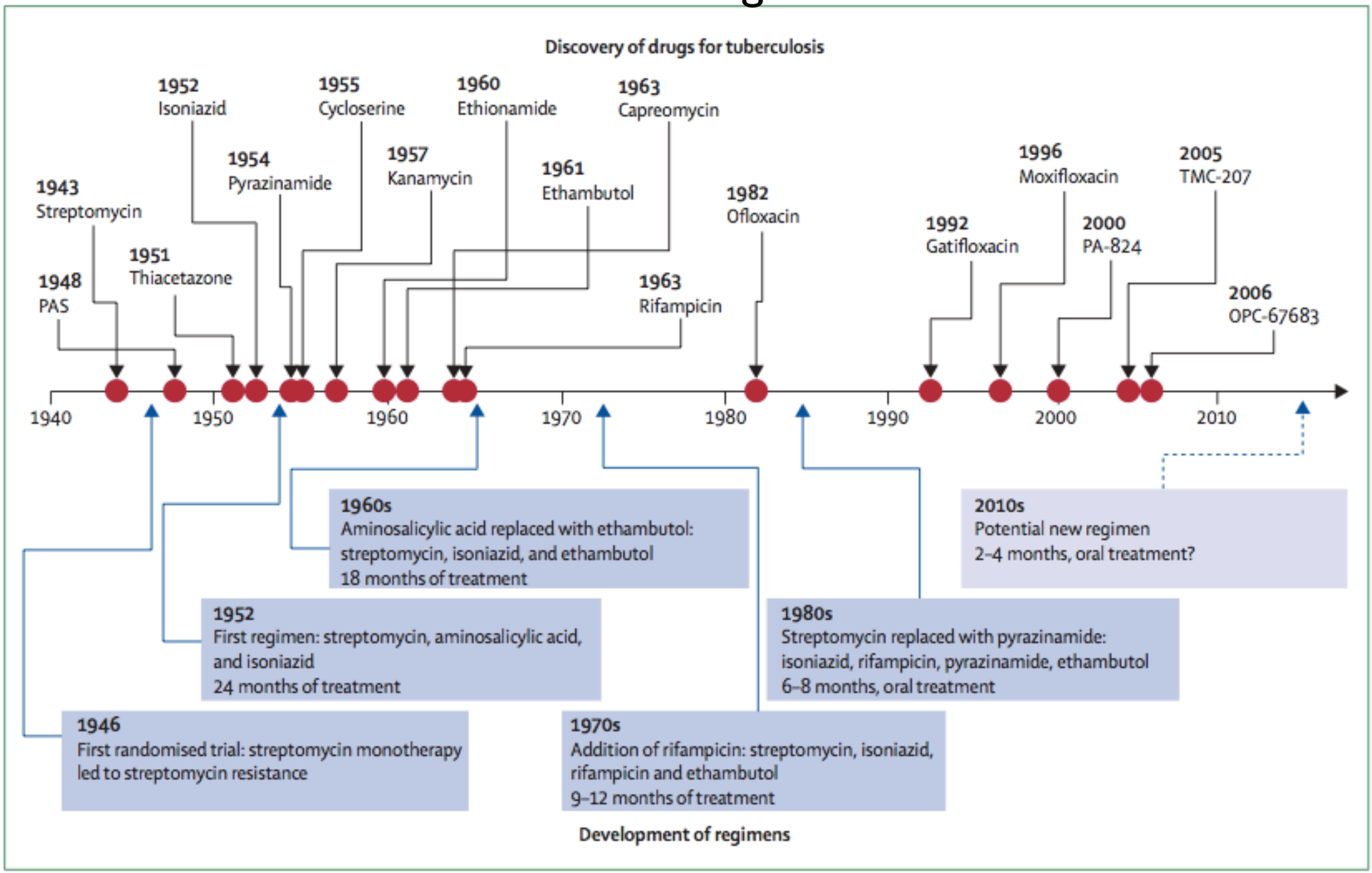



***Figure 2:*** **History of drug discovery and development of treatment regimens for tuberculosis**
Compounds that are in the early-stage of development, but for which there are no human proof-of-concept data, are not shown. Arrow with dashed line represents future regimen. Red dots represent when the drugs were first reported.

| | Drug target | Potential for treatment of MDR and XDR tuberculosis | Potential in shortening treatment |
|---|---|---|---|
| Moxifloxacin, gatifloxacin | DNA gyrase | Pre-existing resistance in clinical isolates; potential for treatment of MDR tuberculosis, but these drugs are not suitable for treatment of XDR disease; high bactericidal activity should assist the prevention of development of resistance to co-administered drugs | Active against drug-persistent culture in vitro; more effective than standard regimen in animal models and phase 2 trials[44-48] |
| Rifapentine | RNA polymerase | Cross-resistance with rifampicin; not suitable for the treatment of MDR and XDR tuberculosis | Active against drug-persistent culture in vitro; more effective than standard regimen in animal models[64] |
| Linezolid, PNU-100480, AZD-5847 | Ribosome | Novel mode of action; active against MDR and XDR tuberculosis | Unknown potential |
| TMC-207 | ATP synthase | Novel mode of action; active against MDR and XDR tuberculosis | Highly active against drug-persistent culture in vitro; more effective than standard regimen in animal models;[57] potential for treating latent tuberculosis infection |
| PA-824, OPC-67683 | Many targets | Reductive activation of prodrug; novel mode of action; active against MDR and XDR tuberculosis | Active against drug-persistent culture in vitro; more effective than standard regimen in animal models;[52] potential for treating latent tuberculosis |
| SQ-109 | Unknown (lead compound ethambutol is a cell-wall synthesis inhibitor) | Unknown mechanism of action; potentially active against MDR and XDR tuberculosis | Unknown potential |

MDR=multidrug resistant. XDR=extensively drug resistant.

***Table 3:*** **Modes of action and potential roles of new drug candidates in future regimens**

# Beyond Antimicrobials

- Vaccines
- Immunotherapy

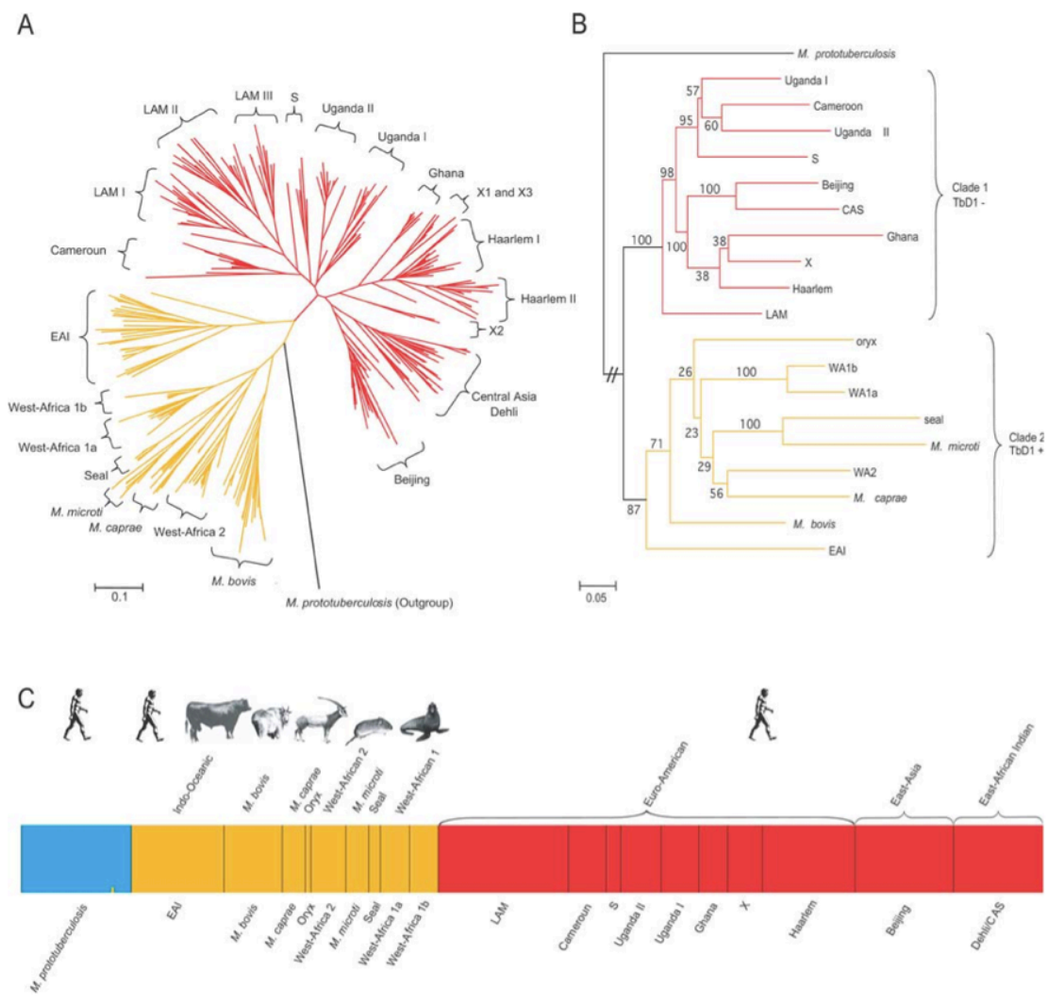
# Best Practices

- Culture-confirmed diagnosis
- Drug susceptibility testing
- If needed- 2nd line drug susceptibility, drug levels
- Directly observed therapy
- Never add a single drug to a failing regimen
- Infection control
- Contact investigation
- Treatment of latent tuberculosis
- Use of rapid testing when appropriate

# Conclusions

- Drug-resistant tuberculosis is a growing problem
- The epidemic is fueled by sub-optimal therapy and control measures
- We currently have, but may be losing, the tools we need to control tuberculosis
- New diagnostic and treatment options will have a positive impact
- Tuberculosis affects everyone but is primarily a disease of poverty

# Understanding drug-resistant tuberculosis




E D I T O R I A L C O M M E N T A R Y

CSE Global Theme Issue on Poverty and Human Development

Extensively Drug-Resistant Mycobacterium tuberculosis: Charles Darwin Would Understand

**Michael D. Iseman**
University of Colorado School of Medicine, Denver

**Clinical Infectious Diseases 2007; 45:1415–6**