Diagnostic tests for TB: a clinician's perspective

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Disclosures

- Chair, Data Monitoring Committee for studies of TMC207 (bedaquiline)
- Scientific advisor to Tibotec – TMC207
  - Tibotec pays my employer for my time
- Participant in a study of interferon gamma-release assays as screening tests among health care workers

Objectives

- Provide clinician's perspective on diagnostic tests for TB
- Review current tests, look for opportunities for better tests
- Key diagnostic decision points
  - Does this patient have TB?
  - Does this patient have drug-resistant TB?
- Will not discuss diagnosis of latent TB

Case #1 – severe illness in an HIV-positive man

- 33 year old man with 5-days of non-productive cough, fevers, dyspnea, profound fatigue
- PMH
  - AIDS, CD4 of 57, started on ART 8 weeks ago
  - Injection drug use (methamphetamine), jailed twice, periods of homelessness
  - From Iowa

Case #1 - continued

- Meds:
  - Tenofovir/FTC, darunavir/R, TMP/SMX
- Exam – alert and oriented, T – 38.5, BP 100/54
  - Temporal wasting, profoundly fatigued
- Labs
  - WBC – 0.4, HCT – 22.5, PLT – 92,000
  - Mildly increased alkaline phosphatase
- Chest x-ray – miliary infiltrates
- Sputum and BAL: AFB smear-negative

Case #1 – clinical questions

- What is the etiology of this acute, severe illness?
  - TB, non-tuberculous mycobacterial infection, fungal disease, malignancy
  - Immune reconstitution syndrome
- What should be done now?
  - Start empiric TB therapy?
  - Start empiric antifungal therapy?
  - Consultation for a biopsy (liver, lung, bone marrow)

Case #2 – prolonged cough in an immigrant

- 52 year old man from Bhutan
- Cough for 4 weeks, small amounts of phlegm, no hemoptysis
- Subjective fevers, no weight loss
- No other household members ill
- PMH
  - Positive TST, no treatment
  - 30 pack-year tobacco history, still smoking

Case #2 – Chest radiograph

Sputums x 3
- Smear-negative
- Cultures pending

Rapid HIV test – negative

No prior chest x-rays available

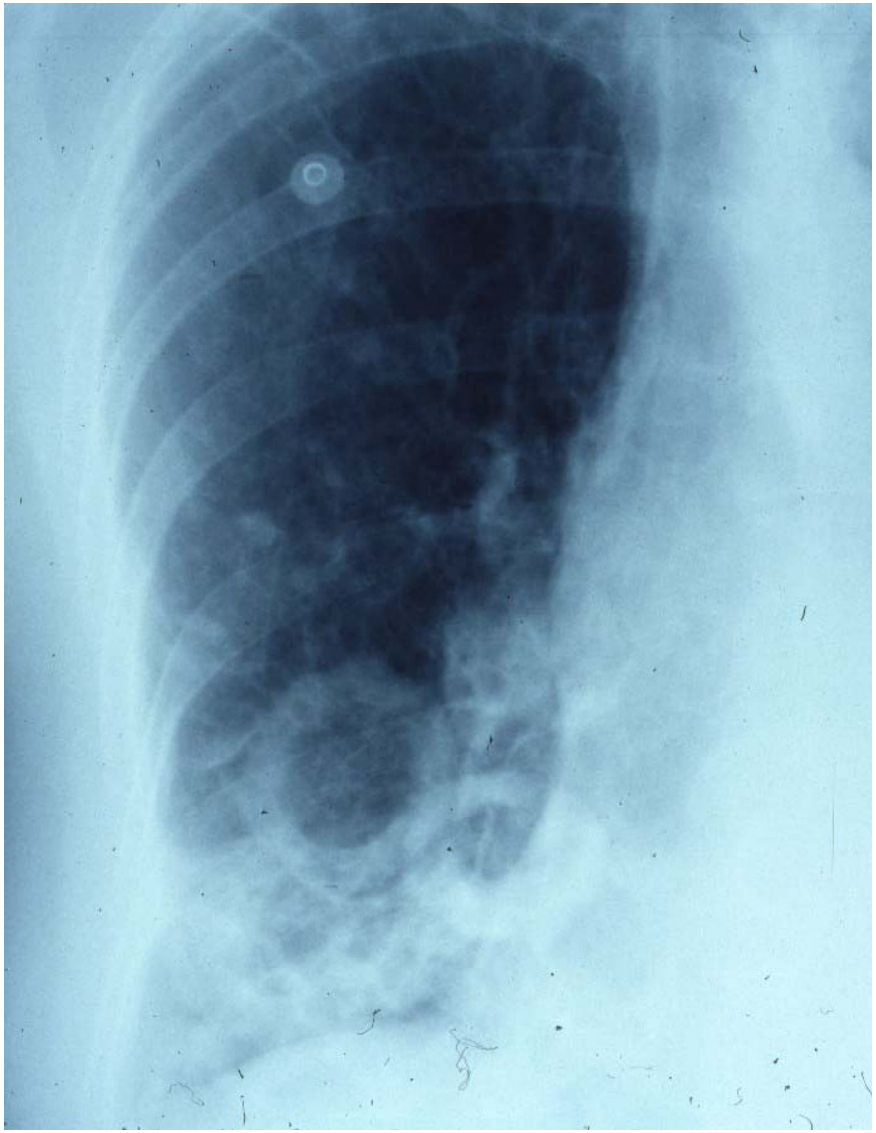
Case #2 – clinical questions

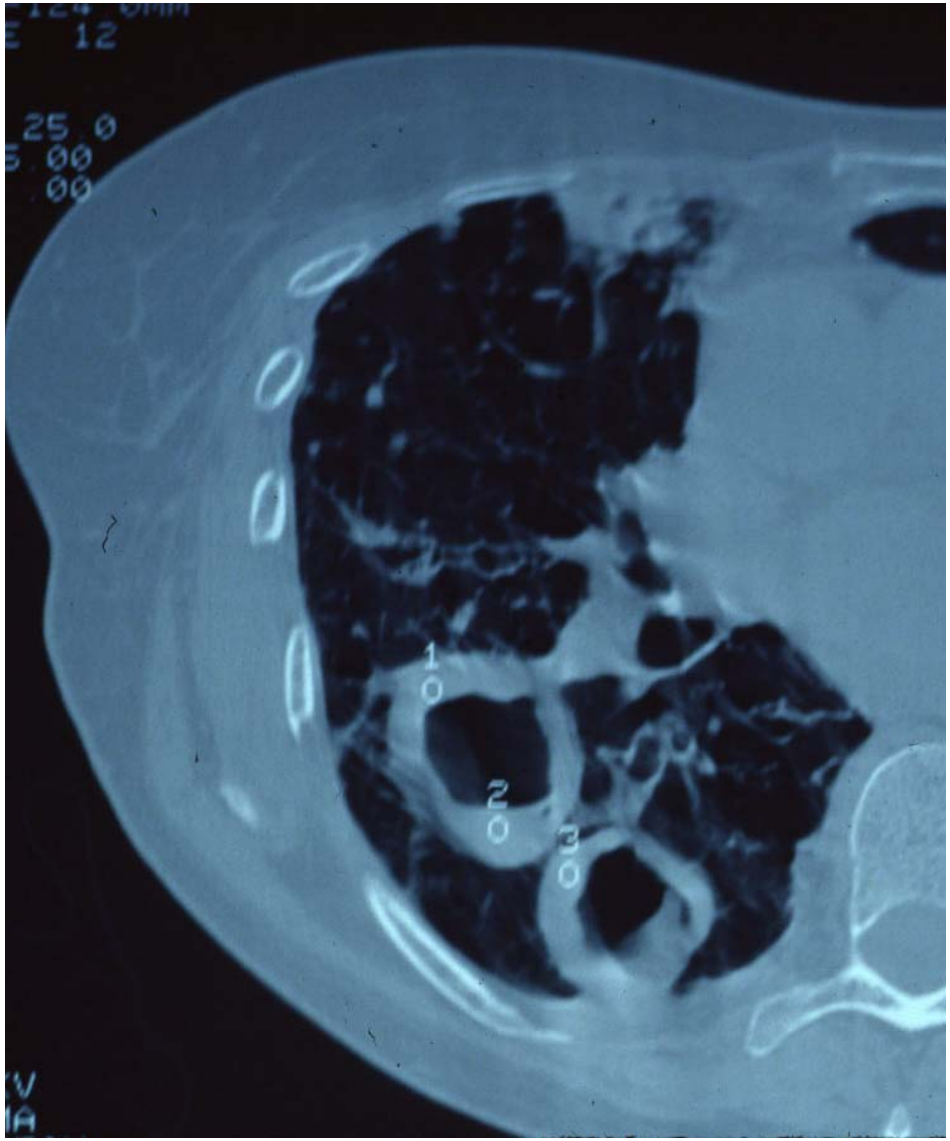
- Is this tuberculosis? Or is it atypical bacterial pneumonia, fungal pneumonia, or the presenting symptoms of lung cancer?
- What should be done at this point?
  - Start TB therapy while awaiting cultures
  - Give a course of therapy for presumed bacterial pneumonia (doxycycline)
  - Consultation with a pulmonary specialist – for bronchoscopy

Case #3 – cough and weight loss in an elderly woman

- 72 year old woman with cough for 4 months
  - Small amounts of phlegm, occasional hemoptysis
  - Weight loss of 15 lbs.
  - No response to amoxicillin-clavulanate
- PMH
  - No known exposure to TB, negative TST while a teacher; currently a volunteer in a kindergarten
  - Lived in Colorado all her life
  - Hypertension, controlled on therapy

Case #2 – Chest radiograph and CT scan





Case #3 - evaluation

- Sputum samples
  - 1-2+ AFB-smear positive
  - Cultures pending
- Clinical questions
  - Is this TB or another mycobacterial infection?
  - Should TB therapy be started?
  - Should a contact investigation be started?
  - What is the message to the school, the parents, the media?

Overview of manifestations of TB in a low-incidence settings (Switzerland)

Manifestation	N (%)
Pulmonary	**158 (63%)**
Smear + / culture +	93 (59%)
Smear - / culture +	58 (37%)
Smear - / culture -	7 (4%)
Extrapulmonary	**137 (54%)**
Adenitis	74 (29%)
Pleuritis	18 (7%)
Abdominal	17 (7%)
Bone-joint	17 (7%)
Genitourinary	16 (6%)
Other	25 (10%)

- 84% foreign-born
- 11% HIV-positive

Kherad O, et al. BMC Infect Dis 2009; 9: 217

Currently available tests for active TB

- Relatively rapid tests (< 3 days)
  - AFB smear
  - Nucleic acid amplification (NAA) tests
  - Tuberculin skin test
  - Interferon gamma release assays (IGRA)
- Mycobacterial culture
- Histopathology

AFB smear and culture in a hospital in a low-incidence setting (UK)

Isolate	AFB smear-positive	AFB smear-negative	Total
M. tuberculosis	98	82	180
M. avium complex	5	25	30
M. kansasii	8	8	16
Other mycobacterium	10	44	54
Culture-negative	2		
Total	128	159	282

- **Sensitivity of smear for culture-positive TB: 54% (98/180)**
- **Specificity of smear for TB: 77% (98/128)**

Taegtmeyer M, et al. Thorax 2008;63:317-21

Factors associated with smear-negative TB

- Age – children have a much higher risk of smear-negative TB
- HIV infection (immunosuppression)
- Extrapulmonary involvement
- Disseminated disease

All groups with an increased risk of rapidly-progressive disease

Yield of microbiological tests among 250 children with suspected pulmonary TB: South Africa

	Smear-positive	Culture-positive	Smear or culture positive
Total	29 (12%)	58 (23%)	62 (25%)
Induced sputum	25 (10%)	51 (20%)	54 (22%)
Gastric aspirate	17 (7%)	38 (15%)	40 (16%)

Zar H, et al. Lancet 2005; 365:130-4

False-positive reading of AFB smear

- Setting – mycobacteriology lab in South Africa
- Study – evaluation of smear and culture results
- Intervention – 190 simulated sputum specimens submitted into standard lab work
- AFB smear: 6 (3.2%) read as positive (all culture-negative)
- AFB culture: 2 (1.1%) reported as positive

Demners AM, et al. Int J Tuberc Lung Dis 2010; 14: 1016-23

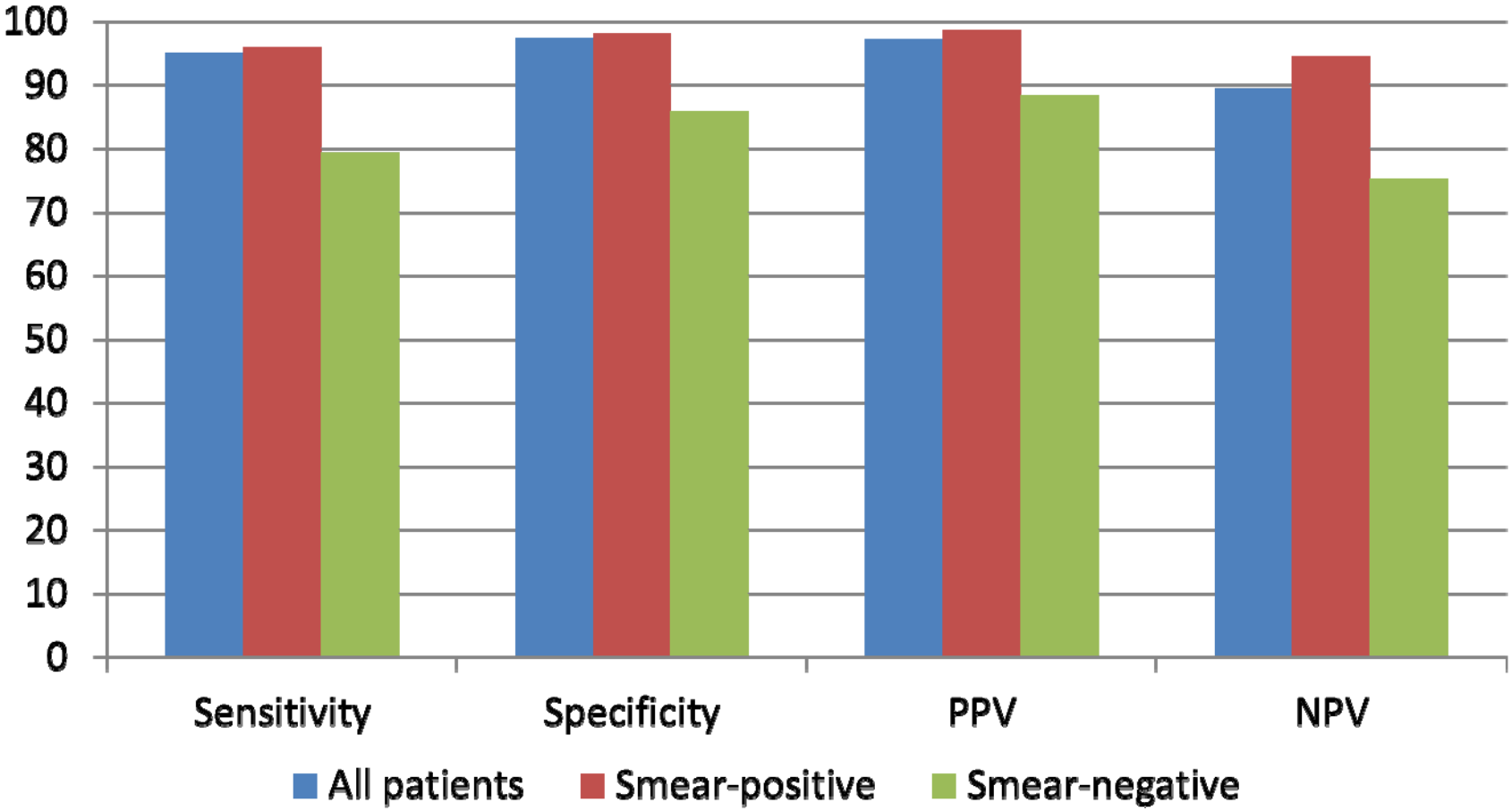
Diagnostic value of sputum smear

- Poor sensitivity
  - Smear-negative TB is common in pulmonary TB, very common in extrapulmonary TB
- Specificity
  - High for mycobacterial disease
  - Relatively low for *M. tuberculosis* in developed countries
  - False-positive results – unusual, but not rare

Clinical problems posed by the test characteristics of sputum smear

- Poor sensitivity
  - Delayed diagnosis among young children, patients with HIV co-infection, extrapulmonary TB
    - May result in clinical progression, even death
  - Delayed diagnosis of smear-negative pulmonary disease in HIV-negative adults
    - Patients may be lost while waiting for culture results
    - Low risk of transmission
- Suboptimal specificity for TB
  - Unnecessary treatment for TB, respiratory isolation, contact investigations

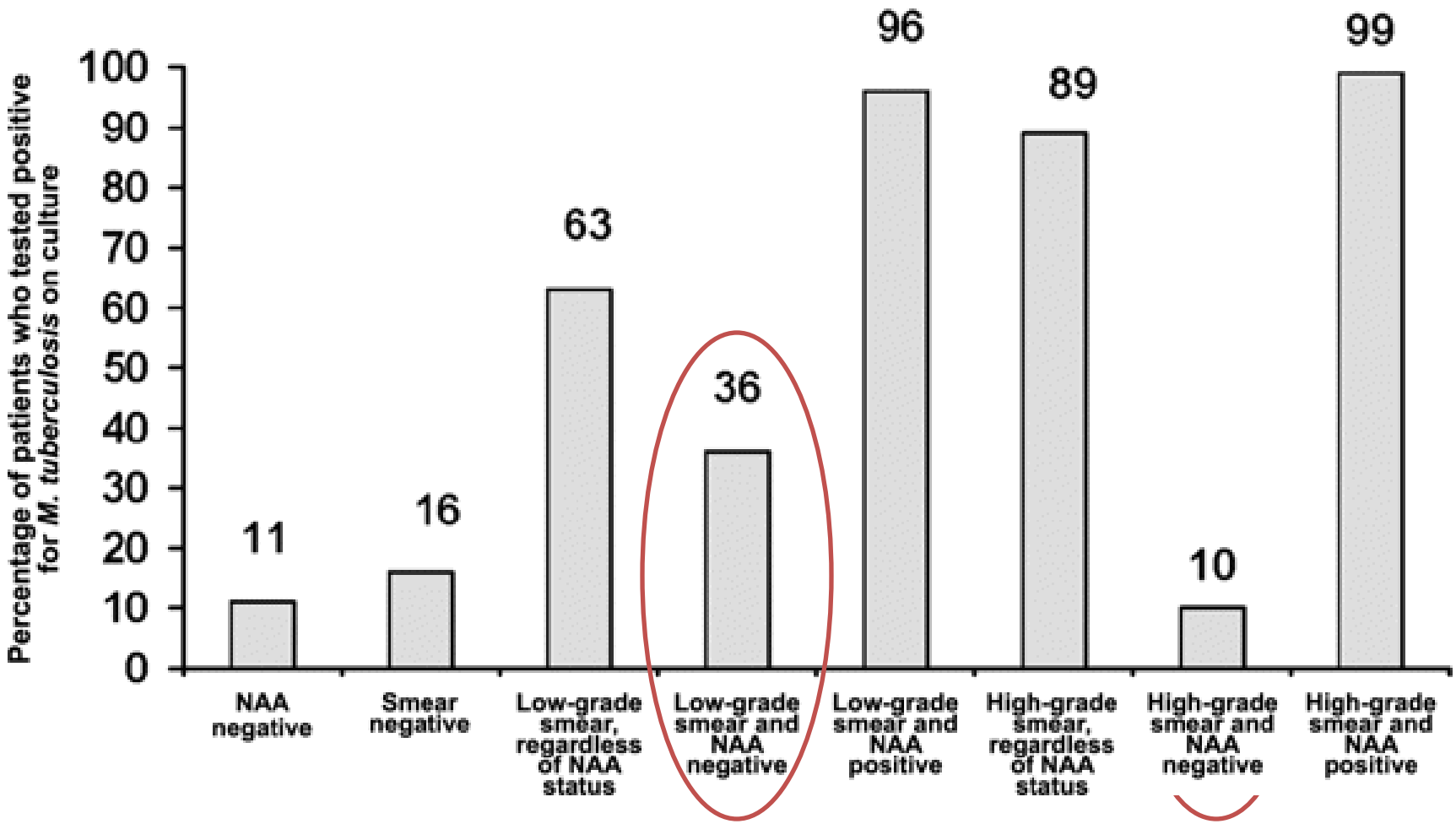
Performance of NAA for respiratory tract specimens: New York City (2000-2004)



N = 2021

Laraque F, et al. Clin Infect Dis 2009; 49: 46-54

% of patients with a positive sputum culture, by NAA result and sputum smear



Laraque F, et al. Clin Infect Dis 2009; 49: 46-54

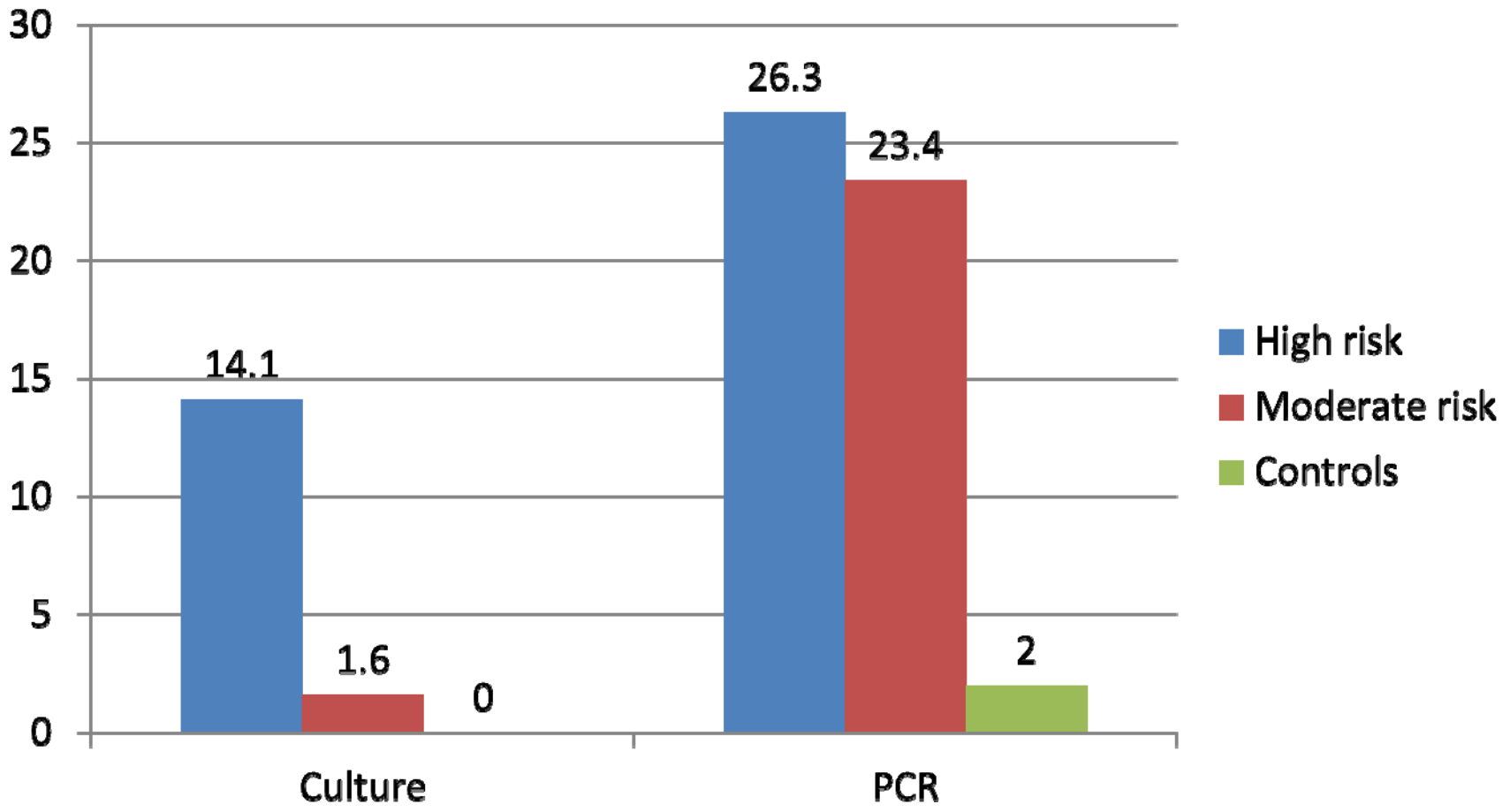
Diagnostic yield among patients with TB meningitis: Denmark (2000-2008)

Test	N, (% of 42 patients)
Positive AFB smear	4 (10%)
Positive nucleic acid amplification test	15 of 26 (58%)
Positive culture	31 (74%)

Median time to positive culture – 20 days, range 7-46 days

Christensen ASH, et al. BMC Inf Dis 2011; 11: 47

Comparative yield of culture and PCR among children at high- and moderate-risk of active TB and controls: Peru



Oberhelman RA, et al. Lancet Infect Dis 2010; 10: 612-20

Effect of NAA results on clinical care – pulmonary TB

Baltimore TB lab – 638 suspects, 270 with *M. tuberculosis* on culture, 173 with NTM

- Sensitivity – 92.3%, specificity – 99.8%
- Management compared to period before NAA testing
- No apparent effect on initial management of smear-positive patients
- Decreased length of unnecessary therapy (6 vs. 31 days)

Guerra RL, et al. Chest 2007; 132: 946-951

Effect of NAA results on clinical care – extrapulmonary TB

Hospital in New York City

- 32 patients with suspected extrapulmonary TB
- 14 diagnosed with TB
  - NAA sensitivity – 86%, specificity – 100%
- NAA result used clinically in two cases
  - One case – positive led to starting therapy
  - One case – negative led to stopping therapy

(Weiner RS, et al. Chest 2005; 128: 102-107)

Clinician's view of NAA testing

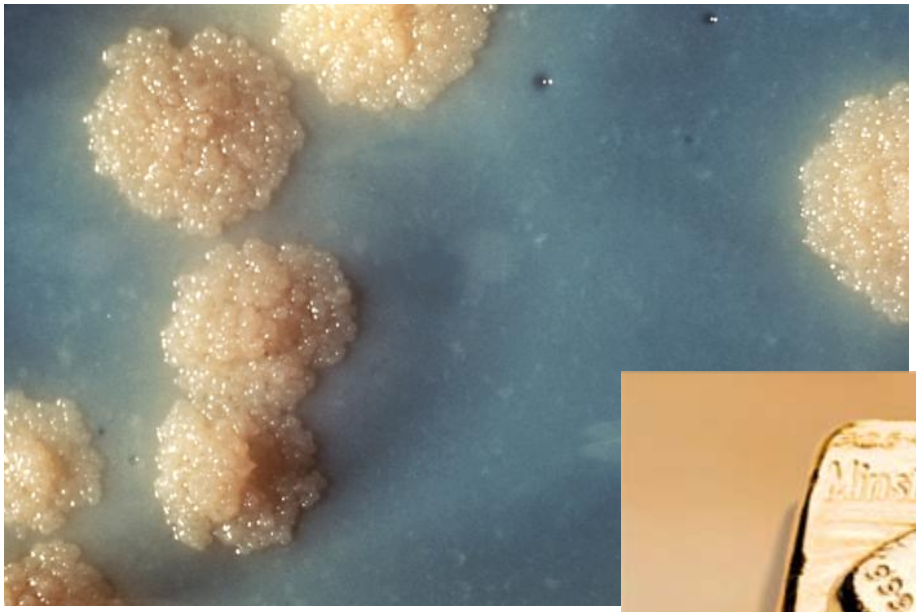
NAA and pulmonary TB

- Greater specificity for smear-positive specimens
  - Useful for smear-positive cases in which TB is not likely (Case #3)
  - Caveat - negative NAA in smear-positive case is not definitive
- Greater sensitivity for smear-negative specimens
  - Added clinical value is moderate
  - Value in children – unclear
- Major limitation of approved tests
  - Lack of assessment of drug-susceptibility

Clinician's view of NAA testing (continued)

- NAA and extrapulmonary TB
  - Greatest clinical need
    - Frequency of smear-negativity
    - Severity of some forms of extrapulmonary TB
  - Challenges
    - Positive result – strongly suggests TB
    - Negative results – insufficient as basis for stopping therapy
    - Additional data needed

Mycobacterial culture – the gold standard?



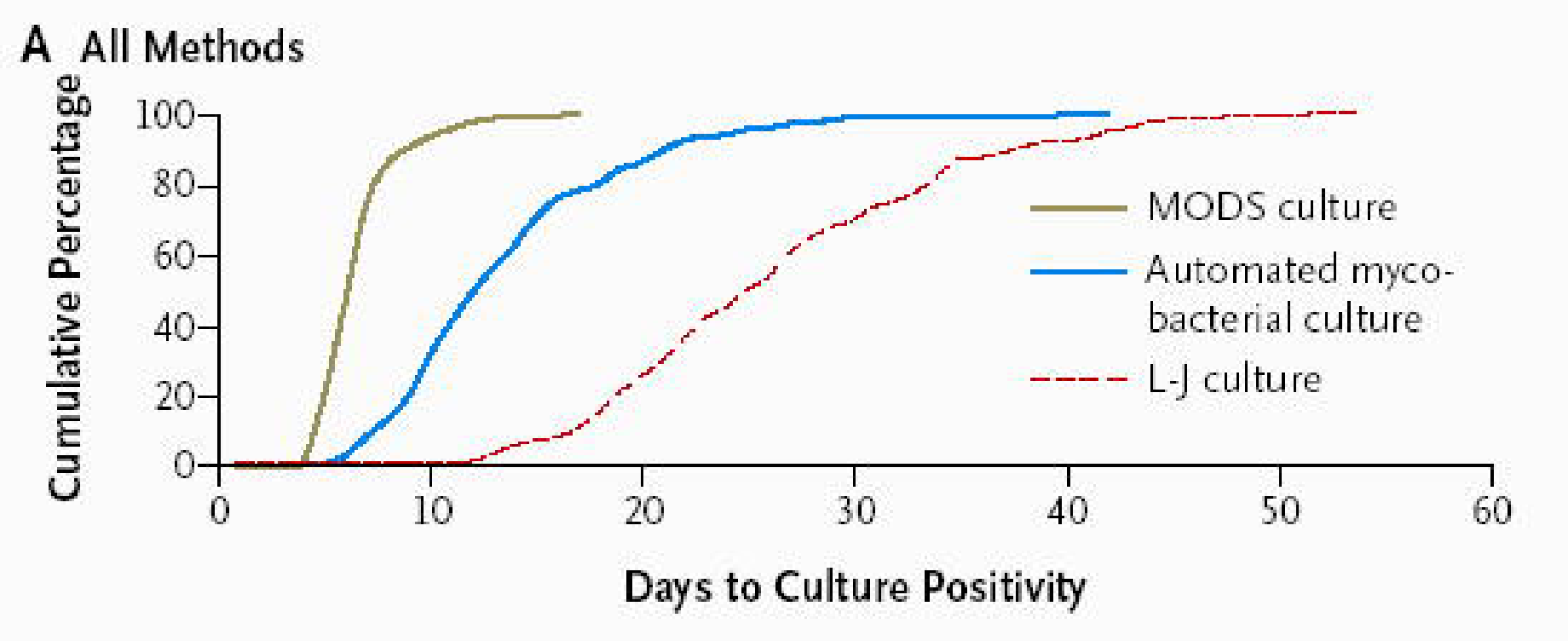
Mycobacterial culture and pulmonary TB

- High sensitivity
  - HIV-positive and HIV-negative
  - Sensitivity uncertain in young children
- High specificity
  - Can accurately distinguish *M. tuberculosis* from NTMs
- Positive culture is usual first step in testing for drug-resistance testing

Limitations of mycobacterial culture

- Time to positive results problematic
  - Decision-making for public health (initial therapy, isolation, contact investigations)
  - Rapidly-progressive forms of TB
  - Drug-resistant TB
- Suboptimal sensitivity
  - Effect of prior (unwitting) therapy
  - Extrapulmonary disease
- Suboptimal specificity
  - Laboratory cross-contamination

Time (days) to culture-positivity among patients with TB



N Engl J Med 2006;355:1539-50

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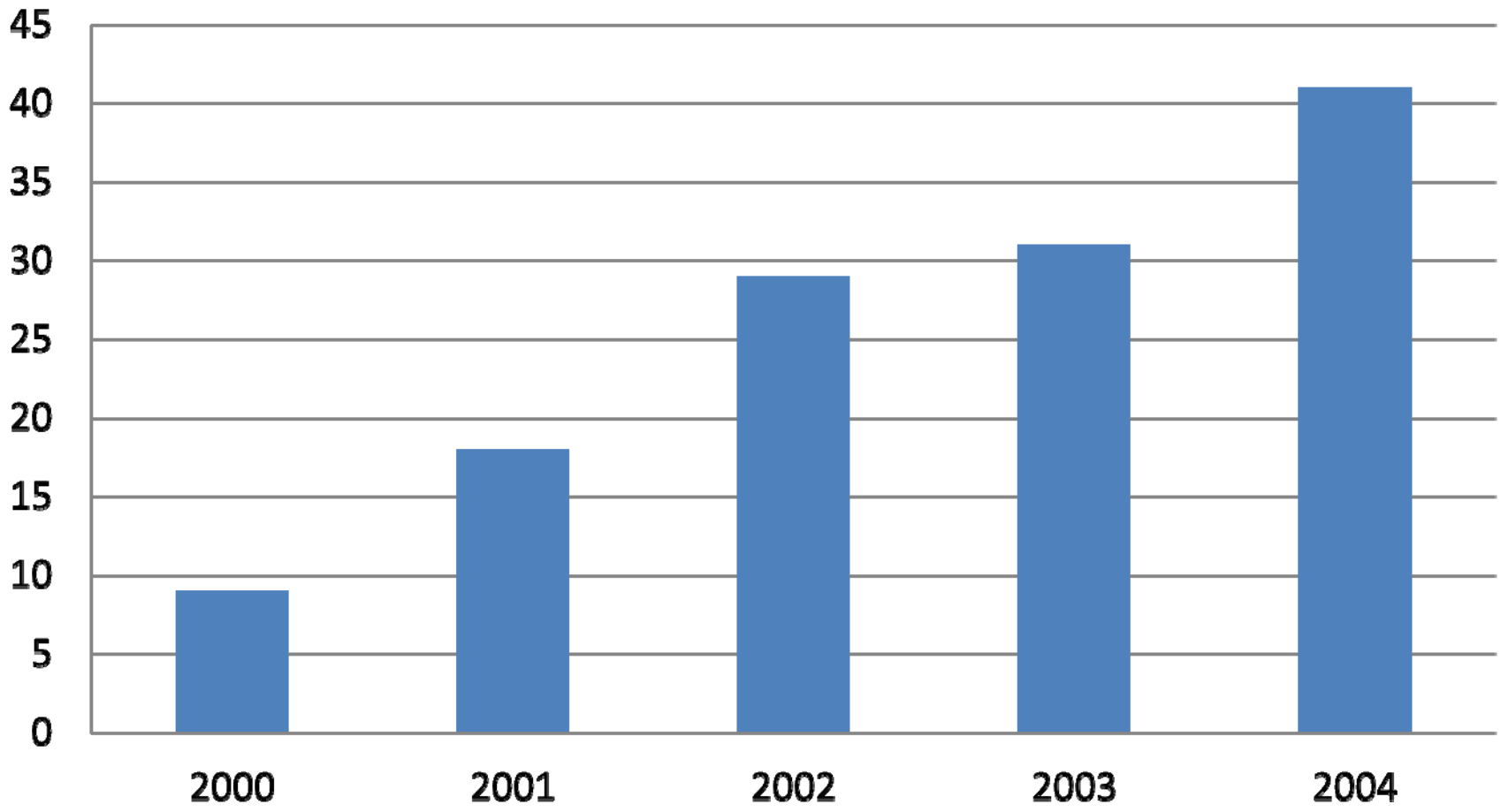
Frequency of fluoroquinolone therapy within 12 months prior to diagnosis of TB: Tennessee



Gaba PD, et al. Arch Intern Med 2007; 2317-2322

Predictors of culture-negative pulmonary TB: Tennessee

Risk factor	Adjusted OR (95% CI)	P
Age	0.95 (0.94-0.97)	<.001
Male sex	0.88 (0.47-1.58)	.62
Black race	0.63 (0.33-1.21)	.16
HIV-positive	0.24 (0.07-0.82)	0.02
Fluoroquinolone exposure	**1.18 (0.55-2.53)**	**.67**
Year of diagnosis	1.18 (0.95-1.45)	.13

Median time from FQ exposure to TB diagnosis – 80 days (IQR 27-250 days)

Gaba PD, et al. Arch Intern Med 2007; 2317-2322

Diagnosis of TB pericarditis: South Africa (1995-2001)

- Prospective study – 233 patients with large pericardial effusions
- Pericardiocentesis
  - Mycobacterial smear and culture (BACTEC 960)
  - LDH, protein, adenine deaminase activity
  - Cytology, WBC count and differential
- Pericardial biopsy if no diagnosis in 7 days
- HIV testing – 84 positive (36%)

Reuter H, et al. Q J Med 2006; 99: 827-39

Final diagnoses – patients with large pericardial effusions

Final diagnosis	N (%)
Tuberculosis	162 (70%)
Malignancy	22 (9%)
Connective tissue disease	12 (5%)
Bacterial pericarditis	4 (2%)
Other	32 (14%)

Reuter H, et al. Q J Med 2006; 99: 827-39

Utility of diagnostic tests for TB pericarditis

Test	N	Sensitivity	Specificity
Positive pericardial fluid culture	203	52%	100%
Histopathology	36	64%	100%
Pericardial ADA (> 40)	212	87%	89%
Pericardial lymphocyte/neutrophil ratio > 1	192	73%	79%
TST > 10 mm	52	89%	56%
PCR for *M. tuberculosis*	48	30%	100%

Patients with culture-negative pericardial TB: smear-positive (3), positive histopathology from biopsy (16), positive culture or histopathology from another site (16), etc.

Reuter H, et al. Q J Med 2006; 99: 827-39

Clinical settings in which mycobacterial culture lacks sufficient sensitivity to "rule out" active TB

- Extrapulmonary TB
  - Pleural, pericardial, ascitic fluid
    - Invasive biopsies often necessary
  - Meningitis
- Suspected pulmonary TB
  - Young children
  - Pretreated patients

Laboratory cross-contamination causing false-positive cultures for *M. tuberculosis*

- 16 studies including > 100 patients with a positive culture for *M. tuberculosis*
  - Literature review (Burman W, et al. Clin Infect Dis 2000; 31: 1390-5), updated to June 2011
- 432 of 13,192 patients (3.0%) had false-positive
- Median percentage/study: 2.9%, IQR: 2.0-4.0%, range: 0-33%
- Of patients with reported clinical outcomes: 55% treated for TB (206/375)

Suboptimal specificity - false-positive cultures

- Found in nearly all studies evaluating > 100 patients (16 of 17)
- Common mechanism – cross-contamination during initial batch processing
- Factor associated with higher rates:
  - Low volume lab (J Clin Micro 2002; 40: 4004-9; Arch Pathol Lab Med. 2001 ;125: 1213-6)
- Clinical relevance
  - Most patients treated for TB
  - Unnecessary hospitalizations, procedures, contact investigations

Clinician's summary of mycobacterial culture

- Excellent test – with significant limitations
- Time to a positive can be problematic
- Suboptimal sensitivity in key situations
  - Extrapulmonary TB
  - Young children
- Suboptimal specificity
  - Frequency of false-positive cultures is not widely appreciated by clinicians
- Cost and complexity of lab procedures needed

Follow-up in the cases

- Case #1 – Severe illness in a man with AIDS
  - Immune reconstitution syndrome related to disseminated *M. avium* complex (liver biopsy)
- Case #2 – Cough in a Bhutanese refugee
  - Smear-negative, culture-positive TB
- Case #3 – Cough, weight loss in an elderly U.S.-born woman
  - *M. avium* complex pulmonary disease

Case# 4 – Young woman with prolonged cough

- 1 month of cough: urgent care clinic, Dx - bronchitis (no CXR), RX - azithromycin
- 3 months: urgent care, Dx - bronchitis (no CXR), Rx - amoxicillin/clavulanate
- 5 months: begins an accelerated nursing program, TST negative (no symptom screen)
- 6 months: self-referral to National Jewish
  - Travel Hx: lived in S. Africa for 6 months, including in KwaZulu-Natal

Case#4 – Chest x-ray

- 3+ AFB-smear positive

Clinical/programmatic questions

- Should an expanded regimen for possible drug-resistant TB be started?
  - If so, should that include agents with predictable activity against “MDR-only” TB or should it include drugs for “pre-XDR”, etc.
- How should close contacts be managed?
  - Should treatment of TST/IGRA+ contacts be held until the degree of resistance in the source case is known?
- What is the message for the public / media?

Case #5 - abdominal pain, fevers in a Sudanese refugee

34 year old man

- 7 weeks of non-bloody diarrhea, fevers, abdominal pain
- Weight loss of 12 kg, severe fatigue

Cachectic, somewhat confused

- T- 36.8 P – 68 BP – 103/62 O2 sat. – 98% (2 L)
- HEENT – no thrush, no nodes

- WBC – 7.2, HCT – 25, PLT – 230
- LP – normal HIV+ CD4 – 6 VL – > 750,000

Initial evaluation

- CXR – retrocardiac infiltrate
- CT abdomen – retroperitoneal adenopathy, ascites, omental caking, colonic wall thickening
- Colonoscopy – diffuse ulcers; CMV positive
- Sputums x 3 – AFB smear-negative, paracentesis fluid – AFB smear-negative
- Treatment – ganciclovir
- Broth AFB culture – positive at 6 days

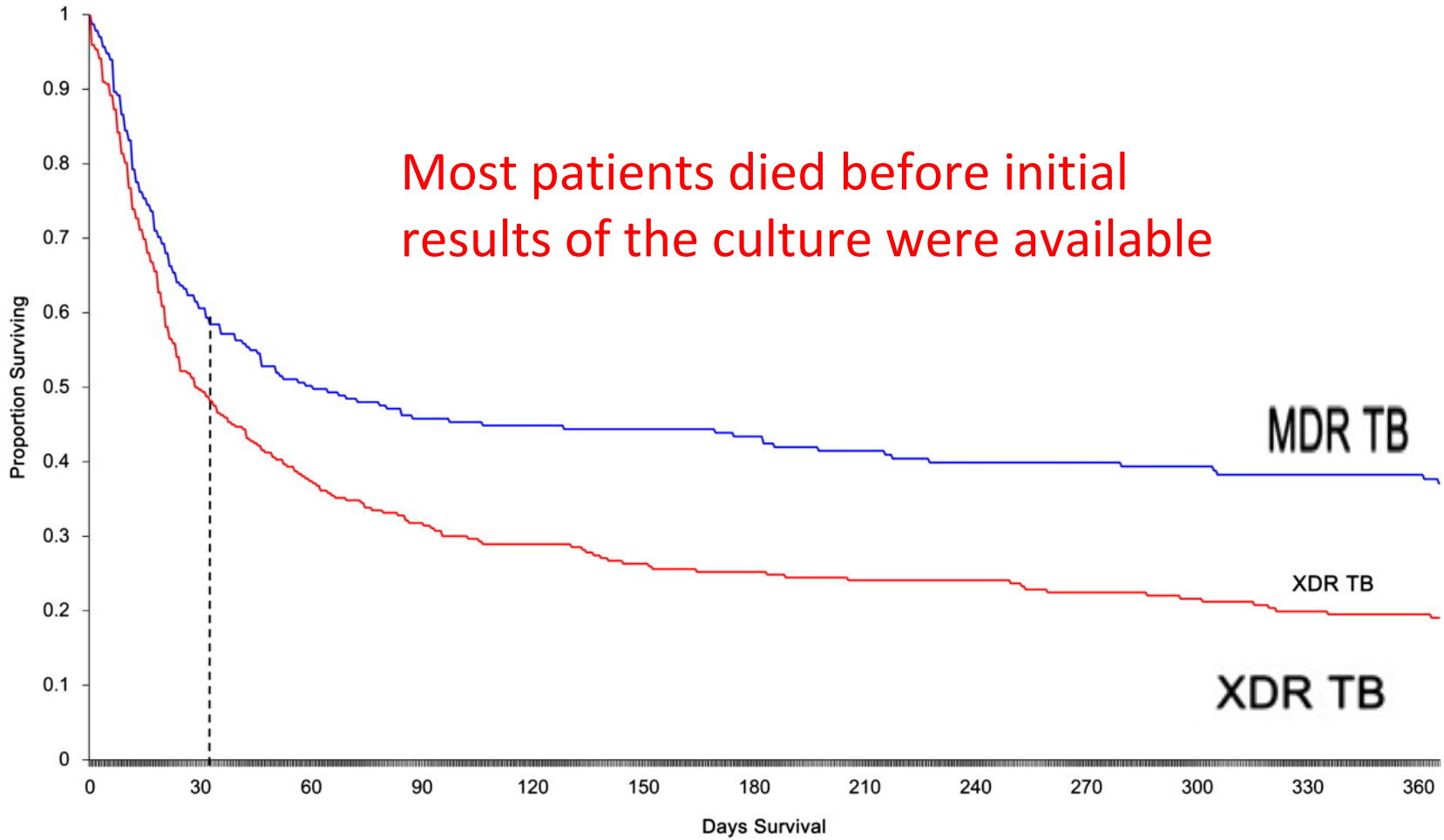
Case #5 – Clinical questions

- Is this drug-resistant TB?
  - If so, what is the breadth of resistance?
- What treatment should be started in this severely ill patient?
  - Standard initial therapy for TB (HRZE)
  - Expanded therapy for TB
  - Antifungal therapy

Pros and cons of phenotypic resistance testing

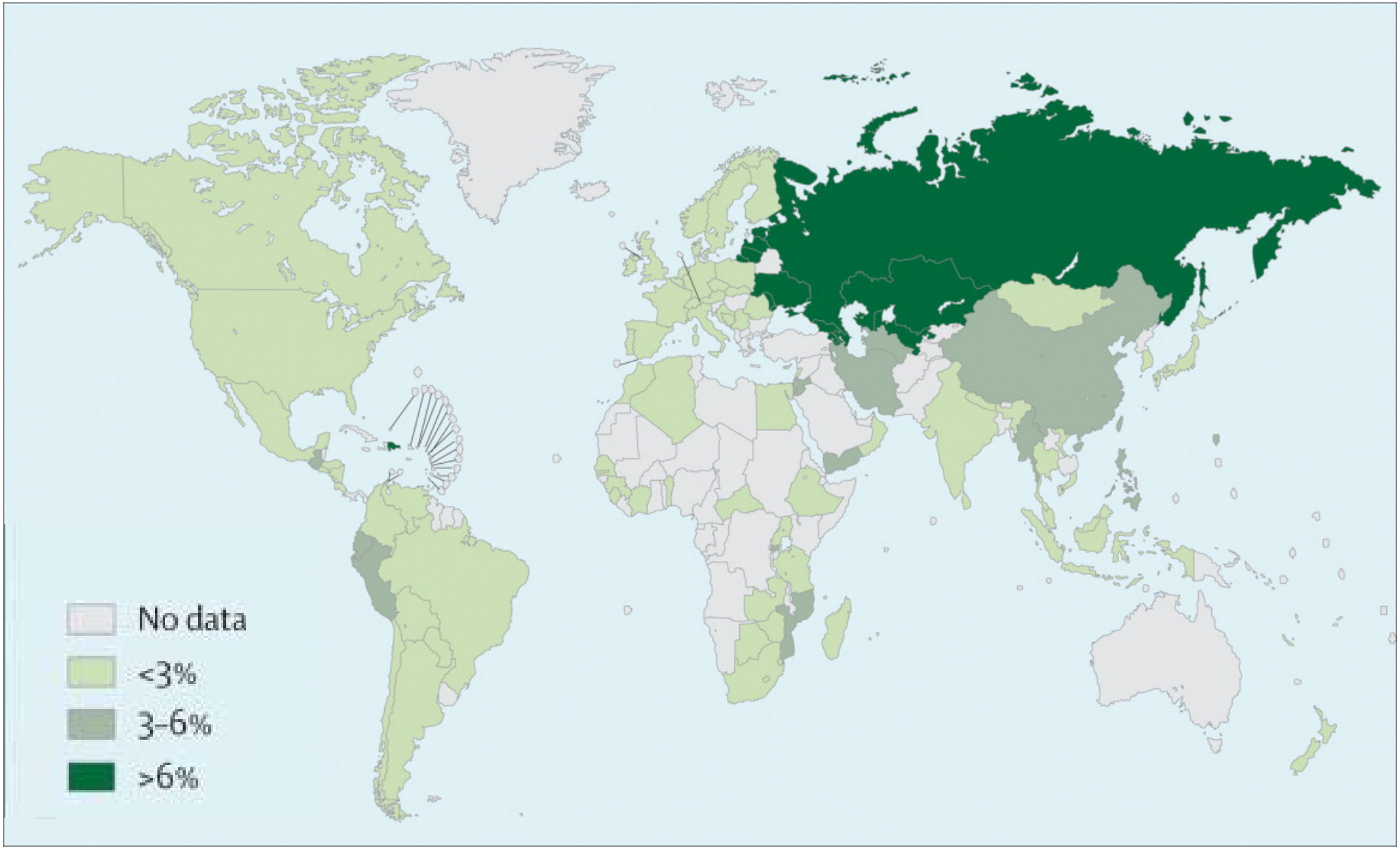
- Time required for results
- Difficulty in obtaining highly reproducible results for drugs other than INH and rifampin
- Uncertainty about the interpretation of *in vitro* measures of resistance to drugs other than INH and rifampin
  - Relationships between *in vitro* activity (MIC, MBC) and clinical activity are class-specific, cannot be assumed
  - Simple pharmacodynamic models (peak/MIC) do not correlate well with clinical activity

Survival of 654 patients with drug-resistant TB, from the time of sputum collection (South Africa)



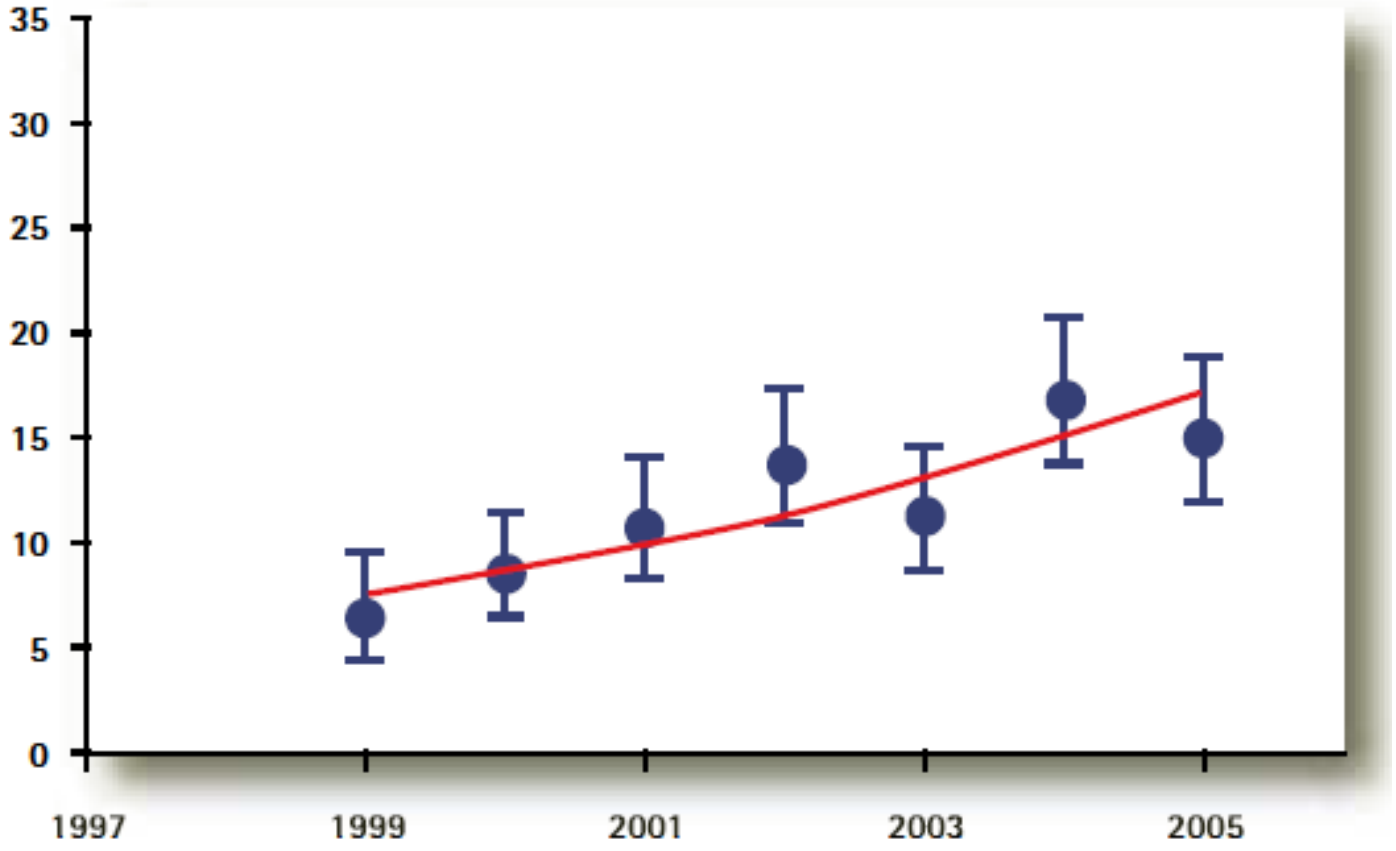
Am J Respir Crit Care Med 2010; 181: 80-86

Prevalence of MDR-TB - 2009



Lancet 2009;373:1861-73

Prevalence of primary MDR-TB in Tomsk Oblast, Russia



WHO: Antituberculosis drug resistance in the world: report #4 (2008)

Prevalence of MDR-TB in Africa over time – 2004, 2008, and current estimate

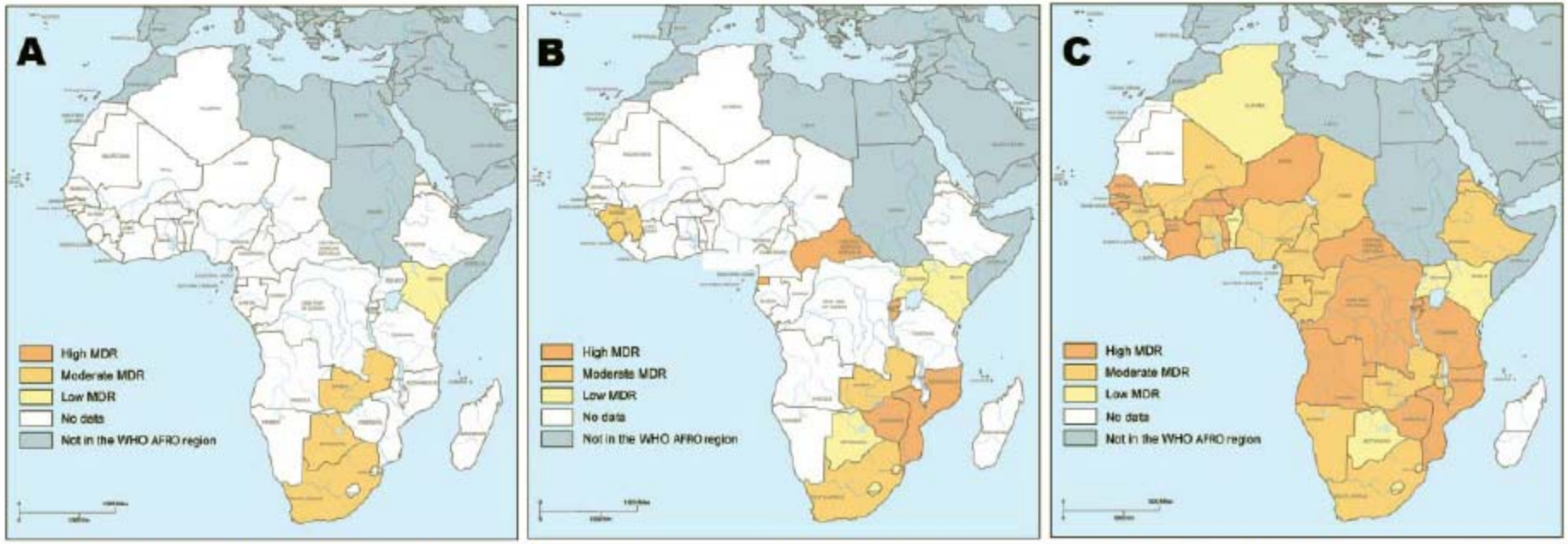


Figure. Prevalence of multidrug resistance (MDR) in Africa among combined tuberculosis cases. A) Data collected from the Third Global Report on Anti-tuberculosis Drug Resistance in the World of the World Health Organization (WHO) published in 2004 (*40*). B) Data from various recent WHO publications, peer-reviewed journal articles, and WHO's Fourth Global Report (*1*). C) Formulaic estimates of Zignol et al. (*11*). AFRO, WHO Regional Office for Africa.

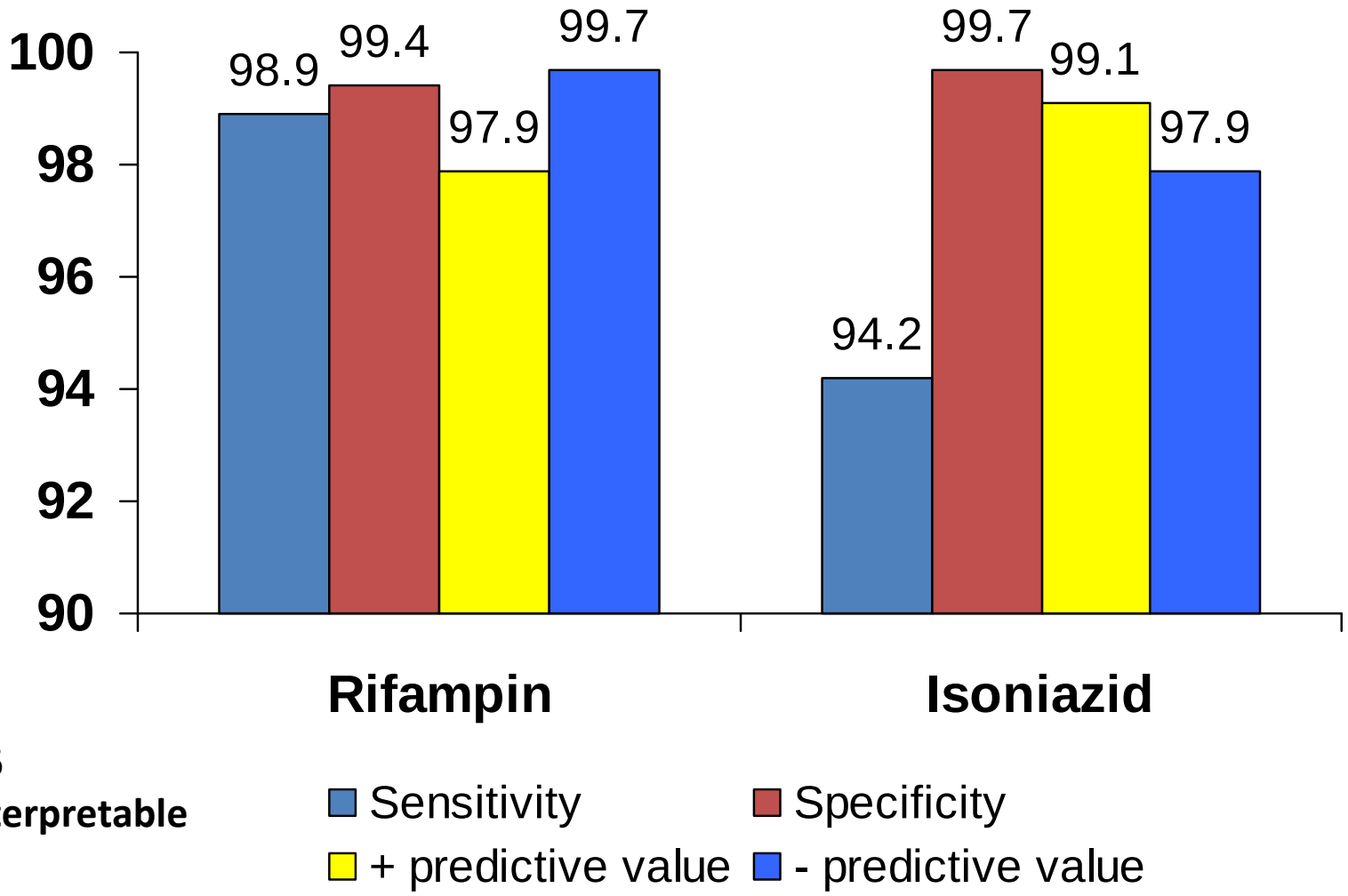
Orange: ≥ 2% MDR among all TB cases

Emerg Infect Dis 2008; 14: 1345-52

Clinician's view of drug-resistance testing

- Global increase in highly-resistant TB creates major challenges for domestic TB control programs
- Critical need for rapid resistance testing
  - Assure appropriate initial treatment for the individual patient
  - Assure appropriate TB control activities – contact investigation, treatment of close contacts, decisions about hospitalization and isolation

Rapid genotypic detection of RIF and INH resistance among smear +sputum specimens – Cape Town



N = 536
97% interpretable results

Am J Respir Crit Care Med 2008;177:787-92

Pros and cons of rapid genotypic drug resistance tests

- Pros
  - Results in < 24 hours
  - High sensitivity and specificity for rifampin resistance
  - Possibility that genotypic testing may clarify resistance to “weaker drugs”
- Cons
  - Possibility of laboratory cross-contamination causing false-positive results
  - Uncertainty about interpretation

Do we underestimate the frequency of drug resistance? Activity of 12RE(7) for retreatment TB, by baseline ethambutol susceptibility

	Resistance to INH and EMB	Resistance to INH alone
Number assessed	15	73
Favorable	5 (33%)	53 (73%)
Failure	10 (67%)	20 (27%)

Acquired resistance in treatment-failure isolates from patients with baseline susceptible isolates

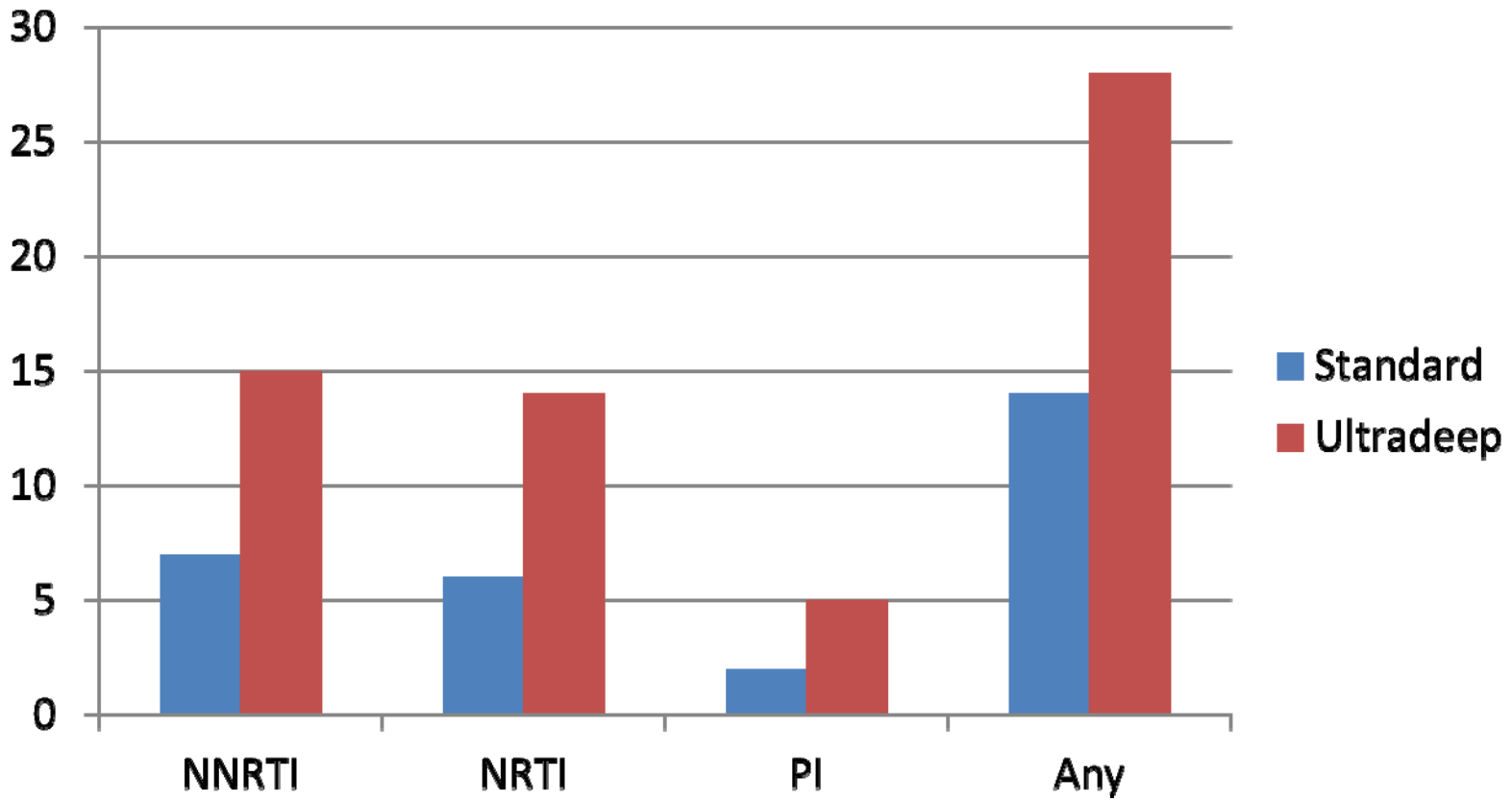
- RIF – 95%, EMB – 60%

Conclusions

- Remarkably poor performance among patients with baseline "EMB-susc." isolates – undetected resistance?

Hong YP. Tubercle 1988 69: 241-53

Frequency of HIV resistance among treatment-naïve patients: standard vs. ultra-deep genotype



Baseline NNRTI resistance predicted failure of NNRTI-based therapy (HR – 2.7)

J Infect Dis 2009;199:693-701

Reasons that resistance may be under-estimated by phenotypic testing

- Lack of detection of specific forms of resistance
  - Specific rpoB mutations (e.g., Asp516Tyr), low-level resistance
  - Not detected in broth systems, correlated with failure/relapse (J Clin Microbiol 2009; 47: 3501-6)
- Minority variants – resistance mutations having a fitness cost
  - May explain worse outcomes in patients with prior treatment, even after adjusting for degree of resistance by phenotypic testing

Summary – rapid resistance tests

- High priority for TB control – low-burden and high-burden settings
- Need is greatest for RIF, but also needed for other drugs
- Genotypic resistance testing may be superior to phenotypic testing
  - Ethambutol, PZA, 2nd-line drugs
  - More data needed
- Genotypic testing likely to be simpler than phenotypic testing

Follow-up on cases

- Case #4 – cavitary pulmonary disease in a woman who lived in South Africa
  - Drug-susceptible TB – diagnosed at 3 days with a rapid test
- Severely ill man with AIDS from the Sudan
  - Multidrug-resistant TB – excellent response to treatment for MDR-TB and HIV disease

Closing thoughts

- Despite favorable epidemiologic trends in the U.S., great need for improved diagnostic tests for TB
- High priorities
  - More sensitive tests for TB: for children, HIV-positive, patients with extrapulmonary-only disease
  - Rapid resistance tests
  - Simpler tests with lower risk of cross-contamination