TB Resistance Testing: Perspective from a Clinician

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POPULATION HEALTH DIVISION
SAN FRANCISCO DEPARTMENT OF PUBLIC HEALTH
DISEASE PREVENTION & CONTROL

Conflict of Interest Disclosure Statement

- Neither I, nor my spouse/partner have financial or other relationships with any commercial interest organizations within the past 12 months.
- Nothing to disclose

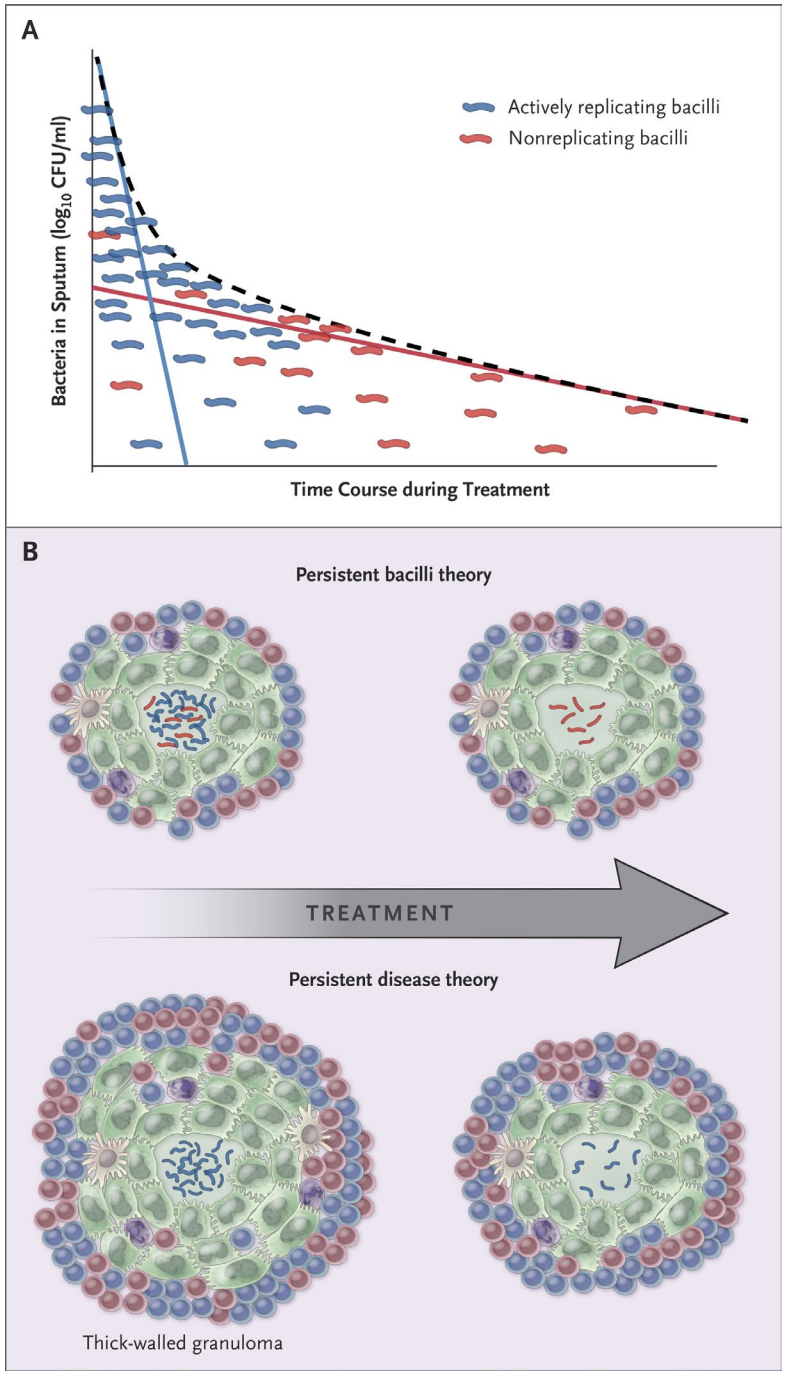
Principles of TB Treatment

CDC and WHO-recommended TB treatment consists of 4 drugs:

isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA)

The goals of multidrug tuberculosis therapy are threefold:

- TB killing: rapidly reduce the number of actively replicating bacilli, thereby decreasing disease severity, death, and halting transmission (INH, RIF, fluoroquinolones like levofloxacin/ moxifloxacin);
- TB sterilizing: eradicate populations of persisting bacilli (can be dormant in granulomas) to prevent relapse after treatment completion (RIF, PZA); and
- Prevent emergence of drug resistance during therapy (EMB)



Biphasic Decline in Viable Bacteria during Treatment for Tuberculosis.

- Panel A shows the time course of decline of viable *Mycobacterium tuberculosis* in a sputum sample from a patient being treated for tuberculosis. The number of bacteria declines at a rapid rate during the early phase of therapy (blue curve), with a less rapid rate of decline during the later phase (red curve). The biphasic pattern that is observed (black dashed curve) suggests that there are bacterial subpopulations that differ in their drug susceptibility. CFU denotes colony-forming units. Panel B shows two proposed explanations for this differential response: persistent bacilli and persistent disease. The first explanation is that bacteria in a replicating state (blue) are more susceptible to drugs than are bacteria in a nonreplicating state (red), which can persist despite drug treatment. The second explanation is that some bacilli are sequestered in thick-walled granulomas, where antibiotics are not able to reach them, resulting in persistent disease.

Horsburgh et al. N Engl J Med 2015;373:2149-216

Practices in TB treatment

- Standard initial regimen: INH, RIF, EMB, PZA
- Once susceptibility known to at least INH and RIF→ EMB is stopped
- PZA is continued for 60 doses – no benefit with longer duration
  - If cavitary disease or < 60 doses of PZA, treatment duration extends from 6 to 9 months to reduce risk of relapse.
- Non-standard drugs if mono resistance or drug intolerance:
  - Fluoroquinolones (levofloxacin or moxifloxacin)
  - Linezolid
  - Amikacin
  - Cycloserine
  - If needed, bedaquiline and/or pretonamid
- Treatment duration can extend to 18-24 months depending on the final drugs in the regimen

2024 SF TB Cases

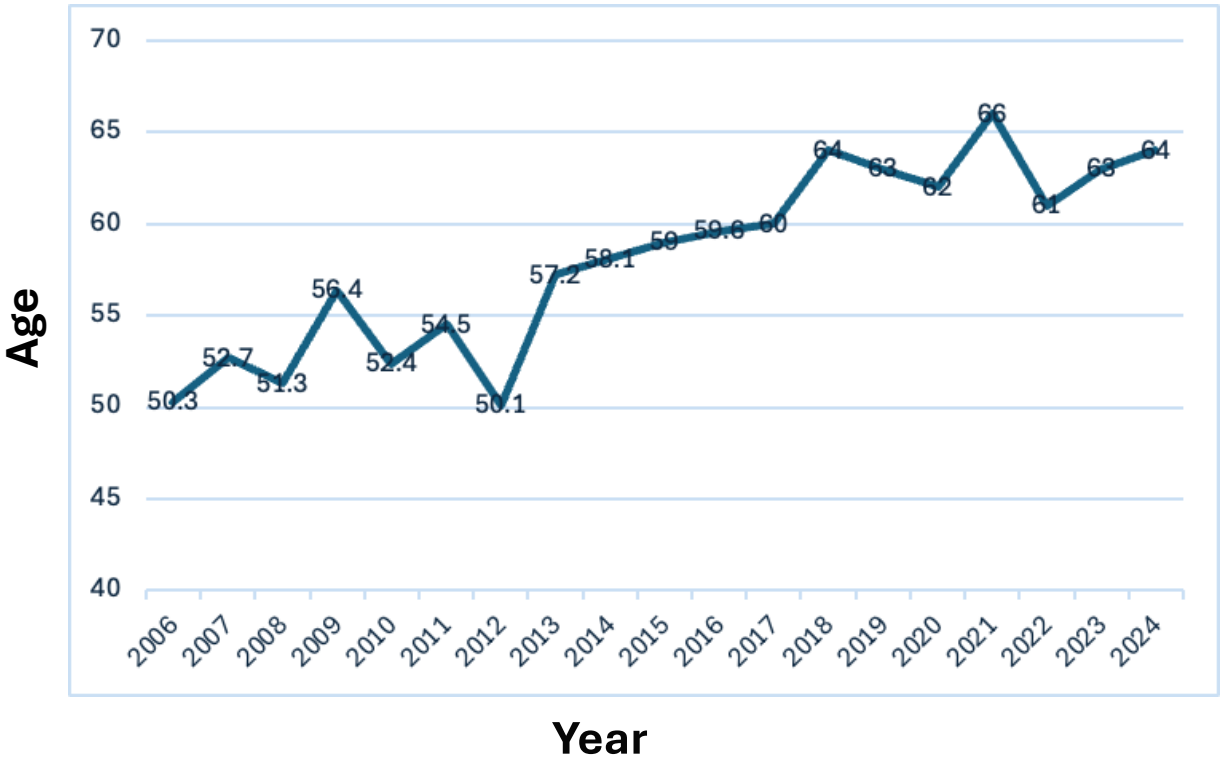
Total: 92 active cases (incidence 11 per 100,000)

- Median age: 64 years (range 0-94)
- Predominantly patients born in China, Vietnam, Philippines, Central America
- 65 (71%) had ≥ 1 comorbid medical condition

  -Diabetes mellitus: 19 (21%)

  - Immunocompromised status: 12 (13%), including HIV: 2 (2%)

TB case median age, 2008-2024



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Challenges of Treating TB in Older Persons

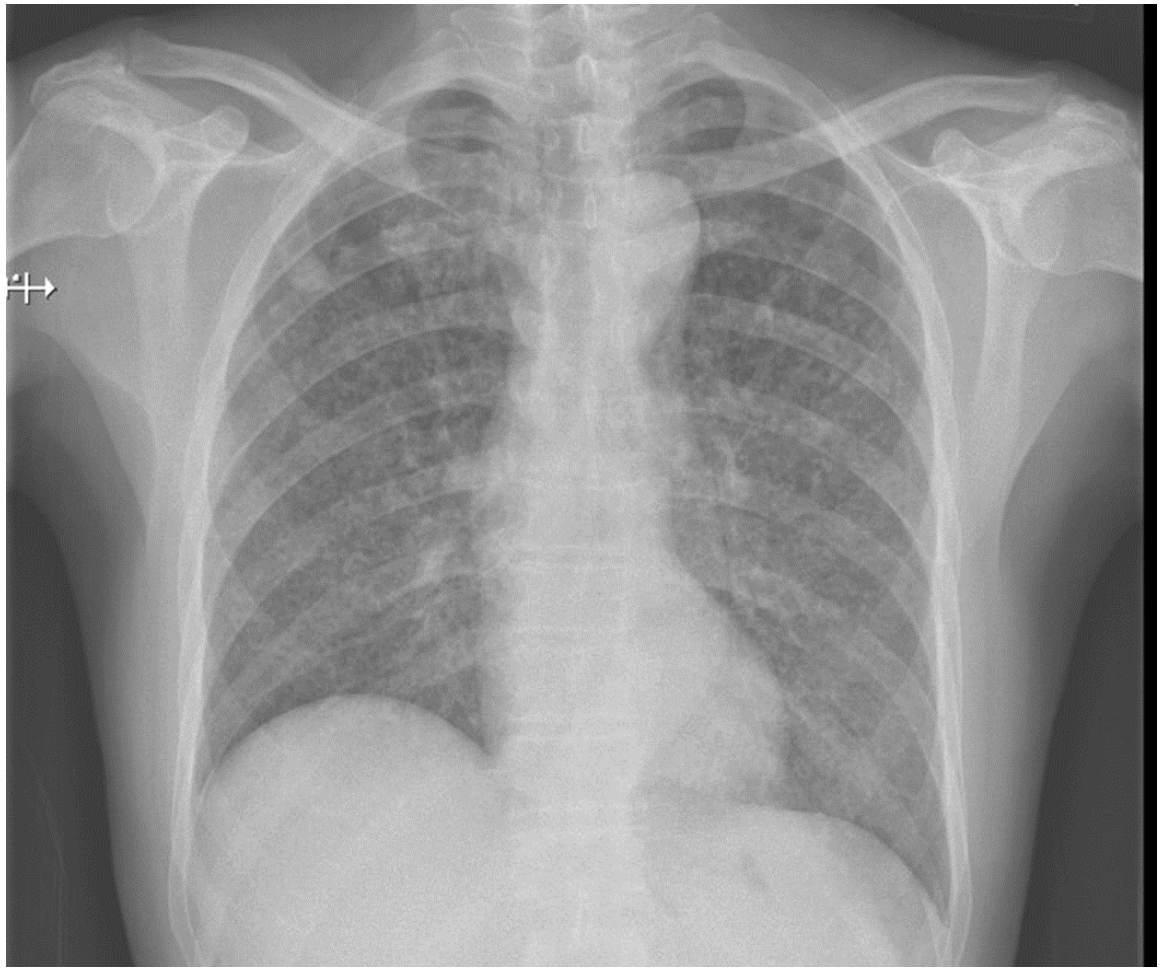
- More co-morbid illnesses
- Polypharmacy and drug-drug interactions with rifamycins
- Decreased renal function--> dosing challenges
- Decreased hepatic function
- Dementia/cognitive decline
  - Difficulty with following instructions during screening exams (vision, hearing, neurologic)
  - Challenges in identifying/reporting side effects
- Increased risk of adverse events/hepatotoxicity

2–122 , Shu CC et al. Int J Tuberc Lung Dis 2013; 17(7):934–939, Hagiya H et al. Epidemiol Infect. 2018 Nov 9;147:e38.

Clinical Case

- 79 year old female from Philippines
- PMH: diabetes, rheumatoid arthritis, chronic kidney disease
- Presented to the hospital with fever, chills, cough x 1 month, weight loss x 6 months and worsening back pain for one year
- Medications: metformin, infliximab (TNF inhibitor), prednisone 10 mg qd
- No prior TB exposure
- Does not smoke, drink or use drugs
- Lives with husband, son, and 2 and 4 year old grandchildren
  - Husband recently received a kidney transplant
  - Son in treatment for opioid addiction, taking methadone

Radiology

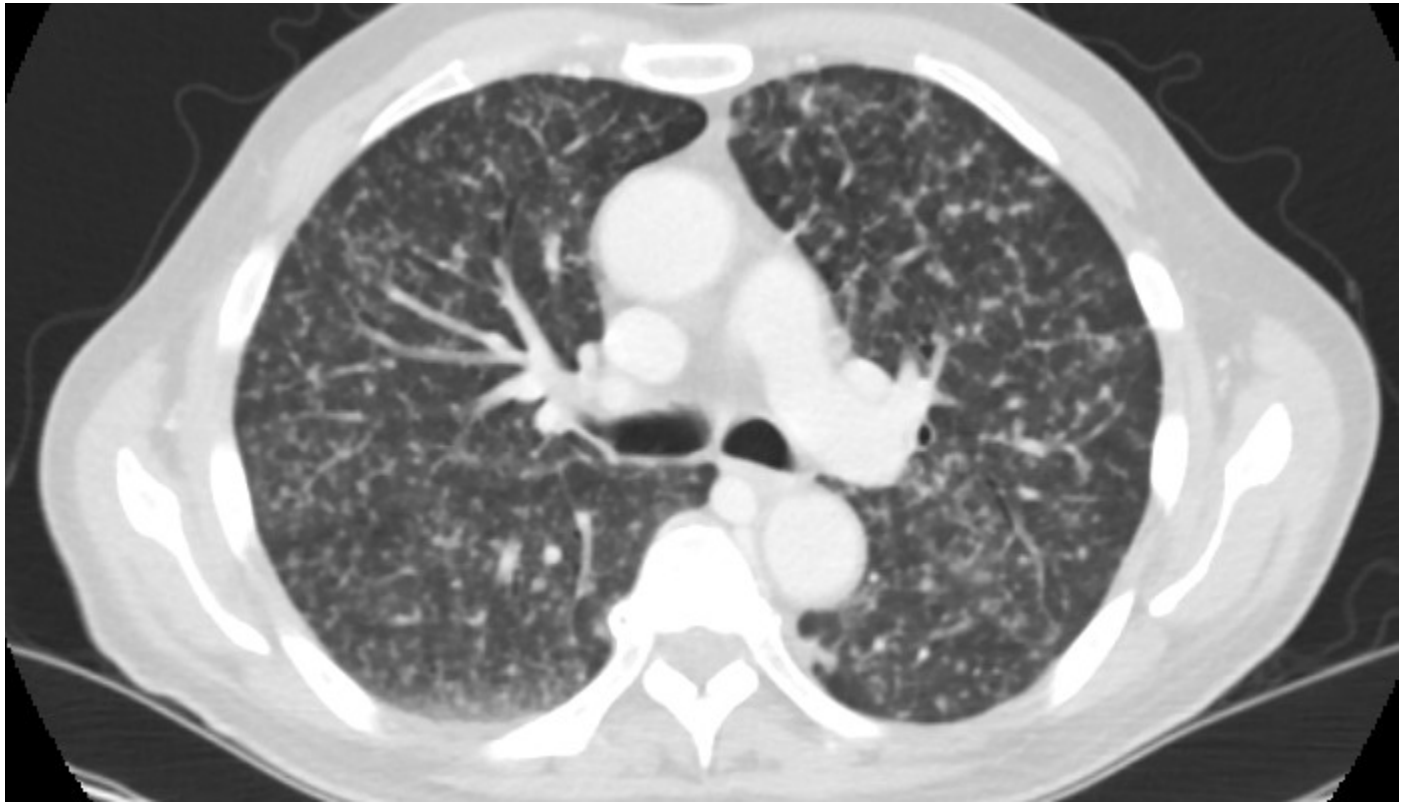


Chest X-ray shows multiple nodules in the right lung and miliary TB “millet seeds” throughout

Radiology

Chest CT:

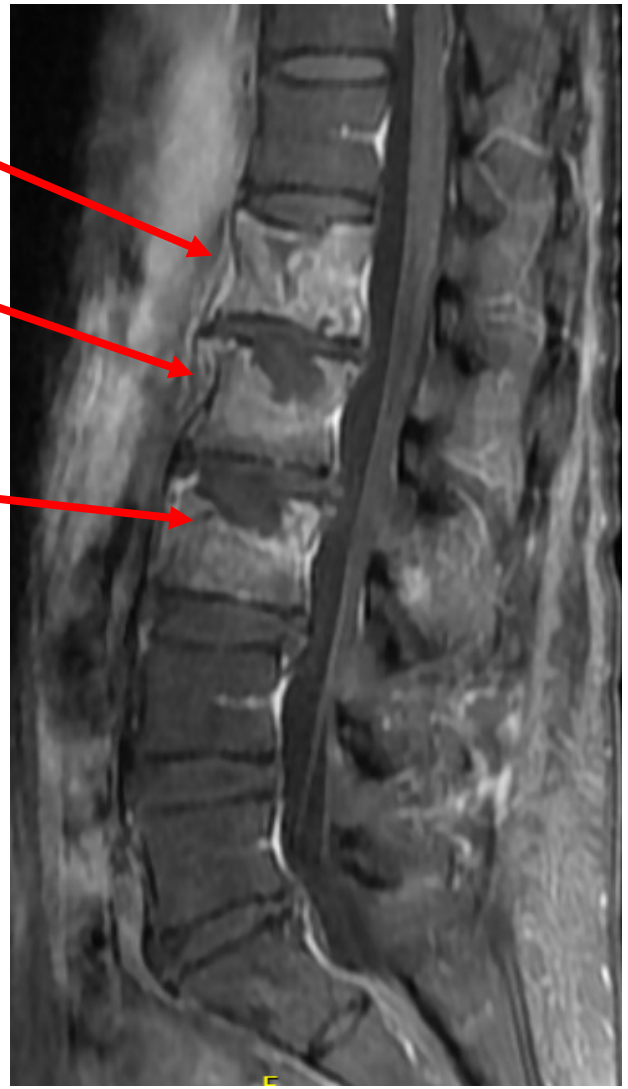
- Innumerable lung nodules
- Large right upper lobe nodules.
- Pathologic fractures in the spine at L1 and L3.

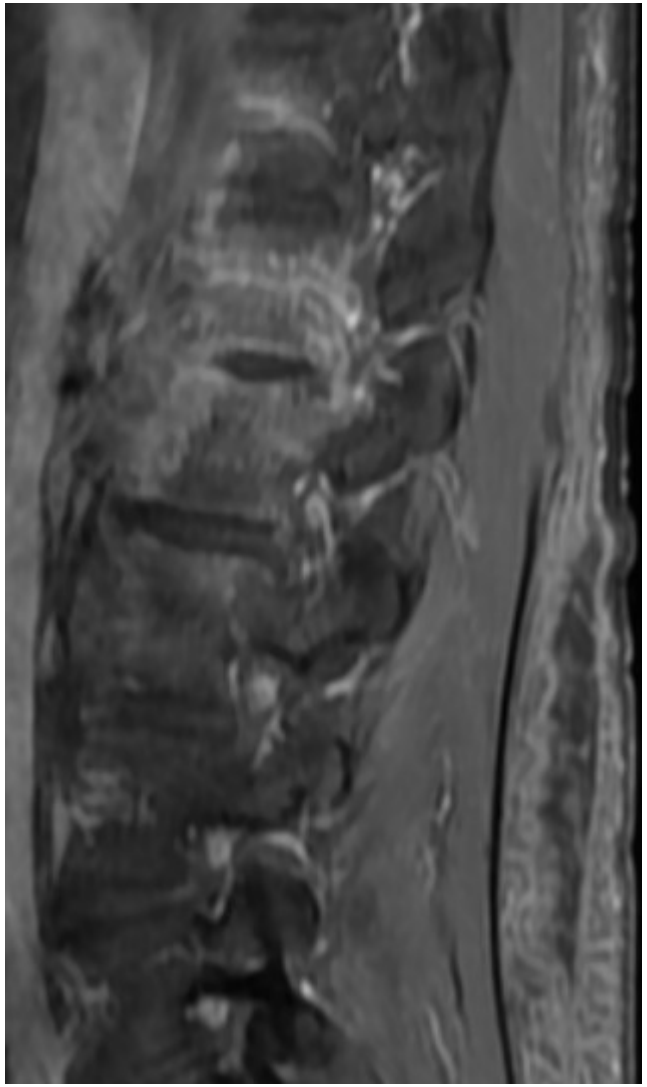


Spine imaging

Spine MRI

- Bony infection in the lumbar L-spine area in multiple consecutive vertebral bones and the vertebral disc spaces





Microbiology studies

Date	Smear	GeneXP	Culture
Day #1 sputa((induced)	Numerous positive	Positive, no RIF resistance	Pending
Day #2 sputa (home)	Numerous positive		Pending
Day #3 sputa (home)	Negative		Pending

Hospital course

- Day #1: Started on INH daily, RIF daily, EMB 3X/week, PZA 3X/week
- Day #5: Complaining of nausea and poor appetite, liver function tests have increased to 3X upper limit of normal (ULN)
- All TB meds held.
- By day #9, symptoms are improved and liver function tests are improving (<2X ULN)
- Patient is rechallenged with RIF x 3 days→ EMB x 3 days→ INH x 3 days.
  - Liver function tests return to normal
  - PZA is thought to be the cause of liver injury
  - A 4th drug, levofloxacin, is added

Hospital course

3rd week of hospitalization:

- Patient is taking her TB medications (INH, RIF, EMB, Levofloxacin) and appetite improved
- Smears: few AFB positive
- Cultures are still no growth to date
- Liver function tests: normal
- White blood cell count has declined since starting TB medications from 8,000 to 1500 (normal 3700-10,000)
- Most likely cause of patient's low white blood cell count is rifampin, which is held.

Clinical Infectious Diseases

BRIEF REPORT

Adverse Events Associated With Treatment for Pan-Susceptible Tuberculosis in San Francisco

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Of 373 patients treated for drug-susceptible tuberculosis, 35.4% (46.2% aged ≥65 years) developed moderate/severe adverse events that required treatment interruption (34.8%), first-line drug discontinuation (26.2%, primarily pyrazinamide), second-line drug initiation (30.0%), and treatment duration up to 3.8 months longer. More safe and effective options are needed, including for the elderly.

Keywords. tuberculosis; TB; adverse events; elderly.

Current treatment recommendations for drug-susceptible tuberculosis (TB) include daily isoniazid, rifampin, pyrazinamide, and ethambutol (HRZE) [1]. TB treatment can be associated with adverse events (AEs) that require resource-intensive management including frequent clinic visits, symptom assessment, and laboratory monitoring. HRZE intolerance can result in longer treatment duration. For example, if pyrazinamide cannot be taken for the first 8 weeks, current guidelines recommend extending treatment duration to 9 months [1]. Likewise, if rifampin is not included in the regimen, duration can extend up to 18 months [2].

In 2020, patients aged ≥65 years had the highest TB incidence in the United States [3]. Compared with their younger counterparts, older patients may have higher risk of AEs including hepatotoxicity, leading to substantial treatment-related morbidity [4–7]. We reviewed TB cases in San Francisco treated over a 5-year period, including in patients aged ≥65 years, to understand the potential challenges of managing antituberculous drug-associated AEs.

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METHODS

We reviewed medical records of TB cases evaluated at the San Francisco Department of Public Health Tuberculosis Clinic from 1 January 2016 through 31 December 2020. We extracted data including clinical course, treatment regimens, symptoms, and laboratory results, which were used to assess drug-associated AEs. Patients with monoresistance to an anti-tuberculous drug were excluded from the denominator for specific drug-associated AEs. Patients with multidrug resistance or who died during treatment were excluded; deaths due to drug-related AEs were not excluded.

All patients underwent diagnostic evaluation, treatment, and management according to national recommendations [1]. Standard dosing included isoniazid 300 mg daily, rifampin 600 mg daily, and weight-based daily dosing of ethambutol (15–20 mg/kg) and pyrazinamide (20–25 mg/kg) [1]. For patients with creatinine clearance <30 mg/dL, ethambutol and pyrazinamide were dosed 3 times per week [1]. Recommended treatment durations were 6 months for culture-positive pulmonary TB, 9 months for cavitary pulmonary disease or for those with delayed mycobacterial culture conversion, and 9–12 months for spinal disease or TB meningitis [1]. Individual regimens were managed depending on clinician discretion. Treatment variations included withholding pyrazinamide (eg, if the patient had a history of gout), replacing rifampin with rifabutin for patients on concurrent medications with drug–drug interactions (eg, rifampin interaction with coumadin or methadone), or using liver-sparing regimens that excluded isoniazid, pyrazinamide, or a rifamycin (eg, in patients with cirrhosis). When an AE developed, standard practice included stopping all TB drugs and rechallenging 1-by-1 to determine if AE recurrence was associated with a specific drug. AEs were deemed to be treatment-related if they resolved after hold of a specific TB medication and/or recurred after reintroduction. Clinician discretion was also used to assess whether an AE was treatment-related or due to another cause. If first-line TB drugs were permanently discontinued, clinician assessment was used to decide whether to start a second-line agent. Options included drugs commonly recommended for drug-resistant TB, including fluoroquinolones (moxifloxacin 400 mg daily or levofloxacin 750 mg daily), linezolid 600 mg daily, or amikacin dosed at 10 mg/kg intramuscularly or intravenously 5 times weekly. The Common Terminology Criteria for Adverse Events (CTCAE) was used to assess drug-associated AEs, with grades 1, 2, and 3 indicating mild, moderate, and severe AEs, respectively; grade 4 indicating life-threatening consequences; and grade 5 indicating death [8].

San Francisco 5-year review

N=373 (132 > age 65 years)

- 35% (46% > 65 years) developed moderate/severe* adverse events
  - PZA: ~20% (~30% > 65 years)
    - No longer routinely recommended in elderly in Japan, Seattle
  - Rifamycin: ~13.5% (~17% > 65 years)
  - INH: ~9% (~12% > 65 years)
- 26-30% required first line drug discontinuation, and started a second line drug (FQ or linezolid)
  - FQs associated with AEs in ~40% (all ages)
- Adverse event(s) associated with treatment up to 3.8 months longer

*Moderate/severe= unable to perform normal daily activities or hospitalized

Hospital Course and Contact Investigation

- Day #30: Patient doing well on INH, EMB, and Levo
- Patient is requesting to be discharged home
  - Good appetite, no fever, but still coughing
  - Anxious because she is going on a family cruise to celebrate her 80th birthday in a month
- Before discharge home, everyone in the household is assessed as part of the contact investigation
  - Husband and son born in the Philippines
  - Both with positive interferon gamma release assay (IGRA)→ diagnosed with latent TB
  - Started on INH for latent TB treatment
  - Cannot take rifampin because of drug-drug interactions with their current medications.
  - The two grandchildren test negative by IGRA for latent TB.

Microbiology studies, day #30

Date	Smear	GeneXP	Culture
Day #1 (induced)	Numerous positive	Positive, no RIF resistance	Culture positive for Mtb*
Day #2 (home)	Numerous positive		Culture positive for Mtb
Day #3 (home)	Negative		Pending
Day #8 (home)	Numerous positive		Pending
Day #10 (home)	Moderate positive		Pending
Day #20 (home)	Few positive		Pending
Day #21 (home)	Few positive		Pending
Day #29 (home)	Negative		Pending
Day #30 (home)	Negative		Pending

*Day #40: Mtb culture DST suggests high and low level INH resistance

Day #46

Whole Genome Sequencing:

INH resistance with katG mutation

Physician:

Specimen Type: LJ

Pregnancy Status:

ICD Code:

ANALYTE	RESULTS and INTERPRETATION
Identification	**DNA of Mycobacterium tuberculosis species detected**
Isoniazid	**Mutation(s) associated with resistance to isoniazid detected**
katG	c.944G>C (p.Ser315Thr)
fabG1	No mutations detected
inhA	No mutations detected
Ethionamide	**No mutations associated with resistance to ethionamide detected**
ethA	No mutations detected
fabG1	No mutations detected
inhA	No mutations detected
Rifampin	**Predicted susceptibility to rifampin**
rpoB	No high confidence mutations detected
Pyrazinamide	**No mutations associated with resistance to pyrazinamide detected**
pncA	No mutations detected
Ethambutol	**No mutations associated with resistance to ethambutol detected**
embA	No high confidence mutations detected
embB	No high confidence mutations detected
Amikacin	**No mutations associated with resistance to amikacin detected**
rrs	No high confidence mutations detected
eis	No mutations detected
Kanamycin	**No mutations associated with resistance to kanamycin detected**
rrs	No high confidence mutations detected
eis	No mutations detected
Capreomycin	**No mutations associated with resistance to capreomycin detected**
rrs	No high confidence mutations detected
tlyA	No high confidence mutations detected

INH and RIF resistance in California TB patients

A	B	C	D	E	F	G	H	I	J

Table 10. Isoniazid (INH)-Resistant, Rifampin (RIF)-Resistant, and Multidrug-Resistant (MDR) Tuberculosis Cases by Country of Origin: California, 2019-2023

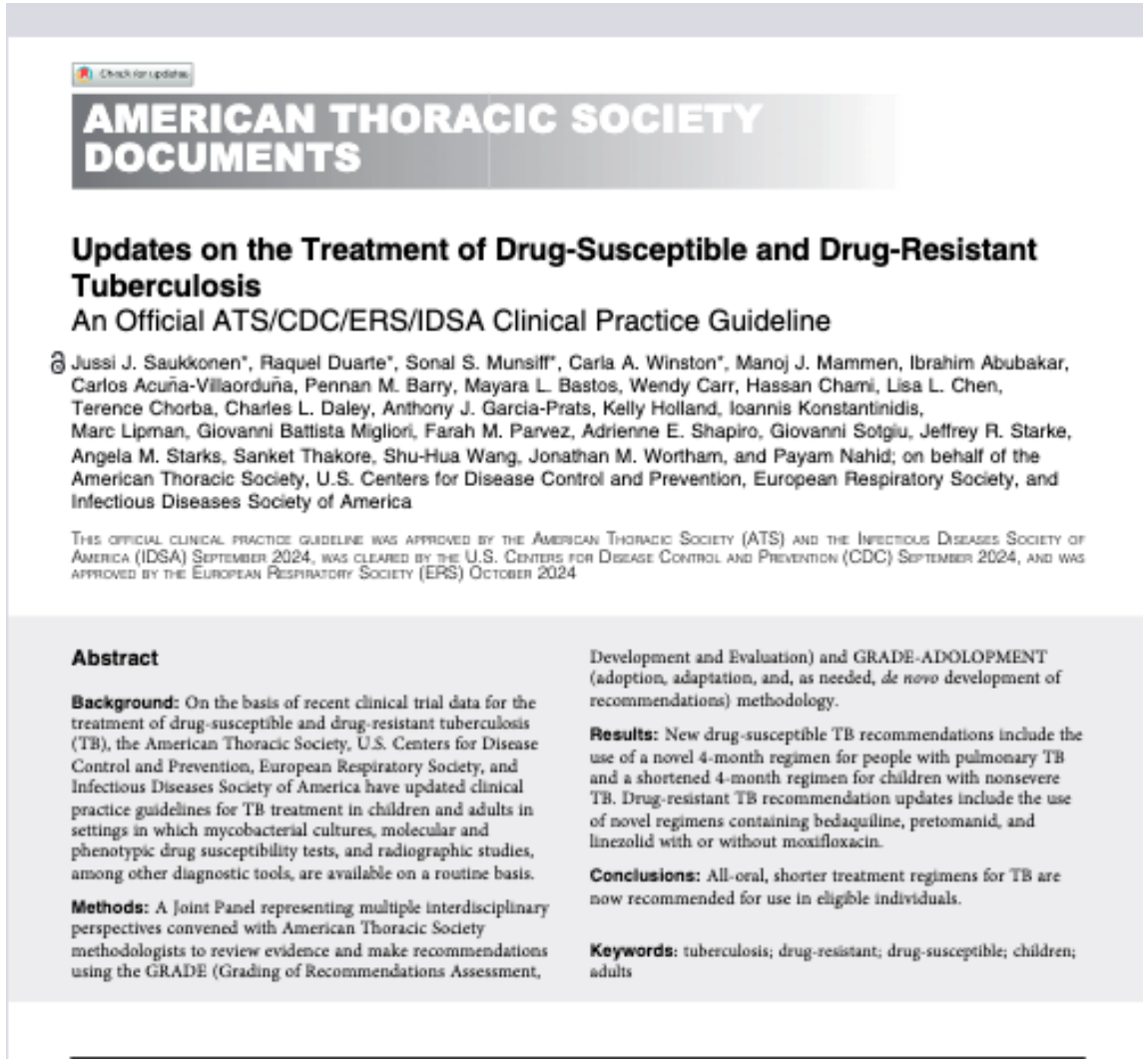
Country of Birth	INH Tested No.	INH Resistant No.	INH Resistant %	RIF Tested No.	RIF Resistant No.	RIF Resistant %	MDR Tested No.	MDR No.	MDR %
Total Cases	7669	776	10.1	7672	106	1.4	7656	86	1.1
Afghanistan	50	2	4.0	50	.	.	50	.	.
Cambodia	93	8	8.6	93	1	1.1	93	.	.
China*	527	42	8.0	527	3	0.6	526	3	0.6
Colombia	31	1	3.2	31	.	.	31	.	.
El Salvador	96	2	2.1	96	.	.	96	.	.
Ethiopia	29	2	6.9	29	1	3.4	29	1	3.4
Guatemala	184	10	5.4	184	2	1.1	184	2	1.1
Honduras	52	3	5.8	52	1	1.9	52	1	1.9
India	415	28	6.7	416	7	1.7	415	6	1.4
Indonesia	28	4	14.3	28	1	3.6	28	1	3.6
Iran	33	1	3.0	33	1	3.0	33	1	3.0
Korea, North	52	8	15.4	51	2	3.9	51	2	3.9
Korea, South	116	21	18.1	116	3	2.6	116	2	1.7
Laos	89	11	12.4	89	4	4.5	89	3	3.4
Mexico	1732	121	7.0	1734	9	0.5	1731	5	0.3
Myanmar	55	6	10.9	55	3	5.5	55	3	5.5
Nepal	30	.	.	30	.	.	30	.	.
Nicaragua	27	5	18.5	27	3	11.1	27	2	7.4
Pakistan	41	7	17.1	41	1	2.4	41	1	2.4
Peru	65	16	24.6	65	8	12.3	65	8	12.3
Philippines	1499	251	16.7	1501	22	1.5	1495	17	1.1
Taiwan	53	9	17.0	53	.	.	53	.	.
Thailand	43	2	4.7	42	1	2.4	42	1	2.4
United States	1160	59	5.1	1162	8	0.7	1160	5	0.4
Vietnam	801	134	16.7	801	15	1.9	799	15	1.9
All Other Countries	368	23	6.3	366	10	2.7	365	7	1.9

. Indicates zero cases or zero percent.
INH=Isoniazid; RIF=Rifampin; MDR=Multidrug-Resistant
Drug susceptibility testing includes both phenotypic testing for persons with positive culture results and molecular testing results when available.
Multi-drug Resistant (MDR) TB is defined as resistant to at least isoniazid and rifampin.
Restricted to countries with at least 25 patients tested for MDR.
Patients from countries with fewer than 25 cases tested may still have elevated risk for drug resistance.
* People's Republic of China. Includes Hong Kong and Macau.

New NTCA De-Isolation Guidelines, July 2024

Patient is now AFB smear negative x 3, can she be discharged off of isolation?

- Guidelines emphasize de-isolation as soon as feasible for patient well-being and more efficient use of public health resources
- Take into account several factors including:
  - Initial smear positivity
  - Burden of TB disease
  - Clinical improvement
  - TB treatment regimen



AMERICAN THORACIC SOCIETY DOCUMENTS

Updates on the Treatment of Drug-Susceptible and Drug-Resistant Tuberculosis

An Official ATS/CDC/ERS/IDSA Clinical Practice Guideline

Jussi J. Saukkonen*, Raquel Duarte*, Sonal S. Munsiff*, Carla A. Winston*, Manoj J. Mammen, Ibrahim Abubakar, Carlos Acuña-Villaorduña, Pennan M. Barry, Mayara L. Bastos, Wendy Carr, Hassan Chami, Lisa L. Chen, Terence Chorba, Charles L. Daley, Anthony J. Garcia-Prats, Kelly Holland, Ioannis Konstantinidis, Marc Lipman, Giovanni Battista Migliori, Farah M. Parvez, Adrienne E. Shapiro, Giovanni Sotgiu, Jeffrey R. Starke, Angela M. Starks, Sanket Thakore, Shu-Hua Wang, Jonathan M. Wortham, and Payam Nahid; on behalf of the American Thoracic Society, U.S. Centers for Disease Control and Prevention, European Respiratory Society, and Infectious Diseases Society of America

This official clinical practice guideline was approved by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) September 2024, was cleared by the U.S. Centers for Disease Control and Prevention (CDC) September 2024, and was approved by the European Respiratory Society (ERS) October 2024

Abstract

Background: On the basis of recent clinical trial data for the treatment of drug-susceptible and drug-resistant tuberculosis (TB), the American Thoracic Society, U.S. Centers for Disease Control and Prevention, European Respiratory Society, and Infectious Diseases Society of America have updated clinical practice guidelines for TB treatment in children and adults in settings in which mycobacterial cultures, molecular and phenotypic drug susceptibility tests, and radiographic studies, among other diagnostic tools, are available on a routine basis.

Methods: A Joint Panel representing multiple interdisciplinary perspectives convened with American Thoracic Society methodologists to review evidence and make recommendations using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) and GRADE-ADOLOPMENT (adoption, adaptation, and, as needed, *de novo* development of recommendations) methodology.

Results: New drug-susceptible TB recommendations include the use of a novel 4-month regimen for people with pulmonary TB and a shortened 4-month regimen for children with nonsevere TB. Drug-resistant TB recommendation updates include the use of novel regimens containing bedaquiline, pretomanid, and linezolid with or without moxifloxacin.

Conclusions: All-oral, shorter treatment regimens for TB are now recommended for use in eligible individuals.

Keywords: tuberculosis; drug-resistant; drug-susceptible; children; adults

SFDPH De-isolation Review Form

- For de-isolation, patient must be on “effective” therapy with a multi-drug regimen to which “the organism is susceptible or anticipated to be susceptible”
- This is difficult to assess in practice without laboratory confirmation

Documentation of Interdisciplinary Care Team Planning for Isolation Transition

Date:_____ ☐ 15 day review ☐30 day review ☐45 day review ☐Other____

1. **Clinical improvement checklist. Meeting every criterion is not necessary, these are suggested parameters to consider.**
   - ☐ decreased cough
   - ☐ weight gain _____ lbs/kg
   - ☐ If available, repeat radiograph imaging (CXR at 1 month or CXR/chest CT at 2 months) is stable, or ideally improved
   - ☐ If smear positive: AFB smears are improving/decreasing in positivity. Initial smear ____; current smear ____
   - ☐ If culture positive: time to culture positivity is lengthening. M. tb initially grew at ____ days; last grew at ____ days)
   - ☐ other reported or observed improvements:_____

2. **Appropriate TB treatment checklist**
   - ☐ Multi-drug regimen (usually 4 drugs) **approved by local TB program** including at least 2 bactericidal drugs (specify: _____); and
   - ☐ Patient is tolerating stable daily therapy ; and
   - ☐ If available, DST or molecular testing (such as WGS, tNGS) shows no evidence of resistance to medications in regimen; and
   - ☐ If performed, drug level testing shows within therapeutic range

3. **Patient impact and capacity to adhere to moderate isolation restrictions:**
   - ☐ Patient can understand the reason for isolation (6-item cognitive screen score: _____; Able to teach-back how TB is transmitted and how to protect others Y/N)
   - ☐ Patient is willing to follow public health instructions to refrain from contact with high-risk individuals or in high-risk settings
   - ☐ High impact of continuing strict isolation (consider employment, housing status, ability to feed themselves and perform family caregiving or other essential obligations)
   - ☐ Patient has the means and supports to be able to maintain moderate isolation

Interdisciplinary Decision: (Select one choice:)	**Fill-in staff responsible for communicating Isolation update to patient(Assigned DCI or RN Case Manager)**	**Care Team Acknowledgment: (Team member Initials)**
☐ Remain under full isolation		RN Case Manager:
☐ transition to **moderate** isolation precautions		Assigned DCI:
		MD/NP:
		Medical Director/TB Controller:

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Back to the patient, day #48

- The patient is currently only on levofloxacin and EMB (one bacteriocidal and one bacteriostatic drug)
  - INH→ resistant
  - PZA→ liver injury
  - RIF→ leukopenia
- The patient is told that without INH and RIF, she should be switched to an MDR regimen with bedaquiline, pretonamid, linezolid and moxifloxacin (BPaLM)
- It will take ≥14 days to obtain the bedaquiline and pretonamid
- Patient's treatment is held
  - Advised to stay in isolation in her home while off TB medications
  - Told she will have to postpone her cruise

Contact Investigation Follow-up

- The patient's husband and son
  - Have been taking INH for latent TB treatment for ~2 months
  - Switched to levofloxacin
  - Advised that they will have to start over again with a 6-9 month course.
- Her two grandchildren are started on window prophylaxis with rifampin since they are living in the same household with a now presumed infectious TB patient, while the patient awaits starting BPaLM

The clinical usefulness of rapid molecular resistance testing on direct specimens for TB drugs cannot be overstated

Rapid molecular testing…

- Overcomes the inherent delay in culture-based drug susceptibility testing, which can take weeks to months—a timeframe during which:
  - Patients may receive ineffective therapy,
  - Experience worse outcomes, and
  - Continue transmitting drug-resistant strains
- Proof-of-concept: Xpert MTB/RIF Ultra significantly reduces time to diagnosis (0.49, 95% CI 0.39 to 0.62) and time to treatment initiation (0.35, 95% CI 0.21 to 0.58)
- Ideal targets: RIF, INH, PZA, fluoroquinolones, linezolid

Otieno JA, Were LM, Lutje V, Scandrett K, Takwoingi Y, Ochodo EA. Impact of rapid nucleic acid amplification tests for tuberculosis on patient outcomes. Cochrane Database Syst Rev. 2025 Dec 18;12(12):CD016194.

Why multiple targets? TB regimens are not always HRZE

- Patients will be treated differently depending on co-morbidities, country of origin, and side effects
  - In SF approximately 20% of patients receive a fluoroquinolone and ~15% receive linezolid
- Patients with chronic liver disease receive drugs that are less hepatotoxic (e.g., rifabutin, EMB, levofloxacin)
- Patients with TB meningitis or who are critically ill may need IV medications (RIF, Levo, Linezolid)
- Patients with MDR-TB may initially get 5-6 second-line TB drugs
- Some patients want to get treatment done quickly → 4-month regimen with INH, rifapentine, moxifloxacin and PZA (HPMZ)

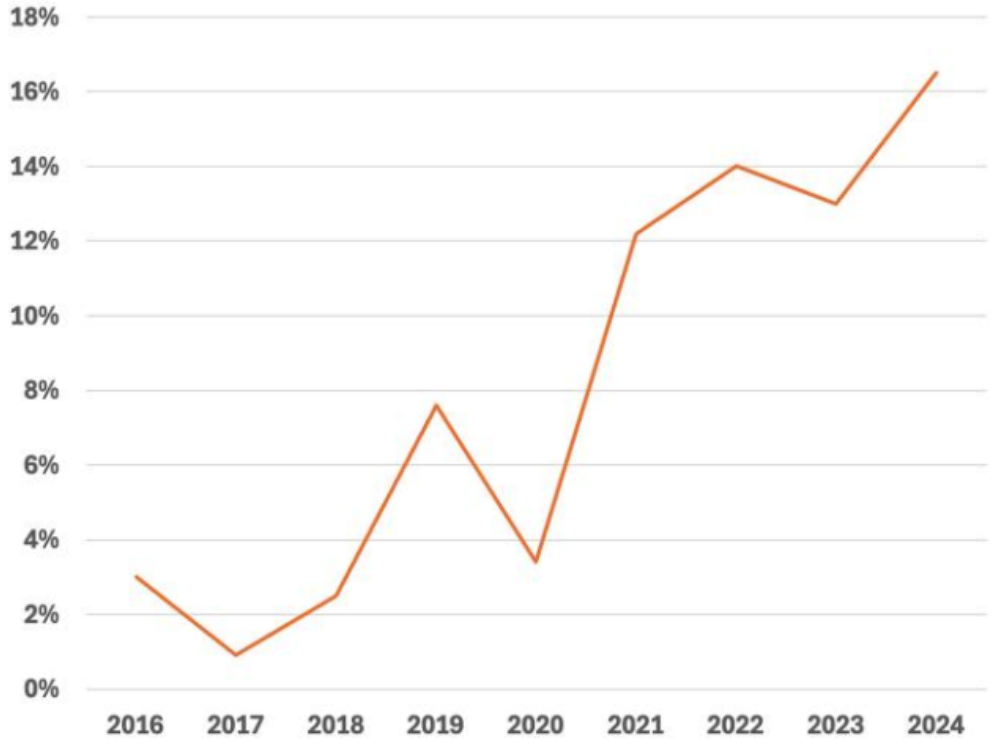
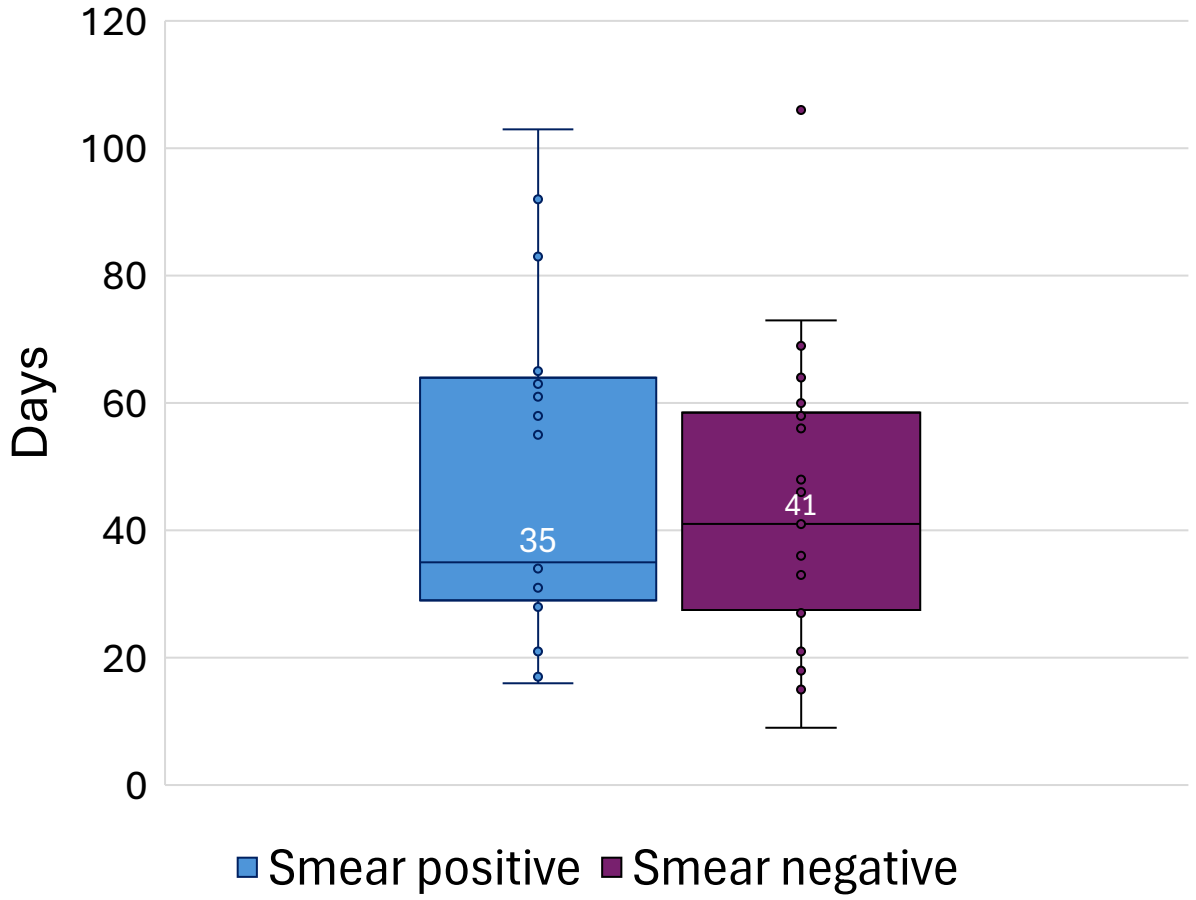


Figure 1. Trend in Linezolid Use for Treatment of Drug-Susceptible Tuberculosis in San Francisco, 2016–2024.

Current Status of Molecular Resistance Testing in SF

- Only available rapid resistance test performed on direct specimens is the geneXP for rpoB mutations.
- WGS requires mature culture
- Average TAT for resistance testing by WGS in SF after submission: ~ 32 days (1-106), depending on
  - Growth of adequate biomass
  - Specimen quantity and quality
  - Staff for packaging, shipping and data entry
  - Courier delays (weather, etc)
- tNGS in development at CA state lab



Summary

- TB clinicians are juggling multiple epidemiologic, clinical and socioeconomic factors when starting treatment
- The laboratory plays a key role throughout
- Early knowledge of resistance can help identify the most effective TB treatment regimen
- Delayed resistance testing results affects TB morbidity, mortality, treatment duration, isolation status, treatment of contacts and use of public health resources
- Even pre-knowledge of patient risk for drug resistance (e.g., with local or regional antibiograms for all TB drugs) would be helpful

Thank you!

TB lab testing: progress and perspective

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Hawaii State Laboratories Division

Conflict of Interest Statement

No Potential Conflicts of Interest

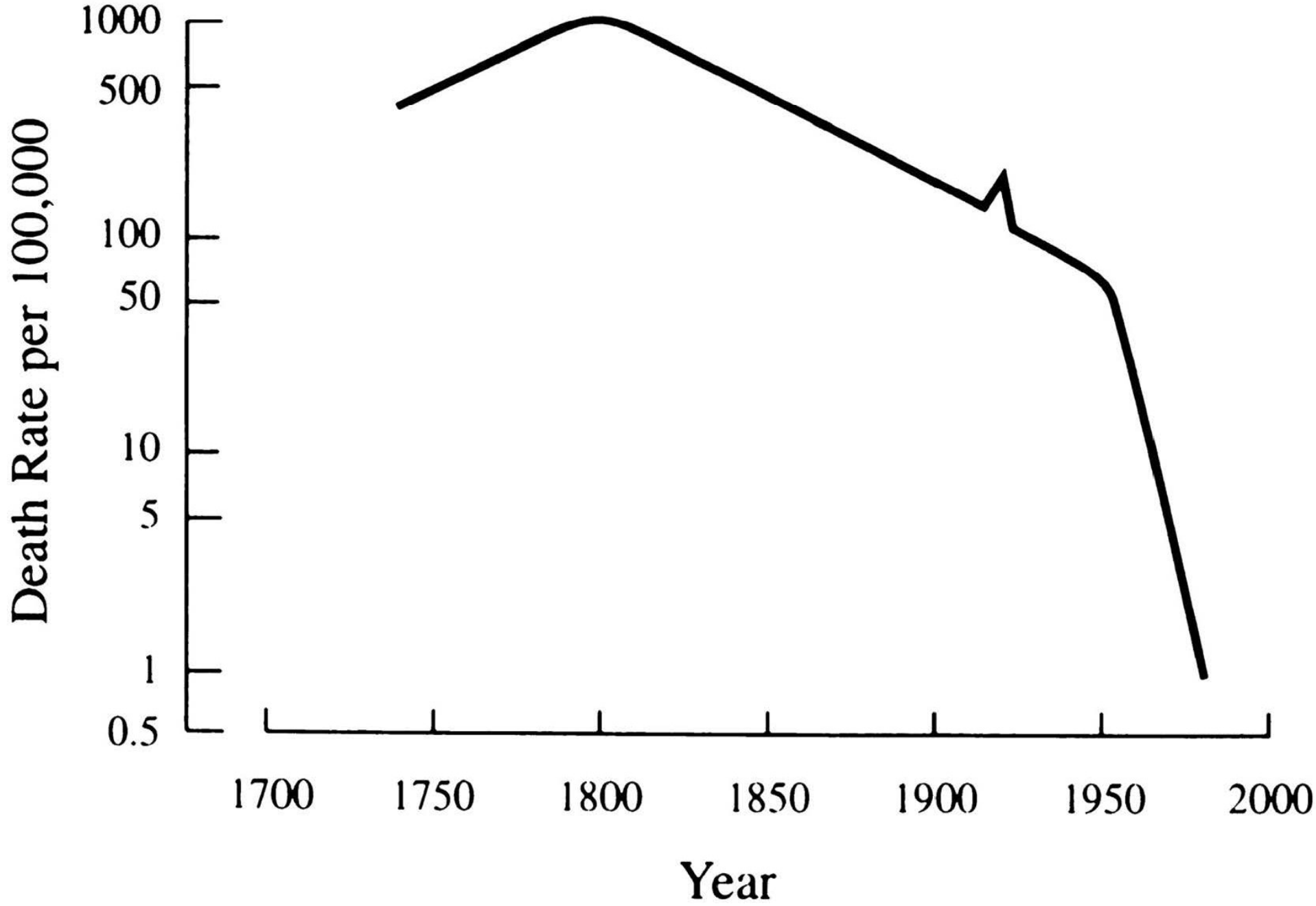
Speaker: Edward Desmond, Hawaii State Department of Health

I have NO conflicts of interest related to this presentation to disclose.

1990 until now: “talk story”

A cultural practice rooted in Hawaiian tradition, involving sharing personal experiences, history, and wisdom in a relaxed way, fostering connection and passing down knowledge across generations.

I want to develop three major themes as I talk story.


Death Rate per 100,000
1000
500
100
50
10
5
1
0.5
1700
1750
1800
1850
1900
1950
2000
Year

Why did TB decline in the first half of the 20th century?

- Improvements in living conditions
- **Better sanitation and housing:**
- Access to clean water, better housing, and improved living conditions reduced the spread of the disease.
- **Improved nutrition:**
- Better diets strengthened the population's immune systems, making them more resistant to infection.
- **Reduced overcrowding:**
- Initiatives like the creation of more public parks and recreational areas helped reduce overcrowding in cities.

Why did TB decline in the first half of the 20th century? (cont'd)

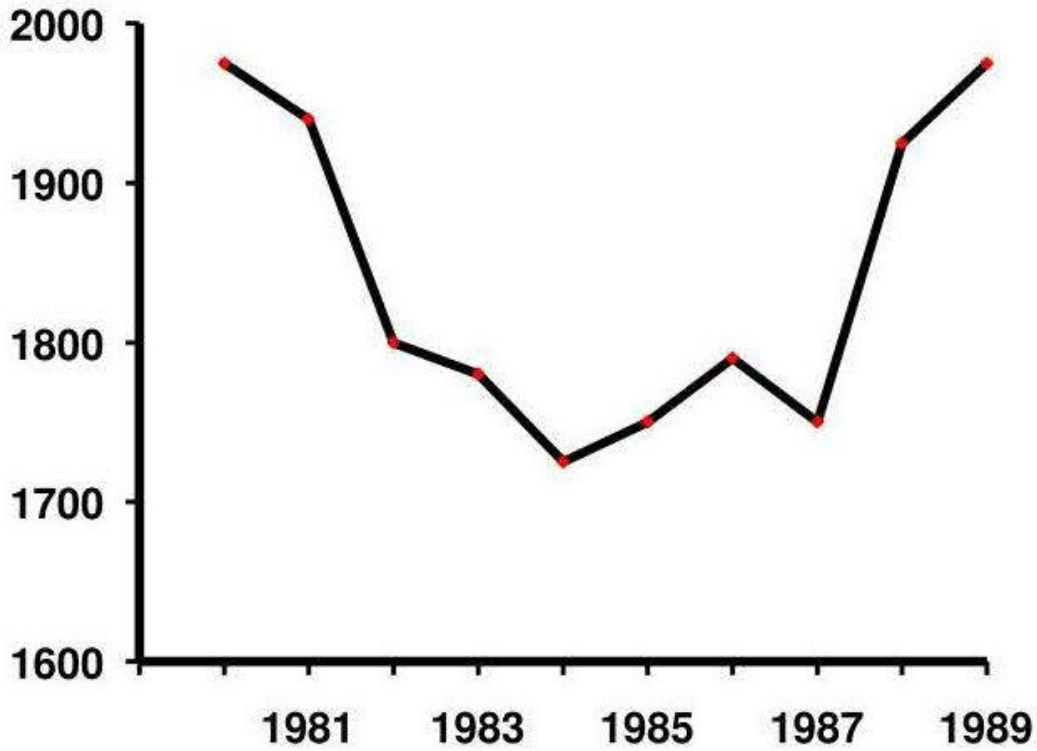
Public health initiatives

- **Public education campaigns:**
- After Robert Koch identified the tuberculosis bacillus in 1882, public health campaigns began to educate people on how the disease spread and how to prevent it, such as practicing good hygiene and avoiding close contact with the sick.
- **Case finding and diagnosis:**
- Doctors and public health organizations worked to find and treat cases early through methods like the tuberculin skin test, which was developed in 1907.
- **Sanatorium treatment:**
- Patients were treated in sanatoriums, where they received rest, nutrition, and other supportive care. Though not a cure, this helped control the disease and reduce transmission.
- **Eradication of bovine TB:**
- The eradication of bovine tuberculosis from cattle eliminated one source of human infection.

Why did TB decline in the first half of the 20th century? (cont'd)

Impact of the 1918 influenza pandemic

- **Harvesting effect:** The 1918 influenza pandemic may have hastened the decline of tuberculosis by disproportionately killing individuals who were already weakened by TB, thus removing them from the population and reducing the pool of infected individuals.



Tuberculosis deaths by year, United States, 1980-1989
(National Health Statistics)

Why did U.S. TB cases go up in the 1980s?

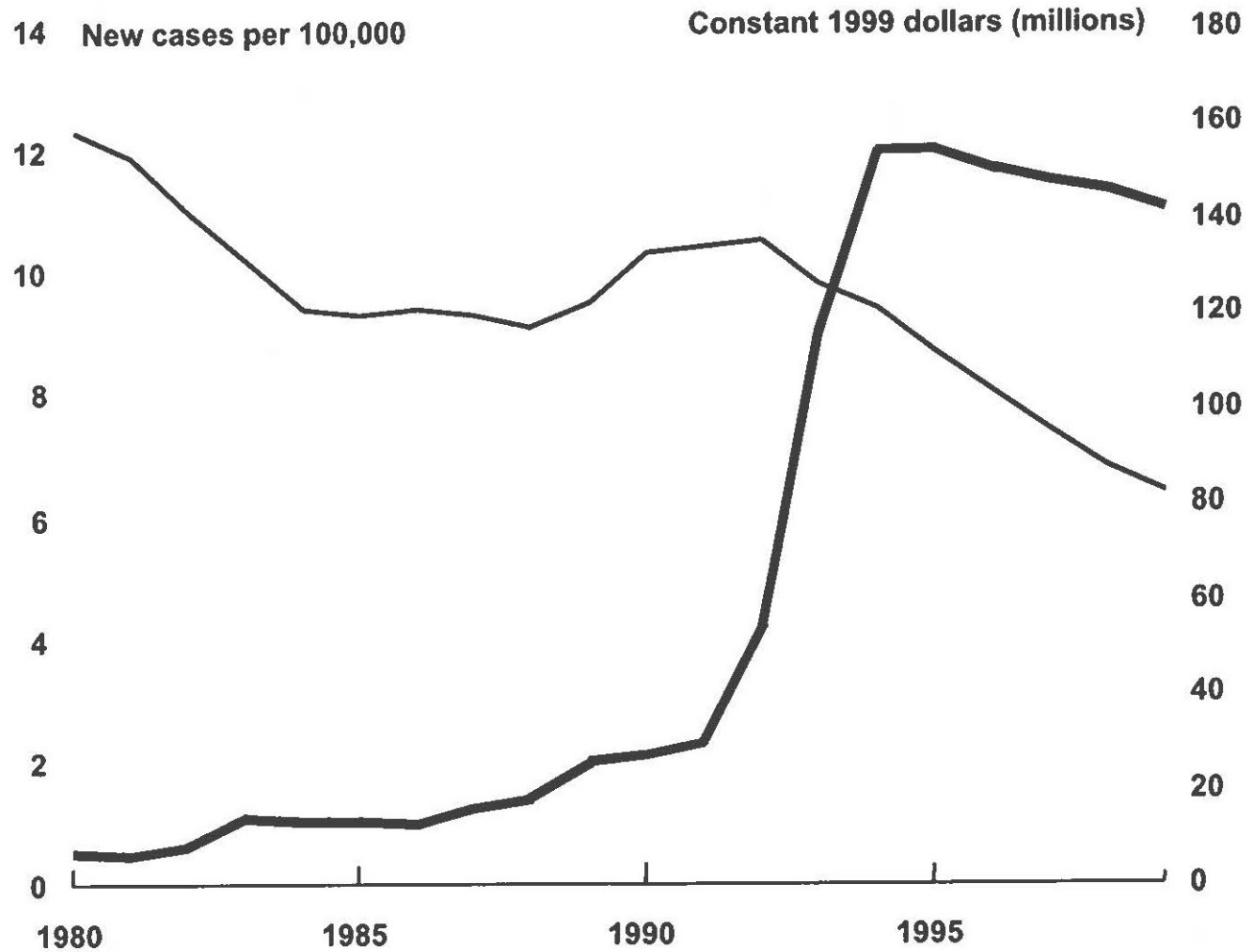
The rise in new TB cases in the late 1980s and early 1990s has been associated with several factors, including

- Emergence of the human immunodeficiency virus (HIV): in mid-1980s, about 2% of TB cases were HIV+, later increasing to 20 to 30%

- Cutbacks in TB programs (subsequent slides)

- MDR-TB outbreaks.

Figure 1: CDC Funding for TB and Case Rate, United States, 1980-99



Impact of “block grants” in the 1980s on public health funding

In the 1980s, the conversion of federal categorical grants into block grants under the Reagan administration had a substantial and largely negative impact on public health. The transition led to significant funding cuts, increases state administrative responsibilities, and a loss of federal oversight for many health services.

Reduced funding: block grants consolidated 57 federal categorical programs into 9 block grants and slashed their combined funding by 25%.

Loss of targeted programs: The shift consolidated dozens of specific, targeted health & prevention initiatives into broader block grants. Programs focused on TB were combined into a larger stream of funds, which diluted their specific focus. This led to significant variations in service levels across the country.

California TB control program, 1990: 1 physician and one secretary (in a state with 30 million population and 5000 cases per year)

Valway, S.E., et al. Am. J. Epidemiol. Outbreak of TB in a New York State prison, 1991

From the abstract:

Eight HIV-positive inmates and one HIV-negative guard, who was immunocompromised with cancer, had multidrug-resistant tuberculosis. Eight died, a median of 28 days after the first culture-positive specimen was collected.

“…laboratory delays contributed to this outbreak.”

[HAART became available beginning in 1996]

Drug susceptibility testing at CA lab when I joined it in 1990

- Culture on solid media LJ and Middlebrook agar
- DST by agar proportion method
- Rigorous QC program for drug susceptibility testing
  - 1988 study demonstrated that individual lots of basal medium (such as Middlebrook 7H10) and supplements (OADC) can vary significantly in their suitability for drug susceptibility testing (DST). Study emphasized that failure to detect these variations through rigorous QC can lead to inaccurate drug susceptibility profiles.
- CA laboratory still practices rigorous QC of medium components to look for lot to lot variations.

Evolving technologies, need for confirmation, turnaround time

Year	Technology	Time to result	Need for confirmation
1990	Culture followed by agar proportion DST	2 months	N.A. (2nd line drugs required an additional month)
1993	Selective broth culture followed by broth culture DST	1 month	N.A. (2nd line drugs required an additional 2 weeks)
2001	Molecular beacons at CDPH for INH, rif	Same or next day	Susceptible results or results for other drug required culture. Silent mutations → occasional false R for rifampin
2009/10	Cepheid GeneXpert for rifampin	Same or next day	Same as above, no INH result

2004 Molecular beacons to detect INH/ Rif resistance directly in specimens

- Sensitivity for INH resistance 83% +/-
  - Positive predictive value excellent
  - Negative predictive value varied based on risk factors, but averaged at 98%
- Sensitivity for rifampin resistance 98%
  - Occasional false indication of resistance due to synonymous/ silent mutation
  - Positive and negative predictive values good, but *confirmatory testing required.*
- Turnaround time for molecular beacons 0-1 day
- Turnaround time for confirmatory testing (broth culture) ~ 2 weeks

Screening vs confirmatory testing

- For molecular beacons testing and pyrosequencing, failure to find a mutation was considered to be a screening result, subject to confirmation by a reference method.
- Finding a mutation in rpoB that could be a silent mutation (particularly position 433) was also considered to require confirmation.
- Despite these limitations, the molecular screening tests resulted in patients with drug-resistant TB being put on appropriate therapy several weeks earlier (Banerjee 2010 J Clin Micro 48:3779)

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2010, p. 3779–3781 Vol. 48, No. 10
0095-1137/10/$12.00 doi:10.1128/JCM.01236-10

Rapid Drug Susceptibility Testing with a Molecular Beacon Assay Is Associated with Earlier Diagnosis and Treatment of Multidrug-Resistant Tuberculosis in California▽

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Received 18 June 2010/Accepted 4 August 2010

To assess the clinical impact of a molecular beacon (MB) assay that detects multidrug-resistant tuberculosis (MDR TB), we retrospectively reviewed records of 127 MDR TB patients with and without MB testing between 2004 and 2007. Use of the MB assay reduced the time to detection and treatment of MDR TB.

Molecular detection of drug resistance means that MDRTB patients convert to culture negative 27 days earlier.

Molecular beacons group Probert, Lin, Desmond, and APHL fellows Khuong and Lo

1990s: state labs reach out to private sector

The majority of clinical specimens for suspected TB were cultured in the private sector or in local public health laboratories.

A significant portion of M. tb identification and DST was also in private sector laboratories.

State laboratories had different and innovative ways to promote use of methods that were quicker than solid medium culture and DST.

1990s: state labs reach out to private sector

New York State lab: “Fast Track”-required a sample from each TB patient to be sent to the State lab in Albany for rapid workup that included BACTEC broth.

California lab: encouraged and promoted “BACTECs by mail” program.

- Encouraged local public health labs to send an inoculated but unincubated BACTEC broth to the State lab for rapid culture, ID, DST
- Collaborated with National Laboratory Training Network in putting on workshops to identify and encourage the use of up to date rapid methods

Wisconsin laboratory: networking. Brought all the TB labs in the state together to learn and encourage use of standard of practice methods

Texas laboratory: laboratories could send smear positive sediments to TX laboratory, where HPLC would be done to identify TB.

Snapshot: early 2000s

- TB case rate had declined by around 50% compared to peak in 1990
- Private sector had stepped up
  - Becton Dickinson: Mycobacterial Growth Indicator Tube
  - Cepheid developed GeneXpert and conducted clinical trial to get FDA clearance
  - Major medical centers had TB expertise and labs with up to date practices
- Public Health sector had stepped up
  - TB Cooperative Agreements
  - CA TB control program had gone from 2 people to ~50
  - CDC-funded National TB Genotyping and Surveillance Network
  - Public Health labs with rapid screening and confirmatory methods
    - CA molecular beacons, CDC Molec Detect Drug Resistance (MDDR)
  - Regional Centers of Excellence with TB consultation

Traditional public health adage

"Before any infectious disease is eliminated, the program to eliminate it will be eliminated."

Elimination of the program is often stepwise and subtle.

Is that kind of subtle erosion happening now?

One type of erosion

- Manufacturers of rapid diagnostic systems do not conduct the clinical trials required for FDA clearance in the United States
- Amount of product to be sold in USA does not lead to timely/ acceptable return on investment (the investment being the cost of the clinical trial)

What we need (but haven't got) Cepheid GeneXpert Ultra: more sensitive for TB detection than MTB-Rif

Cochrane Database Syst Rev 2021 2(2) CD009593 Zifodya et al.

Pulmonary tuberculosis detection: Xpert Ultra pooled sensitivity 90.9% (versus Xpert MTB/RIF pooled sensitivity 84.7%)

Rifampicin resistance detection: Pooled sensitivity 94.9% for Xpert Ultra versus 95.3% for Xpert MTB/RIF.

Specificity of rifampin resistance detection: 99.1% for Xpert Ultra, and 98.8% for Xpert MTB/RIF

What we also need, but haven't got: Xpert MTB/XDR: quick detection of resistance to isoniazid, FQs, injectables, and ethionamide

Cochrane Database Syst Rev 2022 5(5): CDO14841 Pillay et al.

Drug resistance	Sensitivity	Specificity
INH	94.2%	98.5%
FQ	93.2%	98.0%
Ethionamide*	98.0%	99.7%
Amikacin	86.1%	98.9%

*for rifampin –resistant strains, compared with genotypic results
Other drugs: for all strains, compared with phenotypic results

Another gradual shift

Declining TB cases will affect private sector hospitals, clinics, laboratories.

- It may become no longer cost-effective for them to maintain TB expertise or specialized procedures

The bulk of TB drug susceptibility testing will be done by public health laboratories

- We're already there.
- Paradigm shift: public health laboratory → clinical laboratory

Functions of a public health laboratory

- Provide support to a health department program
  - Epidemiology, contact investigation, consultation, control measures
  - The majority of PH laboratory services
  - Turnaround time is sometimes less critical

- Provide clinical laboratory testing to support healthcare providers
  - Usually limited to situations where the private sector doesn't provide the needed services
  - Needs of clinicians must be met
  - Turnaround times may be critical—consult with clinicians!

Evolving technologies, need for confirmation, turnaround time, cont'd

Year	Technology	Time to result	Need for confirmation
2013	Pyrosequencing for INH, rif, injectables, or fluoroquinolones	Next day	Susceptible results, or results for other drugs
2016	Whole genome sequencing	1 month (including growing culture)	If novel mutation found or heteroresistance suspected
2016	Targeted next generation sequencing initial studies (INH, rif, injectables)	4-11 days	If novel mutation is found or for other drugs
Feb 2023	CDC MDDR services 24 amplicons, 15 genes, 12 drugs	8 days	Novel mutation

Clinical laboratories often use algorithms

Available TB lab methods

Method	Advantages	Disadvantages
Acid-fast microscopy	Quick, cheap, indicates infectivity	⬇ sensitivity
Real time PCR	Quick, gives info about drug suscept	May not be as sensitive or specific as culture or NGS
tNGS (targeted next gen seq)	Quicker than WGS	Difficult to validate and maintain supplies
WGS	Reliability	Turnaround time too slow for clinical application
Culture and phenotypic DST	Detect heteroresistance and or phenotypic effects of novel mutations	May take 3 weeks, problems with EMB & PZA

Algorithms, cont'd

Algorithms can be based on risk factors/ clinical history

- Problem: patient may not be aware of or communicate risk factors to health care provider
- Health care provider may not communicate presence or absence of known risk factors to laboratory

Algorithms based on preliminary results or screening tests

- Example: real time PCR results may indicate need for further rapid testing.
- INH/ rif molecular beacons could indicate need for tNGS

What should the screening tests be?

Possible screening tests/ algorithms

- INH molecular beacons assay developed by CA laboratory?
  - Would need to be a laboratory-developed test
  - INH-resistant cultures or specimens are common and should be easy to collect
  - Lin, S-Y G. J Clin Microbiol 2004 42(9):4204-08
- Follow up with rifampin real time PCR
  - Cepheid GeneXpert MTB/Rif (FDA cleared)
  - California molecular beacon LDT (same reference as above)
  - If no indication of INH resistance, then detection of an rpoB mutation becomes suspect, affirming need for sequencing
- tNGS for pyrazinamide? *pncA* gene and promoter
  - Could this be a follow up screen if TB is detected along with R to INH +/- Rif?

tNGS is a limited resource: when should it be used?

- When MDRTB is suspected?
- Patient exposed to MDRTB case, patient has been treated before, patient is from an area with high incidence of DRTB, patient is suffering relapse after initial treatment success
  - Does laboratory know any of this information?

- When a screening method indicates drug resistance?

- Answer should be developed in collaboration with local TB clinicians and TB control program

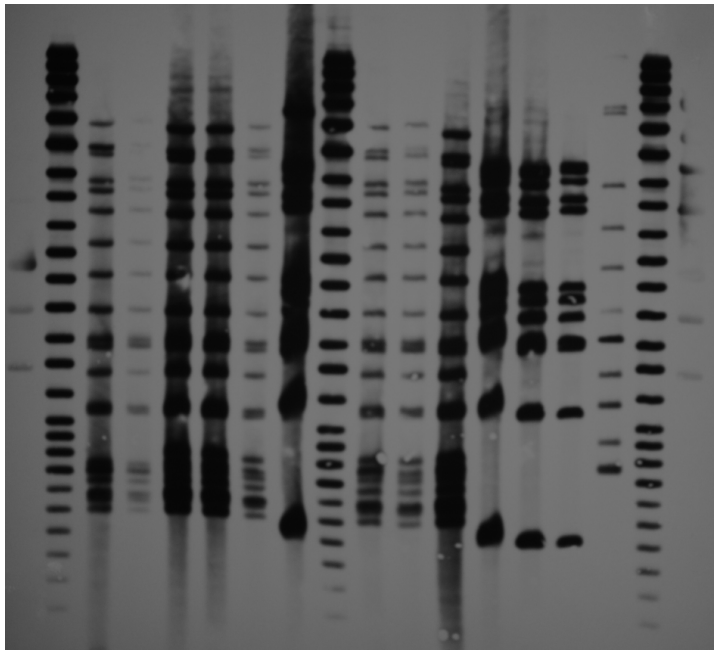
Is targeted NGS accurate enough?

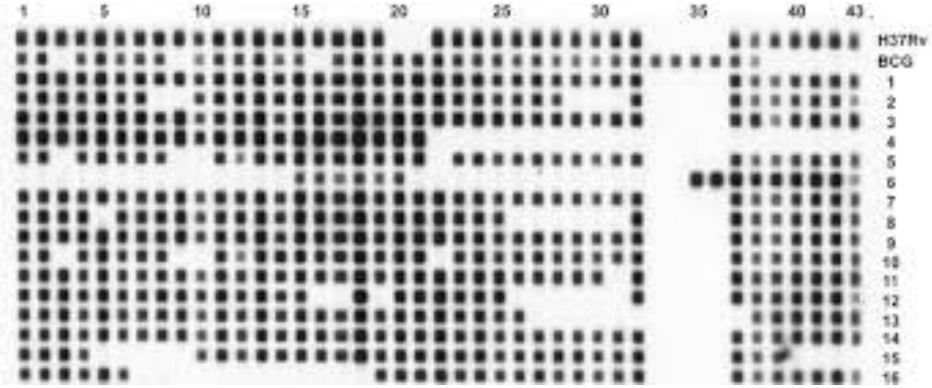
- Offers better depth of coverage than WGS
  - More likely to detect heteroresistance (important for fluoroquinolones)
- Phenotypic/culture-based testing has its problems
  - Reproducibility of PZA and ethambutol testing is poor.
- Knowing which mutation is present can be linked to prediction of MIC
- No laboratory test is perfect; accuracy of tNGS is comparable to other methods

APHL

HAWAII STATE DEPARTMENT OF HEALTH

TB genotyping





IS6110 RFLP and spacer oligonucleotide typing

Matches taken together with pretest probability could confirm epidemiologic linkage or cross-contamination events

Laboratory role in utilization of genotyping by TB control program and laboratories

- TB control programs can vary greatly in their ability to understand and use genotyping results
  - Public health lab can be very useful in explaining and helping with understanding of the uses and limitations of WGS
- Genotyping can guide investigations into suspected laboratory cross-contaminations
  - Scenario: specimens were processed in the same batch in a BSC but patients have no epi connection
  - Lab may have a questionable practice such as:
    - Using a common buffer flask for pouring phosphate buffer for neutralization
    - Including a positive control in each test batch

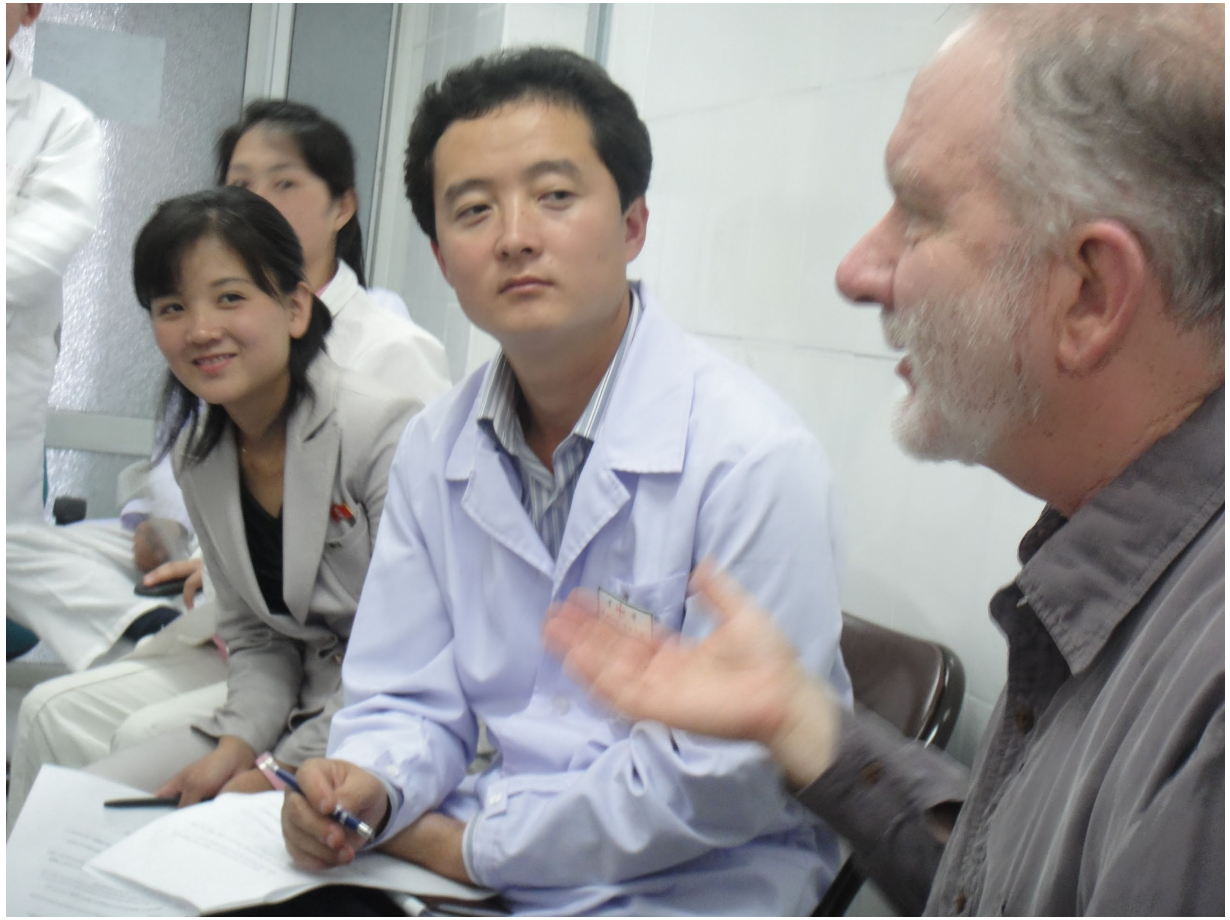
Investigating and advising re lab practices when there has been an apparent cross-contamination

- Experienced PH lab staff member visits lab that has a suspected cross-contam
- Observe practices and recommend improvements

International projects—much cause for modesty

- U.S.-affiliated Pacific Islands→ most effective measure was a local expert (Vasiti Uluiviti)
- North Korea—with Christian Friends of Korea,WHO, Nuclear Threat Initiative, Bay Area TB Consortium, Stanford University→ built, equipped, trained national TB reference lab 😊
  - Foreigners now excluded, NTRL now questionable 😡
- With WHO: USA collaborative team develops international TB lab consultants manual
  - WHO gives completed draft to international team, which completely rewrites it
  - Final rewritten product not used by anybody
  - Many thanks to USA team: publication in Clin Micro Newsletter 2014 36(16) 123-28
  - **Z. Berrada, R. Ferguson, K. Lewis, S. Liska, W. Murtaugh, E. Rider, D. Warshauer, C. Peter**

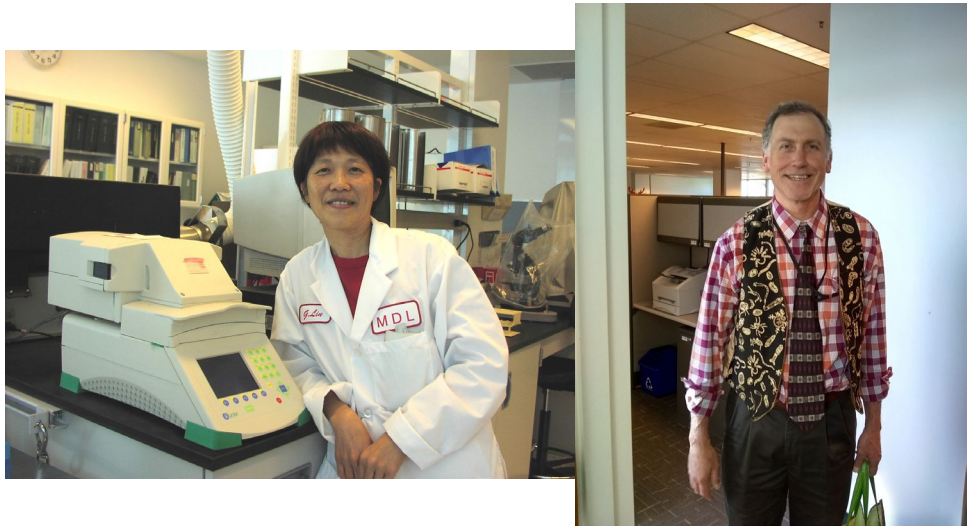
Teaching acid-fast microscopy in U.S. affiliated Pacific islands



Democratic Peoples Republic of Korea

My three themes

1. For TB, your lab is a clinical lab. Communicate with your TB clinicians and find out what they need from you. Turnaround time is a goal, and an algorithm involving rapid screening methods may be called for.
2. Diminishing lab/clinical/epi programs for TB could lead to a resurgence of this dreaded disease. Fight for retaining resources! If companies don't get FDA clearance for needed test systems, develop them yourself as LDTs (in consultation with your clinicians).
3. Reach out to local and private sector laboratories. Promote use of up to date rapid and accurate methods. Provide reference services.



TB drug susceptibility R & D team

TB lab team with multinational origins

TB clinicians in Janice Louie's back yard

I want to thank:

- APHL for their inspired leadership and strong support throughout my career
- The people I have mentored for giving me a sense of purpose
- CDPH Microbial Diseases Laboratory energy, professionalism, and actually doing the work that I have been given credit for
- State of Hawaii Department of Health for their constant support (particularly during the pandemic) and for letting me come here.