

Update on Nontuberculous Mycobacteria

B. Robinson-Dunn, Ph.D., D(ABMM), FIDSA
Oakland Univ./W^m. Beaumont School of Medicine
Beaumont Health System
Royal Oak, MI, USA
Barbara.Robinson-Dunn@beaumont.edu

Conflicts of Interest

- I have none!

Beaumont Hospital



Royal Oak, Michigan



Newsweek

March 11, 2002 \$2.00

92
ISSUES AT A GLANCE
A 7-Page Voters' Guide

TB

Why It's Back
How We Can
Protect Ourselves



Life (in the lab) was easy...

Conventional Classification of *Mycobacterium* spp.

- *M. tuberculosis* complex
- Runyoun classification: mycobacteria classified into groups based on:
 - Rapidity of growth
 - Pigmentation
 - Photochromogenicity (whether the organism changes color after being exposed to light and then reincubated)

Runyon classification-NTM (MOTT)

– Group I-----Photochromogens

M. kansasii

– Group II ---Scotochromogens

M. scrofulaceum

– Group III --Nonchromogens/slowly growing

M. avium complex

– Group IV---Rapid growers

M. fortuitum complex

IN 2003, It All Changed

AND IT BECAME SCARY!

M. tuberculosis complex

- *M. tuberculosis*
- *M. bovis* subsp. *bovis*
- *M. bovis* BCG
- *M. bovis* subsp. *caprae*
- *M. africanum*
- *M. microti*
- *M. canettii*
- *M. pinnipedii*
- *M. mungi*- 2010, epidemic in mongooses
- *M. orygis*- 2012, orig. thought to be subsp. of *M.tb.*
- *M. suricattae*- 2013, from 3 free-living meerkats

Nontuberculous Mycobacteria

Modified Runyon Scheme

- Based on major phenotypic features:
 - Growth rate
 - Pigmentation type
- Groups divided into:
 - Pigmented slow growers
 - Nonpigmented slow growers
 - Pigmented rapid growers
 - Nonpigmented rapid growers
 - Solely environmental species

Tortoli, E. 2003. Impact of Genotypic Studies on Mycobacterial Taxonomy: the New Mycobacteria of the 1990s. *Clin. Micro. Rev.* **16**:319-54.

New Mycobacterial Species

- Modern genetic techniques provide for distinct identifications of new species
 - We have become splitters rather than lumpers!
- Natural reservoir of most of the new strains is the environment (water, aerosols, dust, soil)
- Most are nontuberculous mycobacteria (NTM)
 - Low virulence
 - Rare human to human transmission
 - More than 170 new species!

Genotypic Taxonomy

- Detects highly conserved regions with hypervariable sequences - species-specific deletions, insertions or replacements or single nucleotides are present
 - **16S rRNA** is the primary target for molecular taxonomic studies-two hypervariable regions (A & B)
 - **65-kDa Heat Shock Protein** (*hsp65*) presents hypervariable regions that may be sequenced
 - **Internal Transcribed Spacer** (ITS) is suitable for identifying most mycobacterial species
 - **Mycolic Acid** varies with the species and can be useful for identification
- All of these have contributed to the 170+ species of *Mycobacterium*!

Pigmented Slow Growers

Name	Date	Source	? HIV	Other
<i>M. bohemicum</i>	1998	L.N., resp., skin	N	-
<i>M. celatum</i>	1993	Resp., bld, fecal, bone	Y	Inc. pathogenicity
<i>M. conspicuum</i>	1995	Multiple sites	Y	Cellular Ig def.
<i>M. doricum</i>	2001	CSF	Y	-
<i>M. heckeshornense</i>	2000	Resp.	N	-
<i>M. interjectum</i>	1993	L.N., urine, resp.	Y(?)	-
<i>M. intermedium</i>	1993	Resp.	N	-
<i>M. kubicae</i>	2000	Resp.	N	-
<i>M. lentiflavum</i>	1996	Gastric, resp., urine, L.N., bone	N	Low-level virulence
<i>M. palustre</i>	2002	Resp., L.N.	N	Pig L.N. & water
<i>M. tusciae</i>	1999	L.N.	N	Water

Tortoli, E., 2006, The New Mycobacteria: an Update. FEMS Immunol. Med. Microbiol., **48**:159-178.

Nonpigmented Slow Growers

Name	Date	Source	?HIV	Other
<i>M. arupense</i>	2006	Resp., L.N., tissues	N	-
<i>M. caprae</i>	2003	Resp., pericard.	N	M. tb. complex
<i>M. chimaera</i>	2004	Resp.	N	Male, elderly
<i>M. colombiense</i>	2006	Blood	Y	Columbia
<i>M. florentinum</i>	2005	Resp. fecal, L.N.	Y	<i>M. triplex</i> -like
<i>M. montefiorensis</i>	2003	Captive moray eels	-	Very slow growing
<i>M. pinnipedii</i>	2003	Resp. (seal trainer)	N	Captive & wild seals (M.tb.)
<i>M. sherrisii</i>	2004	Resp.	Y/N	<i>M. simiae</i> -like
<i>M. tilburgii</i>	2005	Resp., G.I.	Y	-

Tortoli, E., 2006, The New Mycobacteria: an Update. FEMS Immunol. Med. Microbiol., **48**:159-178.

Pigmented Rapid Growers

- *M. cosmeticum* 2004
 - Human lesions & the sink drain of a nail salon
 - Undergoing mesotherapy with unknown substance
- *M. lacticola* 2004
 - Catheter related sepsis
 - 4 y.o. female post stem cell transplant
 - Fever resolved when catheter was removed
- *M. manitobense* 2003
 - Posttraumatic wound (ankle)
 - Immunocompetent male

Tortoli, E., 2006, The New Mycobacteria: an Update. FEMS Immunol. Med. Microbiol., **48**:159-178.

Nonpigmented Rapid Growers

Name	Date	Source	?HIV	Other
<i>M. immunogenicum</i>	2001	Keratitis, catheters, skin, urine , synov. fld, BAL	N	Between <i>M. chelonae</i> & <i>abscessus</i> - hypersens. pneumonitis in metal-workers
<i>M. mageritense</i>	1997	Resp., sinus, catheter, surg. wnds	N	1 pt was lg suppressed
<i>M. mucogenicum</i>	1993	Wounds, catheters, hepatitis	N	MCLO, ice & water
<i>M. peregrinum</i>	1992			Split from <i>M. fortuitum</i>
<i>M. septicum</i>	2000	Metastatic hepatoblastoma, blood, CVC	N	Single isolate
<i>M. wolinskyi</i>	1999	Osteo, traumatic cellulitis, surg. wnds	N	Hi MIC to tobra

Tortoli, E. 2006, The New Mycobacteria: an Update. FEMS Immunol. Med. Microbiol., **48**:159-178.

Environmental Species

- *M. botniense* 2000
 - Water
 - Similar to *M. xenopi*
- *M. chlorophenolicum* 1994
 - Formerly a member of *Rhodococcus*
 - Isolated from chlorophenol-contaminated Finnish soils
- *M. cookii* 1990
 - Isolated from sphagnum and water in New Zealand
 - May be responsible for false positive results with *M. bovis* tuberculin in cattle

New Subspecies

- *M. avium* subsp. *avium*
- *M. avium* subsp. *paratuberculosis*
 - Mycobactin dependent
 - Suspected in Crohn's disease in humans
- *M. avium* subsp. *silvaticum*
 - Obligate pathogen of birds
 - ? cause of chronic enteritis in calves
- *M. avium* subsp. *hominissuis*
- *M. bovis* subsp. *caprae*
 - Goats, sheep, a pig and 3 humans in close contact with the goats
 - L.N., pulmonary lesions

A comprehensive list of all validated NTM
species can be found online at:

www.bacterio.cict.fr/m/mycobacterium.html

NTM Diversity

- New species are in both the slowly growing and rapidly growing groups
 - Differ significantly in 16S rRNA
- Number of new species due to
 - Their appearance in persons with AIDS
 - Advent of methods for sequencing DNA
- Many new species are actually subspecies within a larger group

Example: *M. avium* complex includes:

- The following subspecies:
 - ssp. *avium*, *silvaticum*, *hominis-suis*, *paratuberculosis*
- It also includes:
 - *M. intracellulare*
 - *M. arosiense*
 - *M. chimaera******
 - *M. colombiense*
 - *M. marseillense*
 - *M. timonense*
 - *M. bouchedurhonense*
 - *M. ituriense*

How do you determine the significance of NTM isolates?

HINT: it is not up to us in the laboratory to make that decision!

Diagnostic Criteria of NTM Lung Disease

- Minimum evaluation should include:
 - Chest radiograph
 - In the absence of cavitation, a chest high-resolution computed tomography scan
 - Three or more sputum specimens for AFB analysis
 - Exclusion of other disorders such as TB

Microbiologic Criteria of a “Clinically Significant NTM

1. Positive culture results from at least 2 separate expectorated sputum samples OR
2. Positive culture results from at least one bronch wash or bronch lavage OR
3. Transbronchial or lung Bx with mycobacterial histopath features and positive culture for NTM or biopsy showing positive histopath. features and positive sputum or bronchial washing cultures for NTM

Where Do Human Infections with NTM Come From?

Structure & Function of NTMs

- NTMs have an outer membrane
 - Rich in long-chain fatty acids (C_{40} - C_{80} , mycolic acids) and are 40% of the weight of the cell
 - Impermeable to hydrophilic nutrients
 - Resistant to heavy metals, disinfectants and antibiotics
 - Makes the cell extremely hydrophobic so they prefer surface attachment and growth (eg. in biofilms)
 - Also resistant to high temperatures!

Ecology of NTM

- Worldwide distribution in the environment (soil and water)
 - *M. kansasii*, *xenopi* and *simiae* exclusively recovered from municipal water sources
 - with identical methods, NTM isolation rates from the environment are similar in diverse geographic areas
 - *M. bolletii*, *M. massiliense* accounted for 40% of isolates in one study

Roux, A.L., E. Catherinot, F. Ripoll, et al., 2009, Multicenter Study of Prevalence of Nontuberculous Mycobacteria in Patients with Cystic Fibrosis in France, J. Clin. Microbiol. **47**:4124-4128.

Other Fun Facts

- Relatively resistant to low pH
 - MAC can survive exposure to stomach pH
 - Can also survive in the acidic brown-water swamps of the east coast of the U.S. (Battey bacillus)
- Survive phagocytosis by free-living amoeba and protozoa
 - All occupy the same habitat in drinking water distribution systems and in household plumbing
 - Some *M. avium* strains survive and grow in *A. polyphaga* and *A. castellanii*!

Nontuberculous Mycobacterial Habitats

Habitat	Physiological Determinants
Natural waters	Oligotrophic, biofilm formation
Drinking water	Oligotroph., biofilm, R to disinfectants
Households	Oligotroph., biofilm, R to disinfectants, thermal tolerance
Aerosols	Hydrophobic NTM cells enriched in aerosols
Water filters	Oligotroph., biofilm, R to disinfectants & metals
Soils	Oligotroph., particle attachment, amoebae-resisting, humic/fulvic acid growth stimulation
Dusts	Particle attachment

Oligotroph: able to grow using organic material in drinking water or low O₂ conc.

Waterborne NTM

Organism	Water Source
<i>M. abscessus</i>	Tap water
<i>M. avium</i>	Potable water
<i>M. chelonae</i>	Ice and ice machines
<i>M. chimaera</i>	Heater-cooler units
<i>M. fortuitum</i>	Hospital water systems and showers
<i>M. genavense</i>	Tap water
<i>M. mucogenicum</i>	Bathing, tub immersion, electronic faucets, sinks, showers & hospital water systems
<i>M. neoaurum</i>	Hospital water systems
<i>M. phocaicum</i>	Showers
<i>M. porcinum</i>	Ice and ice machines
<i>M. simiae</i>	Tap water

Kanamori, H., D.J. Weber, W. A. Rutala, 2016, Healthcare Outbreaks Associated With a Water Reservoir and Infection Prevention Strategies, Clin. Infect. Dis. **62**:1423-35.

Epidemiology of Pulmonary Non-tuberculous Mycobacterial Infections

Pulmonary NTM Diseases are Increasing

- 1987, CDC estimated 1.8 cases/100,000 population
 - TB cases decreasing
 - ? Waning cross-protective mycobacterial immunity
 - Better diagnoses due to ATS/IDSA guidelines
- A number of studies from U.S, Canada, Great Britain, Australia & Taiwan (1997-2008) cited increasing prevalence
 - In a 10 yr study of Medicare patients, prevalence increased from 20 to 47/100,000

Kendall, B.A., K.L. Winthrop, 2013, Update on the Epidemiology of Pulmonary Nontuberculous Mycobacterial Infections, *Semin. Resp. Crit. Care Med.* 2013; **34**:87-94.

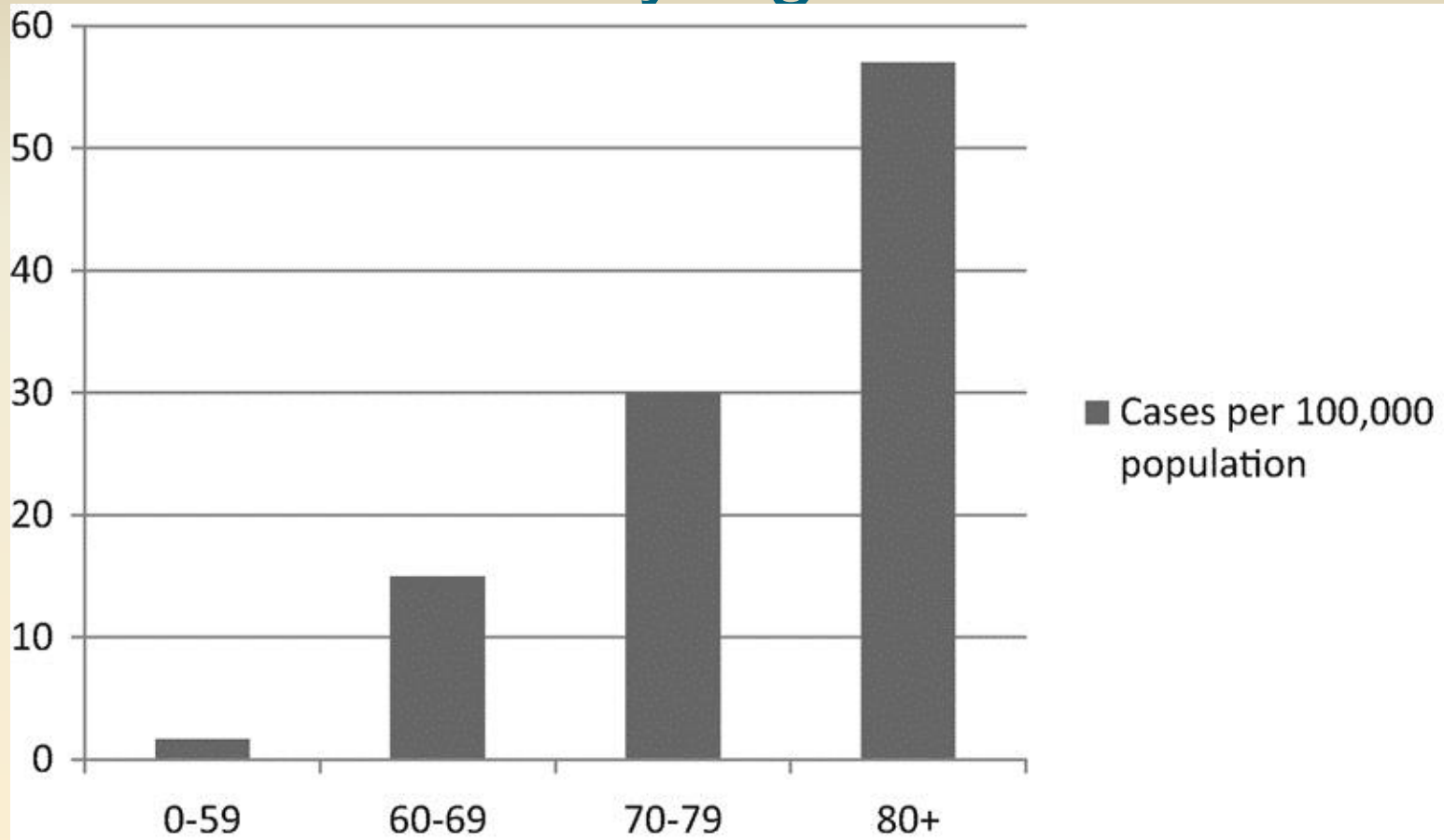
Extent of most NTM Disease (unknown)

- In non-HIV infected persons, NTM are not reportable
- Environmental exposure varies by region and even varies within individual states
- May be difficult to distinguish colonization from contamination from true disease-even with ATS/IDSA guidelines
- NTM disease may be uncommon-or the clinical laboratory may not be able to accurately identify the isolate(s)-(need molecular techniques or MALDI-TOF)
- Risk factors not fully elucidated

Pulmonary NTM Infections

- Two clinical pictures
 - HIV negative patients:
 - looks like TB with very slow progression
 - Mostly elderly patients with predisposing pulmonary conditions
 - Manifestations: range from no symptoms to cavitory disease (rarely)
 - HIV positive patients (severely immunocompromised):
 - Rapid progression
 - CXR may be normal or may reveal hilar or mediastinal adenopathy
 - CD₄ count lower than 100 cells/ μ L
 - HAART has reduced the incidence of these infections
 - Organisms responsible:
 - *M. avium* and *M. intracellulare*
 - In Europe, *M. xenopi* and *M. malmoense*
 - In US, *M. kansasii*
 - In CF patients, *M. abscessus* and closely related species

Ave. Prevalence of Pulmonary NTM by Age



Prevots, D.R., P.A. Shaw, D. Strickland, et al., 2010. Nontuberculous Mycobacterial Lung Disease Prevalence at Four Integrated Health Care Delivery Systems. *Am. J. Respir. Crit. Care Med.*, **182**:970-976.

Clinical Presentation of Pulmonary NTMs

- Study in Oregon found
 - NTM pts more likely to be female, U.S.-born, significantly older (not defined), have COPD, and take immunosuppressive drugs
 - TB pts more likely to be younger, foreign-born, and have lung cavities
 - MAC most frequently isolated
 - Rapidly growing NTMs next most frequent isolates

Prevots, D.R., P.A. Shaw, D. Strickland, et al., 2010. Nontuberculous Mycobacterial Lung Disease Prevalence at Four Integrated Health Care Delivery Systems. *Am. J. Respir. Crit. Care Med.*, **182**:970-976.

Is There Person-to-Person Spread of NTMs?

- 2012, Aitken et al. reported an outbreak of *M. abscessus* ssp. *massiliense* in a lung transplant and cystic fibrosis center.
 - Over an 8 month period, 5 pts with CF treated at the same clinic were infected with identical isolates of this organism. The isolates were the same when compared by molecular methods.
 - Extensive evaluation of the clinic did not find an environmental source
 - The pts had no common environmental or other exposure beyond shared time at the CF center
- Therefore, highly likely there was person-to-person spread!

Aitken, J.L., A. Limaye, P. Pottinger, et al., 2012, Respiratory Outbreak of *Mycobacterium abscessus* Subspecies *massiliense* in a lung Transplant and Cystic Fibrosis Center, *Am. J. Resp. Crit. Care Med.* **185**:231-232.

Lymph Node Infections Due to NTM

- Usually affects cervical lymph nodes in children
- Cervical lymphadenitis etiology
 - Rarely due to *M. scrofulaceum*
 - Commonly due to *M. avium*
 - Incidence of *M. malmoense* is increasing
 - Incidence of *M. lentiflavum* and *M. bohemicum* are also increasing
- May be very difficult to recover NTM from lymph node specimens

Skin and Soft Tissue Infections

- Granulomatous lesions - develop a few weeks after infection-
 - may have ulceration, cellulitis and cutaneous dissemination
 - Contact with contaminated water, fish, trauma or surgical wounds
- *M. marinum* from water or fish
 - Also *M. fortuitum* and *M. chelonae*
- *M. ulcerans* causes Buruli ulcer (Ige necrotizing ulcers, painless, Ige scars)
- *M. haemophilum* in immunocompromised patients-req. hemin for growth
- Post-traumatic infections due to: *M. fortuitum*, *M. chelonae*, *M. abscessus*, *M. goodii* and *M. massiliense*

Bone and Joint Infections

- Infections with NTM originate from trauma or surgical wounds
- Joint function may be severely affected and osteomyelitis may occur
- Predisposing factors are articular rheumatism and steroids
- Species involved: *M. haemophilum*, *M. kansasii*, *M. avium* complex, *M. asiaticum*, *M. flavescens*, *M. szulgai*, *M. xenopi*, *M. thermoresistibile*, *M. goodii*
- In spondylodiscitis, *M. xenopi* is as common as *M. tuberculosis*

Disseminated Infections

- Most are seen in immunocompromised patients
- MAC seen in persons with AIDS and *M. avium* 4X more common than *M. intracellulare*
- *M. genavense* frequently undiagnosed because it doesn't grow well on conventional solid media
- RGM's responsible for catheter-related sepsis
 - *M. abscessus*
 - *M. chelonae*
 - *M. chimaera*
 - *M. fortuitum*
 - *M. mucogenicum*
 - *M. neoaurum*
 - *M. septicum*

Catheter Related Infections due to Rapidly Growing Mycobacteria

Species	Disseminated	CLABSI	Exit Site
<i>M. chelonae</i>	9/18	6/42	6/17
<i>M. abscessus</i>	2/18	0/42	2/17
<i>M. chelonae/absc.</i>	1/18	1/42	0/17
<i>M. fortuitum</i>	2/18	10/42	7/17
<i>M. fluoranthenvivorans</i>	0/18	1/42	0/17
<i>M. immunogenicum</i>	0/18	1/42	1/17
<i>M. mucogenicum</i>	2/18	16/42	0/17
<i>M. neoaurum</i>	0/18	4/42	0/17
<i>M. lacticola</i>	0/18	1/42	0/17
<i>M. septicum</i>	0/18	1/42	0/17
<i>M. hackensackense</i>	0/18	1/42	0/17

Apiwattankul, N., P.M. Flynn, R.T. Hayden, et al. 2015. Infections Caused by Rapidly Growing Mycobacteria spp. in children and Adolescents with Cancer. *J. Ped. Infect. Dis.* 4:104-13.

Mycobacterium chimaera

- Heater–cooler units (HCUs) recently identified as a source of *M. chimaera* causing surgical site infections.
- Commonly used during cardiothoracic surgery to warm and cool a patient's blood during cardiopulmonary bypass
- Patients do not come into direct contact with the water from the H-C unit's tank
- NTM's in the machine can potentially be transmitted during surgery by aerosols from these devices

M. chimaera

- Transmission of *M. chimaera* from HCUs to the surgical field was investigated in an operating room equipped with an ultraclean laminar airflow ventilation system, and bacterial culture sedimentation plates.
- Smoke from the HCU reached the surgical field in 23 s by merging with ultraclean air.

Mycobacterium chimaera

- The HCU produced on average 5.2, 139, and 14.8 particles/min in the surgical field at positions Off, On/oriented toward, and On/oriented away, respectively.
- Culture plates were positive for *M. chimaera* <5 m from the HCU in the test room.
- These experiments confirm airborne transmission of *M. chimaera* aerosols from a contaminated HCU to an open surgical field despite ultraclean air ventilation.

Why does it take so long to identify disease due to *M. chimaera*?

- Patients had various clinical presentations
- Testing requires AFB blood cultures
- Slow growth of *M. chimaera*
- Extended time for identification
 - Most labs stop at *M. avium* complex
 - Need for antimycobacterial susceptibility testing
- Disbelief that this could occur

Specimen Collection (Briefly)

- Work with clinical staff to ensure optimal specimens are collected
 - Sputum: minimum of 3-5 mL
 - Other deep pulmonary specimens
 - Tissues
 - Fluids
- Do **NOT** accept routine orders from the OR's for AFB testing on every specimen
- Do **NOT** accept specimens that are too small just because someone is arguing with you!

Swab Specimens!!!

- Swab specimens will not be processed without prior approval from Infectious Diseases or the Medical/Technical Director of the Clinical Microbiology Laboratory!

Identification of NTM's

- Isolates
 - Conventional biochemicals
 - Niacin, nitrate, catalase, +/- pigmentation; rapidity of growth; growth temperature; urea; aryl sulfatase; tellurite hydrolysis, etc.
 - DNA Probes: *M. tuberculosis complex*, *M. avium complex*, *M. gordonae*, *M. kansasii*
 - Line Probe assay for 16 species + genus: not FDA cleared in U.S.
 - HPLC
 - MALDI-ToF
 - PCR and Sequencing

Definitive Identification

- In U.S., most labs use commercial DNA probe systems-only available for a limited number of species
 - Hologic AccuProbe system targets the 16S rRNA gene with species specific probes and is non-amplified
 - INNO-Lipa Mycobacteria system* (Belgium) targets the internal transcribed spacer interposed between the 16S rRNA and 23S rRNA genes-identifies 18 species
 - GenoType Mycobacterium system* (Germany) targets the 23S rRNA gene and has 3 separate kits:
 - One identifies 44 species + M.tb complex
 - The second identifies 44 species + limited AST (CE marked)

*Not FDA approved

Hybridization Results with Commercial Probes

Species	AccuProbe	INNO-LiPA	GenoType
<i>M. alsiense</i>	Not identified	<i>Mycobacterium</i>	SPsP*
<i>M. arosiense</i>	<i>M. intracellulare</i>	MAIS	<i>M. intracellulare</i>
<i>M. bouchedurhonense</i>	Not tested (NT)	NT	<i>M. intracellulare</i>
<i>M. Ilatzerense</i>	NT	<i>Mycobacterium</i>	<i>M. mucogenicum</i>
<i>M. mantenii</i>	Not identified	MAIS	<i>M. intracellulare</i>
<i>M. paraffinicum</i>	MAC	<i>M. scrofulaceum</i>	SPsP
<i>M. paraseoulense</i>	NT	NT	<i>M. scrofulaceum</i>
<i>M. riadhense</i>	NT	<i>Mycobacterium</i>	<i>M.tb</i> complex
<i>M. sherrisii</i>	NT	<i>M. simiae</i>	<i>M. simiae</i>
<i>M. shigaense</i>	NT	NT	Bands 6, 8, & 16
<i>M. shinjukuense</i>	NT	NT	<i>M. kansasii</i>
<i>M. simulans</i>	Not identified	<i>Mycobacterium</i>	<i>M.tb</i> complex
<i>M. vulneris</i>	MAC (correct ID)	MAC (correct ID)	MAC (correct ID)
<i>M. yongonense</i>	NT	NT	<i>M. intracellulare</i>

*SPsP: Positive with a probe for *M. scrofulaceum*; Tortoli, E., 2014, Microbiological features and clinical relevance of new species of the genus *Mycobacterium*, Clin. Microbiol. Rev., **27**:727-752

MALDI-ToF MS

- Very good results obtained for many common species
- Limiting factors
 - Extent of the database
 - Protocols for sample processing depending on whether growth is from a solid medium or from broth
 - Cost of the instrument but it can be used for identification of isolates in bacteriology and mycology also

MALDI-ToF: Bruker vs. Vitek



- Study performed at Univ. of Washington
 - Developed a safe, simplified protein extraction method to identify *Mycobacterium* spp. on either system for isolates grown on 7H11 agar
 - Both systems reliably differentiated *M.tb* from NTM
 - 198 clinical strains (18 *Mycobacterium* spp.) were correctly identified to species 94.9% of the time using the UW extraction and compared to the augmented database
 - BMX protocol correctly identified 94.4% of the strains
 - Only 79.3% of the strains were identified to species with the nonaugmented Bruker database. This was corrected to 93.9% by lowering the score threshold to ≥ 1.7 .

Mather, C.A., S.F. Rivera, S.M. Butler-Wu, 2014, Comparison of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for identification of Mycobacteria using Simplified Protein Extraction Protocols, J. Clin. Microbiol., **52**:130-138.

Identification of NTM by MALDI-ToF

- Accurate and rapid identification by MALDI-ToF from broth and agar cultures
 - Identification rate and accuracy compared well with sequencing when using the enhanced Library
 - Results from broth culture were better than those obtained from isolates grown on agar
- Could differentiate between most closely related species
 - Comparators were 16S rDNA or *rpoB* gene targets
 - Had some errors with *M. mucogenicum*/*M. llatzerense* and with *M. marinum*/*M. pseudoshottsii*

Buchan, B.W., K.M. Riebe, M. Timke, et al., 2014, Comparison of MALDI-TOF with HPLC and Nucleic Acid Sequencing for the Identification of Mycobacterium Species in Cultures Using Solid Medium and Broth, *Am. J. Clin. Pathol.*, **141**:25-34.

Limitations of MALDI-ToF

- MALDI-ToF requires a greater quantity of organism since there is no pre-amplification step
 - Rapidly growing mycobacteria can often be identified from broth culture (with the appropriate processing)
 - Doesn't work as well with slowly growing mycobacteria and waiting for sufficient growth on solid media may delay the identification process
- Mixed cultures are a problem
- The database is critical to the success of the test!!!!

Buckwalter, S.P., S.L. Olson, B.J. Connelly, et al., 2016, Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of *Mycobacterium* species, *Nocardia* species, and Other Aerobic Actinomycetes, J. Clin. Microbiol., **54**:376-84.

Sequencing

- Identification of NTM by sequencing is on the horizon for most clinical microbiology labs
- Large reference laboratories are now using it for specific identification when necessary
 - Identification of unusual isolates
 - Comparison of relatedness of isolates from widely separate outbreaks
 - Molecular detection of resistance
- Introduction of sequencing kits will probably mirror the introduction of MALDI-ToF into the U.S. and the FDA approval process

Susceptibility Testing (briefly)

Recommended for Slow Growers	Recommended for Rapid Growers
Amikacin	Amikacin
Ciprofloxacin	Cefoxitin
Clarithromycin	Ciprofloxacin
Ethambutol	Clarithromycin
Linezolid	Doxycycline
Moxifloxacin	Imipenem
Rifabutin	Linezolid
Rifampin	Meropenem
Streptomycin	Moxifloxacin
Trimethoprim/sulfa	Trimethoprim/sulfa
	Tobramycin (<i>M. chelonae</i> only)

Broth susceptibility testing only!

Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes, 2011, CLSI, M24-A2, 2nd edition.

Summary: Mycobacteriology

- 170+ new species of *Mycobacterium*
 - Actually 174 species and 13 subspecies of NTM as of March 29, 2016
 - More is becoming known about the ecology and epidemiology of disease due to NTM and clinical significance of isolates
- Labs have improved methods for identification of NTMs and even better methods are on the way
- Isolates are susceptible to antibacterial and some antimycobacterial agents. CLSI has specified the methodology.

References

1. Bodie, E.E., J.A. Cunningham, P. Della-Latta, *et al.* 2008. Epidemiology of nontuberculous mycobacteria in patients without HIV infection, New York City. *Emerg. Infect. Dis.* **14**:390-96.
2. Buchan, B.W., K.M. Riebe, M. Timke, *et al.* 2014. Comparison of MALDI-TOF MS with HPLC and nucleic acid sequencing for the identification of *Mycobacterium* species in cultures using solid medium and broth. *Am. J. Clin. Pathol.*, **141**:25-34.
3. Buckwalter, S.P., S.L. Olson, B.J. Connelly, *et al.* 2016. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Mycobacterium* species, *Nocardia* species and other aerobic Actinomycetes. *J. Clin. Microbiol.* **54**:376-84.
4. CLSI. 2008. *Laboratory detection and identification of mycobacteria; approved guideline*. CLSI document M48-A. CLSI, Wayne, PA 19087-1898.
5. Centers for Disease Control. 2007. Guide to the application of genotyping to tuberculosis prevention and control.
http://www.cdc.gov/tb/genotyping/Chap3/3_CDClab_2description.htm.
6. Griffith, D.E., T. Aksamit, G.A., B. Brown-Elliott, *et al.* 2007. An official ATS/IDSA statement: diagnosis, treatment and prevention of nontuberculous mycobacterial diseases. *Am. J. Respir. Crit. Care Med.* **175**:367-416.
7. Johnson, M.M., J.A. Odell. 2014. Nontuberculous mycobacterial pulmonary infections. *J. Thorac. Dis.* **6**:210-20.
8. Kendall, B.A., K.L. Winthrop. 2013. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. *Semin. Respir. Crit. Care Med.* **34**:87-94.

References (cont.)

9. Leung, J.M., K.N. Olivier. 2013. Nontuberculous mycobacteria in patients with cystic fibrosis. *Semin. Respir. Crit. Care Med.* **34**:124-34.
10. Levesque, S., P.J. Dufresne, H. Soualhine, et al. 2015. A side by side comparison of Bruker Biotyper and Vitek MS: Utility of MALDI-TOF MS technology for microorganism identification in a public health reference laboratory. *PLOS ONE* **10**: e0144878.
11. Mather, C.A., S.F. Rivera, S.M. Butler-Wu. 2014. Comparison of the Bruker Biotyper and the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of mycobacteria using simplified protein extraction protocols. *J. Clin. Microbiol.* **52**:130-8.
12. Pfyffer, G.E., 2015. *Mycobacterium*: general characteristics, laboratory detection and staining procedures, p. 536-569. In Jorgensen, J., et al. (eds.), *Man. of Clin. Microbiol.* 11th ed. ASM Press, Washington, D.C.
13. Tortoli, E. 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. *Clin. Microbiol. Rev.* **16**:319-354.
14. Tortoli, E. 2006. The new mycobacteria: an update. *FEMS Immunol. Med. Microbiol.* **48**:159-178.
15. Tortoli, E. 2009. Clinical manifestations of nontuberculous mycobacterial infections. *Clin. Microbiol Infect.* **15**:906-910.
16. Tortoli, E. 2014. Microbiological features and clinical relevance of new species of the genus, *Mycobacterium*. *Clin. Micro. Rev.* **27**:727-752.

Thank You!

- SWACM for inviting me to speak at this meeting and for the interested microbiologists in the audience
- The fantastic microbiology staff at Beaumont Health System
- My family who puts up with me being away at meetings
- A special thanks and remembrance to Dr. Gerri Hall who invited me to participate in the ASM mycobacteriology workshop (many years ago)!

Questions?