Clinical Chemistry
Trainee Council

PEARLS OF LABORATORY MEDICINE

Non-tuberculous Mycobacteria: Identification and Susceptibility Testing

DOI: 10.15428/CCTC.2013.212084

Nira Pollock, MD, PhD, D(ABMM)
Boston Children's Hospital

www.traineecouncil.org

Non-tuberculous mycobacteria (NTM)

- **Definition**: all species of mycobacteria other than those in the *M. tuberculosis* complex
- >160 species and subspecies catalogued to date
  - http://www.bacterio.cict.fr/m/mycobacterium.html
- Past 15 years: increase in both total number of species and number of species considered to be clinically significant
  - Improved methods for isolation from clinical specimens
  - Increased use of sequencing-based methods
- Opportunistic pathogens in humans (particularly immunocompromised)
- Widely distributed in environment
  - Soil
  - Water (natural and municipal)
- No evidence of human-human or animal-human transmission

NTM clinical disease

- Can cause both asymptomatic infection (colonization) and symptomatic disease in humans
- Various presentations of clinical disease
  - Pulmonary disease (most common)
    - Often associated with structural lung disease (e.g. bronchiectasis, COPD, CF)
    - Can also occur in patients without obvious predisposing factors
  - Lymphadenitis
  - Skin, soft tissue, bone disease
  - Disseminated disease

NTM species most commonly implicated in disease

- More common:
  - *M. avium* complex (MAC)*
  - *M. kansasii*
  - *M. abscessus*
  - *M. chelonae*
  - *M. fortuitum*
  - *M. haemophilum*
  - *M. marinum*
  - *M. scrofulaceum*
  - *M. malmoense*
  - *M. xenopi*
  - *M. celatum*
  - *M. ulcerans* (disease-endemic areas)
  - *M. leprae* (disease-endemic areas)
- Less common:
  - *M. genavense*
  - *M. smegmatis*
  - *M. szulgai*
  - *M. simiae*
  - *M. mucogenicum*
  - *M. immunogenum*
  - *M. nonchromogenicum*
  - *M. terrae complex*
  - *M. asiaticum*

* Most common NTM pathogen in U.S. Includes *M. avium* and *M. intracellulare*.

NTM species: most common associations

- Pulmonary Disease
  - *M. avium* complex (MAC), *M. abscessus, M. kansasii, M. malmoense, M. xenopi*
- Lymphadenitis
  - *MAC, M. malmoense, M. scrofulaceum*
- Disseminated Disease
  - *MAC, M. chelonae, M. haemophilum, M. kansasii, M. abscessus*
- Skin, Soft Tissue, and Bone Disease
  - *M. abscessus, M. chelonae, M. fortuitum, M. marinum, M. ulcerans*
- Specimen Contaminant
  - *M. gordonae* (most common; easily recovered from freshwater, lab faucets, etc.), *M. mucogenicum, M. nonchromogenicum, M. terrae complex*

Adapted from Griffith et al, ATS/IDSA Statement 2007 (AJRCCM); CLSI M48A

Isolating NTM from clinical specimens

- Avoid potential sources of contamination, particularly tap water
- Appropriate clinical specimens:
  - Expectorated or induced sputum (x3), BAL, lung biopsy
  - Body fluid (including stool, urine), abscess fluid, tissue
  - Blood
    - Commercial mycobacterial culture systems
- Specimen processing
  - Manipulations of specimens suspected to contain mycobacteria should be performed either in BSLIII or in BSLII with BSLIII practices
  - Specimens from non-sterile body sites should be digested and decontaminated (NALC-NaOH)
  - 2-5% rate of overgrowth by normal flora is considered optimal

AFB smears

- For primary specimens:
  - Fluorochrome
    - false negatives can occasionally occur with RGM (may be specimen-dependent phenomenon)
  - Kinyoun (carbol fuchsin-based)
  - Ziehl-Neelsen (carbol fuchsin-based)
  - NOT gram stain
- For growth of suspected mycobacteria in culture:
  - Ziehl-Neelsen or Kinyoun
- Semi-quantitative analysis (1+ to 4+)

Culture techniques for NTM recovery

- Cultures should include both liquid and solid media
  - Liquid: higher yield, more rapid results, but more frequently contaminated
    - Media typically contains antibiotics given higher contamination rates
  - Solid: growth rates, colony morphology, separation of mixed species, quantitation
    - LJ (egg-based)
    - Middlebrook 7H10, 7H11 (agar-based)
    - Can add antibiotics (or not) depending on specimen source
- Semiquantitative (0-4+) colony counts recommended
- Some NTMs are fastidious
  - E.g. *M. haemophilum*: media must be supplemented with iron-containing compounds

NTM culture techniques, continued

- Temperature
  - Most clinically significant NTM grow well at 35-37°C
  - *M. chelonae, M. haemophilum, M. ulcerans, M. marinum* grow best at 28-30°C
  - All skin, joint fluid, and bone specimens should be cultured at both 28-30°C and 35-37°C
  - *M. xenopi* requires higher temperature (40-42°C) for optimal growth

NTM culture techniques, continued

- Growth rates in subculture
  - "slowly-growing NTM"
    - Most grow in 2-3 weeks on subculture (*MAC, M. kansasii, M. marinum)*
    - *M. ulcerans* and *M. genovense* can take 8-12 weeks to grow
  - "rapidly-growing mycobacteria" (RGM) grow within 7 days on subculture
    - *M. abscessus, M. chelonae, M. fortuitum, M. immunogenum, M. smegmatis*
- Growth rates from primary specimen
  - Depend on burden of organism in that specimen
  - Liquid media is typically held for 6 weeks and solid media for 8 weeks before being considered culture-negative

Identification of NTM: concepts

- Identify clinical isolates to species level
  - different NTM species have different antimicrobial susceptibility
  - Exception: MAC (differentiation between *M. avium* and *M. intracellulare* is not thought to be clinically significant)
- Context is important. Consider:
  - Clinical setting and host risk factors
  - Pathogenic potential of organism
  - Recovery from multiple specimens or sites
  - Recovery from normally sterile site
  - Quantification of organism
- Communicate with clinician

Identification of NTM: methods

- Phenotypic testing
  - Growth rate
  - Pigmentation
    - Photochromogens, scotochromogens, nonphotochromogens
  - Colony morphology
- Biochemical testing:
  - Time consuming, labor-intensive
  - Does not identify many of the newly described NTM species

Identification of NTM: methods

- Molecular probes
  - *MAC, M. kansasii, M. gordonae*
- High-Performance Liquid Chromatography (HPLC)
  - Analysis of mycobacterial cell wall fatty acids (mycolic acids)
  - Can be used in combination with or in place of biochemical/molecular methods
  - Can be performed using growth on solid media, AFB-positive broth cultures, or even sediments from smear-positive specimens
  - Not definitive for some species of slowly-growing NTM, and not optimal for distinguishing all species of RGM

Identification of NTM: methods

- DNA target sequencing
  - PCR-based amplification of genomic DNA target, followed by sequencing of amplicon
  - 16S rRNA gene
    - Present in all prokaryotes
    - Contains highly conserved AND variable sequences
  - Alternative/additional targets for identification of closely related mycobacterial species: *rpoB, hsp65* (also ITS, 23S rRNA gene, *gyrB*, *dnaA, recA, secA1*)
- PCR Restriction Enzyme Analysis (PRA)
  - PCR target (e.g. *hsp*65 gene), restriction enzyme digest, gel electrophoresis, analysis
- MALDI-TOF MS

Antimicrobial Susceptibility Testing (AST) for NTM: concepts

- Role of AST of NTM for guiding patient management remains controversial
- Correlation between in vitro susceptibility and clinical response varies for different NTM species
- AST should be performed on "clinically significant" isolates that exhibit:
  - Variability in susceptibility to clinically useful antimicrobial agents and/or
  - Significant risk of acquired mutational resistance to one or more of these agents
- Optimal susceptibility breakpoints and testing methods remain controversial for many NTM drug-bug combinations
- Clinician needs to be aware of these limitations

Antimicrobial susceptibility testing of NTM: specifics

- Standard is broth microdilution
- MAC
  - Primary drugs: macrolides (test clarithromycin--predicts azithromycin susceptibility).
  - Secondary drugs: moxifloxacin, linezolid (tentative breakpoints)
  - ? EMB, Rifampin, rifabutin, amikacin, streptomycin (breakpoints not established; data emerging for amikacin)
- *M. kansasii*
  - Primary drugs: rifampin, clarithromycin
  - secondary drugs: amikacin, ciprofloxacin, ethambutol, isoniazid, linezolid, moxifloxacin, rifabutin, streptomycin, sulfonamides (only some have breakpoints)
- *M. marinum*
  - Routine susceptibility testing not recommended; treat empirically and test only if patient fails therapy
- RGM
  - Testing panel includes carbapenems, aminoglycosides, quinolones, clarithromycin, cefoxitin, doxycycline, linezolid, TMP-SMX, all with breakpoints
  - Testing should include at least two readings for clarithromycin (day 3 and day 14) to detect inducible macrolide resistance due to presence of *erm* gene

CLSI M24-A2; Barbara Brown-Elliott, personal communication, Sept. 14, 2013

Acknowledgments

- Barbara Brown-Elliott, UT Health Northeast, Tyler, TX
- Gautam Desai, Beth Israel Deaconess Medical Center/Boston Children's Hospital, Boston, MA
- Paul Elvin, Hinton State Laboratory Institute, Boston, MA

References

- CLSI. *Laboratory Detection and Identification of Mycobacteria; Approved Guideline*. CLSI document M48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
- CLSI. *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline*. CLSI document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
- CLSI. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard– Second Edition*. CLSI document M24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
- Griffith et al, An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases (2007). Am J Respir Crit Care Med 175:367-416.

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: None declared
- **Consultant or Advisory Role:** None declared
- **Stock Ownership:** None declared
- **Honoraria:** None declared
- **Research Funding:** None declared
- **Expert Testimony:** None declared
- **Patents:** None declared

Thank you for participating in this
Clinical Chemistry Trainee Council
Pearl of Laboratory Medicine.

Find our upcoming Pearls and other
Trainee Council information at
www.traineecouncil.org

Download the free *Clinical Chemistry* app
on iTunes today for additional content!

Follow us: