Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes

(largely taken from a presentation prepared by Gail L. Woods, MD and Barbara Brown-Elliott, MS, MT(ASCP)SM)

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Special thanks to Tracy Dooley, CLSI Senior Standards Administrator and Staff Liason for Microbiology
At the conclusion of this talk, you will be able to:

- Discuss the indications for susceptibility testing of MTBC, NTM, & aerobic actinomycetes
- Discuss the methods for susceptibility testing of MTBC, NTM, & aerobic actinomycetes
- Accurately interpret and report results
- Establish and implement a QC program
Program Outline

- *Mycobacterium tuberculosis* complex
- Nontuberculous mycobacteria
- *Nocardia* & other aerobic actinomycetes
Susceptibility Testing for MTBC

- Standard based on proportion methods
  - Agar (INH, RMP, EMB) or radiometric (PZA)

- Resistance:
  - “decrease in susceptibility of sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come into contact with the drug.”
  - >1% of an inoculum of bacterial cells in the presence of a “critical concentration” of anti-TB drug
Critical Concentration

- Adopted by international convention

- Lowest concentration of anti-TB drugs that inhibit 95% of “wild strains” of MTBC that have never been exposed to the drugs, but not inhibiting strains isolated from patients failing to respond to therapy

- Test susceptibility of MTBC to the critical concentration of drug specific for the test method you are using
Susceptibility Testing of MTBC
Unique Aspects

• Testing is performed at 1 (or 2) concentrations
• “Critical” concentrations established years ago, & values may differ depending on test medium
• No uniform consensus regarding clinical relevance of testing other concentrations
• Agar proportion uses %age calculation to determine R or S
• Radiometric method for PZA testing uses drug-specific calculation procedure to determine R or S
Agar Proportion Method

Limitation

• Not rapid
• Broth method with shorter incubation time is recommended standard of practice in industrialized countries
• CDC goal: report results of primary drugs within 15-30 days of receipt of specimen
  – 7-14 days after isolation of MTBC (ideal)
Agar Proportion Method
Current Uses

• Confirm commercial broth system result, if necessary

• Test drugs &/or concentrations of drugs not available in commercial systems

• Standard against which new methods are evaluated & for characterizing in vitro susceptibility of “new” anti-TB drugs
MTBC Susceptibility Testing
Recommended Drugs

• **PREVIOUS:**
  • 2 concentrations of INH (critical & higher);
  • Might consider INH, RMP, EMB only

• **NEW:** Initially test all primary drugs at critical concentration
  – INH, RMP, EMB, PZA
  – PZA resistance more common, not predictable, always requires modification of treatment
### Primary Drugs for FDA-Cleared Commercial Broth Systems

<table>
<thead>
<tr>
<th>Drug</th>
<th>System and Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BACTEC 460TB</td>
</tr>
<tr>
<td>INH</td>
<td>0.1</td>
</tr>
<tr>
<td>RMP</td>
<td>2.0</td>
</tr>
<tr>
<td>EMB</td>
<td>2.5</td>
</tr>
<tr>
<td>PZA</td>
<td>100</td>
</tr>
</tbody>
</table>
Testing Frequency for Primary Drugs

- Initial isolate from every patient
- Repeat if cultures do not convert to negative after 3 months of therapy or earlier if there is clinical evidence of failure
- Confirm if initially resistant, by repeat testing (?) or other means, e.g. molecular
Initial Results Indicate Resistance to ≥1 Drug

- Exclude bacterial contaminant and NTM
  - Examine vial/tube: MTBC usually grows in clumps & broth tends to remain clear
  - Prepare AFB-stained smear: cording suggests MTBC; dispersed distribution or random, loose clumps throughout smear suggests NTM
  - Subculture
Initial Results Indicate Resistance to ≥1 Drug

• Determine need for repeat testing
  – Is molecular testing available?—beacons, line probe or sequencing?
• If repeat or molecular testing planned:
  – Report initial resistance, indicating results preliminary & confirmation in progress
  – Testing secondary agents simultaneously strongly recommended
Second-line Drug Testing

• Indications
  – Resistance to RMP or any 2 primary agents
  – NEW: Do second-line testing when there is mono-resistance to critical concentration of INH if therapy with fluoroquinolone planned
Second-line Drug Testing
Resource-Rich Countries

- Test ≥1 drug from each class plus higher concentrations of INH & EMB
  - Exception is cycloserine: testing not recommended due to technical issues
  - “Each class”: e.g. fluoroquinolones, injectables
- Avoid “piecemeal” approach
- If not done in-house, immediately send to lab with second-line drug testing expertise
Second-line Drugs Listed in M24*

- Capreomycin: 10.0/10.0  (7H10 conc/7H11 conc)
- Ethionamide: 5.0/10.0
- Ethambutol: 10.0/10.0 (higher concentration than primary)
- Kanamycin: 5.0/6.0 (class representative for amikacin)
- Ofloxacin: 2.0/2.0 (class representative for fluoroquinolones)
- PAS: 2.0/8.0
- Rifabutin: 0.5/0.5
  - Some test 1-2 µg/ml; however, clinical significance is unknown
- Streptomycin: 2.0 & 10.0/2.0 & 10.0

*Concentrations (µg/ml) in 7H10 agar/7H11 agar
Second-line Drug Testing
New in M24-A2

• Amikacin added (4 µg/ml in 7H10; agar/breakpoint in 7H11 not established)

• Resistance to kanamycin may not indicate resistance to amikacin
  – Consider testing both aminoglycosides
Second-line Drug Testing
New in M24-A2

<table>
<thead>
<tr>
<th>Fluoroquinolone</th>
<th>7H10 (µg/ml)</th>
<th>7H11 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levofloxacin</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

1. Fluoroquinolone tested should be selected based on consultation with physician treating most resistant TB
2. Testing levofloxacin at 1.0 µg/ml = testing ofloxacin at 2.0 µg/ml
3. # studies with moxifloxacin by agar proportion are limited; more studies needed

ND=not determined
Second-Line Drug Testing in Commercial Broth Systems

- Amikacin: 1.0/1.0
- Capreomycin: 1.25/2.5
- Ethionamide: 2.5/5.0
- Kanamycin: 5.0/2.5
- Levofloxacin: 2.0/1.5
- Linezolid: 1.0/1.0
- Moxifloxacin: 0.5/0.25
- Ofloxacin: 2.0/2.0
- Rifabutin: 0.5/0.5

Appendix A

Concentrations (µg/ml) are interpretive criteria, based on multicenter studies, for **BACTEC 460/MGIT 960** (neither is FDA-cleared for testing second-line drugs)
• Ofloxacin is the class representative
• Test at least 1 of the 3 (consult specialist in managing drug-R TB)
• Moxifloxacin S at 0.25 µg/ml by MGIT 960 = levofloxacin S
• If moxifloxacin R at 0.25 µg/ml by MGIT 960, consider testing at 0.5, 1, 2, and 4 µg/ml to determine level of resistance (studies needed to assess clinical efficacy of moxifloxacin for isolates with MIC of 0.5-4 µg/ml)

*Footnote
Second-line Drug Testing Resource-Limited Countries*

- Prioritize choices of drugs to test
- 1st : INH, RMP
- 2nd : EMB, PZA, streptomycin
- 3rd : amikacin, kanamycin, capreomycin, fluoroquinolone (based on surveillance data)

*Appendix B
Agar Proportion Method
Changes in M24-A2

• Select & prepare stock solution of drugs to test
  – Details in Table 2; example calculation in Appendix C
• Agar medium: 7H10 or 7H11
  – Preparation & plating moved to Appendix D
  – Drug-containing disks moved to Appendix E
  – Liquid drug moved to Appendix F
• Inoculum density for indirect method = 0.5 to 1.0 McFarland standard
• Incubation temperature 35-37°C
Susceptibility Testing of MTBC
Result Reporting: What’s New?

Shortened suggested comments for INH-R, depending on # of concentrations tested

- If test result indicates R at critical concentration to INH, and high conc not tested: “This test result indicates the presence of at least low level resistance to INH.”
- If test results indicate high-level R to INH: “These test results indicate high-level resistance to INH.”
- For both: “A specialist in the treatment of drug-R TB should be consulted concerning the appropriate therapeutic regimen and dosages.”
Susceptibility Testing of MTBC

Quality Control

- Always test pan-susceptible isolate
  - H37Rv (ATCC 27294)
  - H37Ra: avirulent, unique HPLC pattern
- Consider ATCC BAA-812 if testing INH at 2 concentrations
  - R to critical concentration/S to higher concentration
- Avoid working with strains resistant to >2 drugs or exhibiting high-level resistance to single drug
Susceptibility Testing of MTBC
Quality Control

• Storage of controls: prepare suspension in suitable stabilizer, distributed in small aliquots in multiple vials, & maintained at -20C or below (never in self-defrosting freezer)

• Test frequency
  – Pan-S (H37Rv): once each week patient isolates tested
  – R (BAA-812): can be less frequent (eg monthly) unless problem identified
Molecular Detection of Drug Resistance in MTBC

- Commercially available methods
  - Real-time PCR, PCR/line probe
  - Not yet FDA cleared
- Test positive cultures or AFB smear-positive sputum
- Identify MTBC and detect mutations associated with INH & RMP resistance
- Interpret negative mutation results with caution: resistance may be caused by different mutations
- Detection of MTBC DNA does not necessarily = viability
- May perform on mixed/contaminated cultures
Molecular Detection of Drug-R MTBC: Possible Indications

• Patients with wide range of contacts who may have spread infection to many others

• Patients suspected of having drug-R disease
  – History of previous treatment
  – From countries or ethnic groups with increased drug-R
  – Not responding well to treatment
  – Exposed to MDR-TB
Nontuberculous Mycobacteria
Antimycobacterial Susceptibility Testing (AST) of Nontuberculous Mycobacteria (NTM)

Rapidly Growing Mycobacteria (RGM)
~ 70 species
Species grow ≤ 7 days

Slowly Growing Mycobacteria (SGM)
~ 69 species
Species grow > 7 days

More than one half identified since 1990
Rapidly Growing Mycobacteria
Identification to species or group important to determine treatment

Species grow ≤ 7 days
M. fortuitum group
M. chelonae/abscessus group
M. smegmatis group
M. mucogenicum group
Pigmented RGM (~35 species as of 2011)
Rapidly Growing Mycobacteria

- Non-pigmented Pathogens

**M. fortuitum group**
- *M. fortuitum*
- *M. peregrinum*
- *M. senegalense/conceptionense*
- *M. setense*

* Same genetically

Rapidly Growing Mycobacteria

**M. fortuitum** group (formerly Third Biovariant Group)

- *M. porcinum*
- *M. houstonense*
- *M. boeniceki*
- *M. mageritense*

- *M. brisbanense*
- *M. neworleansense*
- *M. septicum*

*Phylogenetically distinct; may be included with*

*M. wolinskyi*

Schinsky, et al., IJSEM, 2004
Rapidly Growing Mycobacteria

- Nonpigmented Pathogens

**M. chelonae / abscessus group**

- *M. chelonae*
- *M. immunogenenum*
- *M. salmoniphilum*
- *M. abscessus subsp. abscessus*
- *M. abscessus subsp. bolletii* (formerly *M. massiliense, M. bolletii*)

Adekambi, et al., IJSEM 2006
Leao, et al., IJSEM 2011

Brown-Elliott, et al., CMR, 2002
Whipps, et al., IJSEM, 2007
Rapidly Growing Mycobacteria

*M. mucogenicicum* group
(formerly *M. chelonae*-like Organism)

*M. mucogenicicum*
*M. aubagnense*
*M. phocaicum*

Adekambi, et al., IJSEM, 2006
Rapidly Growing Mycobacteria

Late Pigmenting Species: *M. smegmatis* group

*M. smegmatis* (formerly *M. smegmatis* sensu stricto)
*M. goodii*
*M. wolinskyi***

*** Non-pigmenting phylogenetically distinct (may be included with *M. mageritense*)

Brown BA, et al., IJSB, 1999
Rapidly Growing 
Mycobacteria

Early Pigmenting Species
(~35 species as of 2010)

*M. aurum/neoaurum*  
*M. bacteremicum*  
*M. flavescens*  
*M. vaccae*  
*M. phlei*  
*M. thermodurabile*  
*M. canariasense*  
*M. cosmeticum*  
*M. monacense*  
*“M. lacticola”*  
*M. psychrotolerans*

* Proven pathogens

Recommendation for Which Isolates to Test

- Follow ATS criteria for respiratory samples
  - Generally multiple (+) samples
  - 2 (+) sputa or 1 Bronch
  - (+) Transbronch / lung bx
  - Single (+) sputum not likely to be significant
- Clinically significant isolates from blood, sterile body fluids, skin and soft tissue
- Repeat susceptibility after 6 months if (+) cultures continue

Griffith, et al., ATS/IDSA Statement 2007
Susceptibility Testing of RGM

- Broth Microdilution is recommended “Gold Standard”
- Match McFarland 0.5 turbidity standard
- May require use of beads to homogenize
- 2 fold dilutions in CAMHB
- Organism concentration $10^5$ CFU/mL or $10^4$ CFU/well in 0.1 mL volume
- **Incubation** 28-30°C / 3 days/room air

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Antimicrobial Susceptibility Testing of RGM (cont’d)

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- Clarithromycin MICs
  - 3 days, (mutational resistance) = Point mutation adenine 2058 or 2059 in 23S rRNA gene
  - Final reading at 14 days
  - Inducible clarithromycin resistance (erm gene) at 14 days
  - Especially *M. abscessus*

Wallace et al., Antimicrob Agents Chemother, 1996
CLSI M24-A2, 2011
Clarithromycin Resistance

*M. fortuitum / M. abscessus*

- rRNA methylase gene
- *erm*(39) *M. fortuitum*
- Confers inducible macrolide resistance
- *erm* (41) *M. abscessus*
- No *erm* gene in *M. chelonae*

**erm Gene in *M. abscessus***

- Approximately 85% *M. abscessus* (subsp. *abscessus*) contain inducible *erm* gene.
- Isolates of *M. abscessus* subsp. *bolletii* (formerly *M. massiliense* and *M. bolletii*) do not contain inducible *erm* gene.

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**erm Gene in M. abscessus**

(Cont'd)

**Clinical Significance**

Patients with isolates containing *erm* gene have delayed treatment response and possible failures compared to those patients whose isolates DO NOT contain functional *erm* gene.


Leao, SC, et al. IJSEM. 2010
Clinical Significance of *erm* Gene in *M. abscessus*

- Treatment response rates in clarithromycin-containing regimens are higher in patients with *M. abscessus* subsp. *bolletii* than those with *M. abscessus* subsp. *abscessus* lung disease.
- Proportion of patients with sputum conversion and negative sputum cultures: 88% with *M. abscessus* subsp. *bolletii* compared to 25% with *M. abscessus* subsp. *abscessus*.
Clinical Significance of *erm* Gene in *M. abscessus* (cont’d)

- *M. abscessus* lung disease has been considered a chronic incurable infection but this may not be true with *M. abscessus* subsp. *bolletii*.
- *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* associated with CF strains

Changes to Broth microdilution RGM MIC breakpoints (µg/mL)

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline/Minocycline</td>
<td>≤ 1</td>
<td>2-4</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Imipenem, Meropenem</td>
<td>≤ 4</td>
<td>8-16</td>
<td>&gt; 32</td>
</tr>
<tr>
<td>TMP-Sulfamethoxazole</td>
<td>≤ 2/38</td>
<td>-</td>
<td>&gt; 4/76</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤ 2</td>
<td>4</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤ 1</td>
<td>2</td>
<td>&gt; 4</td>
</tr>
</tbody>
</table>

CLSI, M24-A2, 2011
Reporting MICs of RGM

- Imipenem
  - New breakpoint ($I = 8-16 \, \mu g/mL$) to allow reporting in all species (MICs more reproducible)
  - Report for *M. fortuitum* group - If MIC $> 8 \, \mu g/mL$ - repeat/confirm

- Tobramycin
  - Report only for *M. chelonae*
  - If MIC $> 4 \, \mu g/mL$ – repeat/confirm

CLSI, M24-A2, 2011
Reporting MICs of RGM

- Amikacin with *M. abscessus*
  - If MIC $\geq 64$ ug/mL – repeat/confirm

- Clarithromycin
  - Trailing endpoints with *M. fortuitum* group – report as “resistant”

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Quality Assurance for RGM
Susceptibility Testing

Initial Validation
Quality Control
Proficiency testing
Parallel Testing (Accredited Labs)
Reference Strains

M. peregrinum ATCC 700686
S. aureus ATCC 29213
*P. aeruginosa ATCC 27853
E. faecalis ATCC 21212

CLSI, M24-A2, 2011
# Changes to RGM QC Ranges (µg/mL)

<table>
<thead>
<tr>
<th>Drug</th>
<th>ATCC 700686</th>
<th>ATCC 27853*</th>
<th>ATCC 29212*</th>
<th>ATCC 29213</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>1-4</td>
<td>64-256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4-32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25-1</td>
<td>0.25-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2-8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>2-16</td>
<td>1-4</td>
<td>0.5-2</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>1-8</td>
<td>1-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>2-16</td>
<td></td>
<td></td>
<td>0.03-0.12</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.12-0.5</td>
<td>1-4</td>
<td>0.06-0.5</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤0.06-0.25</td>
<td>1-8</td>
<td>0.06-0.5</td>
<td>0.015-0.12</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>≤0.25/4.8-2/38</td>
<td>8/152-32/608</td>
<td>≤0.5/9.5</td>
<td>≤0.5/9.5</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2-8</td>
<td>0.25-1</td>
<td>8-32</td>
<td></td>
</tr>
</tbody>
</table>

CLSI M24-A2, 2011
Slowly Growing NTM

*M. marinum*

*M. kansasii*

*M. avium* complex (MAC)

Other NTM
M. marinum

- Routine MICs not recommended
- All untreated strains have same drug pattern
- Acquired mutational resistance is rare
- MICs performed at 3 months if still culture (+)

CLSI, M24-A2, 2011
Clinically Recommended Agents

- Clarithromycin
- RMP
- Doxycycline/Minocycline
- TMP-SMX
- RMP + EMB

CLSI, M24-A2, 2011
<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Breakpoints (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Doxycycline/Minocycline</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&gt;1</td>
</tr>
<tr>
<td><strong>Trimethoprim sulfamethoxazole</strong></td>
<td>&gt;2/38</td>
</tr>
</tbody>
</table>

CLSI, M24-A2, 2011
M. kansasii

- Routine testing of rifampin (RMP) and CLARI only
- RMP susceptibility ≤1 µg/mL
- CLARI ≤8 µg/mL = S
- Test 2o agents only if RMP resistant (treatment failure generally seen only with RMP resistance; testing other TB drugs can be problematic)
- If RMP susceptible, will be rifabutin susceptible (HIV patients on protease inhibitors)

CLSI M24-A2, 2011
### Broth MICs Indicating Resistance for M. kansasii

<table>
<thead>
<tr>
<th>Antimycobacterial Agents</th>
<th>MIC Indicating Resistance (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Agents</strong></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin*</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&gt;1</td>
</tr>
<tr>
<td><strong>Secondary Agents</strong></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ciprofloxacin/Levofloxacin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Linezolid*</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Moxifloxacin*</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>&gt;2/38</td>
</tr>
</tbody>
</table>

*Changes proposed in CLSI, M24-A2, 2011*
M. kansasii
Clinically Recommended Agents

- Clarithromycin, EMB, RMP/RBT
- INH, EMB, RMP/RBT
M. kansasii

Summary of Changes

- Addition of clarithromycin as Primary Agent
- Addition of moxifloxacin and linezolid as secondary agents
- Includes MICs indicating resistance to all agents tested

CLSI, M24-A2, 2011
### Quality Control Ranges of MICs for Testing of *Mycobacterium kansasii* to Rifampin

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acceptable Range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. kansasii</em> ATCC 12478</td>
<td>≤ 1</td>
</tr>
<tr>
<td><em>M. marinum</em> ATCC 927</td>
<td>≤ 0.25-1</td>
</tr>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>0.5-4</td>
</tr>
</tbody>
</table>

CLSI, M24-A2, 2011
**M. avium complex (MAC)**

**Recommendation for which isolates to test:**
- Initial isolates to establish baseline value
- Isolates from patients on prior macrolide therapy
- Isolates from patients who develop bacteremia on macrolide prophylaxis
- Isolates from patients who relapse on macrolides
**M. avium complex (MAC)**

- Clarithromycin: Class drug for macrolides
- Susceptibility evaluated on basis of Clarithromycin MICs *only*
  - Broth based system
- No correlation between *in vitro* susceptibility results for antituberculous agents (RMP, EMB, RBT) with clinical outcome
- Repeat MICs at 3 mos (disseminated); 6 mos (chronic respiratory) MAC
MAC: Clarithromycin Resistance

- Untreated strains MICs ≤ 8 μg/mL - responders
- Relapse strains after treatment failure: MICs ≥ 32 μg/mL - no longer respond to macrolides

CLSI, M24-A2, 2011
MAC: Acquired (Mutational) Resistance to Clarithromycin

- 100% of high level, Clari\textsuperscript{R} isolates have mutations A2058 or A2059 in 23S rRNA gene
- Untreated strains with intermediate/resistant MICs are rare
- I/R untreated strains may indicate “mixed population”
MAC: Acquired (Mutational) Resistance to Clarithromycin (cont’d)

- Closely monitor “I” strains for development of macrolide resistance

- Azithromycin/Clarithromycin have same rRNA mutation (If S to Clari, S to Azi & vice versa)

CLSI, M24-A2, 2011
Macrolide Resistant MAC

- Reasonable to test/For interpretation use tentative breakpoints
  - Moxifloxacin
  - Linezolid – Same as RGM breakpoints
  - No breakpoints established for aminoglycosides (Amikacin, Streptomycin)
  - No 1st line antituberculous agents should be reported
  - Consult expert in treatment of macrolide resistant MAC
<table>
<thead>
<tr>
<th>Method / Antimicrobial</th>
<th>MIC</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth Microdilution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH 7.3-7.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin&lt;sup&gt;1&lt;/sup&gt;</td>
<td>≤ 8</td>
<td>16</td>
<td>≥ 32</td>
<td></td>
</tr>
<tr>
<td>Moxifloxacin&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>≤ 1</td>
<td>2</td>
<td>≥ 4</td>
<td></td>
</tr>
<tr>
<td>Linezolid&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>≤ 8</td>
<td>16</td>
<td>≥ 32</td>
<td></td>
</tr>
<tr>
<td>Radiometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin (pH 6.8)</td>
<td>≤ 16</td>
<td>32</td>
<td>≥ 32</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin (pH 7.3-7.4)</td>
<td>&lt; 4</td>
<td>8-16</td>
<td>≥ 32</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Primary agent  <sup>2</sup>Secondary Agent  <sup>3</sup>Tentative breakpoints

*Changes proposed in CLSI M24-A2, 2011

CLSI, M24-A, 2003
Quality Control Ranges of Broth Microdilution MICs for *Mycobacterium avium* ATCC 700898

<table>
<thead>
<tr>
<th>Macrolide</th>
<th>pH</th>
<th>MIC range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>6.8</td>
<td>1-4</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>7.3- 7.4</td>
<td>0.5-2</td>
</tr>
</tbody>
</table>

CLSI, M24-A2, 2011
Other Slowly Growing NTM

- Too few isolates studied
- No specific susceptibility method recommended
- Generally test as for *M. kansasii* including 2º panel
- Must validate

CLSI, M24-A2, 2011
Other Slowly Growing NTM

*M. terrae/nonchromogenenicum*

*M. simiae*  
*M. xenopi*

*M. szulgai*  
*M. celatum*

*M. lentiflavum*  
*M. malmoense*

Newly described species

CLSI, M24-A2, 2011
Fastidious Species of NTM

No current standardized susceptibility method

*M. haemophilum*
Requires hemin/iron compounds
Agar disk elution/"X" strips*
Broth microdilution/ferric ammonium citrate
Extended incubation 2-3 wks 28-30° C

*Appendix J has a proposed method

CLSI, M24-A2, 2011
Fastidious Species of NTM
(cont’d)

No current standardized method

*M. genavense*
Requires Mycobactin J supplementation
Extended incubation ≥ 6 wks

*M. ulcerans*
Extended incubation 4-6 wks
### Summary: CLSI, M24-A2, 2011

**RGM**

- **Recommended susceptibility method:** Broth Microdilution
- **No antituberculous agents tested**
- **Interpretive criteria for**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Interpretive Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>Doxycycline</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>Imipenem</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Linezolid</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>*Meropenem</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
</tr>
<tr>
<td></td>
<td>*New</td>
</tr>
<tr>
<td></td>
<td>*Minocycline</td>
</tr>
<tr>
<td></td>
<td>*Moxifloxacin</td>
</tr>
<tr>
<td></td>
<td>*TMP/SMX</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
</tr>
</tbody>
</table>

*New*
Summary: CLSI, M24-A2, 2011

Slowly Growing Mycobacteria

- *M. marinum* – Routine susceptibility not recommended
- *M. kansasii* – RMP and clarithromycin susceptibility only except for RMP resistant isolates
- MAC – Clarithromycin susceptibility only except for moxifloxacin, linezolid (tentative breakpoints)
  - Broth method recommended and no testing of TB agents
Summary: CLSI, M24-A2, 2011

QC organisms

RGM:  
- *M. peregrinum*  ATCC 700686  
- *S. aureus*  ATCC 29213  
- *P. aeruginosa*  ATCC 27853  
- *E. faecalis*  ATCC 29212

MAC:  
- *M. avium*  ATCC 700898  
- *M. kansasii*  ATCC 12478  
- *M. marinum*  ATCC 927  
- *E. faecalis*  ATCC 29212

*Modification*
Susceptibility Testing (AST) Caveat

- For labs that encounter NTM infrequently, the recommendation is to refer isolates to an established reference lab for AST
- For labs that elect to do AST, test performance/proficiency must be evaluated initially and maintained regularly
Acknowledgments

CLSI Subcommittee on Antimycobacterial Susceptibility Testing – Gail Woods M.D., Chair

UTHSCT Susceptibility Testing Lab
Richard J. Wallace, Jr., M.D.
Kimberly Kriel
Pamela Newton
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Linda Bridge Mann
Joanne Woodring
Ravikiran Vasireddy
Paula Johnson
Ann McClendon
Teagen Martin
Aerobic Actinomycetes
Aerobic Actinomycetes

• Recommended method: broth microdilution
  – For *R. equi*, use microtiter dilution panels for gram-positive aerobic bacteria & follow guidelines in M7-A8
• Potential problems requiring further studies:
  – Ceftriaxone endpoints difficult to interpret consistently
  – SXT endpoints difficult to interpret consistently
    • Supplement with disk diffusion using sulfisoxazole disk
  – False-resistant results for ceftriaxone & *N. brasiliensis*
  – False-resistant results for imipenem & *N. farcinica*
Aerobic Actinomycetes

- Antimicrobial agents: new in M24-A2
  - For *R. equi* only, include rifampin & vancomycin
- Inoculum suspension
  - Prepare heavy suspension in sterile, deionized water or saline from growth on blood or trypticase soy agar
  - Break up clumps using micropestle or vortexing with glass beads
  - Allow clumps to settle (about 15 minutes)
  - Add supernatant to 2ml water or saline; check turbidity with nephelometer to = density of 0.5 McFarland
Aerobic Actinomycetes

- Inoculate tray, cover with adhesive seal, & place in plastic bag
- Incubation: 35±2C, ambient air
- Read at 72 hr (*R. equi*, 24 hr; *Tsukamurella*, 24-48 hr)
  - If growth <2+, incubate & read daily for up to 5 days
- MIC=lowest concentration that inhibits visible growth, except for SXT
• General recommendation: well with 80% inhibition of growth compared with growth in + control well or well with lowest SXT concentration (if more than + control)

• More practical: dilution showing significant difference in amount of growth compared with + control well or to an adjacent well with a higher drug concentration
Sulfisoxazole Disk Diffusion

- 0.5 McFarland suspension
- Follow guidelines in CLSI M02
- 250 µg disk
- Incubate in ambient air, 35±2°C, 72 hr
- Evaluate growth: should not be confluent; streak marks should be obvious with clear areas between streaks apparent
- Compare to SXT MIC if latter is questionable
Sulfisoxazole Disk Diffusion*

- Zone $\geq 35$mm = Susceptible
- Zone $\leq 15$mm = Resistant
- Zone 16-34 mm uninterpretable (insufficient data)
- If disk diffusion and MIC results are discrepant, retest or send to reference lab

**Reporting Results**

- *Nocardia*: report MIC value & interpretation
  - Compare to those expected for the species (Appendix K)
  - If differ from expected, repeat &/or send to reference lab with expertise
  - Report sulfa-R with caution (most species susceptible)
- *R. equi*: report MIC value & interpretation using breakpoints for *S. aureus* (M100)
  - Tentative pending more data
- Other genera: report MIC value, referring to footnote “a” in Table 9
Breakpoints in this table apply to Nocardia and can tentatively be used for other aerobic actinomycetes. These breakpoints are considered tentative and should be reported as such pending the accumulation of further information.
Aerobic Actinomycetes: QC

- Test appropriate strains weekly or each day test is performed (if less than weekly)
  - *S. aureus* ATCC 29213
  - *P. aeruginosa* ATCC 27853
  - *E. coli* ATCC 35218 (for amoxicillin-clavulanic acid)
- Acceptable ranges for strains in M100
??? QUESTIONS ???