Introduction
Principles of Susceptibility Testing and Antimicrobial Agents

SWACM 2012
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The Goal for this Morning
• Discuss the principles of susceptibility testing from a “different” angle (45 minutes)
• Discuss recent changes to CLSI breakpoints for the Enterobacteriaceae (45 minutes)
• BREAK ~30 minutes
• Discuss recent changes to CLSI breakpoints for Pseudomonas aeruginosa (45 minutes)
• Discuss current events in carbapenem resistance relevant confirmatory methods (30 minutes)

The Goal for the Next 45 Minutes
• Really think about susceptibility testing and what we’re doing
• Brief review of the antibiotics in question and their activity

Susceptibility Testing: What’s the Point?
TO PREDICT THE OUTCOME OF TREATMENT WITH A PARTICULAR ANTIBIOTIC.
Assumptions/Considerations:
1. You are reporting results on a disease causing organism.
2. The physician will see, trust and act on the information you’ve given them.
3. The results actually mean something.
4. Communication of results is efficient and easy to understand.
5. Stewardship!

What’s in an interpretation?
• **Susceptible** – Implies that an isolate is inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.
• **Intermediate** – An isolate that approaches the usually attainable blood and tissue levels and for which response may be lower than for a susceptible isolate. Also includes a buffer zone to account for small differences in testing that would otherwise lead to a major interpretive discrepancy.

What’s in an interpretation?
• **Resistant** – Implies that an isolate is not inhibited by the usually achievable concentrations of the agent with normal dosages.
• **Non-susceptible** – Category used for organisms that only have a susceptible category. This designation does NOT necessarily mean that an isolate has a resistance mechanism. It only means that the result falls outside the range that has been defined for the wild-type distribution.
So how good are we?

<table>
<thead>
<tr>
<th>IDEAL</th>
<th>REALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SUSCEPTIBLE — % Success —</td>
<td>100%</td>
</tr>
<tr>
<td>• RESISTANT — % Success —</td>
<td>0%</td>
</tr>
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</table>

Why not the 100 – 0% Rule?
1. Technical errors?
2. Wrong test?
3. Patient factors?

Things not accounted for by susceptibility testing:
- Drug pharmacokinetics
- Drug delivery to site of infection
- Host response (or lack of)
- Toxin production
- Polymicrobial interactions

Why is this?

The “90-60” Rule

Why not the 100 – 0% Rule?
1. Technical errors?
2. Wrong test?
3. Patient factors?

How Breakpoints are Established
Breakpoints are established by considering 4 different kinds of data.
1. PK/PD data
2. In vitro data
3. Presence or absence of resistance mechanisms
4. Clinical outcome data

The value of an MIC...
The MIC represents an IN VITRO value that “MAY tell a physician the concentration of antimicrobial agent required at the site of infection to inhibit an infecting organism.”

The value of an MIC...
- The MIC does not represent an absolute value!
- The “true” MIC is somewhere between the lowest test concentration that inhibits growth and the next lower concentration.

MIC is between 8 and 16

Shouldn’t we be more precise?
The value of an MIC...
What about the E-test?
Remember....
• The acceptable reproducibility of the test is within ONE
twofold dilution of the actual end point.

QC results to further illustrate the point
• Under the most reproducible conditions we can
generate in the laboratory the allowable MIC
range of S. aureus for vancomycin is 0.5 - 2.
• CMC 2012 QC Data

One more thing to think about...
• Is the difference between MIC’s of 1 and 2 the
same as the difference between 64 and 128?

Where do you go to get your
breakpoints?

FDA Breakpoints (no CLSI breakpoints)
Let's talk about the drugs

Back to Basics: Cell Wall Acting Antibiotics

**β-Lactams**
1. Penicillins
2. Cephalosporins
3. Carbapenems
4. Monobactams

**β-Lactamase inhibitors**
1. Clavulanate
2. Amoxicillin/Clavulanate
3. Sulbactam
4. Anitbiotics
5. Tazobactam
6. Pip/Tazo

- Bactericidal
- Inhibit cell wall synthesis by blocking transpeptidase (Penicillin binding protein or PBP) activity.
- Weak antibacterial activity
- Potent inhibitors of some β-lactamases

Beta-Lactam Spectrum of Activity

**Active**
- Aerobic and anaerobic Gram positive cocci and bacilli
- Aerobic and anaerobic Gram negative bacilli
- Beta-Lactamase inhibitors used to inhibit beta-lactamases produced by anaerobes
- Other organisms:
  - Pasteurella
  - Streptobacillus
  - Treponema pallidum
  - Erysipelothrix

**Inactive**
- Carbapenemase producing Gram negative bacilli
- Ampicillin resistant Enterococci
- Mycoplasma
- Stenotrophomonas maltophilia
- MRSA

*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.

Back to Basics: Other Cell Wall Acting Antibiotics

**Glycopeptides**
1. Vancomycin
2. Teicoplanin

**Lipopeptides**
1. Daptomycin

**Polymyxins**
1. Colistin

- Inhibits assembly of peptidoglycan precursors (murine) — Slowly bactericidal
- Binds to cell membrane and disrupts bacterial cell membrane potential — Rapidly bactericidal
- Penetrate and disrupt cell membranes — Rapidly bactericidal

Spectrum of Activity

**Active**
- Glycopeptides and Lipopeptides
  - Gram positive cocci and bacilli
- Polymyxins
  - Gram negative

**Inactive**
- Glycopeptides and Lipopeptides
  - Gram negatives
- Polymyxins
  - Gram positives
  - Anaerobes
  - Burkholderia
  - Proteus
  - Serratia
  - Providencia

*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.

Major Changes for CLSI

**Enterobacteriaceae**
1. Revised ertapenem DD and MIC interps.
2. Revised ciprofloxacin DD and MIC interps for S. typhi and extraintestinal Salmonella spp.
3. Added guidance for labs which have not adopted new carbapenem breakpoints.

**Pseudomonas aeruginosa**
1. Revised pip, pip/tazo, ticar, ticar/clav DD and MIC interps.
2. Revised/new carbapenem breakpoints

*Revised ciprofloxacin DD and MIC interps for S. typhi and extraintestinal Salmonella spp.*

*Added guidance for labs which have not adopted new carbapenem breakpoints.*
Back to Basics: Protein Synthesis Inhibitors

Oxazolidinones
- Linezolid

Tetracyclines and Chloramphenicol
- Doxy, mino, tetracycline
- Chloramphenicol

Back to Basics: Protein Synthesis Inhibitors

ML5b Antibiotics
- Macrolides
  - Erythromycin, Clarithromycin
  - Azithromycin
- Lincosamides
  - Clindamycin
- Streptogramins
  - Synercid

Spectrum of Activity

Active*
Linezolid
- Gram positives
- Mycobacteria
- Nocardia
- Aerobic and anaerobic Gram positives
- Spirochetes
- Rickettsiae, chlamydiae, mycoplasmas and protozoans

Inactive
- Gram negatives
- Mycoplasmas and ureaplasmas

*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.

Back to Basics: Protein Synthesis Inhibitors

Macrolides
- Aerobic and anaerobic Gram positives and Gram negatives
- Treponemes, mycoplasmas, Chlamydia and rickettsiae
- Aerobic and anaerobic Gram positives
- Some Gram negatives

Lincosamides
- Enterobacteriaceae
- Streptogramins
- Enterococcus faecalis
- Enterobacteriaceae
- Pseudomonas aeruginosa
- Acinetobacter

Aminoglycoside Spectrum of Activity

Active*
- Aerobic Gram positives and Gram negatives
- Mycobacteria

Inactive
- Anaerobes
- MRSA
- S. pneumoniae
- Stenotrophomonas maltophilia
- Burkholderia cepacia

*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.
Back to Basics: DNA Synthesis Inhibitors
Quinolones and Fluorquinolones
• Numerous...
  — Nalidixic acid, Ciprofloxacin, Levofloxacin, Gatifloxacin and Moxifloxacin

Quinolone Spectrum of Activity
Active*
• Aerobic Gram negatives and Gram positives
• Anaerobic Gram negatives and Gram positives
• Mycobacteria
• Mycoplasma, Chlamydia and Treponema

Inactive
• Not considered optimal treatment for anaerobic infection.

*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.

Back to Basics: RNA Synthesis Inhibitors
Rifamycins
— Rifampicin (rifampin), rifabutin, rifapentine

Rifamycin Spectrum of Activity
Active*
• Mycobacterium tuberculosis
• Non-tuberculous mycobacteria
• Gram positive cocci
• Gram negative bacilli
• Some anaerobes
• Neisseria meningitidis (oropharyngeal clearance)
• Other organisms
  — Chlamydia
  — Bartonella
  — E. coli
  — Chlamydia
  — Brucella

Inactive
*In many cases susceptibility testing necessary to confirm activity of specific class of beta-lactam against individual isolates.

Things to know and not test...

<table>
<thead>
<tr>
<th>Organism</th>
<th>Agents that Must not be reported as susceptible</th>
<th>Location in CLSI M100-S21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>1st and 2nd generation cephalosporins, cephamycins and aminoglycosides</td>
<td>Table 2A, Page 42</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>1st and 2nd generation cephalosporins, cephamycins and aminoglycosides</td>
<td>Table 2A, Page 42</td>
</tr>
<tr>
<td>MRSA</td>
<td>Penicillins, beta-lactam/beta-lactamase inhibitor combinations, cephalosporins** and carbapenems</td>
<td>Table 2C, Page 68</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>Aminoglycosides (except high concentrations), cephalosporins, clindamycin and trim/sulfa</td>
<td>Table 2D, Page 84</td>
</tr>
</tbody>
</table>

** Newer “fifth” generation cephalosporins (ceftobiprole and ceftaroline) have activity against MRSA.

Other interesting intrinsic resistance characteristics

<table>
<thead>
<tr>
<th>Vancomycin Resistant Gram positives</th>
<th>Vancomycin Susceptible Gram negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecium</td>
<td>Chryseobacterium</td>
</tr>
<tr>
<td>• Not intrinsically resistant but high percentage are VRE</td>
<td>Sphingomonas</td>
</tr>
<tr>
<td>Erysipelothrix</td>
<td>Elizabethkingia</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td></td>
</tr>
<tr>
<td>Pedicoccus</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
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WARNING: These antimicrobial agent/organism combinations may appear active in vitro but are not effective clinically and should be reported as susceptible.
Other important intrinsic resistance characteristics

**GRAM NEGATIVES**

- *Stenotrophomonas maltophilia* (metallo-beta-lactamase)
  - All beta-lactam antibiotics (except aztreonam)
- *Klebsiella pneumoniae* (class A beta-lactamase)
  - Ampicillin
- *Proteus mirabilis*
  - Nitrofurantoin
- *Proteus vulgaris, Proteus penneri and Morganella*
  - Piperacillin, ampicillin, cefoperazone (3rd), cefuroxime (2nd), cefazolin (1st)
- *Pseudomonas aeruginosa*
  - Bactrim
- *Burkholderia cepacia, Pandorea, Ralstonia pickettii, GC, Neisseria meningitidis, Moraxella catarrhalis, Brucella, Proteus, Providencia and Serratia*
  - Colistin
- *Aeromonas*
  - Ampicillin
- *Achromobacter spp.*
  - Aminoglycosides

Questions?

If you want to sign up...

www.pathologyquestions.com
Appendix: For you information

- Good resources
- Videos of antibiotic mechanisms
- Summary of clinical uses of each antibiotic
- Summary of treatments for common diseases

Good resources

- Medical letter
- Sanford Guide
- CLSI document - M100 - Appendix
- Manual of Clinical Microbiology
  - "Antimicrobial Susceptibilities" sections
- Clinical Microbiology Practices Handbook
  - Section 5 – procedure 5.16
- [http://labtestsonline.org](http://labtestsonline.org)

Beta-lactam Video

[http://www.youtube.com/watch_popup?v=qBdYnRhdWcQ&vq=medium](http://www.youtube.com/watch_popup?v=qBdYnRhdWcQ&vq=medium)

Macrolide Video

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Quinolone Video

Common Clinical Indications for Use

**Azithromycin**
- Legionella
- Chlamydia
- Acute otitis media
- Acute exacerbations of chronic bronchitis
- CAP in low MDR risk patients
- Pertussis
- Mumps in children
- Immunomodulating agent in CF

**Linezolid**
- MRSA
  - Skin and soft tissue infection
  - Pneumonia

**Clindamycin** (not exhaustive)
- S. aureus infections (MRSA and MSSA)
- Skin and connective tissue infections
- Bites, acne, cellulitis, necrotizing fasciitis, toxic shock syndrome
- Bone and joint infections
- Bacterial pneumonias
- Abcesses
- Skin, tissue, and wound, stasis and endocarditis
- Toxic shock syndromes infections caused by Gram positives

**Doxycycline**
- Tick borne disease
  - Ehrlichia, Lyme disease, Typhus, RMSF
- Bacterial pneumonia
- Abscesses
- Brain abscesses
- Bone and joint infections
- Bacterial pneumonias
- Abcesses
- Skin, tissue, and wound, stasis and endocarditis
- Toxin producing infections caused by Gram positives

**Vancomycin**
- Empiric coverage for Gram positive infection
- MRSA
- Recurrent C. difficile infection

**Daptomycin**
- MRSA – non-respiratory
- MRSA – respiratory
- Hospital acquired ventilator associated pneumonia
- MRSA and mixed infections
- Can be nebulized for respiratory infections

**Rifampin**
- Haemophilus influenzae type B and N. meningititis contact prophylaxis
- TB, MAI and other mycobacteria
- Combination therapy
  - Serious staphylococcal infection
  - Brucella infections
  - Bartonella infection
  - Coxiella infection

**Aminoglycosides**
- Synergy with beta-lactam
  - Viridans group streptococci (Gentamicin)
  - Listeria (Gentamicin)
  - Pseudomonas aeruginosa (Pip/Tazo)
  - Enterococcus spp. (high level)
- Tularemia, plague and Brucella
- Complicated UTI
- Serious Gram negative infections

**Cefazolin and Ceftazidime:**
- MSSA infection
  - Rifampin rarely used but predicts susceptibility to oral 1st gen cephs

**Cefotaxime and Ceftriaxone**
- Empiric treatment for meningitis
  - Available added to streptomycin in certain scenarios
- Pneumococcal infection
- N. gonorrhoeae and meningitidis
- Serious Gram negative infection

**Ceftazidime**
- Empiric treatment of fever and neutropenia
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- Serious, resistant Gram negative infections

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Glossary I and II

- Class, subclass, generic name
- Abbreviations / PO, IM, IV