Primer 02

Antibacterial Resistance Primer

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DISCLOSURES

Financial Relationships with Relevant Commercial Interests

• Becton Dickinson Diagnostics – Employee and stockholder/ownership interest
Antimicrobial Susceptibility Tests

• Standardized, reproducible methods for assessing antibiotic activity

• Variety of test methods for “routine” tests have been developed including manual and automated methods that can provide results either rapidly or after overnight incubation.

• Guidelines for performing the tests are defined in the Clinical and Laboratory Standards Institute (CLSI) documents.

• Specialized tests developed for specific applications
  – Beta-lactamase tests (e.g., nitrocephin test; ESBL test, modified Hodge test)
  – Methicillin resistance in Staphylococcus (mecA test)
  – Inducible clindamycin resistance (D test)
CLSI Documents - Examples

- **M2** – Performance Standards for Antimicrobial Disk Susceptibility Tests
- **M7** – Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically
- **M11** – Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria
- **M24** – Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes
- **M27** – Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts
- **M33** - Antiviral Susceptibility Testing: Herpes Simplex Virus
- **M44** – Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts
- **M100** – Performance Standards for Antimicrobial Susceptibility Testing
Antimicrobial Susceptibility Tests

• Two general forms of susceptibility tests are used:
  • Antibiotic dilution in agar (rarely used today) or broth
  • Antibiotic diffusion in agar

• Agar and broth dilution
  • Serial dilutions of antibiotics are added to either broth or agar and then inoculated with the test organism.
  • After incubation for a defined time, the minimum concentration of the antibiotic than inhibits the test organism (MIC) is determined.

• Agar diffusion
  • Antibiotics in a paper disk (e.g. Kirby-Bauer) or strip (E test) is placed onto a lawn of bacteria.
  • After overnight incubation, the size of the area of inhibited bacterial growth is correlated to antibiotic susceptibility or resistance.

• Results of dilution and diffusion tests are directly related.
Broth Dilution Tests

- Broth dilution tests are most commonly performed in microtiter trays. In this example, 8 antibiotics (A-H) are serially diluted. Growth in the lowest antibiotic concentrations is indicated by turbidity (arrow); inhibition of growth by clear wells.

- The endpoint (MIC) is influenced by:
  - Susceptibility test medium
  - Concentration of the test organisms
  - Stability of the antibiotic
  - Incubation conditions (time, temperature, atmosphere)
  - Susceptibility of the organism to the antibiotic

- If all variables are controlled, then the MIC = susceptibility
Kirby-Bauer Disk Diffusion

• A suspension of the test organism is spread over the agar surface and then antibiotic disks are placed on the plate. Areas of inhibited growth form after overnight incubation.

• The size of the zone of inhibited growth is influenced by:
  – Susceptibility test medium
  – Concentration of the test organisms
  – Incubation conditions
  – Rate of growth of the test organism
  – Concentration of the antibiotic in the disk
  – Diffusion of the antibiotic in the agar
  – Susceptibility of the organism to the antibiotic

• Interpretive standards are developed for each antibiotic correlating the size of the zone of inhibition with MIC values when the test is performed under standardized conditions.
E Test

• Essentially an agar diffusion test

• Varying concentrations of an antibiotic are spotted on the commercially prepared strip.

• Diffusion of the antibiotics on a lawn of bacteria establishes a gradient of concentrations; progressively increasing circles of inhibited growth form around each antibiotic spot on the strip merging into an elliptical pattern.

• Point where zone of inhibited growth intersects with the strip is calibrated to correspond to the MIC.
“Automated” Susceptibility Tests

• Commercial companies have automated broth dilution susceptibility tests, combining identification tests with antimicrobial susceptibility tests.
  – BD Phoenix ID/AST system
  – Siemens MicroScan ID/AST system
  – Vitek 2 ID/AST system

• The tests systems, based on broth dilution tests, are widely used, generally accurate, and frequently can provide results rapidly (6-8 hr for many organism-antibiotic combinations)

• One compromise – in order to provide testing of a wide spectrum of antibiotics, only a limited number of antibiotic concentrations are tested.
Antibiotic Resistance: Mechanisms, Susceptibility Patterns, and In Vitro Tests

• Bacterial resistance to specific groups of antibiotics can either be innate or acquired; additionally, expression of resistance can be constitutive (always expressed) or inducible.

• An understanding of resistance mechanisms is important for predicting resistance in bacteria species as well as selecting the appropriate test for determining resistance.

• Mechanisms of resistance, as well as susceptibility patterns and tests, will be presented for the following classes of antibiotics:
  β-lactams     Glycopeptides     Aminoglycosides
  Macrolides    Clindamycin       Tetracyclines
  Linezolid     Quinolones        Lipopeptides
  Streptogramins Trimethoprim-Sulfamethoxazole
Beta-Lactam Antibiotics
Mechanisms of Resistance

• Penicillin-binding proteins (PBP) are bacterial enzymes that are used to build the bacterial cell wall; these are the targets of β-lactam antibiotics.

• Resistance to beta-lactam antibiotics is mediated by 3 general mechanisms:
  – Modification of the antibiotic target
    • PBP over-expression
    • Acquisition of foreign PBP
    • Mutation of PBP
  – Decreased concentration of intracellular antibiotic (permeability barrier or increased efflux); primarily in gram-negative bacteria
  – Enzymatic destruction of the antibiotic – beta-lactamases

• Resistance mediated by beta-lactamases is the most common mechanism.
  – Hundreds of β-lactamases are described with a variety of activities (penicillinases, cephalosporinases, carbapenemases)
  – The most common classification system for β-lactamases is the Ambler system (Classes A-D)
## Beta-Lactamase Summary

<table>
<thead>
<tr>
<th>β-lactamase class</th>
<th>Examples</th>
<th>Encodes resistance to:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Staph penicillinase</td>
<td>Penicillin, ampicillin, amoxicillin</td>
</tr>
<tr>
<td></td>
<td>TEM-1, SHV-2</td>
<td>Penicillin, ampicillin, amoxicillin, 1&lt;sup&gt;st&lt;/sup&gt; gen. cephalosporins</td>
</tr>
<tr>
<td></td>
<td>ESBLs</td>
<td>All penicillins, cephalosporins, aztreonam</td>
</tr>
<tr>
<td></td>
<td>Carbapenemases</td>
<td>All penicillins, cephalosporins, β-lactams/β-lactamase inhibitors, carbapenems, aztreonam</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Metallo-β-lactamases (carbapenemases)</td>
<td>All penicillins, cephalosporins, β-lactam/β-lactamase inhibitors, carbapenems</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>AmpC β-lactamases</td>
<td>All penicillins, cephalosporins, β-lactam/β-lactamase inhibitors, aztreonam</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>OXA carbapenemases</td>
<td>All penicillins, cephalosporins, β-lactam/β-lactamase inhibitors, carbapenems</td>
</tr>
</tbody>
</table>

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Class A Beta-Lactamases

- Staphylococcal penicillinases – active against penicillin; inhibited by β-lactamase inhibitors (e.g., clavulanate)

- Two important β-lactamases found in Enterobacteriaceae are TEM-1 and SHV-1; these are penicillinases with limited cephalosporinase activity.

- Point mutations in the TEM-1 and SHV-1 genes created extended spectrum β-lactamases (ESBLs) with activity against all penicillins, cephalosporins, and aztreonam. Carbapenems and β-lactam/β-lactamases inhibitors remain active.

- Non-TEM, non-SHV ESBLs (CTX-M enzymes) are now more common and are important because they are widely distributed in community-acquired E. coli.

- Carbapenemases (KPC is the most common) also exist in this class with activity against carbapenems and all other β-lactams.
Class A Beta-Lactamases
Staphylococcal β-lactamases

• Encode resistance to penicillin, ampicillin, amoxicillin, and related drugs.

• Organisms producing these enzymes are susceptible to oxacillin, cephalosporins, carbapenems, and β-lactam/β-lactamase inhibitors.

• Found in:
  – Staphylococcus species
  – Neisseria gonorrhoeae
Class A Beta-Lactamases
Extended Spectrum β-Lactamases (ESBL)

- ESBL-producing bacteria (e.g., E. coli, Klebsiella, Proteus) are resistant in vivo to all penicillins, cephalosporins, and aztreonam; may appear susceptible to one or more of these antibiotics in vitro; susceptible to carbapenems and β-lactamase inhibitors.

- Reduced susceptibility to 3rd generation cephalosporins is used as a screening test.

- Confirmation is demonstrated by inactivation of the ESBL by β-lactamase inhibitors. That is, the combination of a cephalosporin + inhibitor is more active (large area of inhibited growth) that the cephalosporin alone. Compare CAZ-CLA (ceftazidime + clavulanate) with CAZ (blue arrows) and CTX-CLA (ceftriaxone +clavulanate) with CTX (green arrows).
Class A Beta-Lactamases

Carbapenemases

• Plasmid: KPC (widely disseminated – K. pneumoniae, Enterobacter, Citrobacter, Serratia, Salmonella, P. aeruginosa); GES (in P. aeruginosa and K. pneumoniae)

• Chromosomal: SME (Serratia); NMC and IMI (Enterobacter)

• KPC (Klebsiella pneumoniae carbapenemase) is the most important carbapenemase in this class; encodes resistance to all penicillins, β-lactam/β-lactamase inhibitors, cephalosporins, carbapenems, and aztreonam

• MICs to carbapenems elevated (1-4 μg/ml) but may not be “resistant” in vitro so not reliably detected by some conventional susceptibility tests

• Detected using Modified Hodge Test
Carbapenemase Test  
Modified Hodge Test

• A lawn of a carbapenem-susceptible bacteria is streaked on a Mueller-Hinton agar plate and a carbapenem (e.g. imipenem) disk is placed in the center. A zone of inhibited growth will develop around the disk.
• The test organism is streaked from the disk to the periphery.
• If the test organism produces carbapenemase, then the imipenem will be inactivated and the indicator organism will grow up to the disk (left; red arrows).
• If the organism does not produce carbapenemase, the indicator organism will be inhibited (right).
Class B Beta-Lactamases
Carbapenemases

• Metallo-β-lactamase; requires zinc for activity

• Many enzymes but New Delhi metallo-β-lactamase (NDM) is the most important carbapenemase in this class.

• Chromosomal: enzymes occur naturally in Stenotrophomonas, Aeromonas, Chryseobacterium

• Plasmid: Transferred between species and genera; found in Klebsiella, Serratia, Escherichia, Citrobacter, Enterobacter, Pseudomonas, Acinetobacter

• Not reliably detected by conventional susceptibility tests

• Detected using combination disk testing
Class B Carbapenemases
Combination Disk Test

• No single test can accurately detect these enzymes so a combination of disk tests is recommended.

• Double Disk Synergy test:
  – Inoculate the test organism on a Mueller-Hinton agar plate (uniform lawn) and place two disks on the plate – imipenem with and without EDTA.
  – After overnight growth the EDTA should bind zinc in the medium that is required for the enzyme to be active; therefore, the zone of inhibited growth will be larger around the imipenem + EDTA disk.

• Aztreonam Disk test:
  – Class B carbapenemases do not inhibit aztreonam activity; therefore, the organism will be susceptible to aztreonam.
Class C Beta-Lactamases

• Produced by almost all gram-negative bacteria; on chromosomes and transferable plasmids.

• Chromosomal class C β-lactamases are particularly important in Enterobacter, Citrobacter, Morganella, Serratia, P. aeruginosa; inducible enzymes so organisms initially look susceptible by in vitro tests but rapidly become resistant clinically.

• Bacteria producing class C β-lactamases are resistant to penicillins, cephalosporins, β-lactams/β-lactamase inhibitors and aztreonam; susceptible to carbapenems.

• First class C β-lactamase described was plasmid-encoded AmpC cephalosporinase in Citrobacter; now widely disseminated among gram-negative bacteria.
Class C Cephalosporinases

• AmpC cephalosporinases are most commonly found in Enterobacter, Citrobacter, and Serratia.

• Bacteria producing class C β-lactamases are resistant to penicillins, cephalosporins, β-lactams/β-lactamase inhibitors and aztreonam; susceptible to carbapenems. These bacteria may initially appear susceptible to 3rd generation cephalosporins but rapidly develop resistance during treatment.

• Testing isolates recovered over time is recommended to detect the development of resistant strains.
Class D Beta-Lactamases

• OXA-type (oxacillin hydrolyzing) β-lactamases found in Enterobacteriaceae, P. aeruginosa, and Acinetobacter.

• Genes frequently on mobile elements (plasmids) so spread rapidly.

• Class D β-lactamases only weakly inhibited by clavulanate so resistant to β-lactam/β-lactamase inhibitors.

• Some ESBLs are OXA β-lactamases so not detected by conventional ESBL test (because they are not inhibited by β-lactamase inhibitors).

• OXA carbapenemases found particularly in Acinetobacter baumannii. Conventional susceptibility tests may not reliably detect these enzymes.
Class D Beta-Lactamases
Carbapenemases

• OXA-type β-lactamases (preferentially hydrolyze oxacillin)

• OXA carbapenemases found in Acinetobacter baumannii

• Present on plasmids or chromosomes

• Encodes resistance to all penicillins, β-lactams/β-lactamase inhibitors, cephalosporins, carbapenems and aztreonam

• No screening test is available; MICs elevated so most are detected by conventional susceptibility test methods; definitive identification of these enzymes by multiplex PCR tests
Laboratory Tests for Beta-Lactamases

• There is a large variety of beta-lactamases, with a wide spectrum of activities, some of which is inducible and not easily detected by traditional susceptibility methods.

• Screening tests with antibiotic supplemented agars (e.g., carbapenem supplemented agar used for detected carbapenem-resistant bacteria in rectal specimens) are sensitive but require 2-3 days before results are available.

• Molecular tests (e.g., PCR tests) are rapid but must be highly multiplexed to detect the most common enzymes which are rapidly evolving.
Staphylococcus
Penicillin Resistance

• >90% of staphylococci produce penicillinase. “Penicillinase-negative” strains may have an inducible enzyme so penicillin-susceptible strains should be confirmed by a β-lactamase test (e.g., nitrocefin test).

• Nitrocefin is a chromogenic cephalosporin that after exposure to penicillinase it changes from yellow to red/pink.

• The test should be performed with inoculum exposed to subinhibitory concentrations of penicillin or cefoxitin and incubated for 1 hour before considered negative.
  – No color change = penicillinase negative = penicillin susceptible
  – Development of pink or red color = penicillinase positive = penicillin resistant
**Staphylococcus**

**Methicillin Resistant**

- Methicillin-resistance in staphylococci is not mediated by β-lactamases; rather, the penicillin binding protein (PBP) target of methicillin and related penicillinase-stable penicillins (oxacillin, nafcillin, cloxacillin, dicloxacillin) is altered.

- Staphylococci resistant to these antibiotics are referred to as “methicillin-resistant” or “oxacillin-resistant”.

- Staphylococci that are methicillin-resistant may appear susceptible to other β-lactams (penicillins, cephalosporins, carbapenems, β-lactam/β-lactamase inhibitors) but these drugs are not effective clinically.
Staphylococcus Testing Recommendations

• Methicillin resistance in staphylococci is mediated by the mecA or mecC gene products.

• Rare strains of staphylococci may be methicillin-resistant by another mechanism; mecA/C negative organisms with oxacillin MICs $> 4$ ug/ml should be reported as resistant (note: oxacillin is tested rather than methicillin because it is more stable).

• The most accurate methods for detecting methicillin resistance is use of screening agars containing oxacillin or PCR tests designed to detect mecA and mecC.
Staphylococcus Methicillin Resistance Testing

- Oxacillin or cefoxitin can be used to screen staphylococci for methicillin resistance.

- *S. aureus, S. lugdunensis:*
  - Oxacillin MIC > 4 μg/ml = resistant to oxacillin
  - Cefoxitin MIC > 8 μg/ml = resistant to oxacillin

- *Other coagulase-negative staphylococci:*
  - Oxacillin MIC > 0.5 μg/ml = resistant to oxacillin
  - Cefoxitin MIC screen test is not reliable

- For serious infections, susceptibility to oxacillin by these screening tests should be confirmed using a test to detect the mecA and mecC genes.
Detection of mecA and mecC genes

• Commercial assays based on PCR amplification and detection of the mecA/C genes are available.

• Results are generally available in 1-2 hours and the assays are highly sensitive for the claimed targets and specific.

• Commercial platforms make these assays easy to perform in all clinical labs.
Staphylococcus Test Report Recommendations

- Penicillin-susceptible staphylococci are susceptible to all penicillins, cephalosporins, carbapenems, and β-lactam/β-lactamase inhibitors.

- Penicillin-resistant, methicillin-susceptible strains are resistant to penicillinase-labile drugs (e.g., penicillin, ampicillin, amoxicillin, piperacillin, ticarcillin) but susceptible to other β-lactams.

- Methicillin-resistant strains are resistant to all β-lactams.

- Therefore, staphylococcal susceptibility results for all β-lactams can be predicted by testing penicillin and oxacillin (used as a substitute for methicillin).
Streptococcus pneumoniae Susceptibility Testing

• B-lactam resistance is mediated by acquisition of new PBP.

• Reliable disk diffusion tests do not exist for β-lactam antibiotics.

• Penicillin resistance can be screened by disk diffusion using an oxacillin disk (1 μg):
  – Organisms with oxacillin zones > 20 mm are considered susceptible
  – Organisms with oxacillin zones < 20 mm need to be tested by MIC test to confirm resistance
  – Penicillin susceptible organisms can be considered susceptible to amoxicillin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftriaxone, and ertapenem.

• Differential interpretive standards exist for some β-lactams for isolates from CSF and nonmeningitis isolates:

<table>
<thead>
<tr>
<th>Patient</th>
<th>MIC (μg/ml) for Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Penicillin</td>
</tr>
<tr>
<td>Meningitis</td>
<td>&gt; 0.12</td>
</tr>
<tr>
<td>Nonmeningitis</td>
<td>&gt; 8</td>
</tr>
</tbody>
</table>
Glycopeptides
Susceptibility Patterns and Resistance

• Glycopeptides (vancomycin, teicoplanin) inhibit cell wall synthesis.

• Innate resistance is mediated by:
  – These large antibiotic molecules cannot penetrate through the pores in the outer membrane of gram-negative bacteria
  – Absence of target on peptidoglycan cross-linking pentapeptide (Lactobacillus, Leuconostoc, Pediococcus, some Enterococcus species)
Glycopeptides
Susceptibility Patterns and Resistance (cont.)

• Acquired resistance is mediated by:
  – Altered target in Enterococcus –
    • 7 forms of resistance are reported with VanA and VanB the most important
    • VanA enterococci are resistant to vancomycin and teichoplanin
    • VanB enterococci are resistant to vancomycin but appear susceptible to teichoplanin; however, use of teichoplanin induces resistance so glycopeptides should not be used.

  – Resistance in staphylococci
    • Low level resistance (glycopeptide intermediate S. aureus, GISA) acquired by changes in cell wall and PBP4
    • High level resistance through acquisition of VanA from Enterococcus (uncommon)
Enterococcus
Vancomycin Testing

• Agar screening test
  – Spot 10^6 CFU onto a BHI agar plate supplemented with vancomycin (6 μg/ml) and incubate for 24 hr.
  – Any growth is considered presumptive vancomycin resistance
  – Confirm with a MIC test; perform motility test and determine pigment production.

• Non-motile, nonpigmented enterococci with elevated vancomycin MICs (generally ≥ 32 μg/ml) have VanA or VanB mediated resistance (epidemiologically significant).

• Intermediate vancomycin susceptibility (MIC 8-16 μg/ml) is generally mediated by VanC and found in E. gallinarum (motile, nonpigmented) or E. casselilavus (motile, yellow).
Staphylococcus
Vancomycin Resistance

• MIC tests must be performed to determine staphylococcal susceptibility to vancomycin; disk testing is not reliable.
  – S. aureus: susceptible MIC ≤ 2 μg/ml; resistant MIC ≥ 16 μg/ml
  – Coag. neg. staph: susc. MIC ≤ 4 μg/ml; res. MIC ≥ 32 μg/ml

• Screening tests to detect vancomycin-resistant S. aureus:
  – Inoculate S. aureus on a brain-heart infusion agar plate supplemented with 6 μg/ml vancomycin
  – Growth on the plate after overnight incubation = presumed reduced susceptibility to vancomycin
  – Confirmed with MIC tests
Aminoglycosides
Susceptibility Patterns and Resistance

• Decreased uptake and increased efflux
  – Positively charged aminoglycosides bind to negatively charged bacterial cell and then are taken into the cell by an oxygen-dependent step.
  – Some organisms (e.g., multidrug resistant Acinetobacter) actively pump aminoglycosides out of cell.
  – Anaerobes and enterococci are intrinsically resistant (low level resistance); however, enterococci susceptible to a high level of aminoglycosides can be treated with the combination of an aminoglycoside and cell wall active antibiotic.

• Modification of ribosomal target
  – Alterations of ribosomal proteins or methylation of rRNA confers high level resistance.

• Aminoglycoside-modifying enzymes
  – Most common form of resistance; found in gram-positive and gram-negative bacteria; confers high level resistance
Enterococcus
Aminoglycoside Screening Test

• Gentamicin test
  – Disk diffusion screening test: if there is no zone of inhibited growth around a high content gentamicin disk (120 μg disk), consider the organism resistant to all aminoglycosides except streptomycin.
  – Broth test: growth of the test organism in BHI broth supplemented with gentamicin (500 μg/ml) = resistance

• Streptomycin test
  – Disk diffusion screening test: if there is no zone of inhibited growth around a high content streptomycin disk (300 μg disk), consider the organism resistant to streptomycin.
  – Broth test: growth of the test organism in BHI broth supplemented with streptomycin (1000 μg/ml) = resistance

• Enterococci with high level resistance by these tests will not have synergistic activity when combined with cell wall antibiotics.
Macrolides and Clindamycin
Susceptibility Patterns and Resistance

• Macrolides (erythromycin, clarithromycin, azithromycin, roxithromycin) inhibit protein synthesis.

• Most gram-negative bacteria are resistant because macrolide entry into the cell is restricted.

• Methylation of ribosome results in macrolide resistance, as well as clindamycin resistance.
  – Resistance may be expressed constitutively (macrolides and clindamycin appear resistant) or be inducible (clindamycin may appear falsely susceptible by in vitro tests).

• Macrolide resistance (but not clindamycin) can also result from active efflux.
Inducible Clindamycin Resistance (D Test)

• Erythromycin (a macrolide antibiotic) and clindamycin (a lincosamide) represent distinct classes of antimicrobial agents that inhibit protein synthesis.

• Macrolide resistance due to alteration in the ribosomal target results in resistance to both macrolides and clindamycin. This resistance can be either constitutive or inducible.
  – If resistance is constitutive, then both erythromycin and clindamycin will appear resistant by the standard tests.
  – If the resistance is inducible, then erythromycin will appear resistant but clindamycin will look falsely susceptible.

• Macrolide resistance due to active efflux may not result in clindamycin resistance.

• The D test is used to detect inducible clindamycin resistance.
Inducible Clindamycin Resistance (D Test)

• The test organism is spread uniformly over the surface of the agar plate; an erythromycin and a clindamycin disk is placed on the plate. Incubate overnight.

• Flattening of the zone of inhibited growth between the disks = inducible clindamycin resistance (top photo).

• If erythromycin does not influence the zone around the clindamycin disk = clindamycin susceptible (lower photo).
Tetracyclines

Susceptibility Patterns and Resistance

• Four tetracyclines are used clinically: tetracycline, minocycline, doxycycline, and tigecycline.

• These drugs are broadly active, interfering with protein synthesis in gram-positive and gram-negative bacteria.

• Bacteria that are intermediate or resistant to tetracycline may be susceptible to doxycycline and/or minocycline.

• Tigecycline is the most active tetracycline, active against tetracycline-resistant MRSA, penicillin-resistant S. pneumoniae, vancomycin-resistant enterococci, and most Enterobacteriaceae, Acinetobacter, Stenotrophomonas and rapidly growing mycobacteria. Providencia, Proteus and Pseudomonas aeruginosa are generally resistant.

• Resistance to the tetracyclines is mediated by:
  – Efflux – many different membrane bound efflux proteins are described; most confer resistance to tetracycline but not minocycline
  – Ribosomal protection – these proteins bind to ribosome and interfere with binding by the tetracyclines.
Linezolid
Susceptibility Patterns and Resistance

• Linezolid is the only member of the family oxazolidinone antibiotics that is used clinically.

• Linezolid is active against gram-positive bacteria including methicillin-resistant staphylococci and vancomycin-resistant enterococci.

• This family of antibiotics is ineffective against gram-negative bacteria because they cannot pass through the outer membrane.

• Linezolid inhibits proteins synthesis in gram-positive bacteria.

• Resistance in gram-positive bacteria is mediated by point mutations in 23S rRNA gene.
  – Described in S. aureus and E. faecium
  – Typically associated with prolonged use of linezolid although resistance has increased so hospital- and community-acquired infections in linezolid naïve patients is reported.
Quinolones
Susceptibility Patterns and Resistance

• The quinolones are a large class of broad spectrum antibiotics with activity against many gram-positive and gram-negative bacteria, Chlamydia, Mycoplasma, and some mycobacteria. MRSA and enterococci are frequently resistant to these antibiotics.

• Quinolones act by inhibiting DNA synthesis by targeting DNA topoisomerase.

• The most common mechanism of resistance is alterations in the topoisomerases. Multiple mutations result in broad spectrum, high-level resistance.

• Resistance can be due to decreased intracellular accumulation of drug either through a permeability barrier or more commonly active efflux of the antibiotic.
Lipopeptide Antibiotics
Susceptibility Patterns and Resistance

• Three lipopeptide antibiotics are used today: colistin (polymyxin E), polymyxin B, and daptomycin.

• The bactericidal activity of the polymyxins is caused by binding to the cytoplasmic membrane of gram-negative bacteria and disrupting permeability. They bind poorly to the membrane in gram-positive bacteria. In general, polymyxins are bactericidal against Pseudomonas aeruginosa, Acinetobacter, Proteus mirabilis, and most Enterobacteriaceae except Proteus, Providencia, and Serratia.

• In contrast, daptomycin is inactive against gram-negative bacteria because it cannot penetrate through the outer membrane. It is active against gram-positive bacteria including methicillin-resistant staphylococci, penicillin-resistant Streptococcus pneumoniae, and vancomycin-resistant enterococci.

• Acquired resistance is mediated by changes in the bacterial membranes.
Streptogramins
Susceptibility Patterns and Resistance

• Quinupristin-dalfopristin (marketed as Synercid) is a member of the antibiotic family, the streptogramins.

• This drug has excellent activity against Staphylococcus aureus (including MRSA) and Enterococcus faecium (including VRE). Enterococcus faecalis is innately resistant.

• The streptogramins inhibit protein synthesis by binding to the 50S ribosomal subunit (dalfopristin) and inducing a conformational change to allow quinupristin to bind, leading to premature release of the peptide chains.

• Acquire resistance is uncommon, most commonly caused by enzymatic modification of the antibiotic molecules.
Trimethoprim-Sulfamethoxazole Susceptibility Patterns and Resistance

• This antibiotic inhibits tetrahydrofolate synthesis that is required for synthesis of some amino acids and purines.

• Intrinsic resistance is caused by a decrease in the intracellular concentration (P. aeruginosa by both permeability barrier and efflux) or the ability to utilize exogenous folate or thymine (Enterococcus, Lactobacillus). These bacteria may appear falsely susceptible by in vitro tests.

• Acquired resistance due to overproduction of target enzymes (primarily dihydrofolate reductase [DHFR]) or modification of DHFR gene that prevents binding of antibiotic. Observed in a number of bacteria.
Susceptibility Patterns

• Because antimicrobial susceptibility results may not be available for 2 or more days after a culture is collected, it is important to know the susceptibility patterns that are seen in a particular hospital setting. Clinical microbiology are required to publish summaries of these results at least annually.

• Although most organisms have predictable patterns of antibiotic susceptibility, variations occur. It is important to recognize when a pattern is unusual and needs to be confirmed by additional testing.

• In some cases, in vitro susceptibility results do not reflect in vivo response to antibiotic treatment for specific organisms. Most commercial susceptibility test systems have “expert” computer programs to recognize these testing errors; however, an infectious disease expert and microbiologist must be able to recognize these discrepancies.
# Unusual Susceptibility Patterns

<table>
<thead>
<tr>
<th>Organism/Group</th>
<th>Unusual Phenotypes</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>Carbapenem – I or R</td>
<td>Determine if carbapenemase producer</td>
</tr>
<tr>
<td>Citrobacter freundii, Enterobacter, Serratia</td>
<td>Ampicillin, cefazolin, or cephalothin – S</td>
<td>Confirm ID/susceptibility; convert to R</td>
</tr>
<tr>
<td>Klebsiella, Salmonella, Proteus vulgaris</td>
<td>Ampicillin – S</td>
<td>Confirm ID/susc; convert to R</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>Carbapenem – S</td>
<td>Confirm ID/susc; convert to R</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>Ampicillin – S</td>
<td>Confirm ID/susc; convert to R</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>Linezolid – R</td>
<td>Confirm ID/susc.</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Ampicillin, linezolid – R</td>
<td>Confirm ID/susc.</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>Linezolid – R; vancomycin – I or R</td>
<td>Confirm ID/susc.</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>Vancomycin – R</td>
<td>Confirm ID/susc.</td>
</tr>
</tbody>
</table>
Erroneous In Vitro Susceptibility Patterns

Some organism-antibiotic combinations may appear active in vitro but are not effective clinically. They should not be reported as susceptible.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antimicrobials that should be reported as not susceptible:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL-producing Klebsiella, Escherichia coli, Proteus mirabilis</td>
<td>Penicillins, cephalosporins, aztreonam</td>
</tr>
<tr>
<td>Salmonella, Shigella</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt; generation cephalosporins, cephamycins, aminoglycosides</td>
</tr>
<tr>
<td>Oxacillin-resistant Staphylococcus spp.</td>
<td>Penicillins, β-lactams/β-lactamase inhibitors, cephalosporins, carbapenems</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>Aminoglycosides (except high conc), cephalosporins, clindamycin, trimethoprim-sulfamethoxazole</td>
</tr>
</tbody>
</table>
Susceptibility Tests and Patterns: Anaerobic Bacteria

• CLSI established standardized susceptibility test methods for anaerobes although these are rarely performed in clinical labs. These include:
  – Agar dilution
  – Broth microdilution
  – E tests

• The lack of testing is because most anaerobes have predictable susceptibility patterns. For example,

<table>
<thead>
<tr>
<th>Always active</th>
<th>Usually active</th>
<th>Variable resistance</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenems</td>
<td>Clindamycins</td>
<td>Penicillin</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>β-lactam/β-</td>
<td>Cephamycins</td>
<td>Cephalosporins</td>
<td>Aztreonam</td>
</tr>
<tr>
<td>lactamase</td>
<td>Tigecycline</td>
<td>Tetracycline</td>
<td>Trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>inhibitors</td>
<td>Metronidazole</td>
<td>Vancomycin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macrolides</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluoroquinolones</td>
<td></td>
</tr>
</tbody>
</table>
Susceptibility Tests and Patterns: Mycobacteria

- CLSI established standardized susceptibility test methods for mycobacteria and related bacteria including:
  - Agar proportion method – a variation of agar dilution testing; if >1% of the inoculum is resistant to a specific concentration of an antibiotic, the organism is considered resistant.
  - Broth dilution testing – growth of mycobacteria in an antibiotic-containing medium is assessed by an automated detection system.
  - Detection of antimicrobial resistance genes – primarily used in reference labs.

- The identification of the mycobacterial species determines which antibiotics may be useful for testing. For example:
  - M. tuberculosis complex – primary drugs: isoniazid, rifampin, ethambutol, pyrazinamide; if resistant then test secondary drugs: ethionamide, fluoroquinolones, amikacin, kanamycin.
  - M. avium complex – clarithromycin, azithromycin.
  - M. kansasii – rifampin, ethambutol, amikacin, clarithromycin; if resistant, test ciprofloxacin and trimethoprim-sulfamethoxazole.
  - Rapidly-growing mycobacteria – amikacin, tobramycin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, sulfamethoxazole.