ANTIBIOTIC RESISTANCE
LauraLe Dyner MD
Pediatric Infectious Disease Fellow
June 2009
Antibiotic Resistance

- Relative or complete lack of effect of an antimicrobial agent
- An organism is able to grow in readily achievable serum concentrations of the antibiotic in question
- Increase in MIC/Decrease in disk diffusion
Minimum Inhibitory Concentration (MIC)

- Lowest concentration of antimicrobial that inhibits the growth of the organism after an 18 to 24 hour incubation period
- Interpreted in relation to the specific antibiotic and achievable drug levels
- Can not compare MICs between different antibiotics
- Discrepancies between in vitro and in vivo
MIC/Disk Diffusion

Antibiotic susceptibility tests

Minimum inhibitory concentration test

Disk diffusion test

Susceptible organism

A

B

Resistant organism

\( \mu g/ml \) antibiotic

10 \( \mu g \) antibiotic in discs
Antibiotic Resistance

- Intrinsic resistance
  - Occurs naturally in all or most strains of that species
  - Chromosomally encoded
  - Gram-negatives are resistant to Vancomycin

- Acquired resistance
  - Results from a mutation in the existing DNA of an organism or acquisition of new DNA
CLASSIFICATION OF ANTIBIOTICS

- AMINOGLYCOSIDES
  - Gentamycin

- B-LACTAMS
  - PENICILLINS
  - CARBAPENEMS
    - Imipenem
    - Meropenem
  - MACROLIDES
    - Erythromycin
    - Clarithromycin
    - Azithromycin

- GLYCOPEPTIDES
  - Teicoplanin
  - Vancomycin

- CEPHALOSPORINS
  - 1st – 4th Generation

- FLUOROQUINOLONES
  - Ciprofloxacin
  - Levofloxacin
  - Nalidixic Acid

- LINCOSAMIDES
  - Clindamycin

- OXAZOLIDINONES
  - Linezolid

- MISCELLANEOUS
  - Metronidazole
  - Tetracyclines
Timeline of Antibiotic Resistance

Antibiotic deployment:
- Sulfonamides
- Penicillin
- Chloramphenicol
- Streptomycin
- Tetracycline
- Vancomycin
- Ampicillin
- Methicillin
- Cephalosporins
- Erythromycin

Antibiotic resistance observed:
- 1930
- 1935
- 1940
- 1945
- 1950
- 1955
- 1960
- 1965
- 1970
- 1975
- 1980
- 1985
- 1990
- 1995
- 2000
- 2005
- Linezolid
- Daptomycin
Factors That Promote Resistance

- Exposure to sub-optimal levels of antimicrobials
- Exposure to broad-spectrum antibiotics
- Exposure to microbes carrying resistant genes
- Lack of hygiene in clinical environments
- Use of antibiotics in foods/agriculture
Inappropriate Antimicrobial Use

- Prescriptions not taken for a total duration of therapy
- Antibiotics for viral infections
- Antibiotics sold without medical supervision
CDC Estimates

Unnecessary Antibiotic Prescriptions

- Ear Infection: 30% (23m)
- Common Cold: 100% (18m)
- Bronchitis: 80% (16m)
- Sore Throat: 50% (13m)
- Sinusitus: 50% (13m)

Totals prescribed per year (in millions)
Consequences of Increased Resistance

- Infections that are difficult to treat
- Increased mortality
- Increased cost of therapy
  - MDR-TB
  - Cost per tablet
    - PCN $0.24
    - Linezolid $86.90
    - 360 x as much

Genetic Basis of Resistance

- Chromosomal
  - Mutation
  - Chromosomally mediated inducible enzymes

- Plasmid
  - Most common genetic basis of resistance
  - Genetic determinants can spread laterally through a population without cell division
  - Interspecies lateral transfer of plasmids
  - Resistance usually involves antibiotic inactivating enzymes (many encoded by transposons)
Selection of Resistant Bacteria

1. Population of Dividing Microbes

2. Process of growth and division produces naturally occurring mutants

3. Microbial population continues to multiply, occasionally giving rise to more mutants

4. Microbes are killed or prevented from growing by antimicrobial except for specific, resistant mutants

5. Mutants continue to grow and divide in the presence of the antimicrobial and begin to spread throughout the environment

'Selective Pressure'

Microbes are Exposed to Antimicrobial Compound
Mechanisms of Resistance

- Enzymatic Inactivation**
- Decreased permeability
- Efflux
- Alteration of target site
- Overproduction of target
ANTIMICROBIAL RESISTANCE IN THE ICU

(1) Decreased Permeability
- Permeability barrier
- Porin channels

(2) Inactivating Enzymes
- Beta-lactamases
- Aminoglycoside modifying enzymes
- Esterases and acetyltransferases

(3) Altered Target Sites
- Penicillin-binding proteins
- DNA gyrase
- Ribosomes
- RNA polymerase and DHFR

(4) Active Efflux
- Outer cell envelope
- Cytoplasmic membrane
Decreased Permeability

- **Outer membrane permeability**
  - Gram-negative bacteria
  - Impedes the entry of hydrophobic antibiotics (nafcillin/erythromycin)
  - Porins
    - Loss of porins leads to increased resistance to β-lactams

- **Inner membrane permeability**
  - Altered proton motive force leading to aminoglycoside-resistance after long term use
Efflux

- Tetracyclines
  - Energy-dependent transporter system
- Fluoroquinolones
- Chloramphenicol
- Macrolides
  - *mef* gene (macrolide efflux)
  - *S pneumoniae, S aureus, S epidermidis*
Target Sites

Bacterial Targets for Current Antibiotics Used in the Clinic

- **Cell wall synthesis**
  - 1955: Vancomycin, Teichoplanin, Bacitracin, Penicillins
  - 1962: Cephalosporins
  - 1985: Monobactams, Carbapenems

- **Folic acid metabolism**
  - 1962: Trimethoprim, Sulfonamides

- **DNA Gyrase**
  - Quinolones

- **DNA-directed RNA polymerase**

- **Protein synthesis (50S inhibitors)**
  - 1959: Rifampin
  - 1950: Erythromycin (Macrolides), Chlormphenicol, Clindamycin
  - 1948: Tetracyclines, Spectinomycin, Streptomycin, Gentamicin, Tobramycin (aminoglycosides)

- **Protein synthesis (30S Inhibitors)**
  - 1947: Chloramphenicol Transacetylase

- **Cell Membrane**
  - Polymyxins
Altered target sites

- Cell wall
  - PBPs: *S pneumo*, *N meningitidis*, MRSA
  - D-Ala-D-Ala target: VRE
    - VanA, VanB, VanC, VanD

- Cell membrane changes

- Ribosomal alterations
  - Tetracyclines, macrolides, lincosamides, aminoglycosides
  - Failure of the antibiotic to bind to the ribosome disrupts the ability to inhibit protein synthesis
Penicillin Binding Proteins (PBP)

- More common resistance mechanism for gram-positive organisms
  - Gram-negative access to PBPs is limited due to the outer membrane
- Target for all $\beta$-lactams
- Found as membrane-bound & cytoplasmic proteins
- Involved in the final stages of peptidoglycan synthesis of cell walls
Inactivating enzymes

- β-lactamases**
- Aminoglycoside modifying enzymes
- Chloramphenicol acetyltransferase
- Macrolide, Lincosamide, & Streptogramin inactivating enzymes
- Tetracycline inactivation
  - *tetX* enzyme
  - Although most tetracycline resistance is mediated by efflux & ribosomal protection
β-lactamases

- Large, diverse family of enzymes
- Wide range of activity
  - ESBLs
  - AmpC β-lactamases
  - Carbapenemases
- Widely dispersed
  - Gram-positive
  - Gram-negative
    - Major mechanism of resistance in gram-negatives
  - Anaerobes
β-lactam Mechanism of Action

- β-lactam antibiotic binds to PBP
- Inhibition of peptidoglycan synthesis
  - Inhibits synthesis of cross-links
- Cell death
**β-lactamases**

- Split the amide bond of the β-lactam ring
β-lactamases: Structural Classes

- **Class A**
  - Plasmid mediated
  - Serine residue at the active site
  - Preferentially hydrolyze penicillins

- **Class B**
  - Chromosomal
  - Metalloenzymes; zinc-binding thiol group
  - Hydrolyze carbapenems, penicillins, & cephalosporins

- **Class C**
  - Chromosomal
  - AmpC
  - Mainly active against cephalosporins

- **Class D**
  - Plasmid mediated
  - Oxacillin-hydrolyzing enzymes
<table>
<thead>
<tr>
<th>Classification</th>
<th>β-Lactamases</th>
<th>Amino Acid</th>
<th>Examples</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambler class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Penicillinases</td>
<td>Serine</td>
<td>TEM-1, SHV, KPC, CTX-M, SME-1</td>
<td>Clavulanate</td>
</tr>
<tr>
<td>B</td>
<td>Metallo-β-lactamases</td>
<td>Zinc</td>
<td>IMP-1, VIM-1</td>
<td>EDTA</td>
</tr>
<tr>
<td>C</td>
<td>Cephalosporinases</td>
<td>Serine</td>
<td>AmpC</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinases</td>
<td>Serine</td>
<td>OXA-1</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Bush-Jacoby-Medeiros group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cephalosporinases</td>
<td></td>
<td>AmpC</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>Penicillinase</td>
<td></td>
<td>PCI</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>Broad-spectrum penicillinases</td>
<td></td>
<td>TEM-1, SHV-1</td>
<td></td>
</tr>
<tr>
<td>2be</td>
<td>Extended-spectrum β-lactamases</td>
<td></td>
<td>TEM-10, SHV-2, CTX-M-type</td>
<td></td>
</tr>
<tr>
<td>2br</td>
<td>Inhibitor resistant</td>
<td></td>
<td>TEMs, IRTs, TEM-30,31</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>Carbenicillin hydrolyzing</td>
<td></td>
<td>PSE-1</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>Oxacillin hydrolyzing</td>
<td></td>
<td>OXA-1 to 11, PSE-2</td>
<td></td>
</tr>
<tr>
<td>2e</td>
<td>Cephalosporinases</td>
<td></td>
<td>FEC-1</td>
<td></td>
</tr>
<tr>
<td>2f</td>
<td>Carbapenemases</td>
<td></td>
<td>KPC-1, KPC-2, SME-1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Metallo-β-lactamases</td>
<td></td>
<td>IMP-1, VIM-1, SPM-1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EDTA = ethylenediaminetetraacetic acid.
<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>Examples</th>
<th>Substrates</th>
<th>Inhibition by Clavulanic Acid*</th>
<th>Molecular Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad-spectrum</td>
<td>TEM-1, TEM-2, SHV-1</td>
<td>Benzylpenicillin (penicillin G), amoxicillin, clavulanate, cefoxitin, cephalosporins (cefotaxime, ceftazidime, and ceftriaxone)</td>
<td>+++</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA family</td>
<td></td>
<td>Substrates of the broad-spectrum group plus doxacillin, methicillin, and oxacillin</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>Expanded-spectrum</td>
<td>TEM family and SHV family</td>
<td>Substrates of the broad-spectrum group plus oximino-cephalosporins (cefotaxime, cefoxime, cefsulodin, and ceftriaxone)</td>
<td>++++</td>
<td>A</td>
</tr>
<tr>
<td>Others (BES-1, GES/IBC family, PER-1, PER-2, SFO-1, TLA-1, VEB-1, and VEB-2)</td>
<td>Same as for TEM family and SHV family</td>
<td></td>
<td>++++</td>
<td>A</td>
</tr>
<tr>
<td>CTX-M family</td>
<td></td>
<td>Substrates of the expanded-spectrum group plus, for some enzymes, ceftime</td>
<td>+++</td>
<td>A</td>
</tr>
<tr>
<td>OXA family</td>
<td></td>
<td>Same as for CTX-M family</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>AmpC</td>
<td>ACC-1, ACT-1, CFE-1, CMY family, DHA-1, DHA-2, FOX family, LAT family, MIR-1, MOX-1, and MOX-2</td>
<td>Substrates of expanded-spectrum group plus cephamycins (ceftotaxime, ceftoxitin, and others)</td>
<td>0</td>
<td>C</td>
</tr>
<tr>
<td>Carbapenemase</td>
<td>IMP family, VIM family, GIM-1, and SPM-1</td>
<td>Substrates of the expanded-spectrum group plus cephamycins and carbapenems (ertapenem, imipenem, and meropenem)</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPC-1, KPC-2, and KPC-3</td>
<td>Same as for IMP family, VIM family, GIM-1, and SPM-1</td>
<td></td>
<td>+++</td>
<td>A</td>
</tr>
<tr>
<td>OXA-23, OXA-24, OXA-25, OXA-26, OXA-27, OXA-40, and OXA-48</td>
<td>Same as for IMP family, VIM family, GIM-1, and SPM-1</td>
<td></td>
<td>+</td>
<td>D</td>
</tr>
</tbody>
</table>

* Plus signs denote relative sensitivity to inhibition.
AmpC

- Confers resistance to
  - Cephamycins (cefotetan, cefoxitin)
  - Oxyimino-\(\beta\)-lactams (ceftriaxone, cefotaxime, ceftazidime)

- Chromosomal in SPACE organisms & are inducible

- Plasmid mediated in other gram-negatives & usually not inducible

- Not inhibited by \(\beta\)-lactamase inhibitors
AmpC
Carbapenemases

- Can hydrolyze penicillins, cephalosporins, monobactams, & carbapenems.

Groups

- Metallo- β-lactamases (MBLs)
  - Confer a high level of resistance
  - *Pseudomonas, Acinetobacter, Enterobacter*

- Serine- β-lactamases
  - Oxacillinases or D β-lactamases (OxaA)
    - Not as diverse
    - *Acinetobacter*
    - Another resistance is usually necessary to raise the MIC

- Class A Carbapenemases
  - *Pseudomonas & Enterobacter*
  - *Klebsiella*: predominant type (plasmid)
ESBL Mediated Resistance

- Contain a number of mutations that allow them to hydrolyze expanded-spectrum \( \beta \)-lactam antibiotics

- Derived from older antibiotic-hydrolyzing \( \beta \)-lactamase enzymes (TEM-1, TEM-2, SHV-1)
  - A single amino acid substitution can give rise to new ESBLs
  - Not as catalytically efficient
  - Inhibited by \( \beta \)-lactamase inhibitors
  - Susceptible to cefoxitin and cefotetan *in vitro* only

- 10%–40% of *K. pneumoniae, E coli* express ESBLs
Distinction between AmpC & ESBL

- The AmpC β-Lactamases are encoded by genes located on chromosomes
  - Often inducible
  - Commonly found in *Enterobacter* sp, *Citrobacter freundii*, *Morganella morganii*, *Serratia marcescens*, and *Pseudomonas aeruginosa*.
  - The genes are not easily transferable to other bacterial species
- ESBLs are encoded by genes located on plasmids, resulting in easy transfer to other bacterial species.
- AmpC β-Lactamases are weakly inhibited by β-Lactamase inhibitors (clavulanic acid) and usually confer resistance to cephapycins
- In contrast, ESBLs are generally well inhibited by β-Lactamase inhibitors and usually retain sensitivity to the cephapycins (in vitro)
# Resistance Against Specific Antibiotics

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Mode of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beta-lactams</strong></td>
<td>β-lactamases&lt;br&gt;Alteration of PBPs</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td>Enzyme modification of the drug - common&lt;br&gt;Ineffective transport&lt;br&gt;Altered ribosomal binding site (30S) – rare</td>
</tr>
<tr>
<td><strong>Macrolides</strong></td>
<td>Decreased permeability of cell wall to drug&lt;br&gt;Alteration in the 50S ribosomal binding site&lt;br&gt;Inactivation by enzymatic hydrolysis (esterase)&lt;br&gt;Efflux pump</td>
</tr>
<tr>
<td><strong>Sulfonamides</strong></td>
<td>Overproduction of PABA</td>
</tr>
<tr>
<td><strong>Trimethoprim</strong></td>
<td>Plasmid mediated dehydrofolate reductases</td>
</tr>
<tr>
<td><strong>Vancomycin</strong></td>
<td>Low frequency of resistance</td>
</tr>
</tbody>
</table>
## Resistance Against Specific Antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cephalosporins</strong></td>
<td>Extended Spectrum Beta Lactamases (ESBL)</td>
</tr>
<tr>
<td></td>
<td>Chromosomal cephalosporinases</td>
</tr>
<tr>
<td><strong>β-Lactamase Inhibitors</strong></td>
<td>Hyperproducers of β-lactamases</td>
</tr>
<tr>
<td></td>
<td>New β-lactamases resistant to inhibitors</td>
</tr>
<tr>
<td></td>
<td>Chromosomal cephalosporinases</td>
</tr>
<tr>
<td><strong>Carbapenems</strong></td>
<td>Porin mutations</td>
</tr>
<tr>
<td></td>
<td>Efflux pump overproduction (excluding Imipenem)</td>
</tr>
<tr>
<td></td>
<td>Zinc Metalloenzymes and other β-lactamases</td>
</tr>
<tr>
<td><strong>Fluoroquinolones</strong></td>
<td>Decreased number of porins in target cells</td>
</tr>
<tr>
<td></td>
<td>Efflux mechanisms</td>
</tr>
<tr>
<td></td>
<td>Mutations in DNA gyrase &amp; topoisomerase</td>
</tr>
</tbody>
</table>
Specific Organisms

- Staphylococcus aureus
  - MRSA
  - VISA
  - VRSA
- Group A (beta-hemolytic) Strep
- Streptococcus pneumoniae
- Enterococcus
  - VRE
- “SPACE” (Serratia, Pseudomonas, Proteus, Acinetobacter, Citrobacter, Enterobacter)
Staph Aureus

- Produce $\beta$-lactamases
  - Preferentially hydrolyze penicillins
  - Encoded for on bacterial plasmids or transposons
- At LPCH approximately 18% of *S. aureus* is susceptible to penicillin or ampicillin
- Methicillin & nafcillin are resistant to penicillinase
- Resistance has increased in recent years
Methicillin Resistant Staph Aureus (MRSA)

- **1959:** Methicillin introduced
- **1961:** MRSA discovered in England
- **1968:** MRSA found in the US (Boston)
- **1974:** 2% of hospital staph infections
- **1981:** MRSA acquired in the community
- **2002:** Community & hospital acquired infections are found to be genetically different
- **2007:** In the US: 94,000 severe infections/yr & 19,000 deaths/yr
MRSA

- Most frequent nosocomial resistant pathogen
- Acquired via MecA
- Chromosomal mutation leading to the synthesis of a new penicillin binding protein
- The protein is inducible & confers resistance to all beta lactam antibiotics
MecA

- Encodes for PBP 2a
  - Weak affinity for methicillin & all β-lactams
- Speculation that the origin was from *coagulase-negative Staph* or *E coli*
- Mobile chromosomal element called staphylococcal cassette chromosome (SCCmec)
  - SCCmec types I, II, and III
    - Multidrug resistant
    - Large cassettes
    - Health-care associated
  - SCCmec type IV and type V
    - Not multidrug resistant
    - Community associated
Clindamycin Resistance: D-test

- Resistance to macrolides (e.g. erythromycin) can occur by two different mechanisms

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Gene</th>
<th>Erythro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinda Efflux</td>
<td>msrA</td>
<td>R</td>
</tr>
<tr>
<td>Ribosome Alt.</td>
<td>erm</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>erm</td>
<td>R</td>
</tr>
</tbody>
</table>
Clindamycin Resistance: D-test
VISA/VRSA

- **VISA:** Vancomycin Intermediate Staph Aureus
  - MIC 4-8 ug/ml
- **VRSA:** Vancomycin Resistant Staph Aureus
  - MIC $\geq$ 16 ug/ml

- Glycopeptide resistance
Group A Strep

- 100% susceptible to penicillin & other beta lactam antibiotics
- Some are resistant to macrolides
  - In a European surveillance, it has been found to be as high as 1/3 resistant
Streptococcus pneumoniae

- Resistance to penicillin due to stepwise mutations in penicillin binding proteins (PBP)
  - Chromosomally mediated
  - Decreased affinity to PBP
  - Can be overcome with high-dose penicillin
  - Alterations in PBPs decrease susceptibility of the organism to other beta lactam antibiotics

- Macrolides
  - Genetic changes to binding target on ribosome-high level can not be overcome = erm(B)
  - Efflux pump-lower level-may be overcome = mef (A)

- Vancomycin is used to treat penicillin & cephalosporin resistant infections
Enterococcus

- Intrinsic resistance
  - Chromosomal
  - Less susceptible to penicillin & ampicillin than other streptococci (MIC 2-8 ug/ml) [by 10-1000 x]
  - Low level of resistance to aminoglycosides
  - Synergy between a cell wall agent & an aminoglycoside

- Acquired resistance
  - Beta lactamase production
  - High level of resistance to aminoglycosides
    - Lose synergy ability as well
  - Glycopeptide resistance (Vancomycin)
Vancomycin Resistant Enterococci (VRE)

- First reported in 1988
- Origins
  - Normal fecal flora
  - Food chain
  - Selection by use of Vancomycin, Cephalosporins, Anaerobic Antibiotics
  - Person-to-person spread
- Produce a new membrane protein to inhibit binding of vancomycin to D-alanyl-D alanine terminus
- Lack of synergy with aminoglycosides
Vancomycin Resistant Enterococci (VRE)

- Vancomycin-susceptible enterococci make cell-wall precursors that have high affinity for vancomycin.
- Vancomycin-resistant enterococci, in the presence of vancomycin, make cell-wall precursors that have low affinity for vancomycin.

Inhibition of cell-wall synthesis
Vancomycin-resistant *Enterococcus* in US hospital intensive care

“SPACE” Organisms

- Gram-negative bacteria have a chromosomal gene for the $\beta$-lactamase, ampC
- SPACE organisms can undergo single-step mutations to constitutive, high-level enzyme production
- If enough ampC beta-lactamase is produced, resistance occurs
Conclusion

- Combat Antibiotic Resistance!
- Narrow antibiotic coverage when possible
- Restrict antimicrobial use
  - At LPCH: Linezolid, Micafungin, Caspofungin
- Track resistance patterns
- Direct Observed Therapy (TB)
Resources

- Centers for Disease Control
- Johns Hopkins Antibiotic Guide
- UpToDate 2009
- Nature
- The 2006 American Academy of Pediatrics Redbook
- Mandell’s
- Prober, Long, & Pickering. Principles & Practice of Pediatric Infectious Disease, 3rd Edition