Overview of Bacterial Resistance

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Objectives
- State the 5 common methods used by bacteria to overcome antibiotic therapy & provide antibiotic-bacterial examples of each.
- Identify 2 natural advantages that bacteria possess in terms of survival.
- Identify 5 consequences of bacterial resistance.
- Define the terms plasmid, transposon, constitutive/induction, ESBL, influx, efflux, PBP, vertical/horizontal transmission, static vs cidal activity, & collateral damage

Overview
- Bacterial resistance
  - Resistance increases morbidity and mortality
  - Pathogens often resistant to multiple antibiotics
  - Results in increased length of stay in hospital and ICU
  - Increases the cost of care
  - Understanding mechanism of resistance helps identify appropriate antibiotic therapy

Antibiotic Behavior
- Bactericidal vs Bacteriostatic
- Concentration Dependent vs Independent
  - Speed & extent of bacterial kill
- Mitigating Circumstances
  - Bacterial concentration and actual bacterial burden
  - Mutational frequency
  - Single vs multiple mechanisms of action and resistance
  - Level of antibiotic exposure
    - Frequency, magnitude, and duration
    - Environment (pH, oxygen tension, etc)
    - Protein Binding
    - Blood vs site of infection concentration
    - Presence of foreign body or device

Bactericidal vs Bacteriostatic
- Definition
  - Bactericidal – Actual bacterial killing
    - MBC / MIC ≤ 4
  - Bacteriostatic – Inhibition of bacterial growth
    - MBC / MIC > 4
  - Tolerance
    - MBC / MIC ≥ 32
  - Optimal therapy for a particular pathogen does not equate to optimal therapy for all
    - Collateral damage resistance scenario
    - Resistance transfer from another vector (vanA to S. aureus)

The Bacterial Advantage
- The modern antibiotic era is ~ 60 years old while bacteria have been here for millennia
- Bacteria have the ability to adapt to the environment, bacteria are natural survivors
- Bacteria spontaneously mutate continuously looking for a selective advantage
- Bacteria have rapid growth rates & can rapidly replace the former generation.
Bacterial Resistance

Mechanisms of Resistance
- Influx-Block Porin Channel
- Efflux- Pump
- Enzyme Inactivation
- Target Alteration
- Metabolic Bypass
- Environment Factors

Mechanisms of Bacterial Resistance
- Influx Alterations
  - Porin channel modification
    - Beta-lactams
    - Fluoroquinolones
    - Aminoglycosides
    - Tetracyclines
  - Environmental changes
    - pH (ionized vs. unionized)
  - Facilitated transport
    - Anaerobic environment prevents transport (i.e., aminoglycosides)

Common Mechanisms of Bacterial Resistance
- Beta-lactamases
  - Enzyme is made up of aminoacids
  - Altering aminoacids sequence changes potency (ESBL)
  - Result of beta-lactamase
    - Open antibiotic beta-lactam ring
    - Regenerate enzyme
  - Several chromosomal and plasmid mediated enzymes
    - Richmond Sykes or Bush Type enzymes

Antibiotic Inactivation
- Chromosomes
  - Enzyme coding is intrinsic to bacteria
- Actual enzyme expression may be:
  - Constitutive- enzyme at constant level of production
  - Inducible- enzyme is only expressed when a specific agent (inducer) is present
  - Certain antibiotics cause bacteria to move from a repressed state into a derepressed state

Bacteria can rapidly replace the former generation
Bacteria naturally mutate or acquire resistance (plasmids)
Mechanisms of resistance are not mutually exclusive
Antibiotic Inactivation

- **Plasmids**
  - Extra chromosomal pieces of DNA
  - With gram negative bacteria, can code for 4 to 5 different bacterial enzymes
  - For gram positives can code for only a couple of bacterial enzymes
  - Externally introduced
  - Promote genetic diversity
  - TEM 1, TEM 2, & SHV 1 most commonly found plasmid mediated beta-lactam enzymes

ESBL

- Mutants of TEM 1, TEM 2, or SHV 1
- Vary by 1 to 4 amino acids substitutions
- Very potent enzymes
  - Hydrolyze 3rd generation cephalosporins & aztreonam
  - Carbapenems & cephamycins not affected
  - Very difficult to detect in laboratory
  - Commonly seen with E.coli & Klebsiella spp.
- ESBL positive strains likely resistant to other antibiotics

Klebsiella spp. ESBL

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM 1</td>
<td>0.125</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>TEM 5</td>
<td>4.0</td>
<td>128</td>
<td>8.0</td>
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<tr>
<td>TEM 12</td>
<td>0.06</td>
<td>4.0</td>
<td>0.25</td>
</tr>
<tr>
<td>SHV 1</td>
<td>0.125</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>SHV 2</td>
<td>64</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>SHV 4</td>
<td>128</td>
<td>128</td>
<td>256</td>
</tr>
</tbody>
</table>

Jacoby G AAC 35:1697-1704, 1991

ESBL

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<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM 1</td>
<td>&lt;0.12</td>
<td>Glu</td>
<td>Arg</td>
</tr>
<tr>
<td>TEM 12</td>
<td>4-32</td>
<td>Glu</td>
<td>Ser</td>
</tr>
<tr>
<td>TEM 10</td>
<td>64</td>
<td>Glu</td>
<td>Ser</td>
</tr>
<tr>
<td>TEM 26</td>
<td>&gt;256</td>
<td>Lys</td>
<td>Ser</td>
</tr>
</tbody>
</table>

Amino Acid Position

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>MIC(mg/L)</th>
<th>104</th>
<th>162</th>
<th>237</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM 1</td>
<td>&gt;0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEM 12</td>
<td>4-32</td>
<td></td>
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<td>TEM 26</td>
<td>&gt;256</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rice, L. Pharmacother 19:1205-1285,1999

ESBL Testing

- Test any gram negative isolate where MIC ≥ 2 mg/L for 3rd generation cephalosporin or aztreonam
  - Cefpodoxime ≥ 8 mg/L
- Test cefotaxime or ceftazidime alone and in combination with 4 mg/L clavulanate
  - Look for ≥3 tube dilution change in MIC
  - ESBL Etest strip
  - Vitek ESBL card
- Inoculum effect with ESBL positive organisms
- Previous attempts to treat ESBL outbreaks with imipenem have generated imipenem resistant species
  - Pseudomonas or Acinetobacter spp.

ESBL Zone of Influence

<table>
<thead>
<tr>
<th>Distance (miles)</th>
<th>Resistance %</th>
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<tbody>
<tr>
<td>0-10</td>
<td>37</td>
</tr>
<tr>
<td>10-20</td>
<td>23</td>
</tr>
<tr>
<td>20-80</td>
<td>9</td>
</tr>
<tr>
<td>80-160</td>
<td>3</td>
</tr>
<tr>
<td>&gt;160</td>
<td>5</td>
</tr>
</tbody>
</table>

Monnet D. Infecc Cont Hosp Epidemiol 18:492-498, 1997

Bacterial Resistance
Antibiotic Inactivation
- Can be spread vertically or horizontally among bacteria
  - Resistance conveyed by
    - Chromosomal mutation
    - Spontaneous natural phenomena
  - Transduction
    - Bacterial phage introduces new DNA into cell
  - Conjugation
    - Sexual transfer of genetic material

Antibiotic Inactivation
- Target alteration
  - Examples
    - Fluoroquinolones- DNA-gyrase
    - Macrolides- 50S ribosome
    - Aminoglycosides- 30S ribosome
    - Beta-lactams- alter penicillin binding proteins (PBP)

Antibiotic Inactivation
- Active efflux
  - Ability to pump antibiotic out of bacteria
  - Prevents a critical concentration of antibiotic from being established within the bacteria
  - Example
    - Fluoroquinolones
    - Tetracyclines

Antibiotic Inactivation
- Metabolic bypass
  - Antibiotic interacts with metabolic pathway & blocks critical step
  - Bacteria substitute an antidote that bypasses the metabolic block
  - Examples
    - TMP/SMX
    - Vancomycin

Vancomycin Resistance
- Glycopeptide unable to bind altered precursor
- Resistance requires 3–9 new genes to direct synthesis of altered wall precursor

Mechanisms of Bacterial Resistance
- Bacteria may possess multiple mechanisms of resistance simultaneously
- Mechanisms of resistance are not mutually exclusive

Bacterial Resistance
Bacterial Resistance

The Antibiotics We Use

- Overt and Covert Use of Antibiotics
  - Hospital vs. Nursing Home vs. Day Care vs. Community
  - Prophylaxis
  - Therapeutic
  - Inappropriate
  - Passed Down the Food Chain (Animal Feed)
- Overuse, misuse, and/or underdosing of antibiotic can precipitate bacterial resistance
- Direct and Collateral Damage
  - Cefazidime – ESBL
  - Cephalosporins, Vancomycin, Anaerobic Agents - VRE

Methods to Promote Optimal Utilization of Antibiotics

- Government & professional organizational programs
- Guidelines
- Antibiotic cycling or rotation
- Restricting antibiotic use
- Antibiotic order forms
- Ongoing Education
- Programs that track antibiotic usage & resistance

Antibiotic Control Program Induced Pseudo-outbreak of Infections

Calfee, D.P. Abstract # 19 IDSA 2000 New Orleans

- Number of nosocomial infections rose after introduction of antibiotic control program
- No specific pathogen identified
- Patient acuity index unchanged
- Mortality rate actually decreased
- Physicians changed diagnostic and prescribing practices to comply with antibiotic program
  - “Squeezing the balloon” syndrome

CAP Treatment Guidelines

- American Thoracic Society
  - First published 1993
  - Revision published Am J Respir Crit Care Med 163:1730-1754, 2001
- Infectious Diseases Society of America
  - First published April 1998
  - Revision published CID 31:347-382, 2000
- Center for Disease Control
  - Arch Intern Med 160:1399-1408, 2000
  - Promote macrolides, doxycycline, or beta-lactams
  - Fluoroquinolones suggested if: 1) Failed previous Tx, 2) Allergy to alternative antibiotics, & 3) PCN MIC > 4mg/L

Wide Overuse of Antibiotic Cited in Study WSJ (September 4, 1997)

- 7,147 medicare patients in 131 hospitals studied in 1995
- 63% of vancomycin orders did not follow CDC guidelines
- No difference in comparing large urban teaching centers to small rural hospitals
- Beth Israel-Deaconess - fliers & lectures did not alter prescribing behavior
Antibiotic Rotation in Neonatal ICU

- Monthly rotation of gentamicin, P/T, & ceftazidime vs unrestricted antibiotic use in side by side ICU’s for 1 yr
- Pharyngeal & rectal samples 3X/week
- PFGE for genetic discordance
- N= 1062 infants, 10.7% (cycled group) vs 7.7% (control group) colonized with resistant gram negative pathogen
- Incidence of nosocomial infection & mortality similar among the two groups
- Rotation of antibiotics had no detectable effect

Influences that Increase Antibiotic Prescribing
July 1998 Survey of all primary care MD’s in Massachusetts
APUA Newsletter Vol 19 2001

- 499 usable responses out of ~6,000 questionnaires (8%)
- 93% agreed that physicians over prescribe antibiotics

Factor | % MD's Factor influenced Rx
--- | ---
Purulent Discharge | 64%
Dx Uncertainty | 62%
Patient request | 55%
Patient satisfaction | 48%
Fever | 47%
Tx Uncertainty | 36%
Payer Policy/Summary | 26%
Time Pressure | 26%
Return visit cost | 20%
Litigation concern | 19%
Payer policy-QA | 13%
Drug promotion | 7%
Resistance concern | 5%
Cost | 2%

Ceftazidime Induced ESBL with Gram Negative Bacteria
Rice, LB Pharmacother 19: 120S-128S, 1999

- Overuse &/or underdosing of ceftazidime may precipitate an ESBL (TEM or SHV) problem with Klebsiella or Enterobacter
- Piperacillin/tazobactam substituted for ceftazidime but Amp-C like enzymes or overproduction of ESBL can inactivate tazobactam
- Treatment of these organisms with imipenem or fluoroquinolones may precipitate multi-resistant A. baumanni

Resistant A. baumanni in ICU
Weingarten, CM Pharmacother 19:1080-1085, 1999

<table>
<thead>
<tr>
<th>Infected</th>
<th>Match</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51 yrs</td>
<td>50 yrs</td>
</tr>
<tr>
<td>Apache II</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Mortality</td>
<td>34%</td>
<td>18%</td>
</tr>
<tr>
<td>LOS</td>
<td>51 days</td>
<td>19 days</td>
</tr>
<tr>
<td>Phar cost</td>
<td>$19K</td>
<td>$3K</td>
</tr>
<tr>
<td>Hosp cost</td>
<td>$128K</td>
<td>$31K</td>
</tr>
</tbody>
</table>

Cost of Nosocomial Infections
Roberts, R SHEA 2001

- Retrospective cohort study of patients at Cook County with hospital acquired infections (HAI-CDC criteria)
  - Randomly selected patients from 1998 discharge database with ≥5 ICD-9 diagnoses
  - Severity of illness measured using Apache III scores
  - Costs calculated from hospital perspective
- 193 (164 medical & 29 surgical patients (41/193 with HAI)
  - Medicine average cost of hospitalization with HAI $24,762 vs. $6,202 without
  - Surgical average cost of hospitalization with HAI $52,422 vs. $20,823 without
  - Confounding variable analysis showed suspected HAI added $13,236 (SE $3,134) & documented HAI added $18,223 (SE $2,225)
**Heptavalent Pneumococcal Vaccine**

Kaiser Permanente Trial

Abstract 1398, ICAAC, San Francisco, 1999

- Wyeth-Lederle Heptavalent CRM 197 given to infants 2,4,6,& 12-15 months
- 37,000 children randomly assigned pneumococcal or meningococcal vaccine
- Double Blind Trial
- October/95-July/98 vaccine efficacy 100%
  - Highly effective in preventing invasive disease & pneumonia
  - Significant impact on otitis media

Lieu, TA et al JAMA 283:1460, 2000

**Preventing Bacterial Resistance: Underdosing**


- Examined relationship between bacterial resistance, kinetics, & MIC in 4 LRTI trials
- N=107 patients, 128 pathogens, & 5 antibiotic regimens
- 32/128 (25%) initially susceptible pathogens developed resistance during therapy
- AUC/MIC ratio < 100 risk factor for developing resistance during therapy
- Underdosing would appear to be a significant risk factor for the development of resistance

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**Strategy**

<table>
<thead>
<tr>
<th>Prevention</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccination</td>
<td>Rapid &amp; Accurate Pathogen ID</td>
</tr>
<tr>
<td>Infection Control</td>
<td>Infection Control</td>
</tr>
<tr>
<td>Formulary Selection</td>
<td>Pharmacokinetic &amp;/or</td>
</tr>
<tr>
<td>Education</td>
<td>Pharmacodynamic dosing</td>
</tr>
<tr>
<td>Antibiotic Rotation</td>
<td>Practice Guidelines</td>
</tr>
<tr>
<td>Antibiotic Program</td>
<td>New Agents</td>
</tr>
</tbody>
</table>

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**Conclusions**

- **Goal:**
  - Promote appropriate use of antibiotics
  - Consider the potential of new antibiotic to induce bacterial resistance in formulary evaluations
  - Promote appropriate pharmacokinetic & pharmacodynamic concepts when dosing antibiotics
  - Develop programs to monitor use of antibiotics & resistance within the hospital & benchmark data
  - Communicate data among colleagues
  - While not primary function can help promote appropriate and aggressive use of infection control measures

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**Stuart Smalley Closing Thoughts**

- Remember:
  - Every time you use an antibiotic, you are conducting your own experiment in Darwinian theory!
  - Wash your hands, there are some bad bugs out there!