Antimicrobial Resistance

Consequences of Antimicrobial Resistant Bacteria

• Change in the approach to the administration of "empiric antimicrobial therapy"
• Increased number of hospitalizations
• Increased length of hospitalization
• Increased morbidity and mortality
  – Emergence of strains totally resistant to all available antimicrobials
• Choice of more expensive or more toxic therapeutic alternatives

Topics to be Covered

• Molecular genetics of antimicrobial resistance
• Mechanisms of antimicrobial resistance
• Dissemination of antimicrobial resistance genes
• Epidemiology of antimicrobial resistance
  – Antibiotic use in the community
  – Antibiotic use in agriculture
  – Antibiotic use in hospitals
• Measures to reduce the spread of antimicrobial resistance

Acquisition of Foreign DNA

• Horizontal gene transfer is common, even between Gram positive and negative bacteria
• Plasmid - transfer of single or multiple resistance genes
• Free DNA - acquisition of resistance genes by naturally transformable species e.g., pneumococcus, neisseria
• Bacteriophage - virus mediated transfer of genes

Molecular Genetics of Antimicrobial Resistance

• Micro evolutionary change - point mutations
  – Beta-lactamase mutation extends spectrum of the enzyme
  – rpoB gene (RNA polymerase) mutation alters rifampin binding site preventing activity
• Macro evolutionary change - rearrangements of segments of DNA
  – Transposons carrying antibiotic resistance genes
• Acquisition of foreign DNA - plasmids, phage etc.
  – Conjugative plasmids can transfer resistance genes between different species

• Cross resistance - often (but not always) a single resistance mechanism confers resistance to an entire class of antibiotics
  – Methicillin resistance confers resistance to all beta-lactams, penicillins and cephalosporins
• Cross resistance among different classes of antibiotics can occur as the result of:
  – Drug efflux pumps
  – Overlapping targets e.g. macrolides and lincosamides share overlapping targets on the ribosome that can be altered by methylation of an adenine residue

Levy, Scientific American
Molecular Genetics of Antimicrobial Resistance

- Co resistance refers to the presence of several resistance mechanisms in the same organism
- Co selection refers to the selection of multiple antibiotic resistance genes when one resistance mechanism is selected. Generally occurs if both genes are regulated by the same promoter
- Integrons are mobile genetic elements present in both Gram positives and negatives that mediate both co resistance and co selection

Enzyme Modifiers - Beta-lactamases

- Splits the amide bond hydrolyzing the β-lactam ring

Integrons

- Coordinately express genes under the control of a single promoter
- Represent “hot spots” for site-specific recombination allowing integration of nonhomologous sequences
- Gene transcription is correlated with the proximity of the gene to the promoter

Antibiotic Degrading Enzymes

β-lactamases

- Gram positive beta-lactamases
  - Primarily found in staphylococci
  - Excreted extracellularly
  - Usually plasmid-mediated, often packaged with other antimicrobial resistant determinants e.g., aminoglycosides
- Gram negative beta-lactamases
  - Large variety of different beta-lactamases carried by many Gram negative species with different spectrum of activity
  - Extended spectrum beta-lactamases - plasmid mediated, broad spectrum K. pneumoniae among the first to carry these ESBLs
  - Secreted into the periplasmic space
  - Can be chromosomal or plasmid
  - Single point mutation can change the substrate specificity

Mechanisms of Antimicrobial Resistance

- Enzymatic modification
  - Beta lactamases
- Decreased accumulation of antibiotic
  - Permeability barriers - outer membrane Gram negatives - PCNs
  - Porin mutations - carbapenems
  - Antibiotic efflux pumps - tetracyclines, macrolides
- Alteration of the drug target
  - Methicillin, vancomycin, macrolides

Alteration of the Drug Target Site Vancomycin

- Vancomycin-modifiable enzymes couple and inactivate drugs. bloodstream highly affinity for vancomycin
- Peptidoglycan-modifiable enzymes also can act as active vancomycin cell wall synthesis

Murray, NEJM '00
Alteration of the Drug Target Site

- Enterococcal resistance to vancomycin - different types but all involve synthesis of altered cell wall precursor side chain that doesn’t bind vancomycin e.g., D-Ala-D-Lactate vs. D-Ala-D-Ala
  - This mechanism may be plasmid-mediated
- Staphylococcal resistance to semisynthetic penicillins
  - Synthesis of a novel penicillin binding protein (2a) with reduced affinity for methicillin
  - Takes over role of other PBPs (which are methicillin susceptible) in cell wall synthesis

Antibiotic Efflux Pumps

- Originally designed to protect organism from toxic material therefore have broad substrate specificity
- There are a variety of different efflux systems used by bacteria although the majority use proton-motive force as the means for efflux
- They can pump out a wide variety of different molecules including tetracyclines, beta-lactams, detergents, macrolides and quinolones
- Transfer of this type of resistance is not easy because of the complex genetic machinery needed for the pump to be functional

Epidemiology of the Transmission of Antibiotic-Resistant Bacteria

Community Acquired Resistant Pathogens: Penicillin-Resistant Pneumococcus

- Historical perspective - 1st large scale outbreak in Durban South Africa - 1977
  - Associated with children < 5 years, measles complicated by pneumonia and antimicrobial therapy
  - Isolates also resistant to tetracycline, chloramphenicol
- Nature of the resistance
  - Decreased affinity for penicillin-binding proteins
  - PB P 2 b
  - Structurally altered cell wall

- Acquisition of resistance
  - Uptake of foreign DNA (e.g. S. mitis) by naturally competent S. pneumoniae
  - Recombination event leading to replacement of susceptible PBP with resistant one
- Clonal dissemination
  - Expansion of a limited number of clones (perhaps as a result of other associated virulence determinants)
  - Association of carriage with young children, crowded settings
  - International spread - vacations in hot spots, selective antibiotic pressure
International Spread of Resistant Clones of Pneumococcus

Antibiotics in Agriculture

<table>
<thead>
<tr>
<th>Subject</th>
<th>Antimicrobial (Pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>3,000,000</td>
</tr>
<tr>
<td>Beef*</td>
<td>3,700,000</td>
</tr>
<tr>
<td>Swine*</td>
<td>10,300,00</td>
</tr>
<tr>
<td>Chicken*</td>
<td>10,500,000</td>
</tr>
<tr>
<td><strong>Total in animals</strong></td>
<td><strong>24,500,000</strong></td>
</tr>
</tbody>
</table>

* Nontherapeutic uses only, 1990’s

Union of Concerned Scientists

Resistance to Pneumococcus and Prescribing Practices

Antibiotics in Agriculture

• Antimicrobials are routinely added to animal feed and water to promote animal growth
  – Rationale is to promote more rapid growth reducing farming expenses
  – Mechanisms are debated although most commonly invoked is the reduction of infections, especially in unsanitary conditions
• Many of the antibiotics used in this setting are of the same class as those used to treat human infections
  – Macrolides, tetracyclines, glycopeptides

The Example of Vancomycin Resistance

• In Europe, E. faecium resistance to vancomycin (VRE) described in 1986
• The use of the glycopeptide, avoparcin, in animal feed is believed to be responsible for the emergence of VRE
• Animal reservoir as a source of VRE is supported by epidemiologic data
  – In Denmark 24 kg of vanco used for humans vs. 24,000 kg for animals
  – VRE found in avoparcin fed animals (dead or alive!)
  – VRE types in animals and humans related by molecular typing
Antibiotics in Agriculture: Effect on Animals and Farmers

Changes in Intestinal Flora of Farm Personnel After Introduction of a Tetracycline-Supplemented Feed on a Farm

- Multiple resistance found in >50% E. coli in chickens receiving tetracycline >10 wks
- A similar observation was made in farm dwellers but not in neighbors over time

Antibiotics in Agriculture: Transmission to Humans

- Penicillin first introduced in the early 1940's followed shortly thereafter by the detection of penicillin resistance
  - Due to beta-lactamase
- Semisynthetic penicillins introduced in the late 1950's followed by the emergence of methicillin-resistance
  - Due to altered penicillin binding protein
  - Epidemic spread of MRSA clones worldwide
  - Vancomycin the sole bactericidal agent to treat these infections
- Emergence of MRSA with reduced susceptibility to vancomycin
  - Altered cell wall “sponge hypothesis”

First Clinical VRSA Reported in USA

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Infections</th>
<th>Percent of Infections Resistant to Methicillin</th>
<th>Percent of Methicillin Resistant Infections Sensitivities to Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>1000</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>10%</td>
<td>90%</td>
</tr>
<tr>
<td>2010</td>
<td>3000</td>
<td>20%</td>
<td>80%</td>
</tr>
</tbody>
</table>

Nosocomial Infections - Staphylococcus aureus

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Antimicrobial Resistance - S. aureus

Lowy, NEJM, '98

First Clinical VRSA Reported in USA

MMWR

Morbidity and Mortality Weekly Report


Staphylococcus aureus Resistant to Vancomycin — United States, 2000
Control of the Spread of Antimicrobial Resistance

- Eliminate the use of antimicrobials in animal feed
- Restrict use of antibiotics for inappropriate indications
  - Upper respiratory infections
- Antibiotic restriction in hospital settings
  - Antibiotic cycling?
- Enforce infection control policies
  - Handwashing etc.
  - Appropriate isolation procedures

So What Should You Know

- Molecular genetics of resistance - terminology
  - Integrons
- Mechanisms of antimicrobial resistance
- Mechanisms of dissemination of antimicrobial resistant strains
- Examples of resistance phenomena in the community, hospital setting
  - Including agricultural settings

Evolution of Antimicrobial Therapy in a Nutshell

Year 2000 a.d. “Here, eat this root.”
Year a.d. 1000 “That root is heaven,
Here, say this prayer.”
Year 1850 “The prayer is superstitious,
Here, drink this potion.”
Year 1520 “This potion is snake oil.
Here, swallow this pill.”
Year 1945 “That pill is ineffective,
Here, take this penicillin.”
Year 1953 “OOPS, bugs mutated.
Here, take this tetracycline.”
Years 1960-1999 Thirty-nine more OOPS’s.
“Here, take this more powerful antibiotic.”
Year 2000 The bugs have said: “Here, eat this root.”