Antibiotics & Resistance

What are antibiotics?

- Antibiotics are molecules that stop bacteria from growing or kill them
- Antibiotics, agents "against life"
  - either natural or synthetic chemicals
  - designed to block some crucial process in bacterial cells
- Why do we use antibiotics?
  - To treat bacterial infections
  - To prevent bacterial infections

Bacteriostatic vs Bactericidal

- **Bactericidal**
  - Rapidly kill the target bacteria
- **Bacteriostatic**
  - Inhibit the growth of bacteria

Spectrum

- **Narrow spectrum**: Some bacteria
  - Gram-positive
  - Gram-negative
- **Broad spectrum**: Both Gram-positive and Gram-negative

Growth vs Time

- Normal growth
- Bacteriostatic
- Bactericidal

Gram-positive?
Gram-negative?
Some bacteria?
**Minimum Inhibitory Concentration (MIC)**
- The minimum concentration of the drug necessary to inhibit the growth of bacteria

**Antibiotic Targets**
- Selective Toxicity
  - Antibiotics should be toxic for bacteria not for the host
  - Antibiotic targets should be present uniquely in bacteria

**Cell wall**: β-lactam antibiotics (penicillins, cephalosporins), glycopeptides (vancomycin)
**Membrane**: polypeptides (polymyxin)
**DNA gyrase**: quinolones, novobiocin
**DNA synthesis**: sulphonamides
**DNA structure**: metronidazole
**RNA-polymerase**: rifampin
**Protein synthesis**: 30S: aminoglycosides, tetracyclines, 50S: chloramphenicol, macrolides, lincosamides

**Inhibition of cell wall synthesis**: Peptidoglycan synthesis

- The gram-positive cell wall
- The gram-negative cell wall

**Nature Reviews Microbiology**
Inhibition of cell wall synthesis

● **β-lactams (Penicillins)**
  - The β-lactam ring is structurally similar to the substrate (D-alanyl-D-alanine) of transpeptidase enzymes

  → Penicillin binds to the transpeptidase
  (they are often referred to as penicillin binding protein (PBP))
  → Preventing the synthesis of peptidoglycan
  → Weakening the peptidoglycan
  → Bacterium bursts

● **Cephalosporins**
  - Action mechanism is similar to penicillins
  - Advantages:
    1. Resistance to penicillinase
    2. Not as allergenic as penicillin
    3. Broad spectrum of activity

Cell membrane inhibitors

● **Polymyxin**
  - Binds to the outer surface of the cell membranes and disrupt the structure
  - Active against Gram-negatives but limitedly used for topical applications

Inhibition of cell wall synthesis

● **Bacitracin**
  - A polypeptide isolated from *Bacillus subtilis* that interact with the bacterial cell wall

● **Vancomycin**
  - Prevent the synthesis of peptidoglycan
  - Used to treat serious staphylococcal infections in humans
  - Binds to D-Ala-D-Ala
**Targeting DNA/RNA**

- **Quinolones**
  - Inhibit DNA gyrase A and topoisomerase IV
  - Selectively block DNA synthesis
  - Totally synthetic antimicrobials

- **Novobiocin**
  - Inhibit DNA gyrase B

- **Metronidazole**
  - Its reduction by ferredoxin
  - Generates toxic free radicals
  - DNA damage

- **Rifampin**
  - Bind to DNA-dependent RNA polymerase of bacteria
  - Inhibit RNA synthesis

**Inhibition of growth by analogues**

- **Sulfonamides**
  - Bacteria require paraaminobenzoic acid (PABA) to form folic acids
  - Eukaryotes can transport folates via membrane transport proteins
  - The sulfonamides are analogues of PABA and compete with it

**Targeting Protein Synthesis**

- **Targeting 30S ribosomal subunit**
  1. **Aminoglycoside antimicrobials**
    - Streptomycin, neomycin, kanamycin, gentamicin, spectinomycin
  2. **Tetracycline**
    - Widely used in veterinary medicine
    - Staining of calcified tissues (teeth and bones)
    - A problem in human medicine

**Targeting Protein Synthesis**

- **Targeting 50S ribosomal subunit**
  1. **Chloramphenicol**
    - A low percentage of humans develop a severe anemia if treated with this drug
    - Strictly prohibited in food-producing animals
  2. **Florfenicol**
    - A structural analog of chloramphenicol w/o the same side effects
  3. **Macrolide**
    - Broad spectrum
    - May eliminate much of the normal flora in the intestines
  4. **Lincosamide**
    - Lincomycin: Commonly used in feed in the US
    - Clindamycin: Commonly used in human medicine
**Antibiotic Targets**

- **Cell wall**: β-lactam antibiotics (penicillins, cephalosporins)  
  glyccopeptides (vancomycin)
- **Membrane**: polypeptides (polymyxin)
- **DNA gyrase**: quinolones, novobiocin
- **RNA polymerase**: rifampin
- **Protein synthesis**:
  - 30S: aminoglycosides, tetracyclines
  - 50S: chloramphenicol, macrolides, lincosamides

**Antibiotic Resistance**

**CDC Warns Of ‘Superbug’ - Drug Resistant Staph Infection Kills More People Than AIDS**

Over 90,000 people per year are developing life-threatening infections caused by the drug resistant staph germ MRSA. The germ is common in hospitals, and is spreading to schools, prisons and locker rooms. 10% of those infected develop flesh eating disease.

In schools in Bedford, Virginia have been shut down following the death of a 17 year old student Ashton Bond, who contracted the deadly disease. Hospitalized for over 3 weeks, all antibiotic treatments failed to halt the germs spread throughout the boys body, finally spreading to his kidneys, liver, lungs and the muscles around his heart.

**Overall Resistance Mechanisms**

- Hydrophobic antibiotics
- Hydrophilic antibiotics

**Transfer of antibiotic resistance genes**

- Natural transformation
- Bacterial transduction
- Bacterial conjugation
How to measure antibiotic resistance?

- Dilution Susceptibility Tests
  - Broth Dilution
  - Agar Dilution

Antimicrobial Susceptibility Tests

- Disk diffusion (Kirby-Bauer) test

What causes antibiotic resistance?

- Use of Antibiotics in Farm Animals

- 90% Growth promoter
- 80% for agriculture
- 50 M lb. of antibiotics / year
**Antibiotic Growth Promoter**

- To improve growth and to prevent disease in animals
- The first report on antibiotic growth promoter [Stokstad et al. 1949]
  - Feed chickens with the fermented mash of *Streptomyces aureofaciens*
  - *S. aureofaciens* can produce tetracyclines

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Level in diet per kilo</th>
<th>Weight (No of survivors) at 25 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>103 g</td>
<td>3</td>
</tr>
<tr>
<td><em>S. aureofaciens</em> 2.5 g</td>
<td>140 g</td>
<td>7</td>
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<tr>
<td><em>S. aureofaciens</em> 5 g</td>
<td>210 g</td>
<td>10</td>
</tr>
<tr>
<td><em>S. aureofaciens</em> 10 g</td>
<td>230 g</td>
<td>11</td>
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**Antibiotic Growth Promoter**

- Antibiotic growth promoters enhance weight gain in swine

**Mechanisms of Growth Promotion by Antibiotics**

- Mechanisms are not known, but gut flora are thought to be involved
- Growth promotion by antibiotics doesn’t occur in germfree animals [Coates et al. 1963]
Enrichment of Resistant Bacteria

- Suppression or elimination of antibiotic-sensitive strains
- Amplification of antibiotic-resistant strains

Ban of Antibiotic Growth Promoter

**Pros**
- Reduced the amount of antibiotics used in animals
- Decreased antibiotic resistance

**Cons**
- Frequency of diseases ↑ → use of therapeutic antibiotics ↑
- Weight gain ↓ → production ↓

Isolation of E. coli & Determination of Antibiotic Resistance

Spread of Antibiotic Resistance Genes in the Environment

TABLE 1: Frequency of antibiotic resistance in E. coli isolates from pigs as the Danish farms and from wild small mammals in the vicinity of these farms and in the geographically matched rural areas

<table>
<thead>
<tr>
<th>Environmental source</th>
<th>tet(A)</th>
<th>tet(C)</th>
<th>tet(E)</th>
<th>tet(H)</th>
<th>tet(M)</th>
<th>tet(O)</th>
<th>sul(I)</th>
<th>sul(II)</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Animal feeding operations</td>
<td>0.77</td>
<td>0.28</td>
<td>0.89</td>
<td>1.00</td>
<td>0.85</td>
<td>0.96</td>
<td>1.00</td>
<td>0.94</td>
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<tr>
<td>Wastewater treatment plants</td>
<td>0.91</td>
<td>0.45</td>
<td>0.36</td>
<td>0.91</td>
<td>0.91</td>
<td>1.00</td>
<td>1.00</td>
<td>0.82</td>
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<tr>
<td>Pristine river</td>
<td>0.08</td>
<td>0.00</td>
<td>0.04</td>
<td>0.25</td>
<td>0.08</td>
<td>0.23</td>
<td>0.04</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

Frequency of detection (FOD*) of antibiotic resistance genes

*FOD = No of detection / No of samples
Avoidance of any unnecessary use of antibiotics

Elimination of nontherapeutic uses

Development of alternatives to improve animal health

- Probiotics/ Competitive exclusion products
  - Prevention of colonization by pathogenic bacteria in the intestines

- In-feed exogenous enzymes
  - Phytases, carbohydrases, etc from fermented bacteria or fungi
  - Improve conversion efficiency of plant-based diets in animals

Vaccination
  - However, many animal diseases still can not be controlled by vaccination

Failure of antibiotic chemotherapy
Extended illnesses and high mortality!

Contact

Antibiotic-Resistant Pathogens from Farm