Antibiotics and Resistance

TOPICS
1. Antibiotics and clinical microbiology
2. Types of antibiotics
3. Mechanisms of action
4. Test for antibiotic sensitivity
5. Antibiotic resistance
6. Solutions

Figure References:

Chemotherapeutic agents

- Chemical agents used to treat disease
- Destroy pathogenic microbes or inhibit their growth within host
- Most are antibiotics
  - microbial products or their derivatives that kill susceptible microbes or inhibit their growth
- Distinct from antimicrobial agents
  - Not intended for therapeutic purposes
  - Naturally occurring and/or synthetic
  - Used to sterilize or inhibit microbial growth

Before “modern” antibiotics metals solutions were used.

- Arsenic
  - Used since antiquity
  - was one of the first antimicrobial compounds and was effective against syphilis.
  - Arsenic is very toxic to the patient however.
- Mercury
  - Very effective antimicrobial agent.
  - Used to sterilize surfaces and kill microbes
  - Still used as preservative in vaccines
- Bacteria can develop really high levels of resistance to metals (5-10 mM!).
General effects of Ab on bacteria

- Interferes with protein/DNA synthesis
- Disruption of cell wall and/or membranes

The Development of Chemotherapy

- Paul Ehrlich (1904)
  - Developed concept of selective toxicity
  - Identified dyes that effectively treated African sleeping sickness
  - One of the first is salvarsan (arsenic containing drug)

- Alexander Fleming accidentally discovered penicillin (1928)
  - Observed penicillin activity on contaminated plate

- Selman Waksman discovered streptomycin (1944)

General Characteristics of Antimicrobial Drugs

- Selective toxicity
  - Ability of drug to kill or inhibit pathogen while damaging host as little as possible

- Therapeutic dose
  - Drug level required for clinical treatment

- Toxic dose
  - Drug level at which drug becomes too toxic for patient (i.e., produces side effects)

- Therapeutic index
  - Ratio of toxic dose to therapeutic dose
Mechanism of Action of Antimicrobial Agents

- Can impact pathogen by targeting some function necessary for its reproduction or survival
- Targeted function is very specific to pathogen → higher therapeutic index

Summary of the effect of antibiotics on cell functions

Different types of antibiotics (Ab)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Producer</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall synthesis inhibitor</td>
<td><em>Pencillium</em></td>
<td>Blocks transpeptidation in peptidoglycan synthesis</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Semi-synthetic</td>
<td>Blocks transpeptidation in peptidoglycan synthesis</td>
</tr>
<tr>
<td>Bacitracin</td>
<td><em>Bacillus subtilis</em></td>
<td>Inhibits isopenyl pyrophosphate dephosphhydration</td>
</tr>
<tr>
<td>Disruption of membrane potential</td>
<td><em>Bacillus brevis</em></td>
<td>Ionophore disrupts cell membrane integrity and function</td>
</tr>
<tr>
<td>Tyrocidine</td>
<td><em>B. polymyxa</em></td>
<td>Disrupts membrane transport and function</td>
</tr>
<tr>
<td>Vancomycin</td>
<td><em>Streptomyces rimosus</em></td>
<td>Potassium-specific transportor</td>
</tr>
<tr>
<td>DNA and RNA synthesis inhibitor</td>
<td><em>S. spheroides</em></td>
<td>Binds to a subunit of DNA gyrase and inhibits action</td>
</tr>
</tbody>
</table>

Different types of antibiotics (Ab)

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<tr>
<th>Antibiotic</th>
<th>Producer</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic inhibitor</td>
<td>Synthetic</td>
<td>Dihydrofolate reductase inhibitor</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Synthetic</td>
<td>Dihydrofolate reductase inhibitor</td>
</tr>
<tr>
<td>Dapsone</td>
<td>Synthetic</td>
<td>Inhibits dihydrofolate synthetase inhibitor</td>
</tr>
<tr>
<td>Sulfaazimide</td>
<td>Synthetic</td>
<td></td>
</tr>
<tr>
<td>Protein synthesis inhibitor</td>
<td><em>S. aureus</em></td>
<td>Blocks transcribing enzyme RNA polymerase</td>
</tr>
<tr>
<td>Rifampin</td>
<td><em>S. aureus</em></td>
<td>Blocks 50S ribosomal subunit and stops peptidyltransferase</td>
</tr>
<tr>
<td>Erythromycin</td>
<td><em>S. erythraeus</em></td>
<td>Inhibits 30S ribosomes, blocks amino acid incorporation into peptide</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>S. griseus</em></td>
<td>Inhibits binding of aminoacyl tRNA to ribosomes</td>
</tr>
<tr>
<td>Tetracycline</td>
<td><em>S. aureus</em></td>
<td>Blocks ribosomes blocking peptidyltransferase</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td><em>S. liquefaciens</em></td>
<td>Binds to the 30S subunit to inhibit translocation</td>
</tr>
</tbody>
</table>

*Microbial Life 2e, Table 7.5 (Part 1)*
Broad vs. Narrow Spectrum

Broad spectrum: affects many different types of bacteria
Narrow spectrum: specific to one particular group of bacteria

How do we determine the level of antimicrobial activity?

- Effectiveness expressed in two ways
  - Minimal inhibitory concentration (MIC)
    - lowest concentration of drug that inhibits growth of pathogen
  - Minimal lethal concentration (MLC)
    - lowest concentration of drug that kills pathogen

- Two techniques are routinely used to determine MIC and MLC

1. Dilution Susceptibility Tests

- Inoculate media containing different concentrations of drug.
- Monitor growth by plate counts or OD 600 nm.
- Plot the OD 600 nm vs. concentration
- The lowest concentration showing no growth is MIC
- The MLC:
  - if broth used, tubes showing no growth can be subcultured into drug-free medium
  - broth from which microbe can’t be recovered is MLC

II. Kirby-Bauer Disk Diffusion Tests

- Disks impregnated with specific drugs are placed on agar plates inoculated with test microbe
- Drug diffuses from disk into agar, establishing concentration gradient
- Observe clear zones (no growth) around disks
Kirby-Bauer Disc Diffusion Results

Zone of inhibition (diameter) used to determine susceptibility or resistance

TABLE 30.13 Diameter of the zone of inhibition as a measure of susceptibility to selected antibacterial agents

<table>
<thead>
<tr>
<th>Antibacterial</th>
<th>Amount on Disk (μg)</th>
<th>Zone Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>28 or less</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>13 or less</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>12 or less</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>14 or less</td>
</tr>
</tbody>
</table>

What factors influence the effectiveness of antimicrobial drugs during treatment?

- Ability of drug to reach site of infection
- Susceptibility of pathogen to drug
- Ability of drug to reach concentrations in body that exceed MIC of pathogen

Factors influencing the MIC in the body during treatment

- Amount administered
- Route of administration
- Pharmacokinetics
  - The fate of a substance in the body:
    - Rate of uptake
    - Rate of clearance (elimination) from body
**Microbial drug resistance**

- Using antibiotics inevitably selects for resistance.
- This is a good thing for microbe.
- This is a big problem for public health.
- Once resistance originates in a population it can be transmitted to other bacteria

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**Post-therapeutic effects of antibiotic dispersion**

Home, daycare, hospital, farm

The individual is an incubator for growing and spreading resistant bacteria

- a) Individual taking antibiotics is a focal point for high concentration of Ab (red) and resistant bacteria (black dots).
- b) Over time, resistance bacteria spread and antibiotics goes into the environment via waste water and disposal
- c) If other people are treated this can lead to higher density of resistant microbes within the environment
- d) Selective process continues during and after therapy.

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**Appearance of drug-resistant bacteria**

**Antibiotic Resistance**
Antibiotic resistance

Natural

- Lack the structural target for an antibiotic
  - Eg. Mycoplasma & Archaea are resistant to penicillin because they lack peptidoglycan
- Antibiotic does not reach target
  - E.g outer membrane of Gram- is impermeable to Penicillin G

Acquired... from antibiotic resistance genes

Table 15.1 Common mechanisms of plasmid-encoded antibiotic resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Mechanism of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td>Synthesis of β-lactamases, enzymes that hydrolytically destroy the antibiotic.</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Synthesis of an enzyme that acylates chloramphenicol, rendering it inactive.</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Synthesis of one of several enzymes that inactivate the antibiotic by acetylation, phosphorylation, or adenylation.</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Synthesis of a membrane protein capable of pumping the antibiotic out of the cell before it can act on the ribosomes.</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Synthesis of an enzyme that methylates bacterial 23S ribosomal RNA; methylated ribosomes cannot bind the antibiotic.</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Synthesis of a mutant, trimethoprim—insensitive form of dihydrofolate reductase.</td>
</tr>
</tbody>
</table>

Antibiotic Resistance Genes

- Degradate antibiotic
  - Penicillinase
- Alter antibiotic
  - Acetylation
- Pump out antibiotic
  - Tetracycline
- Mutate target gene
  - Transpeptidation enzyme

Origin and spread of resistance genes

- Resistance genes can be chromosomal or on plasmids
  - Can exist separate from chromosome or integrated into it
- Chromosomal genes
  - Mutations can arise
    - If mutation rate is of 1 in $10^7$,
    - In $10^{10}$ cells you might have 1000 mutations
  - Mutations might occur in genes encoding proteins targeted by drug
- R plasmids
  - Resistance plasmids
  - Can be transferred to other cells by conjugation, transduction, and transformation
  - Can carry multiple resistance genes
- Gene cassettes
  - Sets of resistance genes
  - Can exist as separate genetic elements
  - Can be part of transposon, integron or chromosome
**Alter the antibiotic**

Aminoglycoside
Kanamycin

![Diagram showing alteration of antibiotic action on ribosome](image)

**Pump out the antibiotic**

Example: Tetracycline and Chloramphenicol resistance
Microbe pumps Tc of Cm out of the cell
(Multi-Drug-Resistant Transporters)

![Diagram showing pumpout of antibiotic](image)

**Example: cell wall targets**

*review peptidoglycan biosynthesis*

Transglycosylation  Transpeptidation

Gram positive like *Streptococcus*

**Penicillin**

- Inactivates transpeptidase enzyme, which is also called a peptidoglycan binding protein (PBP)
- Does not target transglycosylation

**Penicillin resistance**

- Acquire a penicillinase, also called a beta lactamase. These cleave the Beta lactam ring
  - or
- Mutate the PBP
- Acquire an alternative PBP
**Vancomycin: drug of last resort**

- For penicillin, resistance started appearing after ~2 years of its discovery
  - This is a typical time for a resistance mechanism involving one gene.
- Vancomycin are very effective against Gram +.
- However, Vancomycin Resistant Enterococci (VRE) were appearing in hospitals in 1987, 29 years after clinical introduction of Vancomycin.
  - Vancomycin resistance requires five genes
  - Need more time to acquire the necessary mutations
- The genes are on now on plasmids and transposons.
  - These rapidly spread among Enterococci.
- There is little defense against MRSA if it gets these vancomycin resistance genes.

**Vancomycin**

- Ties up the peptide substrate for the PBP.
- Inhibits transpeptidation and transglycosylation reactions

**Vancomycin resistance**

- Reprogram the terminal peptide
  - D-Ala-D-Ala to D-Ala-D-Lac
- The new pentapeptide still works with the PBP
- Vanco binds with 1000X less affinity to D-Ala-D-Lac

**The search for new antibiotics...**

- How do scientists find antibiotics?
- Once you find them how can they be put to use?
  
  **Possible answer:**
  *Nature is the best chemical engineer.*

  **Screen for antibiotic producing microbes.**
Actinomycete was streaked and incubated for several days.

Then, four species of bacteria were streaked:

- Bacillus megaterium
- Micrococcus luteus
- Staphylococcus aureus
- E. coli

What do you do once you find an Ab producing microbe?

- Work with natural products chemist to purify and analyze the antimicrobial agent.
  - Structure/function tests
  - Maybe modify the chemical structure to decrease toxicity in humans
- If the new antibiotic is biologically active *in vivo*:
  - The industrial microbiologist may genetically modify the Ab producing strain to increase yields to levels acceptable for commercial development.
Streptomyces

- The Spartans of Chemical Warfare
- Gram+, filamentous soil bacteria
- Produce spore-forming mycelia
- Almost 50% of all isolated strains produce antibiotics
- Production is coupled to sporulation
- Streptomyces also produce compounds that are active against tumors, fungi, and parasites.
- Many of the metabolites are pigmented
- 1000’s have been reported
  http://genomebiology.com/2002/3/7/REVIEWS/1020/

Antibiotic (Ab) production by Streptomyces species

- Production of Ab is due to secondary metabolic islands (SMILES)
  - Big gene cluster
  - S. coelicolor has 20 SMILES
- Antibiotic producing strain is resistant to its own Abs.
  - Uses a variety of mechanisms to be resistant, mainly efflux pumps

Preventing emergence of drug resistance

- Give drug in high concentrations
- Give two or more drugs at same time
- Use drugs only when necessary
- Possible future solutions
  - continued development of new drugs
  - use of bacteriophages to treat bacterial disease
Augmentin
- Two drugs
- Clavulanate: inactivates beta lactamases
- Amoxicillin: inhibits transpeptidation enzyme (PBP)

**Screen Against VRE**
- Make chemical library of vancomycin
- Screen for hits against VRE
- Biphenyl alkyl substitution on the vancosamine sugar increases potency against VRE 2 fold!

**Summary**
- Antibiotics molecules produced by microbes can inhibit growth of a different species
- Antibiotics function by inhibiting processes that are unique to prokaryotes:
  - cell wall synthesis (Penicillin)
  - translation (Chloramphenicol)
  - transcription (Rifampicin)
- Antibiotic resistance is a big problem and can be mediated by:
  - exclusion of drug
  - drug can’t bind to or penetrate pathogen
  - pump drug out
  - inactivation of drug
    - chemical modification of drug by pathogen
  - alteration of target enzyme
  - use of alternative pathways or increased production of target metabolite

**Arsenic resistance conferred by genes on plasmids and/or chromosome**

- $\text{AsO}_4^{3-}$ or $\text{AsO}_2^-$
- $\text{AsO}_2^-$ or $\text{AsO}_4^{3-}$
- Arsenic pump
- Arsenic resistance conferred by genes on plasmids and/or chromosome
- peri.
- CM
- cyto.
- Arsenic
- ADP + Pi
- ATP
- $\text{ArsB}$
- $\text{ArsA}$
- $\text{ArsC}$
- $\text{ArsRDABC mRNA}$
- $\text{arsR}$
- $\text{arsD}$
- $\text{arsB}$
- $\text{arsC}$