20.201
Bacteria, Antibiotics, and Antibiotic Therapy

October 30, 2013
History of Microbiology

- Spontaneous Generation
  - Aristotle 384-322 B.C.
- Example of maggots arising from spoiled meat
  - Francisco Redi 1626-1697
- Air carried spores that led to microbial growth
  - Louis Pasteur 1822-1895
- Pasteurization
- Vaccines for anthrax and rabies
Bacteria

- Single cell organisms
- Gram-positive and gram-negative
- Ubiquitous in the environment
- Microbiome
- Very rapid growth rates
- Exotoxins and endotoxins
Gram-Staining

Crystal violet for 30 seconds
Water rinse for 2 seconds

Gram's iodine for 1 minute
Water rinse

Wash with 95% ethanol or acetone for 10–30 seconds
Water rinse

Safranin for 30–60 seconds
Water rinse and blot

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Microbiology, 4th Ed., Prescott
Cell Wall

- Provides shape
- Protects against osmotic lysis
- Physical barrier
- Peptidoglycan (Murein)
- NAM-NAG-amino polymer

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Cell Wall
Gram-positive vs. gram negative

Fig. 1-2. Composition of the cell surfaces of gram-positive and gram-negative bacteria.

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Cell Wall
Gram-positive vs. gram negative

Fig. 1.7 Schematic illustration of a gram-negative and a gram-positive bacterial cell wall. Note the presence of an outer membrane (also called outer envelope) in the gram-negative wall and the much thicker peptidoglycan layer in the gram-positive wall.
Gram Positive

- Gram positive bacteria
- Thick peptidoglycan
- Teichoic acids
  - Contain phosphates
  - Impart negative charge

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NAM-NAG-Peptide

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Peptidoglycan

N-Acetylmuramic acid

N-Acetylg glucosamine

Pentaglycine interbridge

Peptide chain

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Gram Negative

- **Lipopolysaccharide**
  - Highly diverse and changing polysaccharides
  - Avoids host detection
  - Limits host interaction with outer membrane
  - Prevents entry of bile salts, antibiotics, and toxicants
  - Prevents loss of nutrients from periplasmic space

- **Transporters and porins**
  - Selectively export and uptake small molecules

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Microbiology, 4th Ed., Prescott
Exotoxins and Endotoxins

- Exotoxins
  - Heat-labile, proteins released into surroundings
  - Can migrate to different cells or tissues
    - Diphtheria toxin, anthrax toxin, cholera toxin

- Endotoxins
  - Heat-stable lipopolysaccharide
  - Outer membrane of gram-negative bacteria
  - Released during lysis or cell division/growth
  - Leads to blood clotting, hemorrhaging and organ failure
Exotoxin: Diphtheria Toxin

- **Corynebacterium diphtheriae**
- **Gram-positive, facultative anaerobe**
- **Diphtheria toxin:**
  - 62 kDa Protein
  - B: Cell surface receptor binding
  - A: Enzymatic region
  - Catalyzes addition of ADP-Ribose to EF2
  - Inhibits translation

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Antibiotic resistance is and will be a problem.
Antibiotic Drug Pipeline

Tomorrow’s Antibiotics: The Drug Pipeline

The number of new antibiotics developed and approved has steadily decreased in the past three decades, leaving fewer options to treat resistant bacteria.

Image is by the [Centers for Disease Control and Prevention](https://www.cdc.gov), and is in the public domain.
How to Target Bacteria?

- Unique processes/proteins
- Cell Wall
- DNA synthesis
  - Single circular dsDNA chromosome
- Ribosomes
- Can you selectively target pathogenic bacteria?
How to Target Bacteria?

Inhibitors of cell wall synthesis:
- Fosfomycin
- Cycloserine
- Vancomycin
- Penicillins
- Cephalosporins
- Monobactams
- Carbapenems
- Ethambutol
- Pyrazinamide
- Isoniazid

Penicillins
Cephalosporins
Carbapenems

Inhibitors of transcription and translation:
- Rifampin
- Aminoglycosides
- Spectinomycin
- Tetracyclines
- Macrolides
- Chloramphenicol
- Lincosamides
- Streptogramins
- Oxazolidinones
- Pleuromutilins

PABA

Peptidoglycan cell wall

Pteridine

THF

DHF

Purines

Pyrimidines

Ribosome

Protein

mRNA

DNA

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Folic Acid Metabolism

- Humans require folic acid in diet and use as a cofactor in the synthesis of amino acids and nucleic acids
- Bacteria make their own folic acid
- Bacteriostatic
Sulfonamides

A  Folic acid

B  PABA analogues

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**β-Lactams**

- Inhibit cell wall polymer crosslinking
- Inhibit transpeptidase
- Bactericidal

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**Normal transpeptidation**

- Two peptidoglycan chains
  - L-Ala
  - D-Ala
  - (L-Gly)$_2$-L-Lys
  - (L-Gly)$_2$-L-Lys
  - D-Ala
  - D-Ala

**Activation step**

- Enzyme-peptidoglycan intermediate
  - L-Ala
  - D-Ala
  - (L-Gly)$_2$-L-Lys
  - (L-Gly)$_2$-L-Lys

**Coupling step**

- Crosslinked peptidoglycan chains
  - L-Ala
  - D-Ala
  - (L-Gly)$_2$-L-Lys

**Penicillin action**

- "Dead-end" enzyme penicillin complex
- β-lactamases cleave this bond

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Golan, Fig. 34-3, 34-6

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Quinolones/Fluoroquinolones

- **Type II Topoisomerase**
- Produce double-strand breaks in DNA
- Quinolones inhibit TopoII before second strand can pass
- Bactericidal

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Inhibiting Protein Synthesis

- Multiple Mechanisms
- Not completely understood
- Tetracyclines, Macrolides, Chloramphenicol, Oxazolidinones are bacteriostatic
- Aminoglycosides are only bactericidal class among the protein synthesis inhibitors
Reactive Oxygen Species

- Observed for bactericidal but not bacteriostatic antibiotics
- NADH depletion
- Dependent on TCA Cycle
- Increased production of superoxide (O$_2^{-}$)
- Damage to iron-sulfur clusters
- Fenton Chemistry

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Reactive Oxygen Species

- Increase in ROS leads to an increase in nucleotide pool damage products ultimately producing DNA damage and cell death
- MutT removes 8-oxo-dGTP from the nucleotide pool
- dnaE911, dinB, & umuDC are DNA polymerases that incorporate 8-oxo-dG

8-oxo-dGTP
\[ \uparrow \text{Mut T} \]
8-oxo-dGMP

Science, 2012, 336, 315-319
Antibiotics

• Unique processes/proteins
• Cell Wall
• DNA synthesis
• Ribosomes
• ROS
• Innate immunity
  • Phagocyes (neutrophils and macrophages)
• Adaptive immunity
Alexander Fleming

- 1928 - Fleming’s discovery of “mold juice”
  - Staphylococcus cultures contaminated with a mold from the genus Penicillium
  - Penicillin was born
“Mold Juice”

- Why would a mold make a bactericidal compound?
- Why would bacteria make bactericidal compounds?
Polyketide Biosynthesis

Fig. 23 The biosynthetic pathway for the fungal polyketide 6-methylsalicylic acid (6-MSA).

- Acetate
- Propionate
- Malonate

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Polyketide Biosynthesis

AT = Acyltransferase
ACP = Acyl carrier protein
KS = Ketosynthase
KR = Ketoreductase
ER = Enoyl reductase
DH = Dehydratase
TE = Thioesterase

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Post-PKB Modifications

P450 Hydroxylation

6-Deoxyerythronolide B

Glycosyl transfer

Methylation

Erythromycin A

Glycosyl transfer Hydroxylation

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Nat. Rev., 3, 2005, 925
Post-PKB - Secondary Metabolites

P450 Reactions

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Bacterial Treatment

- Two main initiatives for human infection
  - Eliminate bacteria
  - Avoid emergence of resistance
- Bacteriostatic vs. Bactericidal
- During preclinical (and sometimes clinical) development the compound’s efficacy can be measured (not the case for many targets)
- Different drug classes require different dosing (i.e., different measurable endpoints)
- MIC = Minimal Inhibitory Concentration
Clinical Bacterial Pharmacology

Acquire data:

• PK (AUC, Cmax, time > MIC, protein binding)
• MIC for bacteria
• Evaluate dosage levels
Population Variation

252 Patients with Community Acquired Infections

CID, 2007, 45, S89
Protein Binding

- *Staphylococcus aureus* mouse model (IP)
- 7 separate structurally similar β-lactams
- All have identical MIC
- Only free drug is pharmacologically active


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Bacterial Kill Rates

Aminoglycoside

Quinolone

β-lactam

Tobramycin

Ciprofloxacin

Ticarcillin

Log10 CFU ml⁻¹

0 2 4 6 8 10

Time (h)

24 MIC

16 MIC

4 MIC

1 MIC

1/4 MIC

Control

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Linking Exposure to Efficacy

Cefotaxime β-lactam
Neutropenic Animals

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PD-Dependence on Kill Curves

\[ K_0 \sim K_1 \sim K_2 \]

\[ K_0 > K_1 > K_2 \]

\(\beta\)-lactams: \( K_0 \sim K_1 \sim K_2 \)

Quinolones, Aminoglycosides: \( K_0 > K_1 > K_2 \)


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Fluoroquinolones: AUC to MIC Matters

q.d. = A dose 1x/day  
80 mg/kg daily dose  
lomefloxacin (fluoroquinolone)  
3 strains of *Pseudomonas aeruginosa*  
Neutropenic Rats

b.i.d. = A/2 dose, 2x/day  
3 strains of *Pseudomonas aeruginosa*  
Neutropenic Rats

q.i.d. = A/4 dose, 4x/day  

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Lomefloxacin (fluoroquinolone)

*S. pneumoniae*  
Model: Mouse thigh infection  

CID, 2007, 45, S89
Emergence of Resistance

- Heterogeneous cell populations
- Hypermutators and persisters
- High mutation rate
- Mutations at $10^{-8}$ to $10^{-6}$ genes per generation
- Rapid growth rate
- Double approximately every 30 minutes
- Readily transfer genetic material
- Improper treatment selects for resistant cultures
Persister Phenotype

- Distinct from resistance
- No expansion in presence of antibiotic
- Population growth upon removal
- Nonhereditary phenotype
- Problematic for “compromised” individuals of the population

**Figure 1. Drug Persistence and Recurrent Infection**

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Genetic Variation

- Point mutations
  - Vertical transmission (germ line)
- Plasmid born (conjugation)
  - Horizontal gene transfer
- Acquire environmental DNA (transformation)
  - Horizontal transmission
- Virus/bacteriophage (transduction)
  - Horizontal transmission
Conjugation

- Horizontal transfer (horizontal gene transfer)
- Plasmid can contain multiple factors that render resistance
- Can be passed between different species and genus

1. Donor
2. F Plasmid
3. Relaxasome
4. F Plasmid

Chromosomal DNA
Pili
DNA Polymerase
Relaxasome Transferasome
F Plasmid
Pili
F Plasmid

1. Donor
2. Recipient

1. Old Donor
2. New Donor

Courtesy of Michael David Jones on wikipedia. Used with permission.
Common Resistance Mechanisms

• Metabolic enzymes
  • β-Lactamase
  • Esterase
  • Acetyltransferase

• Efflux pumps

• Reduce concentration of drug

• Mutations in antibiotic targeted proteins

• Topo II
Vancomycin Resistance

- 9 Genes on a transposon
- All genes can hop in and out
- VanS and VanR are regulatory genes that are only switched on in the presence of Vancomycin

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PNAS, 1999, 96, 289
Hypermutator Phenotype

- In absence of horizontal transfer, the only possible resistance mechanism is mutation
- Mutations in DNA repair mechanisms lead to increased rates of germ line mutations
- Accelerated evolution via promiscuous repair/recombination and rapid duplication
Selective Pressure

Amplification of Resistance

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Dosing Impacts Emergence of Resistance

• Size matters

• Larger the bacterial load, the more likely that resistant populations exist

• Rapid and more intense the treatment the better (in general)

• Minimize time for bacteria to mutate or transfer resistance

• Granulocytes (innate immune system) clear bacteria at appreciable rates

• Co-dependence on antibiotics to limit growth and impact population size

• Evaluation of PK/PD antimicrobial parameters are on a case-by-case basis. More work needs to be done in vivo

• Predictive tools for infection type and virulence are needed