Antimicrobials & Chemotherapy

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Microbiology 1
History of chemotherapy

- **Paul Ehrlich**
  - Coined term chemotherapy
  - Looking for “magic bullet”
  - Won Nobel Prize in Physiology or Medicine, 1908

- **Alexander Fleming**
  - *Penicillium notatum* and *S. aureus*
  - Substances from one microorganism inhibits another
  - Won Nobel Prize in Physiology or Medicine, 1945
Sources of antimicrobial drugs

- **Streptomyces spp**
  - Majority of antibiotics
  - Actinomycetes, a group of filamentous bacteria
  - Ex: amphotericin B, chloramphenicol, e-mycin, neomycin
  - Platensimycin
    - *Streptomyces platensis*
Sources of antimicrobial drugs

- **Bacillus**
  - Ex: Bacitracin (*B. subtilis*), polymixin

- **Penicillium, Cephalosporium**
  - Fungi
  - Ex: Penicillin, griseofulvin, cephalothin

- **Note:** most have some type of sporulation process
Spectrum of antimicrobial activity

- Prokaryotic vs. eukaryotic
  - Targets are:
    - Unique to microorganisms
    - More important in the microbes than in the humans

- Viral infections

- Spectrum of microbial activity

- Broad spectrum antibiotics
  - Advantages
  - Disadvantages
    - Competitive inhibition
    - superinfection
Action of antimicrobial drugs

- Bactericidal vs. bacteriostatic
- Five major modes
  - Inhibition of cell wall synthesis
  - Inhibition of protein synthesis
  - Inhibition of nucleic acid synthesis
  - Inhibition of synthesis of essential metabolites
  - Plasma membrane damage
Modes of action

MAJOR TARGETS OF COMMON ANTIMITROBIAL AGENTS

DNA
- Fluoroquinolones
- Novobiocin
- Nitroimidazoles
- Nitrofurans

CELL WALL
- Beta lactam antibiotics
- Glycopeptides
- Bacitracin

RIBOSOMES
- Tetracyclines
- Aminoglycosides
- Lincosamides
- Macrolides
- Streptogramins
- Chloramphenicol
Major categories of antibiotics

- Inhibitors of cell wall synthesis
  - Beta-lactam compounds
    - Penicillin
    - Cephalosporins
    - Monobactams
    - Carbapenems
  - Other cell wall inhibitors
    - Vancomycin
Beta-lactam compounds

Note the characteristic **beta-lactam ring** which is essential for antibiotic activity. Beta-lactamases cleave the beta lactam ring from the rest of the structure, inactivating antibiotic.
Beta-lactam compounds

• Penicillin
  ◦ Source: *Penicillium chrysogenum*
  ◦ Types
    • Penicillin G
    • Antistaphylococcal penicillins (eg. Nafcillin)
    • Extended-spectrum penicillins (eg. Ampicillin)
  ◦ Activity
    • Active against gram positive organisms, gram-negative cocci, non-β-lactamase producing anaerobes
    • not effective against gram-negative rods
  ◦ Mechanism of action (bactericidal)
    • Interferes with bacterial cell wall synthesis by binding to active site on penicillin-binding protein and preventing cross-linking of peptidoglycans
Beta-lactam compounds

- **Resistance**
  - Inactivation by beta-lactamase
    - 300 identified
  - Modification of the penicillin binding proteins
    - Methicillin resistance in staphylococci
    - Penicillin resistance in pneumococci and enterococci
  - Impaired penetration of drug
    - Gram-negative organisms only
  - Presence of efflux pumps
Beta-lactam compounds

- **Cephalosporins**
  - Similar to penicillins in structure, mechanism of action, and activity
  - 1\(^{st}\) Generation
    - Cephalexin, cefazolin, etc.
  - 2\(^{nd}\) Generation
    - Cefuroxime, cefoxitin, cefaclor, etc.
    - Effective against beta-lactamase producing *H. influenza*
  - 3\(^{rd}\) Generation
    - Ceftazidime, cefotaxime, ceftriaxone, etc.
    - Expanded activity against gram-negative organisms
    - Able to cross blood-brain barrier
  - 4\(^{th}\) Generation
    - Cefepime, etc.
    - More resistant to beta-lactamases
    - Good activity against penicillin resistant streptococci and enterobacter infections.
Beta-lactam compounds

- **Monobactams**
  - **Aztreonam**
    - Resistant to most beta-lactamases
    - Active against gram-negative rods
    - No activity against gram-positives or anaerobes

- **Carbapenems** (e.g., imipenem)
  - Wide spectrum, good activity against gram-negative rods, gram positive organisms, *Ps. aeruginosa*, and anaerobes
  - Resistant to most beta-lactamases
  - Good penetration

- **Beta-lactamase inhibitors**
  - Clavulanic Acid, sulbactam, tazobactam
    - Available in fixed combination with certain penicillins (prevent degradation, but have little or no antibacterial properties)
    - Often used in immunocompromised/immunosuppressed patients and in mixed aerobic/anaerobic infections
Other cell wall inhibitors

- **Vancomycin**
  - **Source:** *Streptococcus orientalis* (actinomycete)
  - **Mechanism of action**
    - Binds to the D-Ala-D-Ala terminus of peptidoglycan pentapeptide preventing elongation and cross-linking.
  - **Activity**
    - Bactericidal for gram-positive organisms
    - Can be used in combination with gentamicin and streptomycin to treat *E. faecium* and *E. faecalis* infections
    - Drug of “last resort”
Major categories of antibiotics

- Inhibitors of protein synthesis
  - Antibiotics that bind to 30S ribosomal subunit
    - Aminoglycosides
      - Streptomycin, gentamicin, amikacin, etc.
    - Tetracyclines
    - Spectinomycin
  - Antibiotics that bind to 50S ribosomal subunit
    - Chloramphenicol, lincomycin, clindamycin
    - Oxazolidinones
      - Linezolid
    - Macrolides
      - Erythromycin
  - Antibiotics that prevent elongation of protein
    - Fusidic acid
Inhibitors of protein synthesis

- Chloramphenicol binds to 50S r-RNA and inhibits formation of peptide bond.
- Erythromycin binds to 50S r-RNA and prevents movement along m-RNA.
- Streptomycin changes shape of 30S r-RNA and causes m-RNA to be read incorrectly.
- Tetracycline interferes with the t-RNA anticodon reading of m-RNA codon.
Aminoglycosides

- Source: *Streptomyces* spp.
- Oldest example = **Streptomycin**
- Mechanisms of action
  - Irreversible inhibitor of protein synthesis
  - Binds to 30S ribosomal subunit
    - Interferes with initiation complex of peptide formation
    - Cause mRNA to be misread, producing toxic or nonfunctional protein
    - Breaks up polysomes into nonfunctional monosomes
- Activity
  - Gram-negative enteric bacteria, especially in bacteremia and sepsis
  - Tuberculosis treatment, and with vancomycin or penicillin for endocarditis
- Resistance
  - Transferase enzyme produced by microbe inactivates aminoglycoside
  - Altered transport protein (mutation) that prevents entry
  - Inability to bind to 30S ribosomal subunit due to altered ribosomal structure (mutation)
Tetracyclines

- **Mechanism of action**
  - Binds reversibly to 30S ribosomal subunit
  - Prevents binding of tRNA to mRNA complex
  - Amino acids addition is blocked

- **Activity**
  - Broad spectrum
  - Active against gram-positive and gram-negative, including rickettsiae, chlamydiae, anaerobes, mycoplasma, and some protozoa

- **Resistance**
  - Efflux pumps
  - Tetracycline blocked from binding to ribosome
  - Enzymatic inactivation of tetracyclines

Caution: Can affect bone growth, cause discoloration of teeth. Not for use in pregnancy or children < 8 yo. Also induces photosensitivity.
Chloramphenicol

• Mechanism of action
  ◦ Binds reversibly to 50S subunit of ribosome

• Activity
  ◦ Bacteriostatic, broad-spectrum antibiotic
  ◦ Active against aerobic, anaerobic gram-positive & gram-negative, and rickettsiae
  ◦ Not effective against chlamydia

• Resistance
  ◦ Plasmid-mediated production of chloramphenical acetyltransferase
Oxazolidinones

- New class of synthetic antimicrobial
  - Linezolid
    - Mechanism of Action
      - Prevents formation of ribosomal complex needed for protein synthesis
      - Binds to 50S ribosomal subunit by unique binding site
    - Activity
      - Gram positive organisms, including anaerobic cocci, corynebacteria, and *L. monocytogenes*
      - Bacteriostatic, except streptococci (bacteriocidal)
    - Issues
      - Save this to treat MDR gram positive bacteria
Macrolides

- **Erythromycin**
  - Source: *Streptomyces erythreus*
  - Clarithromycin and azithromycin are semisynthetic derivatives

- **Mechanism of Action**
  - Binds to 50S ribosomal subunit blocking formation of initiation complexes

- **Activity**
  - Gram-positive organisms, especially pneumococci, streptococci, staphylococci, corynebacteria, mycoplasma, legionella, and some mycobacteria
  - Inhibitory or bactericidal depending on organism
  - Works best at alkaline pH

- **Resistance**
  - Usually plasmid mediated
  - Three mechanisms
    - Efflux pumps
    - Hydrolysis by esterases produced by Enterobacteriaceae
    - Methylase production and alteration of ribosomal binding site
Major categories of antibiotics

- Inhibitors of nucleic acid synthesis and function
  - Inhibitors of RNA synthesis and function
    - Rifampin
    - Rifamycin
    - rifampicin
  - Inhibitors of DNA synthesis and function
    - Quinolones & Fluoroquinolones
Inhibitors of RNA synthesis

**Rifampin**

- **Source**
  - Semisynthetic
  - derivative of rifamycin, produced by *Streptococcus mediterranei*

- **Activity**
  - Gram-positive & gram-negative cocci, enteric bacteria, mycobacteria, chlamydia
  - Use of rifampin as single drug selects for resistance

- **Method of Action**
  - Binds to β subunit of bacterial DNA-dependent RNA polymerase
  - Inhibits RNA synthesis

- **Clinical uses**
  - 600 mg/d orally with INH, ethambutol, or other anti-TB drug
Inhibitor of DNA synthesis

- **Fluoroquinolones**
  - Prototype: Ciprofloxacin
  - **Source**
    - Synthetic
    - Fluorinated analogs of nalidixic acid
  - **Activity**
    - Gram-negative aerobic bacteria
    - Newer agents some efficacy against gram positive
      - Gatifloxocin & moxifloxacin effective against *S. pneumoniae*
  - **Mechanism of action**
    - Inhibit DNA synthesis
    - Inhibit bacterial topoisomerase II (DNA gyrase) and topoisomerase IV
Major categories of antibiotics

- Inhibition of essential metabolites
  - Sulfonamides
  - Trimethoprim
  - Methotrexate
Sulfonamides

- **Structure**
  - Organic sulfur compounds
  - Structural analogs of PABA (p-aminobenzoic acid)
  - Bacteriostatic
  - Ex) Sulfamethoxazole

- **Mechanism of Action**
  - Interfere with conversion of PABA to DHF (dihydrofolate), required by bacteria for production of purines and nucleic acid synthesis

- **Activity**
  - Gram-positive and gram-negative bacteria
  - Nocardia, chlamydia, some protozoa
  - Usually always used with another drug—ie, TMP-SMX
  - Enhances growth of rickettsiae !!

- **Three classes**
  - Oral, absorbable
  - Oral, nonabsorbable
  - Topical
Trimethoprim & TMP-SMX

- **Mechanism of Action**
  - Inhibits bacterial dihydrofolic acid reductase
  - With SMX, sequential blocking of metabolic pathway

- **Activity**
  - TMP-SMX trade name = bactrim
  - Bactericidal
  - *P. jevoreci* pneumonia, shigellosis, systemic salmonella infections, UTI’s, prostatis
  - Active against many respiratory pathogens, CAP

- **Resistance**
  - Plasmid mediated and quickly evolving
Plasma membrane damage

- **Examples:**
  - Polymyxin B
    - Effective against gram-negative, including pseudomonads
    - Bactericidal, topical use only

- **Action:**
  - Act like cationic detergents
  - Alter membrane permeability causing loss of important metabolites
  - Bind and inactivate endotoxins
Antifungal drugs

- Problems:
  - Eukaryotic cells
  - Increase in fungal infections due to immunocompromise and immunosuppression

- Drug categories
  - Systemic antifungal drugs for systemic infections
  - Oral drugs for mucocutaneous infections
  - Topical drugs for mucocutaneous infections
Systemic antifungal drugs

- **Amphotericin B**
  - **Source:** *Streptomyces nodosus*
  - **General characteristics**
    - Amphipathic polyene macrolide
    - Polyene = many double bonds
    - Macrolide = contains lactone ring of 12 or more atoms
    - New formulations include liposomal versions (AmBisome)
  - **Mechanism of action**
    - Binds to ergosterol, a sterol found only in fungal cell membranes
    - Forms pores in membrane which increases cell permeability
  - **Activity**
    - Broadest spectrum of antifungal agents
    - Yeasts (*C. albicans, Cryptococcus neoformans*)
    - *H. capsulatum, C. immitis, B. dermatitidis*
Amphotericin B

**Toxicity**

- **Infusion related toxicity**
  - Immediate and include nausea, vomiting, headache, fever, muscle spasms, hypotension

- **Slower toxicity**
  - Renal damage
  - Can be serious enough to warrant dialysis with prolonged use

- **Liposomal amphotericin B (AmBisome)**
  - Toxicity associated with nonspecific binding to mammalian cholesterol
  - Packaged in lipid so that lipid vehicle becomes reservoir, releasing amphotericin more slowly allowing more specific binding and less toxicity
Systemic antifungal drugs

- **Azoles**
  - Synthetic imidazoles or triazoles
    - Imidazoles: ketoconazole, miconazole, clotrimazole
    - Triazoles: itraconazole, fluconazole, voriconazole
  - **Mechanism of action**
    - Inhibits fungal cytochrome P450 enzymes, reducing ergosterol
    - Imidazoles are less specific than triazoles and exhibit more drug interactions and side effects
  - **Activity**
    - Broad range including candida species, C. neoformans, endemic mycosis, dermatophytes, aspergillus, and amphotericin-resistant fungi
Azoles

1) Itraconazole
   - General characteristics
     - Oral and intravenous
     - Poor penetration into CSF
   - Treatment for
     - dimorphic fungi
     - Dermatophytosis
     - onychomycosis

2) Fluconazole
   - General
     - Water soluble, good CSF penetration
     - High oral bioavailability, also as IV
     - Few liver enzyme interactions
   - Treatment for:
     - Cryptococcal meningitis
     - Candidemia
     - Prophylaxis for bm transplant recipients and AIDS patients

3) Voriconazole
   - General characteristics
     - Newest triazole
     - IV and oral
     - Visual disturbances reported including color blindness, light sensitivity, blurred vision
   - Treatment for:
     - Candida species
     - Dimorphic fungi
     - Invasive aspergillosis
Systemic antifungal drugs

- Echinocandins: eg, **Caspofungin**

**General characteristics**
- Newest class to be developed
- Large cyclic peptides linked to long fatty acid

**Mechanism of Action**
- Inhibits synthesis of $\beta$(1-3)glucan disrupting cell wall

**Activity**
- Salvage therapy for patients with invasive aspergillosis unresponsive to Amphotericin B
- Mucocutaneous candidiasis and bloodstream infections

**Adverse effects**
- Well-tolerated
- Do not prescribe with cyclosporine = elevated liver enzymes
Systemic antifungals: mucocutaneous infections

- Griseofulvin
  - General characteristics
    - Source: species of penicillium
    - Insoluble
    - Microcrystalline form
  - Mechanisms of action
    - Protects new skin from infection by binding to keratin
  - Activity
    - Dermatophytosis
    - Must be applied for weeks to months, especially for nail infections
Topical antifungals

- **Nystatin**
  - General characteristics
    - Polyene macrolide
    - Topical use only due to extreme toxicity
      - Poorly absorbed
  - Activity
    - Candida species such as oropharyngeal thrush, vaginal candidiasis

- **Topical Azoles**
  - Clotrimazole and miconazole
  - OTC
  - Vulvovaginal candidiasis, thrush, dermatophytes, tinea corporis, pedis, cruris
  - Shampoo forms for seborrheic dermatitis and pityriasis versicolor
Antiviral drugs

- Nucleoside and nucleotide analogs
- Other enzyme inhibitors
- Interferons
Antiviral drugs

- Nucleoside (sugar + base) and nucleotide analogs
  - Acyclovir
    - Genital herpes
    - Derivatives
      - Famciclovir, ganciclovir
  - Trifluvidine
    - Herpes keratitis
    - Contains thymine
  - Ribavirin
    - Interferes with viral replication
    - Resembles guanine
  - Zidovudine (AZT)
    - Blocks synthesis of DNA from RNA by RTase
Antiviral drugs

- Other enzyme inhibitors
  - Inhibit enzymes in last stage of viral replication
  - Protease inhibitors
  - Examples: Indinavir, saquinavir
  - Tamiflu
    - Inhibits neuraminidase
    - Effective against influenza

- Interferons
  - Alpha interferon for viral hepatitis infections
On the horizon….LJ001

- Broad spectrum antiviral
- Small amphipathic molecule (for the chemists….aryl methylene rhodanine derivative😊)
- Prevents virus to cell fusion of enveloped viruses; does not interfere with cell to cell fusion
- Efficacy demonstrated against all enveloped viruses, including Ebola and HIV!
  - Why? Cells can repair damage to plasma membrane; virions cannot!

Antiprotzoans

- Quinine
- Artemisinin
- Metronidazole
Antiprotozoal drugs (antimalarialis)

- **Quinine**
  - Chloroquine (*synthetic*)
  - Mefloquine
    - Effective against resistant strains

- **Artemisinin** (*1st line drugs in endemic countries*)
  - Artesunate
    - Sometimes with mefloquine in drug resistant areas
  - Dihydroartemisinin
Antiprotozoal drugs: artemisinin

- Derived from Chinese herb qing-hao 青蒿素 (Artemisia annua)
- drug = Qinghaosu
  - Used over 2000 years in Chinese traditional medicine as antipyretic
  - As antimalarials, effective only against the blood schizonts, not hepatic forms
  - Better tolerated than other antimalarials
  - Not available in US
Antiprotozoan drugs: artemisinin

- Limited bioavailability in natural form
- Semisynthetic analogs improve solubility and antimalarial efficacy
  - Artesunate
    - Water soluble
    - Oral, IM, IV, rectal
  - Artemether
    - Lipid soluble
    - Oral, IM, rectal
AntipROTOZOAL drugs: artemisinin

• Mechanism of action
  ◦ *Plasmodium spp* infect red blood cells
  ◦ Cause chemical reactions that release heme from hemoglobin
  ◦ Heme reacts with a peroxide bond in artemisinin producing reactive oxygen radicals that damage *Plasmodium*
  ◦ Usually prescribed in combination with lumefantrine (benflumetol) because artemisinin alone is only active for one to two hours in vitro
    • Combination drug is called Coartem (Novartis)
Antiprotozoal drugs

- Metronidazole (Flagyl)
  - Parasitic protozoa & obligate anaerobes
  - Interferes with anaerobic metabolism
  - Effective against giardiasis, amoebic dysentery, clostridia
Anti-helminthic drugs

- Niclosamide
- Praziquantel
- Mebendazole
- Ivermectin
- Moxidectin

*Ancylostoma duodenale*
Antihelminthic drugs

- Tapeworms
  - Niclosamide
    - Inhibits ATP production
  - Praziquantel
    - Effects plasma membrane permeability
    - Also effective against fluke

- Mebendazole
  - Ascaris, pinworms, whipworms
  - Effects motility by altering microtubules

- Ivermectin
  - Paralyzes nematodes
Anti-helminthic drugs

- **Moxidectin**
  - Experimental treatment for Onchocerciasis (River Blindness)
    - Eradication efforts by WHO in 1970 only partially successful
    - Used insecticides to kill vector
  - Kills larvae and kills or sterilizes adult worms
  - In Clinical trials in Ghana, Liberia, and Congo until 2012
  - Currently Rx in dogs/cats, cattle for parasite infections
    - Prod by Bayer Animal Health
  - If successful, will be produced for humans by Wyeth
  - Current treatment = ivermectin

Source: Laura McInnis, Reuters Health, 7/1/09
Susceptibility tests

- **Kirby-Bauer test**
  - Zone of inhibition
  - Sensitive, intermediate, resistant

- **E-test**
  - MIC = lowest concentration that inhibits microbial growth
  - Antibiotic gradient

- **Broth dilution**
  - MIC & MBC (minimum bactericidal concentration=how much does it take to KILL the organism)
  - Uses microtiter plates and various dilutions to determine if agent is inhibitory or bactericidal
Susceptibility tests
Drug resistance
Mechanisms of antibiotic resistance

- Plasmids
- Chromosomal
- Examples
  - $\beta$-lactamases
  - Efflux pumps

Why?
- Selective pressure
- Widespread use
  - Unregulated in some countries
- Non-adherence
- Addition to animal feed
- Short generation time
Antibiotics in animal feed

**Purpose**
- Reduce bacterial infections
- Enhance growth
  - Reduction of enterics

**Concern: selective pressure**
- Transfer of resistant strains to humans
  - Salmonella in meat or milk
  - Traced back to farms
Antibiotics and animal feed

- **VRE**
  - Use of vancomycin and avoparcin in animal feed in Europe
  - Importation of bacteria in travelers and imported food
  - Increased used of antimicrobials in hospitals
  - Reduction of VRE-positive samples in Germany by 75% when banned in animal feed

- **Microbial alternatives**
  - Prevent colonization
  - Reduce fecal contamination during processing in slaughterhouses
  - Proper storage and cooking of food
## Resistance and TB; example

- Five first-line antimycobacterial drugs
  - Rifampin, INH, pyrazinamide, ethambutol, streptomycin
- Usually use 3 drugs together…why?

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Duration of treatment</th>
</tr>
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<tbody>
<tr>
<td>INH, rifampin, pyrazinamide</td>
<td>6 months</td>
</tr>
<tr>
<td>INH, rifampin</td>
<td>9 months</td>
</tr>
<tr>
<td>Rifampin, ethambutol, pyrazinamide</td>
<td>6 months</td>
</tr>
<tr>
<td>Rifamin, ethambutol</td>
<td>12 months</td>
</tr>
<tr>
<td>INH, ethambutol</td>
<td>18 months</td>
</tr>
</tbody>
</table>
Drugs and TB

- **Modes of action**
  - INH inhibits mycolic acid synthesis
  - Rifampin inhibits mRNA synthesis
  - Ethambutol interferes with cell wall synthesis
  - Pyrazinamide effective against intracellular tubercle bacteria by unknown mechanism
  - Streptomycin interferes with protein synthesis (often used as 2\textsuperscript{nd} line drug in TB management)
Drugs and TB

- Drugs used in combination due to resistance and ability to decrease duration of treatment.
- Most effective drugs: Rifampin and INH
- 1 bacillus/10^6 is naturally resistant
  - TB lesions can have 10^8 organisms, so some will be resistant
  - Ex) If INH is only drug, some resistant organisms will survive and propagate
Drugs and TB

- With MDRTB and XDRTB, 1\textsuperscript{st} line drugs will not work….must move to 2\textsuperscript{nd} line drugs (less effective), and longer duration of treatment

- Second-line drugs include:
  - Amikacin, aminosalicylic acid, capreomycin, clofazimine, clysosercine, ethionamide, levofloxacine, rifabutin, rifapentine
Artemisinin resistance

• Reports of resistance emerging from Thai/Cambodia border
  ◦ Same location as first reports of antimalarial drug resistance 50 years ago

• Driven by
  ◦ Production and distribution of counterfeit drugs
  ◦ Substandard drug preparation methods
  ◦ artemisinin monotherapy
  ◦ Sale of artemisinin in single doses (requires 6 doses over 60 hours to be effective)
  ◦ Lack of prescription control

NEJM, 2009; 361: 455-67
Future directions

- **Increase new drug development**
  - Extend spectrum of existing drugs
  - Antisense and triplex technology
    - Prevent production of pathogenic protein
  - Antimicrobial peptides
    - Used by animals as defense against microbes

- **Improve drug distribution and production control**
  - Prescription controls in deregulated countries
  - Production controls to ensure that only legitimate, fully effective drugs are distributed
  - Video
    [http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/ucm134359.htm](http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/ucm134359.htm)