Detection and therapeutics

Detection

- Microorganisms can contaminate soil, air, water, fomites (plastic, wood, etc)
  - Viability best in soil, water, fomites, air
- Sampling and detection challenged with:
  - low concentrations
  - Environmental impurities/ inhibitors
  - Loss of sample due to processing efficiency

Contaminated Site Processing

- Sampling strategy development, collection, transportation, extraction and analysis
- Need to know background levels, decay or inactivation of organism, noncultivable (but viable) organisms and the detection limit of analysis technique
- Most commonly studied techniques: DNA extraction (environmental) recovery of organism from fomites, collection of organisms in air

Detection methods

- Category A agents reviewed and categorized by instrumental or environmental limit of detection
- Pure cultures used for validation to determine instrument detection limit
- Microorganisms spiked into environmental matrix processed determined environmental detection limit
- Biosensors, Real Time PCR, ELISA, PCR,

Features of Antimicrobial Drugs

- Selective toxicity
  - Antibiotics cause greater harm to microorganisms than to human host
    - Generally by interfering with biological structures or biochemical processes common to bacteria but not to humans
  - Toxicity of drug is expressed as therapeutic index
    - Lowest dose toxic to patient divided by dose typically used for treatment
      - High therapeutic index = less toxic to patient

Features of Antimicrobial Drugs

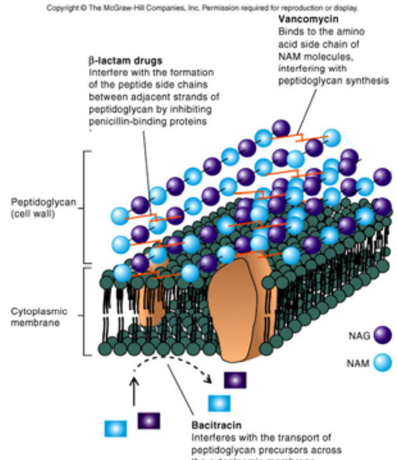
- Antimicrobial action
  - Drugs may kill or inhibit bacterial growth
    - Inhibit = bacteriostatic
    - Kill = bacteriocidal
  - Bacteriostatic drugs rely on host immunity to eliminate pathogen
  - Bacteriocidal drugs are useful in situations when host defenses cannot be relied upon to control pathogen

Features of Antimicrobial Drugs

- Spectrum of activity
  - Antimicrobials vary with respect to range of organisms controlled
    - Narrow spectrum
      - Work on narrow range of organisms
        - » Gram-positive only OR Gram-negative only
    - Broad spectrum
      - Work on broad range of organisms
        - » Gram-positive AND Gram-negative
      - Disadvantage of broad spectrum is disruption of normal flora

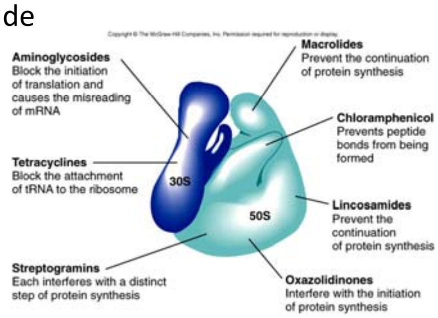
Mechanisms of Action of Antibacterial Drugs

- Inhibition of cell wall synthesis
  - Bacteria cell wall unique in construction
    - Contains peptidoglycan
  - Antimicrobials that interfere with the synthesis of cell wall do not interfere with eukaryotic cell
    - Due to the lack of cell wall in animal cells and differences in cell wall in plant cells
  - These drugs have very high therapeutic index
    - Low toxicity with high effectiveness
  - Antimicrobials of this class include
    - β lactam drugs: Penicillins
    - Vancomycin
    - Bacitracin



Mechanisms of Action of Antibacterial Drugs

- Inhibition of protein synthesis
  - Structure of prokaryotic ribosome acts as target for many antimicrobials of this class
    - Differences in prokaryotic and eukaryotic ribosomes responsible for selective toxicity
  - Drugs of this class include
    - Aminoglycosides
    - Tetracyclins
    - Macrolids
    - Chloramphenicol
    - Lincosamides
    - Oxazolidinones
    - Streptogramins

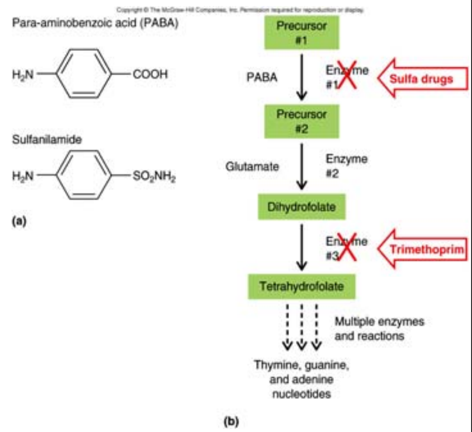


Mechanisms of Action of Antibacterial Drugs

- Inhibition of nucleic acids: fluorquinolones and Rifamycins
- Rifamycins
  - Block prokaryotic RNA polymerase
    - Block initiation of transcription
  - Rifampin most widely used rifamycins
  - Effective against many Gram (+) and some Gram (-) as well as members of genus *Mycobacterium*
  - Primarily used to treat tuberculosis and Hansen's disease as well as preventing meningitis after exposure to *N. meningitidis*
  - Resistance due to mutation coding RNA polymerase
    - Resistance develops rapidly

Mechanisms of Action of Antibacterial Drugs

- Inhibition of metabolic pathways
  - Relatively few
  - Most useful are folate inhibitors
    - Mode of actions to inhibit the production of folic acid
  - Antimicrobials in this class include
    - Sulfonamides
    - Trimethoprim



Mechanisms of Action of Antibacterial Drugs

- Interference with cell membrane integrity
  - Few damage cell membrane
    - Polymixin B most common
      - Common ingredient in first-aid skin ointments
  - Binds membrane of Gram (-) cells
    - Alters permeability
      - Leads to leakage of cell and cell death
    - Also binds eukaryotic cells but to lesser extent
      - Limits use to topical application

Determining Susceptibility of Bacterial to Antimicrobial Drug

- Susceptibility of organism to specific antimicrobials is unpredictable
- Often drug after drug tried until favorable response was observed
  - If serious infection, several drugs were prescribed at one time with hope that one was effective
- Better approach
  - Determine susceptibility
  - Prescribe drug that acts against offending organism
    - Best to choose one that affects as few others as possible

Determining Susceptibility of Bacterial to Antimicrobial Drug

- Acquisition of resistance
  - Can be due to spontaneous mutation
    - Alteration of existing genes
    - Spontaneous mutation called vertical evolution
  - Or acquisition of new genes
    - Resistance acquired by transfer of new genes called horizontal transfer

Determining Susceptibility of Bacterial to Antimicrobial Drug

- Slowing emergence and spread of resistance
  - Importance of an educated public
    - Greater effort made to educate public about appropriateness and limitations of antibiotics
      - Antibiotics have no effect on viral infections
      - Misuse selects antibiotic resistance in normal flora
  - Global impacts of the use of antimicrobial drugs
    - Organisms which develop resistance in one country can be transported globally
    - Many antimicrobials are available as non-prescription basis
    - Use of antimicrobial drugs added to animal feed
      - Produce larger more economically productive animals
      - Also selects for antimicrobial resistant organisms

Vaccines

Several types

- Toxoid-
  - Toxic components- tetanus/ diptheria
- Recombinant
  - Protein in a delivery vector, such as VSV that elicits immune response but does not cause disease
  - Example: Ebola virus (weakened VSV with Glycoprotein from EV)
- Killed
  - Virulent organism which has been inactivated- still can elicit an immune response
  - Example: polio, cholera, rabies, influenza
- Live (Attenuated)
  - Organism alive but missing key virulence components.
  - Can sometimes cause disease in immunocompromised individuals
  - Example: MMR, BCG (TB)

Vaccine development

- Long process
  - discovery, process engineering, toxicology and animal studies to human Phase I, II, and III trials.
  - Sometimes can take more than 10 years, depending on the disease.
  - Human trials:
    - Phase I- safety trials
    - Phase II- “target population” to determine both safety and the stimulation of immune response
    - Phase III: Efficacy trial to large target populations to establish whether a vaccine actually prevents a disease as intended.