CHAPTER 20
ANTIBIOTIC RESISTANCE

WHY IS THIS IMPORTANT?

- The most important problem associated with infectious disease today is the rapid development of resistance to antibiotics
- It will force us to change the way we view disease and the way we treat patients

OVERVIEW

Antibiotic Resistance

- Development of Antibiotic Resistance
- Mechanisms of Resistance
- Clinically Dangerous Resistance
- Resistance to Antivirals and Antiparasitics
- Hope for the Future: Development of New Antibiotics
- Time Line of Antibiotic Resistance
DEVELOPMENT OF ANTIBIOTIC RESISTANCE

- Microbes naturally produce antibiotics
- Bacteria have mechanisms to resist antibiotics
- Increasing amounts of antibiotics creates evolutionary pressure for antibiotic resistance

DEVELOPMENT OF ANTIBIOTIC RESISTANCE: Bacterial Growth and Mutation Rates

- The potential for mutation is considerable
- Bacterial cells that have developed resistance are not killed off when treated with the drug
  - They continue to divide
  - A resistant population is the result
DEVELOPMENT OF ANTIBIOTIC RESISTANCE:
Plasmids and Conjugation

- Bacteria have genes on plasmids
- Plasmids are transferred between bacterial cells and species via conjugation
  - Horizontal gene transfer
  - Resistance islands when genes integrate into the bacterial chromosome

DEVELOPMENT OF ANTIBIOTIC RESISTANCE:
Inappropriate Clinical Use of Antibiotics

- 60% of upper respiratory infections are viral
- Many patients will be given an antibiotic
- Antibiotics are antibacterial, not antiviral
- Unnecessary circulation of antibiotics
DEVELOPMENT OF ANTIBIOTIC RESISTANCE: 
Use of Antibiotics in the Food Chain

- Antibiotics commonly used as feed additives for food animals
- Antibiotics promote growth of food animals
- Such use also contaminates environment
- Creates evolutionary pressure for bacterial resistance

DEVELOPMENT OF ANTIBIOTIC RESISTANCE: 
Immunocompromised Patients

- An important social change is the increase in the number of people who are immunocompromised
  - Necessitates increased use of antibiotics
  - Fosters development of resistance

DEVELOPMENT OF ANTIBIOTIC RESISTANCE: Health Care Facilities

- Hospitals are ideal settings for the acquisition of resistance
  - A population of people with compromised health
  - A high concentration of bacteria, many of which are extremely pathogenic
  - Large amounts of different antibiotics are constantly in use
  - Increased use of antibiotics leads to resistance
DEVELOPMENT OF RESISTANCE: Lifestyle

- There are more large cities in the world today
  - Large numbers of people in relatively small areas
  - Passing antibiotic-resistant pathogens is easier
  - Many large urban populations have poor sanitation

- A person can travel anywhere in the world within 24 hours
  - Often travel with several or many other people in an enclosed space
  - A person infected with the resistant bacteria infects others
  - The process is repeated and the resistant bacteria spread

TIMELINE OF ANTIBIOTIC RESISTANCE

- Resistance to penicillin, streptomycin, chloramphenicol, and tetracycline soon after their introduction
- Almost every known bacterial pathogen has developed resistance to at least one antibiotic
- The more an antibiotic is used, the greater the chance of resistance
TIMELINE OF ANTIBIOTIC RESISTANCE

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<th>Antibiotic</th>
<th>Year Discovered</th>
<th>Year Resistance Observed</th>
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<td>Tobramycin</td>
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<td>1971</td>
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<td>Erythromycin</td>
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SUSCEPTIBILITY TESTING

- Involves culturing the bacteria and exposing it to antibiotics
- Determines the extent to which the drug affects the pathogen

SUSCEPTIBILITY TESTING

- Kirby-Bauer method (disk diffusion):
  - Zone of inhibition surrounds the disk
  - Pathogen described as sensitive, intermediate, or resistant to drug
  - Simple and inexpensive but inadequate for clinical purposes
SUSCEPTIBILITY TESTING

- E test:
  - Gradients of antibiotic on each strip
  - Determines minimal inhibitory concentration (MIC)
  - Lowest concentration that prevents growth

SUSCEPTIBILITY TESTING

- Broth dilution test:
  - Incubate pathogen in a series of wells containing decreasing amounts of antibiotic
  - Bacteria in wells with no growth recultured in antibiotic-free medium
  - Determines minimum bactericidal concentration (MBC)
MECHANISMS OF RESISTANCE

- Bacteria use several mechanisms to become antibiotic-resistant:
  - Inactivation of the antibiotic
  - Efflux pumping of the antibiotic
  - Modification of the antibiotic target
  - Alteration of the pathway

INACTIVATION OF ANTIBIOTIC

- Inactivation usually involves enzymatic breakdown of antibiotic molecules
- A good example is β-lactamase:
  - Secreted into the bacterial periplasmic space
  - Attacks the antibiotic as it approaches its target
  - There are more than 190 forms of β-lactamase

AmpC β-Lactamase

- The bacterial gene AmpC codes for β-lactamase
- Usually turned off and only turned on in the presence of molecule’s β-lactam ring
- Acquired by plasmids and transferred among bacterial species
**AMINOGLYCOSIDE-INACTIVATING ENZYMES**

- Aminoglycoside antibiotics include gentamicin
- Resistance to aminoglycoside antibiotics via enzymes that modify their structure
- Genes encoding these enzymes are found on plasmids, can be transferred among bacteria

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**EFFLUX PUMPING OF ANTIBIOTIC**

- Efflux pumps are found in the plasma membrane of all bacteria, and the outer membrane of Gram-negative bacteria
- Efflux pumping keeps the concentration of antibiotic in the cell below levels that would destroy the cell

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**EFFLUX PUMPING OF ANTIBIOTIC**

- Efflux pumps classified as narrow-spectrum or broad-spectrum
  - Broad-spectrum pumps work on more than one type of antibiotic
- Efflux pumps are active against:
  - β-lactams and fluoroquinolones
  - Greatest activity against tetracyclines
EFFLUX PUMPING OF ANTIBIOTIC

- Genes that code for efflux pumps are located on the chromosome, plasmids, and transposons
  - Readily acquired by non-resistant bacteria
  - Transforms them into resistant bacteria

MECHANISM OF EFFLUX PUMPS

- Efflux pumps have four mechanisms of operation
- Three of them use counterflow:
  - Antibiotic is pumped out
  - Cations are pumped in at the same time
  - Also used to remove antiseptic and disinfectant substances

- The fourth pump mechanism is one-way transport that consumes ATP
  - There is no simultaneous import of cations
  - It is relatively rare
TETRACYCINE EFFLUX PUMPS

- Efflux pumps coded for by the *Tet* gene family are in Gram-positive and Gram-negative bacteria
- Pumps are inducible and only made when tetracycline is present
- Tetracycline is pumped out of the cell and cations are imported

OTHER MECHANISMS OF KEEPING ANTIBIOTICS OUT

- Some bacteria reduce the permeability of their membranes as a way of keeping antibiotics out
  - They turn off production of porin and other membrane channel proteins
  - Seen in resistance to streptomycin, tetracycline, and sulfa drugs

MODIFICATION OF ANTIBIOTIC TARGET

- Bacteria can modify the antibiotic’s target to escape its activity
- Bacteria must change structure of the target but the modified target must still be able to function. This can be achieved in two ways:
  - Mutation of the gene coding for the target protein
  - Importing a gene that codes for a modified target
PENICILLIN-BINDING PROTEINS (PBPs)

- Bacteria have PBPs in their plasma membranes
  - These proteins are targets for penicillin
- MRSA (Methicillin-resistant *Staphylococcus aureus*) has acquired a gene (*mecA*) that codes for a different PBP
  - It has a different three-dimensional structure
  - It is less sensitive to penicillins
- MRSA is resistant to all β-lactam antibiotics, cephalosporins, and carbapenems
  - It is a very dangerous pathogen
    - Particularly in burn patients
- Production of insensitive PBPs is an example of operon function at the genetic level
  - Gene coding for an insensitive PBP is kept switched off by a repressor protein
  - Absence of the repressor protein allows insensitive PBP to be made
- Modified PBP does not attach to any penicillin molecules
  - Cell wall is constructed correctly, even in the presence of antibiotic
This type of antibiotic resistance can accumulate to very high levels

- When MRSA was treated with the fluoroquinolone ciprofloxacin:
  - Resistance increased from 5% to more than 85% in one year

-Streptococcus pneumoniae also modifies PBP
  - It can make as many as five different types of PBP
  - It does this by rearranging, or shuffling, the PDB genes
    - Referred to as genetic plasticity
    - Permits increased resistance

-Bacterial ribosomes are a primary target for antibiotics
  - Different antibiotics affect them in different ways
  - Resistance can be the result of modification of ribosomal RNA so it is no longer sensitive
  - Some organisms use target modification in conjunction with efflux pumps
    - Resistance is even more effective
ALTERATION OF A METABOLIC PATHWAY

- Some drugs competitively inhibit metabolic pathways
- Bacteria can overcome this method by using an alternative pathway

CLINICALLY DANGEROUS RESISTANCE: MRSA

- MRSA: Methicillin-resistant *Staphylococcus aureus*
  - Three or four resistance islands on the chromosome
  - 20+ additional gene clusters on plasmids
  - Approximately 7% of the total *S. aureus* genome codes for antibiotic resistance

- MRSA has different resistance mechanisms:
  - β-lactam antibiotic resistance
  - Erythromycin resistance (via ribosome modification)
  - Aminoglycoside resistance (via antibiotic-altering enzymes)
  - Tetracycline resistance (via efflux pumps)
**CLINICALLY DANGEROUS RESISTANCE: MRSA**

- MRSA infections originally seen in health care settings only
- Now common in the general community
- Spread by skin contact

**CLINICALLY DANGEROUS RESISTANCE: VREs**

- VREs: Vancomycin-resistant enterococci
- For example, *Enterococcus faecalis*
- Leading cause of endocarditis and indwelling catheter infections

**CLINICALLY DANGEROUS RESISTANCE: VREs**

- Genetic resistance to vancomycin involves five tandem genes working in sequence
  - Changes the structure of peptidoglycan so it is no longer affected by the antibiotic
- These resistance genes are easily transferred by plasmids or transposons
  - Resistance to vancomycin can rapidly spread
Bacteria that are part of the normal flora are becoming more dangerous due to resistance.

- *E. coli* is part of the normal flora of the large intestine.
- It has become more involved with systemic and localized infections.
- Antibiotic-resistant *E. coli* infections are now being seen throughout the world.

Re-emerging diseases were previously under control but are now posing clinical issues.

- Caused by bacteria resistant to antibiotics.
- Tuberculosis is now multi-drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB).

Antibiotics eliminate natural bacteria, allowing opportunistic pathogens to colonize.

- *Clostridium difficile* becomes a superinfection in the intestinal tract.
- Resistant to antibiotics and difficult to treat.
RESISTANCE TO ANTIVIRALS AND ANTIPARASITICS: Antiviral Resistance

- Progeny viruses have mutated resistance
- More likely in immunocompromised patients treated with long-term antiviral therapy
- Combination therapy (multiple drugs) used for HIV, HBV, and influenza
- Resistance to oseltamivir in 2009 H1N1

RESISTANCE TO ANTIVIRALS AND ANTIPARASITICS: Parasitic Resistance

- Malaria parasite is now resistant to all antimalarials
- Resistance to chloroquine
- Resistance to sulfadoxine-pyrimethamine
- Best treatment is combination therapies with artemisinin derivatives
DEVELOPMENT OF NEW ANTIBIOTICS

- DNA and RNA Analysis
- Structural Analysis
- Auxiliary Targets
- Automated Synthesis and Screening
- Virulence Factors
- Further Investigation of Known Antibiotic Compounds
- Phage Therapy

DEVELOPMENT OF NEW ANTIBIOTICS

- Bacterial genes that code for potential antibiotic targets can be identified by:
  - DNA sequencing
  - RNA microarray analysis

DEVELOPMENT OF NEW ANTIBIOTICS

- Bacterial structures that are potential antibiotic targets can be identified by
  X-ray crystallography
  - Bacterial ribosomes
  - Bacterial efflux pumps
DEVELOPMENT OF NEW ANTIBIOTICS

- Auxillary targets that may help antibiotics:
  - Proteins that assemble the waxy coat in *Mycobacterium tuberculosis*
  - RNA helicase proteins required for proper folding of the RNA molecule

DEVELOPMENT OF NEW ANTIBIOTICS

- By using computers:
  - Automated synthesis of molecules could produce new antibiotic compounds
  - Automated screening could evaluate 50,000 compounds in one day

DEVELOPMENT OF NEW ANTIBIOTICS

- New antibiotic targets could be virulence factors:
  - The lipid A component of the LPS layer in Gram-negative bacteria
  - Proteins that bacteria use to avoid destruction by phagocytic enzymes
DEVELOPMENT OF NEW ANTIBIOTICS

- Further investigation of known compounds could lead to development of new antibiotics
  - Lantibiotics, antibacterial peptides produced by Gram-positive bacteria
  - Defensins, antibacterial peptides produced as part of the innate immune response
  - Drosocin and apidaecin, antibacterial peptides produced by insects

DEVELOPMENT OF NEW ANTIBIOTICS

- Phage therapy:
  - Bacteriophage viruses attack only bacteria
  - Currently being investigated for therapeutic potential

TESTING OF ANTIBIOTICS

- Many compounds are antibacterial
- Therapeutic use requires selective toxicity
- Potential toxicity to host or side effects need investigation
- Cost of a new drug ranges from $100 million to $500 million (US) and five to ten years of testing and development