CHAPTER 19
ANTIBIOTICS

WHY IS THIS IMPORTANT?

- Antibiotics have drastically reduced the number of deaths due to infection.
- They have changed the face of health care.

OVERVIEW

Microbiology: A Clinical Approach © Garland Science
HISTORICAL PERSPECTIVES

- The discovery of the first antibiotic was an accident.
  - Alexander Fleming accidentally contaminated a plate with a fungus.
  - He observed a clearly defined region of no bacterial growth where the fungi had contaminated the plate.
  - The area around the fungus was eventually referred to as a zone of inhibition.

HISTORICAL PERSPECTIVES

- It is estimated that over 80 million prescriptions are written in America each year.
- 12,500 tons of antibiotics are produced annually.
  - 25-50% is fed to livestock to increase the rate of weight gain.
- From 1900 to 1980, mortality from infectious diseases dropped from 797 per 100,000 persons to 36 per 100,000 persons.
HISTORICAL PERSPECTIVES

- No major discoveries of natural antibiotic substances have occurred for several years.
  - Efforts have now shifted to modifying existing antibiotics.
  - Searching in new places for potential antibiotics has also gained in prominence.

ANTIBIOTICS ARE PART OF BACTERIAL SELF PROTECTION

- Many antibiotics are produced by microorganisms as part of their survival mechanism.
  - They keep other organisms away.
  - They protect the supply of nutrients and oxygen.

- Microorganisms that produce these substances have molecular mechanisms to control production and prevent self-destruction.
  - Naturally produced antibiotics are products of secondary metabolic pathways.
    - These pathways are not turned on all the time.
    - Continuous production could adversely affect the organism.
**ANTIBIOTICS ARE PART OF BACTERIAL SELF PROTECTION**

- Organisms protect themselves in several ways:
  - Some bacteria restrict antibiotic production to the stationary phase.
  - Others keep the intracellular concentrations at low levels.
    - They regulate rates of production and export.

- Antibiotic molecules are exported in an inactive form.
  - They become activated by extracellular enzymes.
  - Some microorganisms modify their own cell walls to ensure their safety.

**ANTIBIOTIC SPECTRA**

- The first molecules that inhibited bacterial growth were natural products.
- Over time, these natural molecules have been modified.
- Several types of semi-synthetic antibiotics have been derived from these molecules.
**ANTIBIOTIC SPECTRA**

- The original natural molecules used by humans as antibiotics have a very narrow spectrum.
  - Penicillin activity is restricted to Gram-positive bacteria.
- Natural molecules can be chemically modified making it possible to broaden their spectrum.
- Antibiotics are classified as either broad-spectrum or narrow-spectrum.

**PENICILLINS**

- The structure of penicillin is useful as a template for the development of an entire group of antibiotics (more than 50 so far).
- In its native form, penicillin is composed of a core four-sided ring structure.
  - The beta (β)-lactam ring
PENICILLINS

- All forms of penicillin contain this ring.
- Derivatives contain additional specific structures:
  - Side chains attached to the ring
  - Chemically changing the side chain can change:
    - Antimicrobial activity
    - Resistance to stomach acid
    - Overall half-life in body

- There are semi-synthetic forms of penicillin.
  - They are created through modifications that can be made in a laboratory.
  - Chemists can create and modify side chains.
    - This produces new forms of penicillin.
**PENICILLINS**

- Natural penicillin has a very narrow spectrum.
- Chemically modifying penicillin broadens the spectrum.
- Semi-synthetic penicillins can be further modified to increase the efficiency of inhibiting bacterial growth.
- Ampicillin can be modified to mezlocillin or azlocillin.

**CEPHALOSPORINS**

- The same kind of manipulation can be seen with cephalosporin family.
- Modification of the natural molecule has resulted in several generations of semi-synthetics.
- Modifications result from changing the side chains leaving core intact.
- Modifications also change reactivity patterns and spectrum.

**RESISTANCE TO ANTIBIOTICS**

- Bacteria are constantly finding ways to counteract antibiotics.
- One of the most important bacterial defense mechanisms is the production of enzyme β-lactamase.
  - It cleaves open the β-lactam ring.
  - It inactivates the molecule.
- Organisms that produce β-lactamase are resistant to penicillin.
RESISTANCE TO ANTIBIOTICS

- One way to overcome penicillin resistance is to combine penicillin drug with molecule that protects the penicillin.
  - This diminishes or impedes β-lactamase activity.
  - An example of this is potassium clavulanate.

RESISTANCE TO ANTIBIOTICS

- Several combinations have been developed:
  - Amoxicillin + potassium clavulanate = Augmentin® or Timentin®
  - Imipenem + cilastatin = Primaxin®
  - Ampicillin + sulbactam = Unasyn®

ANTIBIOTIC TARGETS

- A fundamental criterion of antibiotics for medical use is selective toxicity.
  - The antibiotic should be destructive to the disease-causing organism but have no effect on the human host.
  - The first antibiotic discovered was most selectively toxic.
ANTIBIOTIC TARGETS

- Many chemicals are useful in restricting bacterial growth.
  - They are also inherently toxic.
  - They cannot be used therapeutically.
- Many antibiotic molecules are toxic if administered at high concentrations.
- Toxicity necessitates extensive testing.
  - It can take years and cost millions of dollars.

ANTIBIOTIC TARGETS

- Antibiotic targets can be subdivided into five major groups:
  - The bacterial cell wall
  - The bacterial plasma membrane
  - Synthesis of bacterial proteins
  - Bacterial nucleic acids
  - Bacterial metabolism
BACTERIAL CELL WALL

- It is the most appealing target for antibiotics.
  - Found in bacteria but not humans
  - Meets the criterion of selective toxicity.
- It is found in both Gram-positive and Gram-negative bacteria.

BACTERIAL CELL WALL

- The cell wall is built by many enzymatic reactions.
  - These enzymes can be used as targets of antibiotic molecules.
- The cell wall is made up of the peptidoglycan molecules NAG and NAM.
  - They are cross-linked through activity of transglycosylase and transpeptidase enzymes.
  - Many antibiotics inhibit the activity of these two enzymes.
    - Results in improper cell wall cross-linking
    - Organism is not able to withstand environmental pressures.

BACTERIAL CELL WALL: Penicillins

- Penicillin-binding proteins (PBPs) are involved in the construction of the cell wall.
- β-lactam ring of penicillin binds to these proteins.
- New cell wall continuously built during active growth
  - Penicillin prevents the formation of an intact cell wall.
  - Penicillin is most effective during this phase.
BACTERIAL CELL WALL: Penicillins

- Gram-negative bacteria have markedly less peptidoglycan.
  - They are normally less sensitive to penicillin.
- Reactivity of penicillin against Gram-negative bacteria has been enhanced by synthetically modifying the core structure.

BACTERIAL CELL WALL: Cephalosporins

- Cephalosporins have similar activity to penicillins.
  - They prevent the construction of a stable cell wall.
- Cephalosporins have a much greater affect on Gram-negative bacteria than penicillins.
  - They are naturally broader spectrum antibiotics.
  - They are not susceptible to the β-lactamase enzymes.

- There are multiple generations of cephalosporins.
  - More than 70 versions are in use.
  - They are one of the most widely prescribed antibiotics.
- Mechanism of action similar to that of penicillin but cephalosporin penetrates through porin channels.
BACTERIAL CELL WALL: 
Cephalosporins

- Side chains can be modified to increase the spectrum of reactivity.
- Cephalosporins are frequently used both preoperatively and postoperatively.
  - Frequent use has increased resistance.

BACTERIAL CELL WALL: 
Carbapenems

- Carbapenems contain a β-lactam ring like penicillin.
  - They also inhibit the synthesis of bacterial walls.
- The β-lactam ring of carbapenems contains a double bond.
  - This prevents β-lactamase cleaving the ring.
- Carbapenems have a very broad spectrum of antibacterial activity.
- Two are approved for clinical use in humans.
  - Both are useful against *Pseudomonas* species

BACTERIAL CELL WALL: 
Monobactams

- Monobactams have a different ring structure.
  - They cannot be recognized by β-lactamase.
  - They are effective in overcoming bacterial resistance.
BACTERIAL CELL WALL: Glycopeptide Antibiotics

- Glycopeptide antibiotics are derived from Streptomyces organisms.
  - Vancomycin is a glycopeptide antibiotic.
- Glycopeptide antibiotics have serious side effects.
  - Toxicity level reduced in recent years by improving purification.
- They inhibit cell wall synthesis by forming a complex with the substrates that make up peptidoglycan.
- They cannot penetrate the porins of Gram-negative cells.
  - Narrow spectrum antibiotics restricted to Gram-positive bacteria.

BACTERIAL CELL WALL: Glycopeptide Antibiotics

- Glycopeptide antibiotics work on different parts of peptidoglycan to penicillin.
- There are now *S. aureus* strains resistant to vancomycin.
  - Referred to as VRSA
  - These organisms are very dangerous.

BACTERIAL CELL WALL: Isoniazid and Ethambutol

- These antibiotics are used against bacteria with modified cell walls.
- *Mycobacterium* species (cause TB and leprosy) are a good example.
  - Their cell walls are modified by incorporation of mycolic acids.
- Isoniazid very effective against these organisms.
  - Inhibits the synthesis of mycolic acid.
**BACTERIAL CELL WALL:**

**Isoniazid and Ethambutol**
- Ethambutol is given in concert with isoniazid.
  - Inhibit the incorporation of mycolic acid into cell wall
- The treatment of choice for tuberculosis is a combination of isoniazid, ethambutol, and rifampin.
  - Combinations of drugs lower the potential for development of resistance

**BACTERIAL CELL WALL:**

**Polypeptide Antibiotics**
- Used topically for superficial infections by Gram-positive organisms.
  - *Staphylococcus* and *Streptococcus*.
- They inhibit binding between NAG and NAM.
  - Prevents formation of linear strands of peptidoglycan

**BACTERIAL PLASMA MEMBRANE**
- The plasma membrane is involved with important physiological functions.
  - It is a prime target for antibiotics.
  - Any disruption of the membrane destroys the bacteria.
- Unfortunately the structure of the bacterial plasma membrane is similar to the eukaryotic plasma membrane.
  - This does not allow for selective toxicity.
SYNTHESIS OF BACTERIAL PROTEINS

- Disruption in the production of protein is devastating to a bacterial cell.
- Ribosomes of prokaryotes are not the same as those in the cytoplasm of eukaryotes.
  - This allows for selective toxicity.

SYNTHESIS OF BACTERIAL PROTEINS

- Eukaryotic cells have mitochondria that contain ribosomes.
  - These are the same as the ribosomes in prokaryotic cells.
  - There is antibiotic interference in eukaryotic cell function if antibiotics given in excessive amounts.

SYNTHESIS OF BACTERIAL PROTEINS: Antibiotic Action

- Antibiotics act at different sites on bacterial ribosomes.
  - Spectinomycin, kanamycin, streptomycin, and tetracycline target the 30S subunit.
  - Clindamycin, chloramphenicol erythromycin, clarithromycin, azithromycin target the 50S subunit.
  - Some antibiotics interfere in peptide elongation.
  - Some antibiotics interfere with decoding the message.
SYNTHESIS OF BACTERIAL PROTEINS: Antibiotic Action

- Some antibiotics (streptomycin) upset accuracy of the translation.
- Chloramphenicol totally blocks binding of tRNA.
- Erythromycin blocks the approach to the peptide exit tunnel.
  - Also blocks assembly of the 50S subunit

SYNTHESIS OF BACTERIAL PROTEINS: Synercid®

- *Streptomyces* species make pristinamycin and streptogramin.
  - They work synergistically to inhibit translation at the 50S subunit.
  - Both have been synthetically modified.
  - They make up the antibiotic Synercid®.
    - Approved for the treatment of vancomycin-resistant enterococci

SYNTHESIS OF BACTERIAL PROTEINS: Tetracyclines

- Tetracyclines have been used since the late 1940s.
  - They are bacteriostatic.
  - They block the arrival of tRNA at the A site.
  - They have been in use for so long many bacteria are resistant.
    - Use has steadily declined
SYNTHESIS OF BACTERIAL PROTEINS: Aminoglycosides

- Aminoglycosides have been in use since the 1940s.
  - They target the 16S RNA portion of the 30S ribosomal subunit.
  - Gentamicin is potent against Gram-negative organisms.
  - It is not very effective against Gram-positive bacteria.
  - It is used in combination with β-lactam antibiotics.
  - Produces significant renal toxicity and ototoxicity.

SYNTHESIS OF BACTERIAL PROTEINS: Linezolid

- Linezolid is a totally synthetic antibiotic.
  - Blocks protein synthesis by occupying the P site
  - Very active against Gram-positive bacteria
  - Very active against vancomycin-resistant enterococci

BACTERIAL NUCLEIC ACIDS

- DNA and RNA are universal components.
  - Their structure in bacteria is no different from their structure in humans.
  - This does not allow for selective toxicity.
  - Two families of synthetic compounds can target bacterial nucleic acids.
  - Rifamycins and quinolones
  - Both act against DNA replication and DNA repair.
**BACTERIAL NUCLEIC ACIDS: Quinolones**

- Quinolones target bacterial topoisomerases.
  - Bacterial topoisomerases are different to those in eukaryotic cells.
  - They are an excellent target.
  - Block the movement of the replication fork
  - Used in the treatment of:
    - Urinary tract infections
    - Osteomyelitis
    - Community-acquired pneumonia and gastroenteritis
    - Anthrax

**BACTERIAL NUCLEIC ACIDS: Rifamycins**

- Rifamycins bind to RNA polymerase and prevent it from functioning.
  - Binding occurs away from the active site.
  - Blocking RNA polymerase means no protein synthesis.
    - This is lethal.
  - Rifampin is the only rifamycin in use.
    - It is used only in combination therapy.
    - Resistance develops rapidly if it is used alone.

**BACTERIAL METABOLISM**

- Two targets for inhibiting bacterial growth are:
  - Production of nucleic acid precursors
  - Metabolic pathways that occur at the plasma membrane.
- Several pathways exclusive to bacteria:
  - Interruption selectively inhibits bacterial growth.
  - This allows for selective toxicity.
BACTERIAL METABOLISM: Folic acid

- A good example is the metabolism of folic acid.
- One of the intermediates in the folic acid pathway is para-aminobenzoic acid (PABA).
  - Normal enzyme action incorporates PABA into the pathway.
  - Sulfa drugs competitively inhibit this activity:
    - The enzyme is fooled into incorporating the sulfa molecule.
    - Incorporation of sulfa stops the pathway.
    - This is a lethal event.

- Action against the folic acid pathway has selective toxicity.
  - Bacteria synthesize folic acid.
  - Humans obtain folic acid through diet.
**BACTERIAL METABOLISM:**

**Folic acid**

- Sulfa drugs have been in use longer than any other antibacterial agent.
- Sulfamethoxazole is usually used in combination with trimethoprim to treat urinary tract infections.
  - Both of these drugs block a step in folic acid metabolism.
  - These drugs been used for a long time.
    - Bacterial resistance continues to increase.
    - Their effectiveness continues to decrease.

**ANTIVIRAL DRUGS**

- Viruses pose a different set of problems for antibiotic therapy.
  - They are obligate intracellular parasites.
  - Drugs that can eliminate the virus are dangerous to non-infected cells.
    - This makes selective toxicity difficult.
  - Many viruses difficult to grow.
    - It is difficult to test potential antiviral drugs.

- Many acute viral infections have a short duration.
  - They are essentially over before antibiotics could be of any therapeutic use.
  - The lack of rapid tests means it is difficult to differentiate between various viral infections.
  - Successful antiviral drugs must eliminate all virions.
    - The escape of even one virion could restart the infectious cycle.
ANTIVIRAL DRUGS

- The first antibiotic to be used against viruses was the sulfa drug derivative thiosemicarbazone.
- In 1960s amantadine developed for use against influenza.

ANTIVIRAL DRUGS:

Acyclovir

- Acyclovir is a specific and nontoxic drug.
- It is highly effective against both genital and oral herpes simplex infections.
  - Also been used with some success in treatment of varicella-zoster (chickenpox and shingles).
- It can be taken intravenously or orally or used topically.

ANTIVIRAL DRUGS:

Acyclovir

- Acyclovir is a nucleoside analog of guanine produced as a prodrug.
  - It is activated by enzymes once in the patient’s body.
  - These enzymes are found only in infected cells.
  - Acyclovir has selective toxicity.
- It works by blockade and termination of viral DNA replication.
- New variations (Valtrex® and Famvir® ) are very effective and widely used.
**ANTIVIRAL DRUGS:**

**Ganciclovir**

- Ganciclovir is a derivative of acyclovir.
  - Developed to treat cytomegalovirus infections
  - A less toxic oral derivative has been produced.
  - Used effectively for cytomegalovirus in immunocompromised patients

**Foscarnet**

- Foscarnet acts against DNA replication by inhibiting:
  - The binding site of hepatitis B viral DNA polymerase
  - HIV reverse transcriptase.
- It is used to treat herpes infections.

**Ribavirin**

- Ribavirin is a nucleoside analog and is highly toxic.
  - The mechanism of action is still unclear.
  - It has been used for Lassa fever and Hantavirus infections.
**ANTIVIRAL DRUGS:**

Amantadine

- Amantadine has been around for some time.
  - The first highly specific potent antiviral agent used against influenza A
  - Targets a viral protein and inhibits uncoating.
- Influenza A virus frequently mutates the protein target of amantadine.
  - The virus becomes resistant.
- Influenza B does not contain the target protein and is unaffected by amantadine.

**ANTIFUNGAL DRUGS**

- The emergence of diseases that render a host immunocompromised has led to increased secondary fungal infections.
- Drugs used for fungal infection have selective toxicity issues.
  - Fungi are eukaryotes.
  - Attacking common targets can cause serious side effects.

**ANTIFUNGAL DRUGS:**

Polyenes

- Polyenes are produced by the soil bacterium *Streptomyces*.
- They interact with sterols and increase the permeability of the plasma membrane.
- They must be used with caution because of side effects.
  - Amphotericin B has high renal toxicity.
ANTIFUNGAL DRUGS:
Azoles

- Azoles (imidazole and triazole) inhibit the production of sterols.
- Clotrimazole and miconazole are derivatives of imidazole.
  - Sold without a prescription
  - Routinely used topically against athlete’s foot and vaginal yeast infection

Ketoconazole is a broad spectrum derivative used for systemic fungal infections.
  - Can be taken orally
  - Less toxic than amphotericin B
- Fluconazole and itraconazole are the least toxic azoles.
  - Widely used for systemic fungal infections

ANTIFUNGAL DRUGS:
Griseofulvin

- Griseofulvin is produced by a species of the fungus *Penicillium*.
- It is administered orally and is effective for superficial fungal infections.
- It seems to react with keratin.
  - This blocks the formation of microtubules.
  - This inhibits mitosis in the fungi.
ANTIFUNGAL DRUGS:
Other Antifungal Antibiotics

- Flucytosine interferes with DNA and RNA synthesis.
  - It is taken up preferentially by fungi.
  - Has a high level of toxicity in kidney and bone marrow.
- Pentamidine seems to bind to fungal DNA.
  - Used in the treatment of *Pneumocystis* pneumonia.

DRUGS FOR PROTOZOA

- The development of drugs for parasitic infections has lagged behind.
  - Parasitic infections do not occur often in developed nations.
  - There is no money in it.
- Two widely used anti-parasitic drugs are:
  - Quinine
  - Metronidazole.

DRUGS FOR PROTOZOA:
Quinine

- Quinine has been used as a treatment for malaria since the 1600s.
- It has been chemically modified into several synthetic forms.
  - Chloroquine has been widely used.
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<th>DRUGS FOR PROTOZOA: Quinine</th>
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<td>With use of a drug, the malarial parasite develops resistance.</td>
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<td>Other quinine derivatives are made:</td>
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<td>- Mefloquine</td>
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<td>- Diiodohydroxyquin</td>
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<tr>
<td>- Used for treatment of intestinal amebic disease</td>
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<td>- Found to be toxic to the optic nerve.</td>
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<th>DRUGS FOR PROTOZOA: Metronidazole</th>
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<tr>
<td>Metronidazole is one of the most widely used anti/protozoan drugs.</td>
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<td>- Sold under the name Flagyl®</td>
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<td>- It is the drug of choice for:</td>
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<td>- Vaginitis resulting from <em>Trichomonas vaginalis</em></td>
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<td>- Giardiasis</td>
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<td>- Amebic dysentery.</td>
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<td>- It interferes with anaerobic metabolism.</td>
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<th>DRUGS FOR HELMINTHS</th>
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<td>Anti-helminthic drugs have also been largely ignored until recently.</td>
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<td>- Affected populations were not found in developed countries.</td>
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<td>- The popularity of sushi has led to an increase in tapeworm infestations.</td>
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<td>- Increased world travel has also increased helminth infections.</td>
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DRUGS FOR HELMINTHS:
Tapeworm and Flukes

- Niclosamide is the choice of treatment for these infections.
  - Inhibits the production of ATP
- The broad-spectrum anti-helminthic praziquantel is also effective against tapeworms.
  - Increases the permeability of plasma membranes
- Praziquantel is also the drug of choice for fluke diseases.
  - It induces muscle spasms.
  - This exposes antigenic sites for attack by the host immune system.

DRUGS FOR HELMINTHS:
Pinworm and Ascariasis

- Mebendazole is used against these infections.
  - It disrupts microtubule formation.
  - This affects the motility of the worm.
- Pyrantel pamoate and ivermectin are also used.
  - They paralyze the worm.
  - This induces the worm to exit the body.

DEVELOPMENT OF NEW ANTIBIOTICS

- Most of the naturally occurring antimicrobial products have already been found.
- We are left with synthetic modification of existing drugs.
- A promising method for new antibiotics is to identify novel microbial structures or functions.
  - These can be used as potential new targets.
  - DNA mapping can identify genes coding for products that could be targets.
NEW TARGETS FOR BACTERIA: Chromosome Mapping

- Mapping and analysis of bacterial chromosomes identifies open reading frames.
  - Some are seen only in prokaryotes.
  - These could code for essential components.
  - These components could then be used as targets.

NEW TARGETS FOR BACTERIA: Microarray Analysis

- There are other routes for identifying new targets.
- Microarray analysis uses messenger RNA.
  - The most abundant copies are identified.
  - The products they code for may be required for survival.
  - These could be potential new targets.

NEW TARGETS FOR BACTERIA: Auxiliary Targets

- Molecular biological techniques are used to look for auxiliary targets.
  - Targets that may be associated with old targets
  - Bacterial ribosome can also be re-examined.
  - Blockading the channels would be a lethal event
  - Bacterial efflux pumps can also be similarly targeted.
**NEW TARGETS FOR BACTERIA: Virulence Factors**

- Virulence factors or microbial survival mechanisms are possible targets.
  - Inactivation of proteins used to avoid destruction by phagocytic enzymes

**NEW TARGETS FOR BACTERIA: Peptide Fragments**

- New techniques allow rapid efficient production of synthetic molecules that might be antibacterial.
- Rapid screening of potential antibacterial compounds is performed by:
  - Computer analysis of bacterial genomes
  - Computer-generated construction of chemical fragments.

**NEW TARGETS FOR BACTERIA: Peptide Fragments**

- Several antibiotic compounds are composed of peptide fragments and disrupt the bacterial plasma membrane.
  - Bacitracin, gramicidin S, and polymyxin B
  - Maginins – produced by frogs
  - Defensins – produced by humans
- So far, over 500 known peptides produced by multicellular organisms have antibiotic actions.
NEW TARGETS FOR VIRUSES

Molecular technologies are also used for development of antiviral drugs.
- Target-based screening permits a high throughput of possible compounds.
  - Used with bioinformatics, combinatorial chemistry, computer-based design
  - Can evaluate as many as 50,000 compounds in single day
  - All compounds can be stored in libraries for future use

NEW TARGETS FOR VIRUSES

- Combinatorial chemistry can produce thousands of compounds in a day including:
  - All possible combinations of a basic set of modular components
  - These can be combined into testing programs that search for potential hits.

NEW TARGETS FOR VIRUSES

- Genetic and genome sequencing are also used.
  - Many viral genomes completely sequenced
  - High-density arrays of DNA fragments are used to assess which genes are expressed
  - Identification of gene sequences always switched on points to possible targets
THE COST OF RESEARCH AND DEVELOPMENT

- The costs of drug development are not trivial even though thousands of compounds can be rapidly identified.
  - Thousands of promising compounds may yield only one candidate.
  - Rigorous and expensive testing is required before a new drug can be brought to market.

THE COST OF RESEARCH AND DEVELOPMENT

- Candidate compounds have to pass tests for:
  - Toxicity
  - Allergic effects
  - Mutagenicity
  - Carcinogenicity
- The estimated cost of a new drug is between $100 million to $500 million.
- Development can take as long as 5-10 years.

TESTING OF ANTIBIOTICS

- Development of new antibiotics follows a very stringent highly regulated pathway.
  - Selective toxicity required to keep the public safe
  - The need for product safety two-edged sword.
    - It requires a great deal of time and money.
TESTING OF ANTIBIOTICS

- Several testing systems can be used to evaluate new compounds.
  - Kirby-Bauer is the most widely used.
  - The E-test is more advanced.

TESTING OF ANTIBIOTICS: Kirby-Bauer Test

- An agar plate is covered with known pathogen.
- Filter-paper disks impregnated with known concentrations of the compound.
  - They are placed on agar.
  - Zones of inhibition can be identified.
- The method is also used to compare the relative effectiveness of different compounds.
  - Large zone of inhibition does not necessarily mean a more powerful compound.
  - Differences in diffusion rates of similar compounds
  - Zones are evaluated using standardized tables.
TESTING OF ANTIBIOTICS: Kirby-Bauer Test

- The resistance of specific organisms can be classified as:
  - Sensitive
  - Intermediate
  - Resistant
- The Kirby-Bauer test is inadequate for most clinical purposes.

TESTING OF ANTIBIOTICS: E-test

- The E test is a more advanced diffusion test.
  - Permits determination of the minimal inhibitory concentration (MIC).
    - The lowest antibiotic concentration that prevents growth
  - Uses plastic-coated strips containing gradients of antibiotic concentrations.
    - After incubation MIC can be read from the scale
TESTING OF ANTIBIOTICS: Broth Dilution Test

- Kirby-Bauer and E-test show which compounds inhibit pathogen growth.
  - They cannot determine between microbicidal and microbistatic.
- The broth dilution test used for this purpose.
  - Permits determination of the minimal bactericidal concentration (MBC)

A specific organism is incubated in decreasing amounts of antibiotic.
- Wells that show no growth are recultured in nutrient broth medium.
- Growth in this medium indicates the test compound is microbistatic.
- No growth in this medium indicates the test compound is microbicidal.

Dilution tests are highly automated.
- Additional testing uses colorimetric methods
  - Used to determine serum concentrations
  - Important because drugs are toxic at high concentrations