5. BACTERIAL GENETICS

5.1 DNA: THE GENETIC MATERIAL

The bacterial cell is the smallest self-contained living entity governed by genetic information, deoxyribonucleic acid (DNA). Bacteria do not possess a nucleus like eukaryotes, but a structure called a **nucleoid,** which lacks a nuclear membrane and appears by electron microscopy to be free of ribosomes. Genetic studies, as well as electron microscopic analysis, have shown that most prokaryotes possess one giant, covalently closed, circular chromosome.

5.1.1 Plasmids

Plasmids are a diverse group of extrachromosomal genetic elements. Like the bacterial chromosomal DNA, they can autonomously replicate and as such are referred to as **replicons**. Plasmids are usually circular double-stranded molecules of DNA. However, *Borrelia burgdorferi*, the causative agent of Lyme disease, and the related *B. hermsii* are unique among all eubacteria in that they possess linear plasmids. Plasmids usually do not encode essential functions for bacteria and are thus unnecessary for the growth of the microorganism. Plasmids do, however, carry additional genetic information that is responsible for the appearance of new phenotypic properties in a bacterial cell. For example, plasmids **(R plasmids) may confer high levels of antibiotic resistance and encode the production of bacteriocins or toxins, as well as contain genes that may provide the bacteria a unique advantage in metabolizing some substrates. Over 90% from hospital acquired strains present plasmidic resistance: *Escherichia, Salmonella, Shigella, Proteus, Providencia, Klebsiella, Serratia, Pseudomonas, Acinetobacter, Vibrio, Yersinia, Pasteurella, Campylobacter, Haemophilus, Neisseria, Bacteroides, Staphylococcus, Streptococcus, Bacillus, Clostridium, Corynebacterium.*** Plasmides in Gram negative bacteria are bigger than in Gram positive bacteria.

Large plasmids, such as the **fertility factor F** found in *E. coli* or the resistance transfer factor (RTF) are often able to mediate their own transfer from one cell to another by a process called **conjugation** (see the following). These conjugative plasmids encode all the necessary factors for their transfer. Some smaller plasmids that are not conjugative can, however, be mobilized, meaning that they possess the necessary sequences to allow their transfer but they do not themselves encode for the necessary transfer proteins. Finally, some plasmids are more sedentary and do not transfer at all. **Plasmids can be transferred into a bacterial cell by means other than conjugation, such as transformation, transduction, or incorporation in the cellular chromosome** as discussed later.

Damage to DNA

Since DNA conveys genetic information, there is no molecule in a living organism whose integrity is as vital to the cell as is its DNA. Cells must be able to replicate DNA very accurately. Furthermore, accidental damage to DNA must be minimized by the elaboration of efficient DNA repair systems. These damage-containment systems are so important for the life of a cell that a bacterium may devote a large percentage of its genome to specify and control the enzymes involved.

5.1.2 Mutations Affecting the DNA

A mutation can be defined as any change in the base sequence of the DNA. Many types of mutations affect a single base. Such mutations have no effect on the replication and transcription processes. However, base substitutions responsible for DNA sequence changes exert damaging effects on the next generation, since any changes in the DNA sequence are transferred from parent to daughter cell.

Many mutations occur spontaneously in nature; however, mutations can also be induced by **physical or chemical agents**. Among the physical agents used to induce mutations in bacteria are: heat (which results in deamination of nucleotides), ultraviolet light (which causes pyrimidine dimer formation)

and ionizing radiation such as x-ray (which may be responsible for opening a ring of a base or single- or double-strand breaks in the DNA).

Chemical agents that also are mutagens can be grouped into three classes. The first class consists of nucleotide base analogues. A second class of chemical mutagens includes the frameshift mutagens, which usually cause the addition or deletion of a single base.

1.2 GENE EXCHANGE IN PROKARYOTIC CELLS

The exchange of genetic material between bacterial cells may occur by one of three mechanisms:

1. **transformation,** which results in the acquisition of new genetic markers by the incorporation of exogenous or foreign DNA;
2. **transduction**, which is the transfer of genetic information from one bacterium to another by a bacteriophage;
3. **conjugation**, which is the mating or quasi-sexual exchange of genetic information from one bacterium (the donor) to another bacterium (the recipient). Conjugation occurs with most, if not all, eubacteria. Conjugation usually occurs between members of the same species but has also been demonstrated to occur between prokaryotes and cells from plants, animals, and fungi.

Many bacteria, especially many pathogenic bacterial species, are quite promiscuous, meaning that the bacteria frequently exchange DNA. This genetic information exchange produces additions to the recipient genome. The transferred DNA can be either integrated into the recipient chromosome or stably maintained as an extrachromosomal element (plasmid) and passed on to daughter bacteria as an autonomously replicating unit.

5.2.1 Transformation

Transformation was the first mechanism of genetic transfer to be discovered in bacteria. **In 1928 Frederick Griffith** first observed transformation in the study of pneumococcal infection of mice. Griffith, an English microbiologist, made the observation that pneumococcus virulence was related to the presence of a surrounding polysaccharide capsule. The bacteria possessing a polysaccharide capsule appeared smooth (S) when grown on agar plates and possessed the ability to kill mice. In comparison, colonies appearing with rough edges (R) were unencapsulated and nonlethal. He then discovered that a mixture of killed S plus live R bacteria was lethal. When bacteria were isolated from this lethal mixed infection, it was found that the R bacteria had been replaced or "transformed'" to S bacteria, now able to make a virulent capsular polysaccharide. His studies led to the identification of DNA as the transforming principle some 15 years later by Oswald Avery, Colin MacLeod, and Maclyn McCarty.

Both gram-positive and gram-negative bacteria can take up and stably maintain exogenous DNA. Transformation systems can be classified in two groups. The first group includes those systems in which competence occurs naturally, competence being the ability of a cell to interact with exogenous DNA, leading to its uptake. Naturally occurring competence has been described for bacteria, including *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus* species, and *Neisseria* species.

5.2.2 Conjugation

Genetic transfer in *E. coli* was first reported by Lederberg and Tatum in 1946, when they observed sexlike exchange between two mutant strains of *E. coli* K12. It was later demonstrated that conjugation requires cell-to-cell interaction and that the exchange of genetic material is always unidirectional, with the genetic information moving from male to female cells. Many different conjugative plasmids have been found in several gram-negative bacterial species such as *E. coli* and *Bacteroides* species, as well as in some gram-positive bacteria including *Streptococcus*, *Streptomyces*, and *Clostridium*. Many of these large conjugative plasmids specify colicins or antibiotic resistances. One of the best known conjugative plasmids, called the **fertility factor or F**, is 94 kb in size and is found in *E. coli*. The **male donor is defined as F^+ the female recipient cell is termed F^-.** Many of the F^-like plasmids that are found in gram-negative bacteria exhibit a conjugative mechanism very similar to the F plasmid.

The conjugation process, as described here for the F plasmid, can be divided into four stages. **Conjugation initiates** with contact between the donor (F^+) and recipient cells (F^-). The F plasmid is responsible for the synthesis of sex-specific pili; these pili are probably involved in the recognition of suitable recipient cells and thus allow a close interaction between donor and recipient cells. The F pilus may also cause **wall-to-wall contact** between the pairs by retraction, leading to the formation of a **cytoplasmic bridge**. These extensive surface contacts may generate a signal that results in the initiation of plasmid replication and DNA transfer. The DNA that is transferred by conjugation is not a double helix but a single-stranded molecule. Finally, the transferred single-stranded DNA is **recircularized** and its complementary strand synthesized. The F plasmid is defined as conjugative because it carries all the genes necessary for its own transfer. An important property of F is its ability to integrate into the bacterial chromosome, generating a **Hfr cell or a high frequency of recombination.** Such integration of the F plasmid involves breakage and rejoining of both molecules of DNA. Analysis of some of the Hfr strains indicated that they result from a recombination process between a homologous insertion sequence present on both the F plasmid and the chromosome. However, since F is integrated into the chromosome, only part of the plasmid sequence is initially transferred, followed by chromosomal DNA and sometimes, but only rarely, the remainder of the F plasmid. It would take 100 minutes at 37° C to transfer the complete male genome to the female recipient. However, the fragile connection between the mating pairs is usually broken and the transfer aborted before being completed – explaining why, usually, only the chromosomal sequences adjacent to the integrated F are transferred. Therefore a mating between a Hfr strain and a F^- strain usually leaves the recipient F^-.

Conjugative R (antibiotic resistance) plasmids have been found in **gram-positive bacteria** such as *Streptococcus*, *Streptomyces*, and *Clostridium*. However, conjugation in gram-positive bacteria does not seem to rely on the presence of pili. Instead, the mating pair is brought together by the presence of an **adhesin molecule** present on the surface of the donor cell. Moreover, some bacteria, such as *Streptococcus faecalis*, produce a peptide sex pheromone, which is thought to elicit a mating response leading to the expression of the adhesin in the donor cells.

5.2.3 Transduction

Genetic transfer by transduction is **mediated by bacteriophages**. Bacteriophages are parasitic viruses of bacterial cells, using their energy-generating systems and protein synthesizing factors, as well as their amino acids. A bacteriophage genome can be DNA or RNA, either of which may be single- or double-stranded. The bacteriophage is composed by: a head (protected by a capsid), a tail (covered by a contractile sheath), and a base plate with tail fibers. The viral genome is protected from nucleases or other harmful substances by a protein shell, called a coat or capsid. During the process of bacterial infections, only the nucleic acid component is introduced into the bacterium, with the capsid remaining outside the microorganism. Bacteriophage life cycles are either lytic or lysogenic. **The lytic cycle** results in lysis of bacterial cells. The lytic cycle begins with the adsorption of the phage particle to specific receptors on the bacterial cell surface, followed by the injection of the bacteriophage nucleic acid into the cell. The bacteriophage DNA then takes over the entire bacterial machinery, instructing it to synthesize viral structures. The infected bacterium may lose its ability to synthesize its own RNA and DNA. Inside the bacterial host, the components are assembled into phage particles. With most phages, the bacterium undergoes lysis, releasing mature bacteriophages into the surrounding medium. Thus bacteria infected with filamentous phages can produce viral particles for very long periods. A phage capable of only lytic growth is called a **virulent phage**.

Bacteriophage infection by certain phages may also result in **lysogeny**. Under these conditions, bacteriophage entry does not result in bacterial cell lysis. As with the lytic process, the infection is initiated with the phage binding to specific receptors on the cell surface and injecting its DNA through the cell wall. However, instead of turning the cell into a "phage factory," the phage DNA inserts into the bacterial genome, where it replicates as an integral part of the chromosome as the cell grows. A bacteriophage possessing a lysogenic life cycle is referred to as a **temperate phage**. After many generations, a lysogen can revert to its virulent state, leading to lysis and bacteriophage production by a mechanism called induc-

tion. Lysogeny can also result in a phenotypic change in the bacterial host. For example, only those strains of *Corynebacterium diphtheriae* that are lysogenic for a β-prophage or related temperate phages produce the diphtheria toxin.

Several phages are known in which host DNA can be packaged into the viral capsid. These phages are called transducing phages. Transduction is denned as the transfer of bacterial DNA from one cell to another by means of a bacteriophage infection. **Transduction processes** fall broadly into two classes: **specialized and generalized**. In specialized transduction, a hybrid phage-bacterial genome is generated when a prophage genome is improperly excised from its host chromosome, dragging along some adjacent bacterial genes.

5.2.4 Conjugative Transposons

Transposons are segments of DNA able to move from one position to another in the genome or from the chromosomal DNA to a plasmid or the reverse. They were first discovered in *E. coli*. Since then, many transposable elements have been characterized. The transposons found in bacteria can be divided into three classes: insertion sequences, complex transposons and phage-associated transposons.

Insertion Sequences

The insertion sequence (IS) elements are the simplest transposons. They range in length from 150 to 1500 base pairs and possess inverted repeats of 15 to 40 base pairs at their ends. The IS elements are normal constituents of bacterial chromosomes and can integrate into plasmid and phage genomes. The ISs carry only the genetic information necessary for their own transfer (i.e., the gene coding for the transposase). The ISs can be detected if their insertion leads to interruption or inactivation of genes or if they turn on the expression of adjacent genes.

Complex Transposons

The group of conjugative plasmids known as R factors has received considerable attention from the medical community. The R plasmids are composed of two functionally distinct parts: the resistance transfer factor and the transposon carrying genes for various kinds of drug resistances. These resistance plasmids are **the most common cause of acquired antibiotic resistance in infecting bacteria** and therefore represent a considerable threat to chemotherapy. The transposons carried by these conjugative plasmids can be divided into two general categories or types.

6. CONTROL OF MICROORGANISMS

The inhibition of growth and the destruction of pathogenic microorganisms are accomplished by either **physical or chemical means**.

6.1 NONSELECTIVE METHODS

Nonselective methods of microbial control are applied only to inanimate objects (fomites); other methods display selective toxicity and may be used in vivo.

The use of disinfection and sterilization (as a scientific procedure) methods originated over 100 years ago, when Joseph Lister introduced the concept of aseptic surgery. Since that time, the implementation of effective sterilization and disinfection methods remains crucial in the control of nosocomial infections.

Sterilization refers to the removal of all forms of life, including bacterial spores. By definition, there are no degrees of sterilization – it is an **all-or-nothing process**. Chemical or physical methods may be used to accomplish this form of microbial removal.

Disinfection refers to the **removal of pathogenic organisms** but does **not necessarily** include removal of bacterial or other **spores**. **Physical or chemical methods** may be employed, but most disinfectants are chemical agents applied to inanimate objects.

A disinfectant that is applied to living tissue is referred to as an antiseptic.

Factors that influence the degree of killing of organisms play a significant role in the selection and implementation of the appropriate method of disinfection. They are:

- Types of organisms
- Number of organisms present
- Concentration of disinfecting agent
- Amount of organic soil present
- Nature of surface to be disinfected

Types of Organisms

Organisms vary greatly in their ability to withstand chemical and physical treatment. This variety is due to the biochemical composition of the cells and the protective mechanism afforded by the constituents. For example, spores have coats rich in proteins, lipids, and carbohydrates as well as cores rich in dipicolinic acid and calcium, all of which offer protection to spores. Cell walls of mycobacteria are rich in lipids, which may account for their resistance to chemical and environmental stresses, particularly desiccation. By contrast, viruses containing lipid-rich envelopes are more susceptible to the effects of detergents and wetting agents.

Number of Organisms

It referred to as the microbial load. If number of organisms is plotted against the time they are exposed to the killing agent (exposure time) logarithmically, the result is a straight line. The death curve is logarithmic. Because the microbial load is most likely composed of organisms with varying susceptibilities to killing agents, not all the organisms die at the same time. The microbial load determines the exposure time. In general, higher numbers of organisms require longer exposure times.

Concentration of Disinfecting Agent

The concentration of a disinfecting agent is also important. Agents vary substantially among manufacturers, and it is important that manufacturers' instructions on preparation, dilution, and use be followed very carefully. Proper concentrations of disinfecting agents ensure the inactivation of target organisms and promote safe and cost-effective practices.

Organic Soil Present
Organic soil, such as blood, mucus, and pus, affects killing activity by actually inactivating the disinfecting agent. In addition, by coating the surface to be treated, organic soil prevents full contact between object and agent. For optimal killing activity, instruments and surfaces should be cleansed of excess organic material prior to disinfection.

Nature of Surface to Be Disinfected
Certain medical instruments are manufactured of biomaterials that exclude the use of certain disinfection or sterilization methods because of possible damage to the instruments. An example is endoscopic instruments, which are readily damaged by the heat generated in an autoclave. Alternative methods must be used for this class of instruments.

Medical materials should be categorized into three device classifications:

- Critical materials
- Semicritical materials
- Noncritical materials

Critical materials are those that invade sterile tissues or enter the vascular system. These materials have the greatest chance of producing infection if contaminated and require sterilization.

Semicritical materials come into contact with mucous membranes and require high-level disinfection agents.

Noncritical materials come into contact with intact skin and require intermediate-level to low-level disinfection.

High-level disinfectants have activity against bacterial endospores, whereas **intermediate-level** disinfectants have tuberculocidal activity but not sporicidal activity. Finally, **low-level disinfectants** have a wide range of activity against microorganisms but do not demonstrate sporicidal or tuberculocidal activity.

6.1.1 Physical methods

Potentially pathogenic microorganisms in the environment are reduced in number (in disinfection) or totally eliminated (in sterilization) usually with physical methods.

1. Heat is employed in a variety of methods to reduce or eliminate microorganisms from heat-stable materials.
   a. **Pasteurization** destroys pathogenic microorganisms by rapid heating of a substance to 71.7°C for 15 seconds followed by rapid cooling. Pasteurization is not sterilization, because not all microorganisms are susceptible to it. This technique has eliminated food-borne diseases such as gastrointestinal tuberculosis and Q fever.
   b. **Dry heat of 160° C** sterilizes a material exposed for 2 hours. Vegetative cells are destroyed within the first few minutes, but 2 hours are needed to kill all microbial spores. Dry-heat sterilization chars organic compounds and causes excessive evaporation of liquid materials.
   c. **Moist heat** (i.e., steam) **of 121° C under a pressure of 15 psi** (pressure per square inch) is the most effective means of sterilizing heat-tolerant liquids. Heat-resistant spores are killed in less than 15 minutes in small volumes of liquid; volumes in excess of 500 ml require longer periods of time for equilibration to the sterilization conditions. An autoclave, which essentially is an industrial pressure cooker, is used for moist-heat sterilization.
   d. **Radiation** of varying wavelengths in the electromagnetic spectrum is used for disinfection and sterilization of heat-labile materials.
      i. **Ultraviolet (UV) light** between the wavelengths of 250 and 270 nm is absorbed by nucleic acids.UV light damages microbial cells by disrupting hydrogen bonds and causing thymine dimers to form in DNA. This structural alteration of the DNA often results in lethal frameshift

mutations.UV light has limited application for sterilization because of its poor penetrating energy and its absorption by glass and water.

ii. **Gamma radiation** and **x-rays** are forms of ionizing radiation that effectively sterilize many materials but must be used cautiously because of their potential danger to human cells. These forms of radiation cause the formation of free radicals, which chemically react with proteins and nucleic acids to cause cell death. Gamma radiation is used extensively for sterilization of plastic materials and is receiving renewed interest in the United States as a means of preserving foods.

e. **Microwaves** have been used in the microbiology laboratory for rapid resterilization of media that have been stored for extended periods of time. Sterilization in this case, however, is the result of heat produced by the radiation rather than a direct effect of the microwaves.

f. **Filtration of liquids and gases** through natural or synthetic materials is an effective means of removing bacteria and eukaryotic microorganisms. Membrane filters with a pore size of 0.2 μm effectively remove all bacteria but do not sterilize the liquid or gas; they do not retain most viruses. A practical limitation of filtration is that flow rate decreases as viscosity of the liquid increases and pore size of the filters decreases.

6.1.2 Chemical methods

Include disinfectants and antiseptics, which are nonspecific for the cells they affect, and antibiotics and synthetic antimicrobial agents, which have a selective toxicity.

1. **Nonselective chemicals** that control the growth of microorganisms on inanimate objects are referred to as **disinfectants**; those applied to human tissue are known as **antiseptics**. These chemicals ideally should effectively kill all microorganisms (including viruses), be soluble in water for ease of preparation and application, have a low toxicity for humans, and be reasonably economical.

   a. **Alcohols** (e.g., ethanol, isopropanol, glycerol, benzyl alcohol) are effective antiseptics when used as 50 % - 70 % aqueous solutions (activity is greater in the presence of water). Alcohols precipitate proteins and solubilize lipids present in cell membranes; as solvents, they also effectively clean human tissue. When properly used, alcohols kill the vegetative cells of many bacteria, mycobacteria, some fungi and lipid – containing viruses. They do not, however, affect microbial spores, fungi, and most viruses.

   b. **Halogens**, particularly iodine and chlorine, are widely employed as antiseptics and disinfectants.

      i. **Iodine** compounds are the most effective halogens available for disinfection. The iodine reacts with hydroxyl groups and inactivates proteins by precipitation, oxidizes essential enzymes. It is microbicidal against virtually all organisms including spore-forming bacteria and mycobacteria. Neither the concentration of iodine nor the pH of the iodine solution affect the microbicidal activity, although the efficiency of iodine solutions is increased in acid solutions because more free iodine is liberated. Iodine acts more rapidly than other halogen compounds or quaternary ammonium compounds. However, the activity of iodine can be reduced in the presence of some organic and inorganic compounds, including serum, feces, ascitic fluid, sputum, urine, sodium thiosulfate, and ammonia. Thud skin surfaces must be cleaned before iodine compounds are used as disinfectant. Elemental iodine can be disolved in aqueous potassium iodide or alcohol, or complexed with a carrier. The latter compound is referred to as an iodophor (iodo – iodine and phor – carrier). Povidone iodine (iodine complexed with polyvinylpyrrolidone) is used most commonly, relatively stable and nontoxic to tissues and metal surfaces, but expensive compared with other iodine solutions.

      ii. **Chlorine** gas reacts with water to form hypochlorous acid, which in turn reacts with water to form hydrochloric acid and hydrogen peroxide. Both of these compounds are strong oxidants that kill microbial cells. Household bleach (5.25 % sodium hypochlorite) is another source of hypochlorous acid and an effective disinfectant. Chlorine can exert its effect by the irreversible oxidation of SH groups of essential enzymes. Hypoclorites are believed to interact with cytoplasmic components to form toxic N-chloro compounds, which interfere with cellular

metabolism. The efficacy of chlorine is inversely proportional to the pH, with greater activity observed at acid pHs. This is consistent with greater activity associated with hypochlorous acid rather than hypoclorite ion concentration. The activity of chlorine compounds also increases with concentration (e.g., a twofold increase in concentration results in a 30% decrease in time required for killing) and temperature (e.g., a 50% to 60% reduction in killing time with a 10°C increase in temperature). Organic matter and alkaline detergents can reduce the effectiveness of chlorine compounds. These compounds demonstrate good germicidal activity, although spore-forming organisms are tenfold to a thousandfold more resistant to chlorine than vegetative bacteria.

c. **Aldehydes are alkylating agents** that react with the amine, sulfhydryl, and carboxyl groups of proteins and small organic molecules to kill microorganisms. Formaldehyde (8 %) and glutaraldehyde (2 %) have limited use because of their noxious vapors.
d. **Heavy metals** are effective as antimicrobial substances because of their ability to precipitate proteins and other organic molecules. Silver nitrate, copper sulfate, and merbromin (Mercurochrome) are widely used as antiseptics. Lead, arsenic, and inorganic mercury rarely are employed as disinfectants because they are concentrated by human tissues and cause cell death.
e. **Phenols** and their substituted derivatives are highly effective disinfectants. Low concentrations are used as antiseptics.
   i. Phenols function by denaturing proteins and disrupting cell membranes.
   ii. Phenol no longer is used widely, but phenolic derivatives such as carbolic acid and lysol are common disinfectants. Hexachlorophene is an excellent antistaphylococcal antiseptic available by prescription.
f. **Cationic detergents** contain alkyl groups that interact with membrane lipids to disrupt the cytoplasmic membrane of bacteria. Quaternary ammonium compounds are bactericidal but have a low toxicity for mammalian cells and can be used as effective antiseptics. Cationic detergents are inactivated by low pH solutions, phospholipids, organic compounds, and metal ions.
g. **Gases** of various types have been employed as disinfectants since ancient times. Sulfur dioxide is used as a food preservative. Ethylene oxide and propylene oxide used under pressure are effective sporicides for the sterilization of plastic materials.

6.2 SELECTIVE AGENTS (ANTIBIOTICS)

Selective agents that inhibit the growth of or kill microorganisms include **naturally occurring antibiotics, semisynthetic antibiotics, and some synthetic chemical compounds**. Many of these agents are used in the treatment of infectious diseases because of their low toxicity for mammalian cells.

a. Agents

i. **Antibiotics** are natural substances produced by an organism to kill or inhibit the growth of another organism. Their effectiveness as therapeutic agents is limited by their toxicity for human cells. **Semisynthetic antibiotics** generally are synthetic derivatives of naturally occurring antibiotics (e.g., penicillins, cephalosporins, kanamycins, sisomicins, tetracyclines, rifamycins, lincomycins, and bleomicins).
ii. **Chemotherapeutic agents** are chemicals that are used for treating infectious diseases. Frequently these agents are analogs of microbial cell constituents or substrates.

The attainable serum level of a drug is dependent on the dosage of drug administered, the host's body weight, the route and schedule of administration of the drug, and the rate of elimination.

1. CHEMOTHERAPY OF BACTERIAL INFECTIONS

This part will provide an overview of the mechanisms of action and antibacterial spectrum of the most commonly used antibiotics, as well as common mechanisms of bacterial resistance. Terminology appropriate for this discussion is summarized bellow:

- **antibacterial spectrum**: range of activity of a compound against microorganisms. A broad – spectrum antibacterial drug can inhibit a wide variety of both gram-positive and gram-negative bacteria, whereas a narrow spectrum drug is active only against selected organisms;
- **antimicrobial (bacteriostatic) activity**: activity of a chemotherapeutic agent tested in the laboratory and expressed as the lowest concentration at which the drug inhibits multiplication of the organism (minimum inhibitory concentration, or MIC);
- **bactericidal activity**: ability of a chemotherapeutic agent to kill a microorganism; expressed as the minimum bactericidal concentration (MBC);

Fleming first noted that the mold *Penicillium* prevented the multiplication of staphylococci. A concentrate from a culture of this mold was prepared, and the remarkable activity and lack of toxicity of the first antibiotic, penicillin, was demonstrated. Later, in the 1940s and 1950s, streptomycin and the tetracyclines were developed and were followed rapidly by additional aminoglycosides, semisynthetic penicillins, cephalosporins, quinolones and other antimicrobials. All greatly increased the range and effectiveness of antibacterial agents.

Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable ability to develop **resistance to these agents**. Thus antibiotic therapy will not be the predicted magic bullet against infections but rather one weapon, albeit an important one, against infectious diseases. It is also important to recognize that because resistance to antibiotics is frequently not predictable, **the physician must rely on clinical experience for the initial selection of empirical therapy**. The results of in vitro antimicrobial susceptibility testing are valuable for selecting chemotherapeutic agents, active against the infecting organism.

Extensive work has been performed to **standardize the testing methods** and improve the clinical predictive value of the results. Despite these efforts, the in vitro tests are simply a measurement of the effect of the antibiotic against the organism. Selection of an antibiotic and the patient's outcome are influenced by a variety of interrelated factors, including the pharmacokinetic properties of the antibiotic, drug toxicity, and the patient's general medical status.

The five basic sites of antibiotic activity are summarized:

I. inhibition of cell wall synthesis (penicillins, cephalosporins, cephamycins, carbapenems, monobactams, β-lactamase inhibitors, vancomycin, bacitracin, isoniazid, cycloserine, ethionamide);
II. alteration of cell membranes (plymixins);
III. inhibition of protein synthesis (aminoglycosides, tetracyclines, chloramphenicol, macrolides, clindamycin);
IV. inhibition of nucleic acid synthesis (rifampin, quinolones, metronidazole);
V. antimetabolites (sulfonamides, trimethoprim, dapsone).

6.2.1 Inhibition of cell wall synthesis

By far the most common site of antibiotic activity is interference with bacterial cell wall synthesis. The majority of the cell wall active antibiotics are classified as **β-lactam antibiotics** (e.g., penicillins, cephalosporins, cephamycins, carbapenems, monobactams, and β-lactamase inhibitors), so named because they share a common β-lactam ring structure.

Other antibiotics that interfere with construction of the bacterial cell wall include **vancomycin, bacitracin, and the antimycobacterial agents** (isoniazid, cycloserine, and ethionamide).

6.2.1.1β-lactam Antibiotics

Mode of Action

Synthesis of the bacterial cell wall is catalyzed by specific enzymes (e.g., transpeptidases, carboxy-peptidases, and endopeptidases). These regulatory proteins are also called **penicillin binding proteins (PBPs)** because they can be bound by β-lactam antibiotics. When growing bacteria are exposed to these antibiotics, the antibiotic binds to the PBPs in the cell membrane, synthesis of the cell wall peptidoglycan layer is inhibited, and autolytic enzymes are released that degrade the preformed cell wall, resulting in bacterial cell death. Thus the β-lactam antibiotics generally act as bactericidal agents.

Spectrum of Activity

Penicillins. Penicillin compounds are highly effective antibiotics with extremely low toxicity. The base compound is an organic acid with a β-lactam ring obtained from culture of the mold *Penicillium chrysogenum*.

Penicillin G is incompletely absorbed because it is inactivated by gastric acid. Thus it is used mainly as an intravenous drug for serious infections with penicillin-sensitive organisms (e.g., streptococci, gonococcus).

Penicillin V is more resistant to acid and is the preferred oral form for treatment of susceptible streptococci.

Penicillinase-resistant penicillins such as nafcillin and cloxacillin are used to treat infections caused by penicillinase-producing staphylococci.

Ampicillin was the first penicillin active against gram-negative bacilli, although the spectrum was limited.

However, **parenteral penicillins** (e.g., carbenicillin, ticarcillin, piperacillin) have been now been developed that can be effective against a broad spectrum of gram-negative bacteria including *Klebsiella*, *Enterobacter*, and *Pseudomonas*.

Cephalosporins and Cephamycins. The cephalosporins are β-lactam antibiotics derived from 7-aminocephalosporanic acid, which was originally isolated for a *Cephalosporium* mold. The cephamycins are closely related to the cephalosporins, except that cephamycins contain oxygen in place of sulfur in the dihydrothiazine ring, rendering the antibiotics more stable to β-lactamase hydrolysis. The cephalosporins and cephamycins have the same mechanism of action as the penicillins but have a wider antibacterial spectrum, are resistant to many β-lactamases, and have improved pharmacokinetic properties.

Biochemical modification of the basic antibiotic molecules results in significant improvements in antibiotic activity and pharmacokinetic properties.

The activity of the **first-generation** antibiotics (e.g. cefalexin, cephalotinis) is **similar to that of ampicillin**.

Many of the **second-generation** antibiotics (e.g., cefaclor, cefuroxime, cefoxitin and cefotetan) have **expanded activity to include *Haemophilus influenzae***, an important pediatric pathogen, and cefoxitin and cefotetan are active against ***Bacteroides fragilis***, an important anaerobic pathogen.

The third-generation antibiotics (ceftazidime, cefotaxime, cefoperazon**)** further extend the antibacterial spectrum to **include virtually all *Enterobacter*iaceae and *Pseudomonas aeruginosa*.**

Unfortunately, with these refinements the second- and third-generation antibiotics were frequently less active against gram-positive cocci. Furthermore, all cephalosporin-type antibiotics are ineffective against penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus*, as well as *Enterococcus*, and *Listeria*. In addition, organisms such as *Enterobacter*, *Serratia*, and *Pseudomonas* can develop resistance during therapy with the cephalosporins and then display cross-resistance to all β-lactam antibiotics.

Other β-Lactam Antibiotics. Several β-lactam antibiotics have slightly different biochemical structures from the penicillins and cephalosporins but have similar potent antibacterial activity.

Imipenem is a carbapenem with excellent in vitro and in vivo activity against aerobic and anaerobic gram-positive and gram-negative bacteria.

Aztreonam, a monobactam, is a narrow-spectrum antibiotic with activity specific for gram-negative bacilli (e.g., *Enterobacter*iaceae, *Pseudomonas*).

Finally, **β-lactamase inhibitors** (e.g., clavulanic acid,sulbactam) are relatively inactive by themselves but have been combined with some penicillins (e.g., ampicillin, amoxicillin, ticarcillin) to treat infections caused by β-lactamase producing bacteria. This latter group of antibiotics will irreversibly bind and inactivate bacterial β-lactamases, permitting the companion drug to enter the cell and disrupt bacterial cell wall synthesis. They have indications in: sinusitis, otitis media, urinary tract infections (UTI), lower respiratory tract infections (LRT) and polimicrobian infections. Exemples:

- **Clavulanic Acid with amoxicillin** (augmentin, amoksiklav), oral, parenteral administration
- **Clavulanic acid with ticarcilin** (timentin) intravenous administration
- **Sulbactam with ampicillin** (Unasyn), oral, parenteral administration
- **Tazobactam with piperacilin** (Tazocin, Zosyn), parenteral administration

They are responsible for **alergic reactions**:

- 0.7% - 10% of all alergic reactions after antimicrobial therapy
- anafilactic shock: 0.004% - 0.04%
- death: 0.001% (1/100000)

Mechanisms of Resistance

Three general mechanisms of resistance occur:

- failure of the antibiotic to penetrate through the outer membrane,
-failure to bind to the target site (penicillin binding proteins),
-and hydrolysis of the antibiotic by β-lactamases.

Despite the initial success of penicillin G against staphylococci, resistance mediated by β-lactamase hydrolysis developed rapidly. Unfortunately this resistance was not restricted to the early β-lactam antibiotics. Simple point mutations in the genes for the initial β-lactamases (enzymes with a narrow spectrum of activity and present in many bacteria) have now rendered these enzymes active against most penicillins and cephalosporins, including the broad spectrum agents. Because these potent β-lactamases are present on plasmids and can be exchanged among bacterial species, the utility of β-lactam antibiotics may be severely limited in the future.

6.2.1.2 Glicopeptides- Vancomycin, Teicoplanin

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

Vancomycin, obtained from an actinomycete, is a complex glycopeptide that interferes with cell wall synthesis in growing gram-positive bacteria. Vancomycin acts by sterically interfering with elongation of the peptidoglycan chain by interacting with the terminal D-alanyl-D-alanine present on the pentapeptide side chains of the peptidoglycan precursors.

Vancomycin is **inactive against gram-negative bacteria** because the molecule is too large to pass through the outer membrane and reach the peptidoglycan target site.

Vancomycin is used for the **management of infections with oxacillin-resistant staphylococci (**Meticilin R *S. aureus*- **MRSA**), *Clostridium difficile*, and other gram-positive bacteria that are resistant to β-lactam antibiotics.

There are big molecules, with only intravenous administration. Vancomycin is nefrotoxic, teicoplanin less.

Resistance among gram-positive bacteria is uncommon but has been reported for rare isolates of *Enterococcus* (Vanco-resistant enterococcus –**VRE**) and *Staphylococcus (*Vanco- intermediate resistant *S.aureus*- **VISA***)*.

6.2.1.3 Bacitracin

Mode of Action, Spectrum of Activity, and Mechanism of Resistance

Bacitracin, another cell wall-active antibiotic, is a mixture of polypeptides **used topically for skin infections caused by gram-positive bacteria.** It inhibits cell wall synthesis by interfering with dephosphorylation of the lipid carrier responsible for moving the peptidoglycan precursors through the cytoplasmic membrane to the cell wall. It may also damage the bacterial cytoplasmic membrane and inhibit RNA transcription. Resistance is most likely due to failure of the antibiotic to penetrate into the bacterial cell.

6.2.1.4 Cycloserine, Ethionamide, and Isoniazid

Modes of Action, Spectrum of Activity, and Mechanisms of Resistance

Cycloserine, ethionamide, and isoniazid are antibiotics useful for the **management of some mycobacterial infections.** Cycloserine inhibits two enzymes that catalyze cell wall synthesis, D-alanyl-D-alanine synthetase and alanine racemase. Ethionamide and isoniazid interfere with mycobacterial replication at multiple levels. Although these drugs inhibit synthesis of cell wall components, the exact mode of action has not been defined. Resistance is mediated by either reduced drug uptake into the bacterial cell or alteration of the target sites.

6.2.2 Alteration of cell membranes

Another mechanism of antibiotic action is alteration of the bacterial cell membranes.

The polymyxin class of antibiotics is an important example of this activity. These antibiotics consist of cationic branched cyclic decapeptides that destroy the cytoplasmic membranes of susceptible bacteria.

Members of this class of antibiotics include **polymyxin B and colistin**. These antibiotics are **active against gram-negative bacteria**; however, serious **nephrotoxicity** has limited their use chiefly to the **external treatment of localized infections** such as external otitis, eye infections, and skin infections with sensitive organisms. The detergent-like activity of the polymyxins is prevented when the antibiotic is unable to penetrate through the outer cell wall to the inner cytoplasmic membrane. Other antibiotics acting on the cell membrane include the antifungal polyene antibiotics (e.g., amphotericin B, nystatin).

6.2.3 Inhibition of protein synthesis

The second largest class of antibiotics consists of those whose primary action is to inhibit protein synthesis.

6.2.3.1 Aminoglycosides

Modes of Action

The aminoglycoside antibiotics consist of aminosugars linked through glycosidic bonds to an aminocyclitol. These antibiotics exert their effect by passing through the bacterial membranes and cell wall to the cytoplasm, where they inhibit bacterial protein synthesis by irreversibly binding to the ribosomes. Secondary effects, such as induction of faulty translation and disruption of bacterial membranes, have also been documented. Aminoglycosides can bind to several sites on the ribosome including the interface between the 30S and 50S subunits as well as to the individual subunits.

Spectrum of Activity

The aminoglycosides **are bactericidal antibiotics** due to irreversible binding to ribosomes and are **commonly used to treat serious infections caused by many gram-negative bacilli and some gram-positive organisms**. **Streptococci and anaerobes are resistant to aminoglycosides**.

Gentamicin and tobramycin have a broad spectrum of activity, with tobramycin being slightly more active against *Pseudomonas aeruginosa*.

Netilmicin is reported to be less ototoxic than either gentamicin or tobramycin, but netilmicin also has less antibacterial activity. All three aminoglycosides are used to treat systemic infections caused by susceptible gram-negative bacteria, including the Enterobacteriaceae and *Pseudomonas*. Because enzymatic modification of amikacin is rare, this aminoglycoside is used to treat infections caused by gram-negative bacteria that are resistant to other aminoglycosides.

All three aminoglycosides are used to treat systemic infections caused by susceptible Gram negative bacteria (GNB) like *Enterobacteriaceae* and *Pseudomonas*.

Because enzymatic modification of **amikacin** is rare, it is used to treat infections caused by gram-negative bacteria that are resistant to other aminoglycosides.

Streptomycin has been used for the treatment of tuberculosis, tularemia, and streptococcal endocarditis (when combined with a penicillin). Although **kanamycin** was one of the first aminoglycosides with broad activity against gram-negative bacteria, it is now rarely used because it is inactive against *Pseudomonas*.

Spectinomycin (bacteriostatic) reversibly interferes with m-RNA interaction with the 30S ribosome. It is structurally similar to the aminoglycosides but does not cause misreading of mRNA. Has a **narow spectrum of activity** and it is used in the treatment of penicillin-resistant *Neisseria gonorrhoeae.* Resistance is rare in *Neisseria gonorrhoeae.*

Mechanisms of Resistance

Resistance to the antibacterial action of aminoglycosides can develop in one of three ways: mutation of the ribosome binding site, decreased antibiotic uptake into the bacterial cell, and enzymatic modification (e.g., acetylation, phosphorylation) of the antibiotic. Resistance caused by alteration of the bacterial ribosome is relatively uncommon, except in members of the genus *Enterococcus*.

Because the synergistic combination of an aminoglycoside with a cell wall-active antibiotic is required for the killing of these important gram-positive cocci, this resistance is clinically significant. Resistance caused by inhibition of transport into the bacterial cell is occasionally observed with *Pseudomonas* but more commonly seen with anaerobic bacteria. This is because aminoglycoside uptake by the cell is oxygen dependent. Enzymatic phosphorylation, adenylation, or acetylation of the amino and hydroxyl groups of the aminoglycoside are the most common mechanisms of resistance. The differences in antibacterial activity among the aminoglycosides are due to their relative susceptibility to these enzymes.

Synergy - The aminoglycosides synergize with beta-lactam antibiotics, which inhibit cell wall synthesis and increase the permeability of the aminoglycosides.

Adverse reactions: **aminoglycosides are nefro & ototoxic.**

6.2.3.2 Tetracyclines

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

The tetracycline antibiotics (e.g., tetracycline, minocycline, doxycycline) are **broad-spectrum, bacteriostatic antibiotics** that inhibit protein synthesis in bacteria by blocking the binding of tRNA to the 50S ribosomal subunit. Because the interaction between antibiotic and ribosome is weak, tetracycline antibiotics are bacteriostatic.

Tetracyclines are effective in **treatment of *Mycoplasma pneumoniae* infections, cholera, rickettsial disease, brucellosis, chlamydial urethritis, as well as gonorrhea, uncomplicated urinary tract infections, and acne.**

Resistance to the tetracyclines is primarily due to increased efflux of the antibiotic from the cell. The gene encoding for this mechanism is on a transferable plasmid. Less commonly, resistance can also be the result of chromosomally mediated alteration of the cell surface proteins.

Adverse effects: destruction of normal intestinal flora, gastroenteral troubles (diarea, nausea, vomiting, abdominal pain), staining and impairment of the structure of bone and teeth, suprainfections (with C. difficile, S.aureus, Candida), hepatotoxicity, nefrotoxicity.

Tetracyclines are **not advised for pregnant women and children.**

6.2.3.3 Chloramphenicol

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

Chloramphenicol has a broad antibacterial spectrum similar to that of tetracycline but is considered the drug of choice only for treatment of typhoid fever. The reason is that, in addition to interfering with bacterial protein synthesis, chloramphenicol disrupts protein synthesis in human bone marrow cells and can produce blood dyscrasias such as aplastic anemia (1 case per 24,000 treated patients). Chloramphenicol exerts its effect on bacterial protein synthesis by binding to the 50S subunit and blocking peptide bond formation.

Resistance to chloramphenicol is observed in bacteria producing chloramphenicol acetyltransferase, which catalyzes acetylation of the β-hydroxy group of chloramphenicol. Less commonly resistant strains have altered permeability or ribosomal proteins.

Adverse effects: chloramphenicol it is toxic (bone marrow suppression) but is used in the treatment of bacterial meningitis.

6.2.3.4 Macrolides

Mode of Action, Spectrum of Activity, and Mechanism of Resistance

Erythromycin, a macrolide antibiotic, is a **bacteriostatic** organic base used mainly to treat pulmonary infections caused by *Mycoplasma*, *Legionella*, *Chlamydia*, *Campylobacter*, and gram-positive organisms on patients allergic to penicillin.

Spectrum of activity - Gram-positive bacteria.

The antibiotic disrupts protein synthesis by binding to the 50S ribosomal subunit.

Bacterial resistance to erythromycin develops by modification of ribosomal 23S RNA, which in turn prevents binding by the antibiotic.

Modification of the macrolide structure has led to the development of newer agents including **azithromycin and clarithromycin**. These macrolides are noteworthy because they have better pharmacological properties, as well as improved antibacterial activity.

6.2.3.5 Clindamycin

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

Clindamycin, like chloramphenicol and the macrolides, blocks protein synthesis by binding to the 50S ribosome. It inhibits peptidyl transferase by interfering with binding of the amino acid-acyl-tRNA complex.

Clindamycin is **active against staphylococci and anaerobic gram-negative bacilli** but generally inactive against aerobic gram-negative bacteria.

Bacterial resistance is mediated by induction of an enzyme that methylates the 5OS ribosomal RNA. Because both erythromycin and clindamycin can induce this enzymatic resistance (also plasmid-mediated), cross-resistance between these two classes of antibiotics is observed.

6.2.3.6 Fusidic acid

It is bacteriostatic, activ on the majority of Gram positive bacteria and Gram negative coccus, MRSA. Facultatively active on: *G. lamblia, P. falciparum, Mycobacteria*. Indications: staphylococcal infections (osteomielitis) in local aplications It has a good oral absorbtion.

6.2.4 Inhibition of nucleic acid synthesis

Another mechanism of antibacterial activity involves inhibition of synthesis of bacterial DNA and RNA.

6.2.4.1 Rifampin

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

Rifampin, a semisynthetic derivative of rifamycin B produced by *Streptomyces mediterranei*, binds to DNA-dependent RNA polymerase and inhibits initiation of RNA synthesis.

Rifampin is **bactericidal for *Mycobacterium tuberculosis*** and is **very active against aerobic gram-positive cocci, including staphylococci** (including MRSA strains) and streptococci. Because resistance can develop rapidly, rifampin is usually combined with one or more other effective antibiotics. Alteration of the polymerase leads to rifampin resistance.

6.2.4.2 Quinolones

(nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin, lomefloxacin, sparfloxacin)

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

The quinolones are **synthetic chemoterapeutic agents** that **inhibit bacterial DNA gyrases** or topoisomerases, which are required to supercoil strands of bacterial DNA into the bacterial cell. Nalidixic acid was used to treat urinary tract infections caused by a variety of gram-negative bacteria, but resistance to the drug developed rapidly. This drug has now been replaced by newer, more active quinolones such as norfloxacin, ciprofloxacin, and ofloxacin.

DNA gyrase consists of alpha and beta subunits, with binding of the quinolones to the alpha subunit. Alteration of this subunit is the principal mechanism of bacterial resistance, although decreased drug uptake has also been observed. Decreased uptake is mediated by changes in porin proteins on the bacterial surface. Both resistance mechanisms are chromosomally mediated.

Spectrum of activity – Gram positive coccus, Gram negative bacteria, including Pseudomonas, urinary tract infections (UTIs).

Nalidixic acid was used to treat urinary tract infections caused by a variety of gram-negative bacteria, but resistance to the drug developed rapidly. This drug has now been replaced by newer, more active quinolones such as **norfloxacin, ciprofloxacin, and ofloxacin.**

6.2.4.3 Metronidazole

Mode of Action, Spectrum of Activity, and Mechanisms of Resistance

Metronidazole was originally introduced as an oral agent for treatment of *Trichomonas vaginalis*. It is also effective in treatment of amebiasis, giardiasis, and serious anaerobic bacterial infections (including *Bacteroides fragilis*) but has no significant activity against aerobic or facultatively anaerobic bacteria. The antimicrobial properties of metronidazole appear to be mediated by a partially reduced intermediate, which results in DNA breakage. A decreased rate of reduction of metronidazole to its active form has been observed in resistant strains of bacteria (e.g., *Bacteroides fragilis*).

6.2.5 Antimetabolites

The final mechanism of antibiotic activity is illustrated by the sulfonamides, trimethoprim, and the antileprosy drug, dapsone. The sulfonamides compete with p-aminobenzoic acid, preventing synthesis of folic acid that is required by certain microorganisms. Because mammalian organisms do not synthesize folic acid (required as a vitamin), **sulfonamides** do not interfere with mammalian cell metabolism. Dapsone's activity is at the same site as the sulfonamides. **Trimethoprim** has a high affinity for dihydrofolate reductase, and competitively prevents conversion of dihydrofolate to tetrahydro-folate. This blocks the formation of thymidine, some purines, methionine, and glycine. Trimethoprim is commonly combined with sulfamethoxazole to produce a synergistic combination active at two steps in the synthesis of folic acid.

Sulfonamides are **effective against a broad range of gram-positive and gram-negative organisms,** such as *Nocardia*, *Chlamydia*, and some protozoa. Short-acting sulfonamides such as sulfisoxazole are among the drugs of choice for treatment of acute urinary tract infections caused by susceptible bacteria such as *Escherichia coli*.

Trimethoprim-sulfamethoxazole is effective against a large variety of gram-positive and gram-negative microorganisms and is the drug of choice for acute and chronic urinary tract infections. The combination is active in infections caused by *Pneumocystis carinii*, bacterial infections of the lower respiratory tract, otitis media, and uncomplicated gonorrhea.

6.2.6 The new introduced antibiotics

Tabel 1: New introduced antibiotics

Antibiotic	Class	Spectrum	Clinical indications	Comments
Linezolid	Oxazolidinone	GPC including MRSA/ PRSP	HAP , SSI, including DFI	Oral/ parenteral
Daptomycin	Lipopeptid Cyclic	GPC including MRSA	Complicated SSI	1/day
Quinupristin-dalfopristin	Streptogramină	GPC including MRSA	Complicated SSI	VRE (E.faecium) bacteriemia
Tigecycline	Glycylcyclină	GPC inclusiv MRSA	IAI and SSI	MDRO: Acinetobacter /ESBLs
Ceftobiprole*	Cephalosporină	CGP including MRSA, PRSP şi GNB	Pneumonia, complicated SSI	Resistant to many beta lactamases
Dalbavancin*	Lipoglycopeptid	GPC including MRSA	complicated SSI, catheter related bacteriemia	1/week
Telavancin*	Lipoglycopeptid	GPC including MRSA	Complicated SSI	Bactericid

Legend: PRSP:Penicilin –R Str. Pneumoniae pneumonia ,DFI: diabetic footh infection, IAI:iintraabdominale infecţtion, HAP: hospital acquired pneumoniia, SSI: subcutaneous infections, MRSA, BLSE, MDR

6.3 COMBINATION OF ANTIBIOTICS. BACTERICIDAL AND BACTERIOSTATIC ANTIBIOTICS

An infection should, if possible, be treated with a single antibiotic agent. Combination therapy is, however, administered when a synergistic effect can be achieved. Furthermore, drug combinations are used when a broader antibacterial spectrum, a delay in the development of resistance, or an increase of bactericidal efficacy is required.

The activity of an antibiotic combination can markedly differ depending on the individual components concerned. **Indifference** occurs when the activity of the combination is equal to that of the more active component. **Addition** takes place when the activity of the combination is equal to the sum of activities of the individual components. **Synergism** (potentiation) is observed when the activity of the combination is significantly higher than the sum of activities of the individual components. **Antagonism** occurs when the activity of the combination is lower than that of the more active component. The activity of an antibiotic combination is largely dependent on the bactericidal or bacteriostatic properties of the individual components.

6.4 BACTERIAL RESISTANCE

The phenomenon of bacterial resistance was detected by P. Ehrlich at the beginning of the chemotherapy era in 1909. When the microorganisms continue to proliferate at a therapeutically achievable antibiotic concentration, i.e. when the minimal inhibitory concentration (MIC) is higher in vitro than in vivo attainable serum or tissue concentration, bacterial resistance is present. This is caused by the lack of

penetration of the antibiotic into the bacterial cell (permeability barrier), by modification of the "target" in the cell so that it is insensitive to the antibiotic, or, very importantly, by inactivation of the antibiotic due to the action of a bacterial enzyme.

Not only the various species of bacteria but also the bacterial strains of a species differ with respect to their antibiotic susceptibility. Even within a bacterial population there are variants with different susceptibility. Susceptible and resistant microorganisms can readily be distinguished by means of the disc test, the antibiotic concentration normally attainable in the blood of the patient being regarded as the susceptibility limit.

The following types of resistance are recognized:

1. **Natural resistance** which is due to a permanent genetically determined insensitivity of a bacterial species towards a certain antibiotic (example: all *Pseudomonas aeruginosa* strains are resistant to penicillin G).
2. **Primary resistance** in which some of the existing strains of a bacterial species are resistant, while others are susceptible (example: 30 – 60% of all *E. coli* strains are resistant to tetracycline).
3. **Secondary resistance** which is due to mutation or transfer of resistance in the case of individual organisms of an antibiotic-susceptible population. In the development of resistant variants, antibiotics function as selecting agents: only the resistant organisms and their descendants survive under the "selection pressure" of an antibiotic so that the infection is finally maintained by a resistant population. Secondary resistance may develop at different rates. A distinction is made between a rapid increase of resistance (one-step mutation) as, for example, with streptomycin, and a slow increase (multi-step mutation) as, for example, with penicillin. With the latter, several consecutive mutation steps are required for the emergence of resistance.
4. **Transferable resistance** which is due to the transfer of genetic material, of either chromosomal or extrachromosomal origin from one bacterial cell to another. Three types of genetic transfer mechanisms have been found: transformation, involving transfer of "naked" DNA; transduction, in which the DNA from the donor is carried to the recipient inside a phage; and conjugation, which requires contact of donor and recipient cells in which the genetic material is transferred through a channel between the two mating cells. The genes for drug resistance are carried not only on the chromosome but also on extrachromosomal elements or plasmids, which are called R factors.

6.5 ANTIMICROBIAL SUSCEPTIBILITY TESTS

Several standardized techniques have been developed for determining the susceptibility of bacteria to antimicrobial agents. The exact correlation between in vitro test results and the in vivo efficacy of a drug has yet to be established. In general, the attainable serum level of a drug should be two to four times greater than its minimal inhibitory concentration (MIC) for a specific bacterial isolate in order for the drug to be deemed an effective chemotherapeutic agent.

A. The Kirby-Bauer disk diffusion method is a highly standardized test for determining the susceptibility of bacteria to an antimicrobial agent.

1. The standard medium is Mueller-Hinton agar, with or without sheep blood, poured to a depth of 4 mm in a Petri dish.
2. The plate is inoculated by streaking the entire surface in three planes with a sterile cotton swab dipped into a standardized inoculum. The bacterial inoculum is prepared from an 18-hour broth culture of the microbe to be tested and is standardized with sterile physiologic saline (i.e., 0.85% sodium chloride) to contain 10^5 bacteria/ml.
3. Standard commercial paper disks containing known amounts of the antimicrobial agents to be tested are placed on the surface of the agar. The plate is incubated in an inverted position at 35° C for 18 hours.
4. The diameter of the zone of inhibition produced by the drug is measured for each disk. The zone diameter obtained experimentally is compared with the standard zone diameter provided by the disk

manufacturer, and the bacterial isolate is designated susceptible, intermediately susceptible, or resistant.

5. Zone diameters are designated susceptible or resistant by regression-line analysis of each zone diameter versus the MICs of thousands of bacterial isolates for each drug with the knowledge that serum levels of a drug must be 2-4 times the MIC to be effective. These data are accumulated by the manufacturer of the antimicrobial agent before its approval for clinical use.

B. Broth dilution techniques determine the MIC and the minimal bactericidal concentration (MBC) of an antimicrobial agent for a bacterial isolate.

C. The E-test uses a standardized inoculum of bacteria which is swabbed onto the surface of a Mueller-Hinton agar plate. E-test strips containing a continous gtradient of antimicrobial agent concentractions are placed on the surface. After overnight incubation, an elliptical zone of inhibition forms as the antimicrobial agent inhibits growth. The MIC is read where growth intersects the E-test strip.

7. THE NORMAL FLORA

Medical microbiology is the study of the interactions between animals (primarily humans) and microorganisms such as viruses, bacteria, fungi, and parasites. Microorganisms play a critical role in human survival. The normal population of endogenous organisms **participates in the metabolism of food products, provides essential growth factors, protects against infections with highly virulent microorganisms, and stimulates the immune response**. In the absence of these organisms, life as we know it would be impossible.

Microorganisms that are commonly found on or in body sites of healthy persons are termed normal flora. The different body sites may have the same or different normal flora, depending upon local conditions. The microorganisms that colonize an area for months or years at a time are termed **resident flora**. This is in contrast to **transient flora**, which are present at a site temporarily. Transient flora come to visit but not usually to live or stay.

Organisms that constitute the normal flora can be classified as **parasites** (live at the expence of the host), **symbionts** (benefit the host) **or comensals** (have neutral effect on the host).

A number of **factors** determine which microorganisms are able to colonize the various body sites: the nutritional status of the site, oxidation – reduction potentials, antibody and other antibacterial substances, pH, and interference by already established organisms.

The microbial flora in and on the human body is in a continual state of flux determined by such varied factors as age, diet, hormonal state, health, sanitary conditions, and personal hygiene. It is important that changes in our physical well-being can drastically disrupt the delicate balance that is maintained among the heterogeneous organisms that coexist within us. For example, hospitalization can lead to replacement of normally avirulent organisms in the oropharynx with potentially invasive gram-negative bacilli.

Two important points must be emphasized:

(1) care should be taken to maintain the normal balance of microbes, and

(2) an important distinction exists between colonization (also called infection) with a pathogenic organism and disease.

The normal microbial flora controls the proliferation of pathogenic organisms by a variety of methods including competition for nutrients or receptors on host cells, production of bacteriocins (small-molecular-weight proteins that are bactericidal for other organisms), and stimulation of the immune response. Thus the pathogenic organisms residing in or on the body are controlled by the normal commensal organisms.

However, if the normal flora is disrupted (e.g., by broad-spectrum antibiotics) or if the pathogenic organisms are introduced into a normally sterile environment, then disease can be produced. For example, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and many gram-negative bacilli can be found as part of the normal oropharyngeal flora. However, when these organisms are introduced into the lower respiratory tract by aspiration of oral secretions and when local immunity is unable to contain them, bronchopulmonary disease develops.

An understanding of medical microbiology requires knowledge not only of the different classes of microbes but also of their propensity for causing disease. Some organisms (**opportunistic pathogens**) will not cause disease except in immunocompromised patients under conditions that favor the growth of the organism (e.g., *Staphylococcus epidermidis* disease at the site of an intravascular catheter).

At the other end of the spectrum, some organisms (**strict pathogens**) are always associated with disease (e.g., *Mycobacterium tuberculosis*, *Shigella* species, *Neisseria gonorrhoeae*).

Between these two extremes are the majority of organisms (**facultative pathogens**) associated with disease (e.g., *Staphylococcus aureus, Escherichia coli, Candida albicans*).

The microbial population that colonizes the human body is numerous and diverse.

7.1 RESPIRATORY TRACT AND HEAD

Mouth, Oropharynx, and Nasopharynx

The upper respiratory tract is colonized with numerous organisms. **Anaerobic bacteria** are the most common, including *Peptostreptococcus, Fusobacterium, Prophyromonas, Bacteroides, Actinomyces*. **Aerobic organisms** such as viridans group streptococci, coagulase-negative staphylococci, nonpathogenic *Neisseria*, and *Haemophilus* species (not *H. influenzae* B) are also common. These organisms are all relatively avirulent and are rarely associated with disease, unless they are introduced into normally sterile sites (e.g., sinuses, middle ear, brain).

Potentially pathogenic organisms can also be found in the upper airways, including group A streptococci, *Streptococcus pneumoniae, Staphylococcus aureus, Neisseria meningitidis, Haemophilus influenzae* B, *Moraxella catarrhalis*, and Enterobacteriaceae. It must be remembered that **the isolation of these organisms from an upper respiratory tract specimen does not define their pathogenicity**. Their involvement with a disease process must be demonstrated to the exclusion of other pathogens. For example, with the exception of group A streptococci, these organisms are rarely responsible for pharyngitis even though they can be isolated from patients with this disease.

The neonate's mouth contains microorganisms found in the birth canal. These include lactobacilli, corynebacteria, staphylococci, micrococci, gram-negative enteric rods, yeast, and aerobic, microaerophilic, and anaerobic streptococci. This flora disappears in 2-5 days and is replaced by the oral flora of the mother and hospital personnel.

Most of the anaerobic flora appears after the emergence of teeth and the formation of gingival crevices. Formation of plaque results in bacterial counts as high as 10^{11} organisms/g.

The nasopharynx of the neonate is sterile at birth. However, it becomes colonized with the indigenous flora of the mother and hospital staff within 2-3 days. The carriage rate of pathogens such as *S. pyogenes, S. pneumoniae,* and *Haemophilus influenzae* approaches 100%. Carriage rates among older children are lower than those for infants but higher than those for adults.

Ear

The most common organism found to colonize the outer ear is coagulase-negative *Staphylococcus*. Other organisms colonizing the skin have been isolated from this site, as well as potential pathogens such as *Streptococcus pneumoniae, Pseudomonas aeruginosa,* and the Enterobacteriaceae. This latter group of organisms has also been associated with disease at this site.

Eye

The surface of the eye is colonized with coagulase-negative staphylococci, as well as rare numbers of organisms found in the nasopharynx (e.g., *Haemophilus* spp., *Neisseria* spp., viridans streptococci).

Lower Respiratory Tract

The larynx, trachea, bronchioles, and lower airways are generally sterile, although transient colonization with upper respiratory secretions may occur following aspiration. Acute lower airway disease is usually caused by the more virulent bacteria present in the mouth (e.g., *S. pneumoniae, S. aureus, H. influenzae,* and members of the Enterobacteriaceae such *Klebsiella*). **Chronic aspiration** may lead to a polymicrobial disease with anaerobes as the predominant pathogens, particularly peptostreptococci and anaerobic gram-negative bacilli. Fungi such as *Candida* can cause lower airway disease, but organisms must be demonstrated in tissue to exclude simple colonization. In contrast, the presence of the dimorphic fungi (e.g., *Histoplasma, Coccidioides, Blastomyces*) is diagnostic because colonization with these organisms does not occur.

7.2 GASTROINTESTINAL TRACT

The gastrointestinal tract is colonized with microbes at birth and remains the home for a diverse collection of organisms throughout the life of the host. Although the opportunity for colonization with new

organisms occurs daily with ingestion of food and water, the population remains relatively constant unless exogenous factors such as antibiotic treatment disrupt the balanced flora.

Stomach

This area is generally colonized with small numbers of acid-tolerant organisms such as the lactic acid-producing bacteria, lactobacilli and streptococci. ***Helicobacter pylori***, a cause of gastritis and ulcerative disease, is also commonly found in this area. The microbial population can dramatically change in numbers and diversity in patients receiving drugs that neutralize or reduce the production of gastric acids.

Small Intestine

In contrast with the anterior portion of the digestive tract, the small intestine is colonized with many different bacteria, fungi, and parasites. Most of these organisms are **anaerobes**. **Common causes of gastroenteritis (e.g. *Salmonella, Campylobacter*)** can be present in small numbers as asymptomatic residents; however, their detection in the clinical laboratory is generally indicative of disease production.

Large Intestine

More microbes are present in this site than anywhere else in the human body. It has been estimated that more than 10^{11} bacteria per gram of feces can be found, with anaerobic bacteria in excess by a thousandfold. Many of these **anaerobes** are the relatively avirulent bifidobacteria and eubacteria. However, the peptostreptococci and the anaerobic gram-negative bacilli (particularly *Bacteroides fragilis* group) are common. Members of the family **Enterobacteriaceae** and the **enterococci** are the most common facultative anaerobes present in the large intestine. Antibiotic treatment can rapidly alter this population, with the proliferation of antibiotic-resistant organisms such as enterococci, *Pseudomonas*, and fungi. *Clostridium difficile* can also grow rapidly in this situation, leading to disease ranging from diarrhea to pseudomembranous colitis. Exposure to other enteric pathogens, such as *Shigella*, enterohemorrhagic *Escherichia coli*, and *Entamoeba histolytica* can also rapidly disrupt the colonic flora and produce significant intestinal disease.

7.3 GENITOURINARY SYSTEM

In general, the anterior urethra and vagina are the only anatomical areas of the genitourinary system that are colonized with microbes. Although the urinary bladder can be transiently colonized with bacteria migrating upstream from the urethra, these should be rapidly cleared by the bactericidal activity of the uroepithelial cells and the flushing action of voided urine. The other structures of the urinary system should be sterile except when disease or an anatomical abnormality is present. Likewise, the uterus should also remain free of organisms.

Anterior Urethra

The indigenous population of the urethra consists of a variety of organisms, with lactobacilli, corynebacteria, and coagulase-negative staphylococci the most numerous. These organisms are relatively avirulent and are rarely associated with human disease. In contrast, the urethra can be colonized with **enterococci, Enterobacteriaceae, and *Candida*** – all of which can invade the urinary tract and lead to significant disease. Organisms such as ***Neisseria gonorrhoeae*** **and *Chlamydia trachomatis*** are common causes of urethritis and can persist as asymptomatic colonizers of the urethra. The isolation of these organisms in clinical specimens should always be considered significant, irrespective of the presence or absence of clinical symptoms.

Vagina

The microbial population of the vagina is dramatically influenced by hormonal factors. Newborn girls are colonized with lactobacilli at the time of birth, and these bacteria predominate for approximately 6 weeks. After that time, the levels of maternal estrogen have declined and the vaginal flora changes to include staphylococci, streptococci, and Enterobacteriaceae. When estrogen production is initiated at puberty, the microbial flora again changes. Lactobacilli reemerge as the predominant organisms, and many other organisms are also isolated, including staphylococci (*S. aureus* less commonly than the coagulase-negative species), streptococci including group **B *Streptococcus*, enterococci, *Gardnerella vaginalis*, *Mycoplasma* and the related *Ureaplasma*, Enterobacteriaceae, and a variety of anaerobic bacteria**. Although ***Neisseria gonorrhoeae*** and ***Chlamydia trachomatis*** are common causes of genital disease, a

significant proportion of disease develops when the balance of vaginal bacteria is disrupted, resulting in decreases in lactobacilli and increases in ***Mobiluncus*** **and** ***Gardnerella***. ***Mycoplasma hominis*** may also be involved in this process, and ***Trichomonas vaginalis, Candida albicans,*** **and** ***Torulopsis glabrata*** are certainly important causes of vaginitis. Although herpes simplex virus and papillomavirus would not be considered normal flora of the genitourinary tract, these viruses can establish persistent infections.

The urogenital tract of the **neonate** is sterile, but the nonpathogenic flora of diphtheroids, staphylococci, micrococci, and nonhemolytic streptococci begins developing within the first 24 hours. The neonatal vagina is colonized by lactobacilli as long as vaginal glycogen deposits persist as the result of estrogen passively transferred from the mother.

Alkaline conditions in the vagina prior to puberty enhance the growth of a different microbial flora. At puberty, estrogen causes deposition of glycogen, which is fermented by lactobacilli, producing an acidic environment and promoting growth of the flora typical of an adult vagina.

7.4 SKIN

Although many organisms come into contact with the skin surface, this relatively hostile environment does not support the survival of most organisms. Coagulase-negative staphylococci and less commonly ***Staphylococcus aureus*****, corynebacteria, and propionibacteria** are the most common organisms found on the skin surface. ***Clostridium perfringens*** is isolated on the skin of approximately 20% of healthy individuals, and the fungi ***Candida*** **and** ***Malassezia*** are also found on skin surfaces, particularly in moist sites. **Streptococci** can transiently colonize the skin, but the volatile fatty acids produced by the anaerobe propionibacteria are toxic for these organisms. Gram-negative bacilli do not permanently colonize the skin surface (with the exception of ***Acinetobacter*** and a few other less common genera), because the skin is too dry.

Most of the indigenous flora of the skin inhabit the stratum corneum and the upper portions of hair follicles. Deeper parts of the hair follicle and sebaceous glands usually serve as reservoirs of smaller numbers of microbes, which replace those lost from the skin in washing. The microbial flora of the hair is similar to that of the skin. The number of microbes on the skin generally is 10^3-10^4 organisms/cm^2 but may be as high as 10^6 organisms/cm^2 in moist areas such as the groin. Some of the fatty acids found on the skin probably are bacterial products that prevent colonization by other bacteria. Effective cleaning of the skin can reduce the bacterial count by 90%.

7.5 BLOOD AND INTERNAL TISSUES

Blood and internal tissues are sterile under normal circumstances. Evidence now supports the contention that most individuals experience transient bacteremia nearly every time they brush their teeth or have a bowel movement. **Medical manipulations (e.g., catheterization) also may induce transient bacteremia or the presence of organisms in other tissues.**

The normal flora has beneficial effects. The development of immunologic competence depends upon the normal flora. The immune system is constantly "primed" by contact with the normal flora. Animals born and raised in a germ-free environment have a poorly functioning immune system. Exposure to otherwise innocuous organisms can be fatal to such animals. The normal flora produces conditions at the microenvironmental level that block colonization by extraneous pathogens. When the composition of the normal flora is altered (e.g., by antibiotic therapy with broad spectrum antibiotics), other organisms capable of causing disease may fill the void. *Candida albicans* may greatly multiply and cause diarrhea or infections in the mouth or vagina. *Clostridium difficile* produses a colitis as a result of its proliferation following antibiotic therapy.

7.6 *BACTERIAL ASSOCIATIONS*

An organism living and multiplying within the living human body is termed a **parasite**, the body in this instance being a host. When harmless to the host the parasite is termed a **commensal,** when harmful, a **pathogen**. Under certain conditions, commensals may become pathogens, and pathogens may assume a commensal role. Organisms living on dead matter are termed **saprophytes**. When both host and parasite

mutually benefit the association is often called symbiosis. This same term is used by some authorities irrespective of whether benefit occurs to both partners, but satellitism is the more correct term in where only one partner benefits.