Lectures in microbiology: Antimicrobial drugs
4th stage in biology
Dept. Microbiology
College of Education for PureSciences
University of Basrah
Date: Wensday
Time: 8.30-10.30AM

Antimicrobial drugs:

When the body,s defense normal defenses cannot prevent or overcome a disease, It can be treated by chemotherapy with antimicrobial drugs. Antimicrobial drugs act by killing or interfering with the growth of microorganisms. Unlike disinfectants, antimicrobial drugs must often act within the host without damaging the host. This is known selective toxicity.

Antibiotics: is an antimnicrobial agent usually produced naturally by bacteria or fungi. It is a substance produced by microorganisms that in small amounts inhibit another microorganisms.

In 1928, Alexander Fleming observed that the growth of the bacterium Staphylococcus aureus was inhibited in the area surrounding the colony of a mold that had contaminated a petri dish. The mold was identified as Penicillium notatum, and the active compound was named penicillin.

The spectrum of antimicrobial activity:

Some drugs have a narrow spectrum of microbial activity, Penicillin G , for example, affect gram-positive bacteria but very few gram-negative bacteria. Antibiotics that affect a broad range of gram-positive and gram-negative bacteria are therefore called broad-spectrum antibiotics.

The action of antimicrobial drugs:

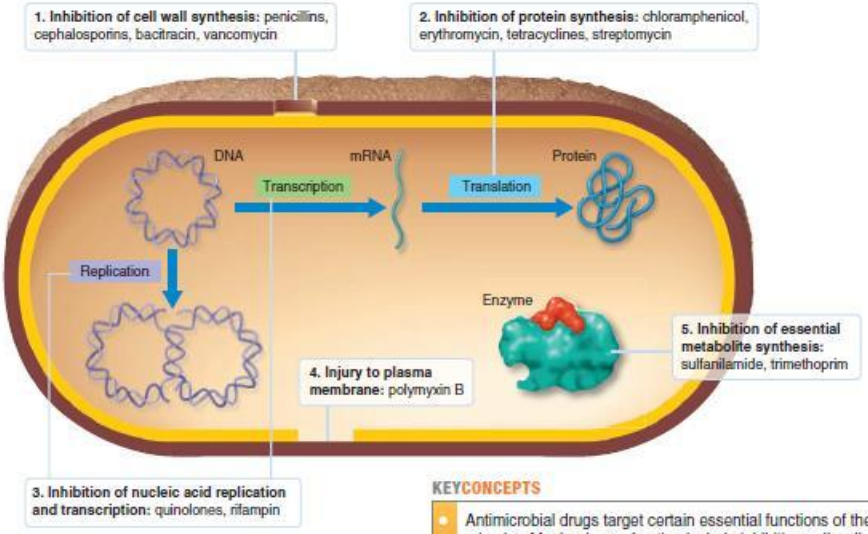
The antimicrobial drugs are either bactericidal (they kill microbes directly) or bacteriostatic (they prevent microbes from growing)

TABLE **20.3** Antibacterial Drugs

Drugs by Mode of Action	Comments
INHIBITORS OF CELL WALL SYNTHESIS	
Natural Penicillins	
Penicillin G	Against gram-positive bacteria, requires injection
Penicillin V	Against gram-positive bacteria, oral administration
Semisynthetic Penicillins	
Oxacillin	Resistant to penicillinase
Ampicillin	Broad spectrum
Amoxicillin	Broad spectrum; combined with inhibitor of penicillinase
Aztreonam	A monobactam; effective against gram-negative bacteria, including *Pseudomonas* spp.
Imipenem	A carbapenem; very broad spectrum
Cephalosporins	
Cephalothin	First-generation cephalosporin; activity similar to penicillin; requires injection
Cefixime	Fourth-generation cephalosporin; oral administration
Polypeptide Antibiotics	
Bacitracin	Against gram-positive bacteria; topical application
Vancomycin	A glycopeptide type; penicillinase-resistant; against gram-positive bacteria
Antimycobacterial Antibiotics	
Isoniazid	Inhibits synthesis of mycolic acid component of cell wall of *Mycobacterium* spp.
Ethambutol	Inhibits incorporation of mycolic acid into cell wall of *Mycobacterium* spp.

Drugs by Mode of Action	Comments
INHIBITORS OF PROTEIN SYNTHESIS	
Chloramphenicol	Broad spectrum, potentially toxic
Aminoglycosides	
Streptomycin	Broad spectrum, including mycobacteria
Neomycin	Topical use, broad spectrum
Gentamicin	Broad spectrum, including *Pseudomonas* spp.
Pleuromutilins	
Mutilin, retpamulin	Inhibit gram-positive bacteria
Tetracyclines	
Tetracycline, oxytetracycline, chlortetracycline	Broad spectrum, including chlamydias and rickettsias; animal feed additives
Macrolides	
Erythromycin	Alternative to penicillin
Azithromycin, clarithromycin	Semisynthetic; broader spectrum and better tissue penetration than erythromycin
Telithromycin (Ketek)	New generation of semisynthetic macrolides; used to cope with resistance to other macrolides
Streptogramins	
Quinupristin and dalfopristin (Synercid)	Alternative for treating vancomycin-resistant gram-positive bacteria
Oxazolidinones	
Linezolid (Zyvox)	Useful primarily against penicillin-resistant gram-positive bacteria
Glycylcyclines	
Tygecycline	Broad spectrum, especially MRSA and *Acinetobacter*
INJURY TO THE PLASMA MEMBRANE	
Polymyxin B	Topical use, gram-negative bacteria, including *Pseudomonas* spp.
Lipopeptides	
Daptomycin	To treat MRSA infections
INHIBITORS OF NUCLEIC ACID SYNTHESIS	
Rifamycins	
Rifampin	Inhibits synthesis of mRNA; treatment of tuberculosis
Quinolones and Fluoroquinolones	
Nalidixic acid, nofloxacin, ciprofloxacin	Inhibit DNA synthesis; broad spectrum; urinary tract infections
Gatifloxacin	Newest generation quinolone; increased potency against gram-positive bacteria
COMPETITIVE INHIBITORS OF THE SYNTHESIS OF ESSENTIAL METABOLITES	
Sulfonamides	
Trimethoprim-sulfamethoxazole	Broad spectrum; combination is widely used

Major Action Modes of Antimicrobial Drugs



KEY CONCEPTS

- Antimicrobial drugs target certain essential functions of the microbe. Mechanisms of action include inhibiting cell wall synthesis, inhibiting protein synthesis, inhibiting nucleic acid synthesis, injuring the plasma membrane, or inhibiting synthesis of essential metabolites.
- The antimicrobial drug must not interfere with essential functions of the microbe's host.

example that sometimes occurs is overgrowth by the yeastlike fungus *Candida albicans*, which is not sensitive to bacterial antibiotics. This overgrowth is called a **superinfection,** a term that is also applied to growth of a target pathogen that has developed resistance to the antibiotic. In this situation, such an antibiotic-resistant strain replaces the original sensitive strain, and the infection continues.

CHECK YOUR UNDERSTANDING

- Identify at least one reason why it is so difficult to target a pathogenic virus without damaging the host's cells. 20-3
- Why are antibiotics with a very broad spectrum of activity not as useful as one might first think? 20-4

The Action of Antimicrobial Drugs

LEARNING OBJECTIVE

20-5 Identify five modes of action of antimicrobial drugs.

Antimicrobial drugs are either **bactericidal** (they kill microbes directly) or **bacteriostatic** (they prevent microbes from growing). In bacteriostasis, the host's own defenses, such as phagocytosis and antibody production, usually destroy the microorganisms. The major modes of action are summarized in Figure 20.2.

Inhibiting Cell Wall Synthesis

Penicillin, the first antibiotic to be discovered and used (if one does not also consider the sulfa drugs), is an example of an inhibitor of cell wall synthesis.

Recall from Chapter 4 that the cell wall of a bacterium consists of a macromolecular network called peptidoglycan. Peptidoglycan is found only in bacterial cell walls. Penicillin and certain other antibiotics prevent the synthesis of intact peptidoglycan; consequently, the cell wall is greatly weakened, and the cell

TABLE 20.2 The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs

	Prokaryotes				*Eukaryotes*			
	Mycobacteria*	**Gram-Negative Bacteria**	**Gram-Positive Bacteria**	**Chlamydias, Rickettsias†**	**Fungi**	**Protozoa**	**Helminths**	**Viruses**
Isoniazid	←							
Penicillin G			←					
Ketoconazole					←			
Niclosamide (tapeworms)							←	
Streptomycin	←	←	←					
Mefloquine (malaria)						←		
Acyclovir								←
Tetracycline	←	←	←	←				
Praziquantel (flukes)							←	

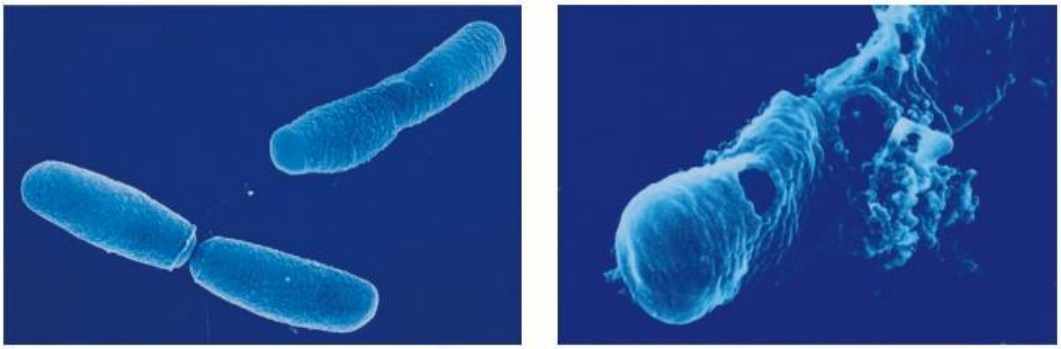
*Growth of these bacteria frequently occurs within macrophages or tissue structures.
†Obligately intracellular bacteria.

undergoes lysis (Figure 20.3). Because penicillin targets the synthesis process, only actively growing cells are affected by these antibiotics—and, because human cells do not have peptidoglycan cell walls, penicillin has very little toxicity for host cells.

Inhibiting Protein Synthesis

Because protein synthesis is a common feature of all cells, whether prokaryotic or eukaryotic, it would seem an unlikely target for selective toxicity. One notable difference between prokaryotes and eukaryotes, however, is the structure of their ribosomes. As discussed in Chapter 4 (page 94), eukaryotic cells have 80S ribosomes; prokaryotic cells have 70S ribosomes.* The difference in ribosomal structure accounts for the selective toxicity of antibiotics that affect protein synthesis. However, mitochondria (important eukaryotic organelles) also contain 70S ribosomes similar to those of bacteria. Antibiotics targeting the

*The 70S ribosome is made up of a 50S and a 30S unit. The S stands for Svedberg unit, which describes the relative rate of sedimentation in a high-speed centrifuge. The apparent arithmetic error here has often puzzled students. However, you can think of a Svedberg unit as a unit of size rather than weight. Therefore, the combination here of 50S and 30S is not the same as combining 50 grams and 30 grams.



(a) Rod-shaped bacterium before penicillin.

(b) The bacterial cell lysing as penicillin weakens the cell wall.

Figure 20.3 The inhibition of bacterial cell synthesis by penicillin.

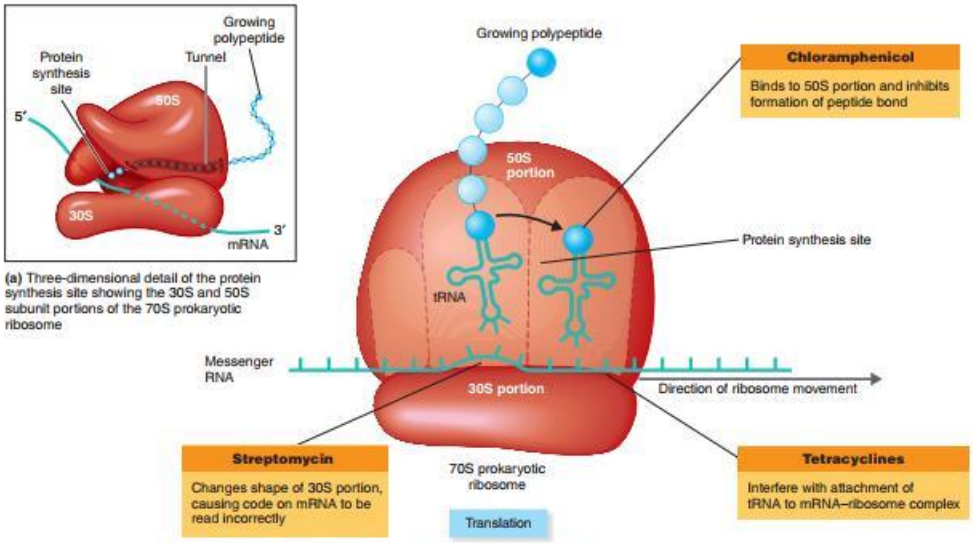


Figure 20.4 The inhibition of protein synthesis by antibiotics. **(a)** The inset shows how the 70S prokaryotic ribosome is assembled from two subunits, 30S and 50S. Note how the growing peptide chain passes through a tunnel in the 50S subunit from the site of protein synthesis. **(b)** The diagram shows the different points at which chloramphenicol, the tetracyclines, and streptomycin exert their activities.

Q Why do antibiotics that inhibit protein synthesis affect bacteria and not human cells?

70S ribosomes can therefore have adverse effects on the cells of the host. Among the antibiotics that interfere with protein synthesis are chloramphenicol, erythromycin, streptomycin, and the tetracyclines (Figure 20.4).

Injuring the Plasma Membrane

Certain antibiotics, especially polypeptide antibiotics, bring about changes in the permeability of the plasma membrane; these changes result in the loss of important metabolites from the microbial cell.

Some antifungal drugs, such as amphotericin B, miconazole, and ketoconazole, are effective against a considerable range of fungal diseases. Such drugs combine with sterols in the fungal plasma membrane to disrupt the membrane (Figure 20.5). Because bacterial plasma membranes generally lack sterols, these antibiotics do not act on bacteria.

Inhibiting Nucleic Acid Synthesis

A number of antibiotics interfere with the processes of DNA replication and transcription in microorganisms. Some drugs with this mode of action have an extremely limited usefulness because they interfere with mammalian DNA and RNA as well.

Inhibiting the Synthesis of Essential Metabolites

In Chapter 5, we mentioned that a particular enzymatic activity of a microorganism can be *competitively inhibited* by a substance (*antimetabolite*) that closely resembles the normal substrate for the enzyme (see Figure 5.7, page 118). An example of competitive inhibition is the relationship between the antimetabolite sulfanilamide (a sulfa drug) and ***para*-aminobenzoic acid (PABA)**. In many microorganisms, PABA is the substrate for an enzymatic reaction leading to the synthesis of folic acid, a vitamin that functions as a coenzyme for the synthesis of the purine

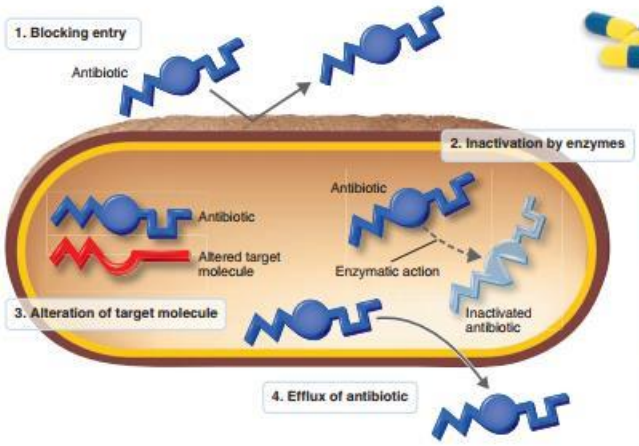
Resistance to Antimicrobial Drugs

LEARNING OBJECTIVE

20-17 Describe the mechanisms of drug resistance.

One of the triumphs of modern medicine has been the development of antibiotics and other antimicrobials. But the development of resistance to them by the target microbes is an increasing concern. To illustrate this concept, human populations often have a relative resistance to diseases to which they have been exposed for many generations. For example, when Europeans first colonized tropical climes, they proved highly susceptible to diseases to which they had never been exposed, although the local populations were relatively resistant. Antibiotics represent, in a sense, a disease for bacteria. When first exposed to a new antibiotic, the susceptibility of microbes tends to be high, and their mortality rate is also high; there may be only a handful of survivors from a

Bacterial Resistance to Antibiotics



KEYCONCEPTS

- There are only a few mechanisms of microbial resistance to antimicrobial agents: blocking the drug's entry into the cell, inactivation of the drug by enzymes, alteration of the drug's target site, efflux of the drug from the cell, or alteration of the metabolic pathways of the host.
- The mechanisms of bacterial resistance to antibiotics are limited. Knowledge of these mechanisms is critical for understanding the limitations of antibiotic use.

population of billions. The surviving microbes usually have some genetic characteristic that accounts for their survival, and their progeny are similarly resistant.

Such genetic differences arise from random mutations. These mutational differences can be spread *horizontally* among bacteria by processes such as conjugation (page 282) or transduction (page 234). Drug resistance is often carried by plasmids or by small segments of DNA called transposons, which can jump from one piece of DNA to another (Chapter 8, page 237). Some plasmids, including those called resistance (R) factors, can be transferred between bacterial cells in a population and between different but closely related bacterial populations (see Figure 8.28a, page 236). R factors often contain genes for resistance to several antibiotics.

Once acquired, however, the mutation is transmitted by normal reproduction, and the progeny carry the genetic characteristics of the parent microbe. Because of the rapid reproductive rate of bacteria, only a short time elapses before practically the entire population is resistant to the new antibiotic.

Bacteria that are resistant to large numbers of antibiotics are popularly designated as **superbugs**. Although the most publicized of superbugs is MRSA (page 568), superbug status has also been assigned to a range of bacteria, both gram-positive and gram-negative. Often cited among these are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and species of *Enterobacter*. Faced with infections by such pathogens, medical science has only limited treatment options.

Mechanisms of Resistance

There are only a few major mechanisms by which bacteria become resistant to chemotherapeutic agents. See Figure 20.20. At least one clinically troublesome bacterium, *Acinetobacter baumanii,* has developed resistance by means of all five of the major target sites illustrated in Figure 20.20.

Enzymatic Destruction or Inactivation of the Drug

Destruction or inactivation by enzymes mainly affects antibiotics that are natural products, such as the penicillins and cephalosporins. Totally synthetic chemical groups of antibiotics such as the fluoroquinolones are less likely to be affected in this manner, although they can be neutralized in other ways. This may simply reflect the fact that the microbes have had fewer years to adapt to these unfamiliar chemical structures. The penicillin/cephalosporin antibiotics, and also the carbapenems, share a structure, the β-lactam ring, which is the target for β-lactamase enzymes that selectively hydrolyze it. Nearly 200 variations of these enzymes are now known, each effective against minor variations in the β-lactam ring structure. When this problem first appeared, the basic penicillin molecule was modified. The first of these

penicillinase-resistant drugs was methicillin (see page 568), but resistance to methicillin soon appeared. The best-known of these resistant bacteria is the widely publicized pathogen MRSA, which is resistant to practically all antibiotics, not just methicillin (see the box on page 423). In a recent year, the CDC ascribed 19,000 deaths to this pathogen. In hospital patients, invasive infections with MRSA can cause as much as 20% mortality. Also, *S. aureus* is not the only bacterium of concern; other important pathogens, such as *Streptococcus pneumoniae*, have also developed resistance to β-lactam antibiotics. Furthermore, MRSA has continued to develop resistance against a succession of new drugs such as vancomycin (the "antibiotic of last resort"), even though this antibiotic has a mode of action against cell wall synthesis that is totally different from that of the penicillins. These highly adaptable bacteria have even developed resistance against antibiotic combinations that include *clavulanic acid*, specifically developed as an inhibitor of β-lactamases (see page 568). At first, MRSA was almost exclusively a problem in hospitals and similar health-related settings, accounting for about 20% of bloodstream infections there. However, it is now the cause of frequent outbreaks in the general community, is more virulent, and affects otherwise healthy individuals. These strains produce a toxin, a leukocidin, that destroys neutrophils, a primary innate defense against infection. In consequence, the descriptive terminology now differentiates *community-associated MRSA* from *health care–associated MRSA*. There is an obvious need for rapid tests to detect MRSA bacteria (generally from nasal swabs) so that infections can be isolated and transmission reduced. The most promising of these are based on PCR technology and yield good results within 1 or 2 hours.

Prevention of Penetration to the Target Site within the Microbe

Gram-negative bacteria are relatively more resistant to antibiotics because of the nature of their cell wall, which restricts absorption of many molecules to movements through openings called porins (see page 86). Some bacterial mutants modify the porin opening so that antibiotics are unable to enter the periplasmic space. Perhaps even more important, when β-lactamases are present in the periplasmic space, the antibiotic remains outside the cell, where the enzyme, which is too large to enter even through an unmodified porin, can reach and inactivate it.

Alteration of the Drug's Target Site

The synthesis of proteins involves the movement of a ribosome along a strand of messenger RNA, as shown in Figure 20.4. Several antibiotics, especially those of the aminoglycoside, tetracycline, and macrolide groups, utilize a mode of action that inhibits protein synthesis at this site. Minor modifications at this site can neutralize the effects of antibiotics without significantly affecting cellular function.

Interestingly, the main mechanism by which MRSA gained ascendancy over methicillin was not by a new inactivating enzyme, but by modifying the penicillin-binding protein (PBP) on the cell's membrane. β-Lactam antibiotics act by binding with the PBP, which is required to initiate the cross-linking of peptidoglycan and form the cell wall. MRSA strains become resistant because they have an additional, modified, PBP. The antibiotics continue to inhibit the activity of the normal PBPs, preventing their participation in forming the cell wall. But the additional PBP present on the mutants, although it binds weakly with the antibiotic, still allows synthesis of cell walls that is adequate for survival of MRSA strains.

Clinical Case

It would take 12 days to kill 200 cells at 4°C and 60 minutes at 23°C. The gentamicin is more effective at the warmer temperature, but the tissues will deteriorate too quickly at this temperature. Hence, the corneas are stored at 4°C to preserve the tissue even though gentamicin is less effective at 4°C.

How might storage of the corneas in gentamicin have contributed to these infections

559 570 579 **581** 584 585

Rapid Efflux (Ejection) of the Antibiotic

Certain proteins in the plasma membranes of gram-negative bacteria act as pumps that expel antibiotics, preventing them from reaching an effective concentration. This mechanism was originally observed with tetracycline antibiotics, but it confers resistance among practically all major classes of antibiotics. Bacteria normally have many such efflux pumps to eliminate toxic substances.

Variations of Mechanisms of Resistance

Variations on these mechanisms also occur. For example, a microbe could become resistant to trimethoprim by synthesizing very large amounts of the enzyme against which the drug is targeted. Conversely, polyene antibiotics can become less effective when resistant organisms produce smaller amounts of the sterols against which the drug is effective. Of particular concern is the possibility that such *resistant mutants* will increasingly replace the susceptible normal populations. Figure 20.21 shows how rapidly bacterial numbers increase as resistance develops.

Antibiotic Misuse

Antibiotics have been much misused, nowhere more so than in the less-developed areas of the world. Well-trained personnel are scarce, especially in rural areas, which is perhaps one reason why