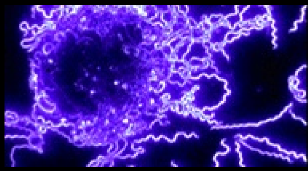

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BACTERIOLOGY - CHAPTER SIX

ANTIBIOTICS - PROTEIN SYNTHESIS, NUCLEIC ACID SYNTHESIS AND METABOLISM

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TEACHING OBJECTIVES

To describe the mode of action of antibacterial chemotherapeutic agents

To discuss antibiotic susceptibility testing

To review the mechanisms by which bacteria express resistance to antibiotics

Fig 1 Antibiotic susceptibility testing

MAJOR PRINCIPLES AND DEFINITIONS

SELECTIVITY

Clinically effective antimicrobial agents all exhibit selective toxicity toward the bacterium rather than the host. It is this characteristic that distinguishes antibiotics from disinfectants. The basis for selectivity will vary depending on the particular antibiotic. When selectivity is high the antibiotics are normally not toxic. However, even highly selective antibiotics can have side effects.

THERAPEUTIC INDEX

The therapeutic index is defined as the ratio of the dose toxic to the host to the effective therapeutic dose. The higher the therapeutic index the better the antibiotic.

CATEGORIES OF ANTIBIOTICS

Antibiotics are categorized as bactericidal if they kill the susceptible bacteria or bacteriostatic if they reversibly inhibit the growth of bacteria. In general the use of bactericidal antibiotics is preferred but many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic antibiotic is used the duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria. If possible, bactericidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defenses are relatively ineffective at these sites and the dangers imposed by such infections require prompt eradication of the organisms.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The basic quantitative measures of the *in vitro* activity of antibiotics are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (*i.e.* colonies on a plate or turbidity in broth culture) under standard conditions. The MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time. Figure 1 illustrates how to determine the MIC of an antibiotic.

For an antibiotic to be effective the MIC or MBC must be able to be achieved at the site of the infection. The pharmacological absorption and distribution of the antibiotic will influence the dose, route and frequency of administration of the antibiotic in order to achieve an effective dose at the site of infection.

In clinical laboratories, a more common test for antibiotic susceptibility is a disk diffusion test (figure 1). In this test the bacterial isolate is inoculated uniformly onto the surface of an agar plate. A filter disk impregnated with a standard amount of an antibiotic is applied to the surface of the plate and the antibiotic is allowed to diffuse into the adjacent medium.. The result is a gradient of antibiotic surrounding the disk. Following incubation, a bacterial lawn appears on the plate. Zones of inhibition of bacterial growth may be present around the antibiotic disk. The size of the zone of inhibition is dependent on the diffusion rate of the antibiotic, the degree of sensitivity of the microorganism, and the growth rate of the bacterium. The zone of inhibition in the disk diffusion test is inversely related to the MIC.

The test is performed under standardized conditions and standard zones of inhibition have been established for each antibiotic. If the zone of inhibition is equal to or greater than the standard, the organism is considered to be sensitive to the antibiotic. If the zone of inhibition is less than the standard, the organism is considered to be resistant. Figure 1 also illustrates how the disk diffusion test is done and Figure 2 illustrates some of the standard zones of inhibition for several antibiotics.

KEY WORDS
Selectivity
Therapeutic Index
Bactericidal
Bacteriostatic
MIC
MBC
Disk Diffusion Test
Antibiotic Synergism
Antibiotic Antagonism
Antimicrobial
Cross Resistance
Multiple Resistance

COMBINATION THERAPY
Combination therapy with two or more antibiotics is used in special cases:

- To prevent the emergence of resistant strains
- To treat emergency cases during the period when an etiological diagnosis is still in progress
- To take advantage of antibiotic synergism.

Antibiotic synergism occurs when the effects of a combination of antibiotics is greater than the sum of the effects of the individual antibiotics. Antibiotic antagonism occurs when one antibiotic, usually the one with the least effect, interferes with the effects of another antibiotic.

ANTIBIOTICS AND CHEMOTHERAPEUTIC AGENTS
The term antibiotic strictly refers to substances that are of biological origin whereas the term chemotherapeutic agent refers to a synthetic chemical. The distinction between these terms has been blurred because many of our newer "antibiotics" are actually chemically modified biological products or even chemically synthesized biological products. The generic terms to refer to either antibiotics or chemotherapeutic agents are antimicrobic or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents.

Figure 2

Zone diameter interpretive standards and approximate MIC correlates used to define the interpretive categories

Antimicrobial agent (amount per disk) and organism	Zone diameter (nearest whole millimeter) for each interpretive category				Approximate MIC correlates (micro gm/ml) for:	
	R	I	MS	S	R	S
Ampicillin (10 micro gm)						
Enterobacteriaceae	≤11	12-13		≥14	≥32	≤8
Staphylococcus spp.	≤28			≥29	beta-Lactamase	≤0.25
Haemophilus spp.	≤19			≥20	≥4	≤2
Enterococci	≤16		≥17		≥16	

Other streptococci	≤21		22-29	≥30	≥4	≤0.12
Chloramphenicol (30 micro gm)	≤12	13-17		≥18	≥25	≤12.5
Erythromycin (15 micro gm)	≤13	14-17		≥18	≥8	≤2
Nalidixic acid (30 micro gm)	≤13	14-18		≥19	≥32	≤12
Streptomycin (10 micro gm	≤11	12-14		≥15		
Tetracycline (30 micro gm)	≤14	15-18		≥19	≥16	≤4
Trimethoprim (5 micro gm)	≤10	11-15		≥16	≥16	≤4

a Adapted from the October 1983 document (M2-T3) of the NCCLS. Refer to the most current MCCLS documents for updates and changes.

b R, Resistant; I, intermediate; MS, moderately susceptible; S, susceptible. An I result should be reported since it indicates an equivocal test result that may require further testing. When designated in the table, an MS result should be reported to indicate a level of susceptibility that should require the maximal safe dosage for therapy. Strains in the MS category are susceptible and not intermediate.

c Approximate MIC correlates used for the definition of the resistant and susceptible categories. Theses correlates should not be used for the interpretation of antimicrobial dilution test results.

PROTEIN SYNTHESIS AND SITE OF ACTION OF ANTIMICROBIALS THAT INHIBIT PROTEIN SYNTHESIS

INITIATION OF PROTEIN SYNTHESIS

Figure 3 illustrates the initiation of protein synthesis and the site of action of antimicrobials that inhibit this process.

ELONGATION

Figure 4 illustrates the process of elongation and the site of action of antimicrobials that inhibit this process.

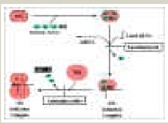


Fig 3 Antibiotics that act at the level of protein synthesis initiation

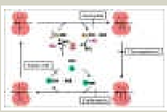


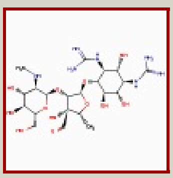
Fig 4 Antibiotics that act at the level of the elongation phase of protein synthesis

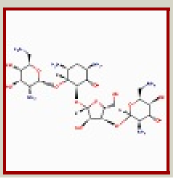
INHIBITORS OF PROTEIN SYNTHESIS

The selectivity of these agents is a result of differences in the prokaryotic 70S ribosome and the 80S eukaryotic ribosome. Since mitochondrial ribosomes are similar to prokaryotic ribosomes, these antimetabolites can have some toxicity. They are mostly bacteriostatic.

ANTIMICROBIALS THAT BIND TO THE 30S RIBOSOMAL SUBUNIT

Aminoglycosides (bactericidal)
Streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and neomycin (topical)


Streptomycin



a. Mode of action
The aminoglycosides irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-tRNA), so that no further initiation can occur. The aminoglycosides also slow down protein synthesis that has already initiated and induce misreading of the mRNA.

b. Spectrum of Activity
Aminoglycosides are active against many gram-negative and some gram-positive bacteria. They are not useful for anaerobic bacteria, since oxygen is required for uptake of the antibiotic,

Neomycin

or for intracellular bacteria.

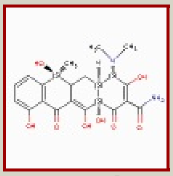
c. Resistance
Resistance to these antibiotics is common

d. Synergy
The aminoglycosides synergize with β-lactam antibiotics such as the penicillins. The β-lactams inhibit cell wall synthesis and thereby increase the permeability of the bacterium to the aminoglycosides.

Tetracyclines (bacteriostatic)
Tetracycline, minocycline and doxycycline

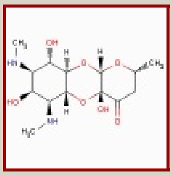
a. Mode of action
The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

b. Spectrum of activity -
These are broad spectrum antibiotics and are useful against intracellular bacteria


Tetracycline

c. Resistance
Resistance to these antibiotics is common

d. Adverse effects
Destruction of normal intestinal flora often occurs, resulting in increased secondary infections. There can also be staining and impairment of the structure of bone and teeth


Spectinolycin

Spectinomycin (bacteriostatic)

a. Mode of action
Spectinomycin reversibly interferes with mRNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA

b. Spectrum of activity -
Spectinomycin is used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*

c. Resistance
This is rare in *Neisseria gonorrhoeae*

ANTIMICROBIALS THAT BIND TO THE 50S RIBOSOMAL SUBUNIT

Chloramphenicol, lincomycin, clindamycin (bacteriostatic)

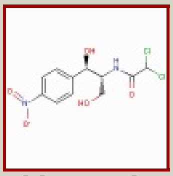
a. Mode of action
These antimicrobials bind to the 50S ribosome and inhibit peptidyl transferase activity.

b. Spectrum of activity

- Chloramphenicol - Broad range
- Lincomycin and clindamycin - Restricted range

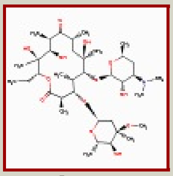
c. Resistance
Resistance to these antibiotics is common

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


Chloramphenicol

Macrolides (bacteriostatic) - Erythromycin (also azithromycin, clarithromycin)

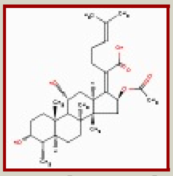
a. Mode of action
The macrolides inhibit translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal 23S RNA.


Erythromycin

b. Spectrum of activity
Gram-positive bacteria, *Mycoplasma, Legionella*

c. Resistance
Resistance to these antibiotics is common. Most gram-negative antibiotics are resistant to macrolides.

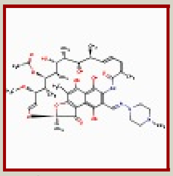
ANTIMICROBIALS THAT INTERFERE WITH ELONGATION FACTORS


Fusidic acid

Fusidic acid (bacteriostatic)

a. Mode of action
Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

b. Spectrum of activity
Fusidic acid is only effective against gram-positive bacteria such as *Streptococcus*, *Staphylococcus aureus* and *Corynebacterium minutissimum*.


Rifampin

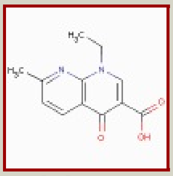
INHIBITORS OF NUCLEIC ACID SYNTHESIS AND FUNCTION

The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

INHIBITORS OF RNA SYNTHESIS AND FUNCTION

Rifampin, rifamycin, rifampicin (bactericidal)

a. Mode of action
These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.


Nalidixic acid

b. Spectrum of activity
They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis

c. Resistance
Resistance to these antibiotic is common.

d. Combination therapy
Since resistance is common, rifampin is usually used in combination therapy

INHIBITORS OF DNA SYNTHESIS AND FUNCTION

Quinolones - nalidixic acid, ciprofloxacin, oxolinic acid (bactericidal)

a. Mode of action
These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

b. Spectrum of activity -
These antibiotics are active against Gram-positive cocci and are used in urinary tract infections

c. Resistance
This is common for nalidixic acid and is developing for ciprofloxacin

ANTIMETABOLITE ANTIMICROBIALS

INHIBITORS OF FOLIC ACID SYNTHESIS

The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their own folic acid. In

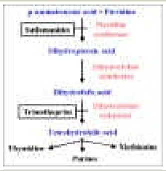
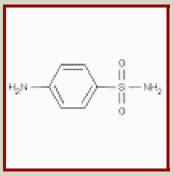
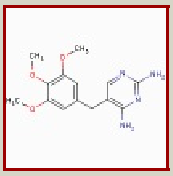
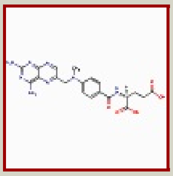
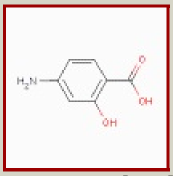


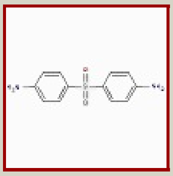
Fig 5
Folic acid metabolism

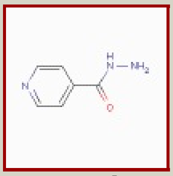

Sulfanilamide


Trimethoprim


Methotrexate


Amino salicylic acid


Dapsone


Isoniazid

contrast, mammalian cells use folic acid obtained from food.

Figure 5 summarizes the pathway of folic acid metabolism and indicates the sites at which antimetabolites act.

Sulfonamides, sulfones (bacteriostatic)

a. Mode of action
These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

b. Spectrum of activity
They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance
Resistance to these antibiotics is common

d. Combination therapy
The sulfonamides are used in combination with trimethoprim. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

Trimethoprim, methotrexate, pyrimethamine (bacteriostatic)

a. Mode of action
These antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.

b. Spectrum of activity
They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance
Resistance to these antibiotics is common

d. Combination therapy
These antimicrobials are used in combination with the sulfonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

ANTI-MYCOBACTERIAL AGENTS

Anti-mycobacterial agents are generally used in combination with other antimicrobials since treatment is prolonged and resistance develops readily to individual agents.

Para-aminosalicylic acid (PSA) (bacteriostatic)

a. Mode of action
This is similar to sulfonamides

b. Spectrum of activity
PSA is specific for *Mycobacterium tuberculosis*

Dapsone (bacteriostatic)

a. Mode of action
Similar to sulfonamides

b. Spectrum of activity
Dapsone is used in treatment of leprosy

Isoniazid (INH) (bacteriostatic)

a. Mode of action
Isoniazid inhibit synthesis of mycolic acids.

b. Spectrum of activity -
INH is used in treatment of tuberculosis

c. Resistance
Resistance has developed

ANTIMICROBIAL DRUG RESISTANCE

PRINCIPLES AND DEFINITIONS

Clinical Resistance
Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety *in vivo*. Resistance to an antimicrobial can arise:

- By mutation in the gene that determines sensitivity/resistance to the agent
- By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene.

Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, *i.e.* the antibiotic selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

Cross Resistance
Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

MECHANISMS OF RESISTANCE

Altered permeability of the antimicrobial agent
Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

Inactivation of the antimicrobial agent
Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

Altered target site
Resistance can arise due to alteration of the target site for the antimicrobial agent.

Replacement of a sensitive pathway
Resistance can result from the acquisition of a new enzyme to replace the sensitive one.

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