جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

المضادات الحياتية

المرحلة الثالثة

الفصل الثاني

ا.م.د. سهاد سعد

م. د. حنين مؤيد

2022-2021

Antibiotics
lecture 1

Introduction

1

Introduction

- The word "antibiotics" comes from the Greek anti ("against") and bios ("life").
- The noun “antibiotic” was suggested in 1942 by Dr. Selman A. Waksman.
- In 1943, an American, Dr. Selman A. Waksman, discovered a drug called streptomycin. It originated from microbes found in soil and was a cure for many intestinal diseases. He discovered 20 other antibiotics, including Neomycin, Actinomycin (Nobel prize 1952).
- Most microbiologist distinguish two groups of antimicrobial agents used in the treatment of infectious disease.

2

Antibiotic: Chemical produced by a microorganism that kills or inhibits the growth of another microorganism

Antimicrobial agent: Chemical that kills or inhibits the growth of microorganisms

chemotherapeutic agents: which are chemically synthesized

3

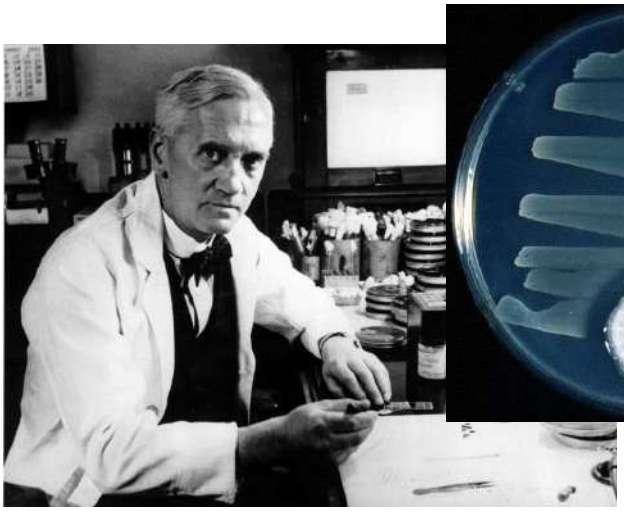
Ehrlich's Magic Bullets

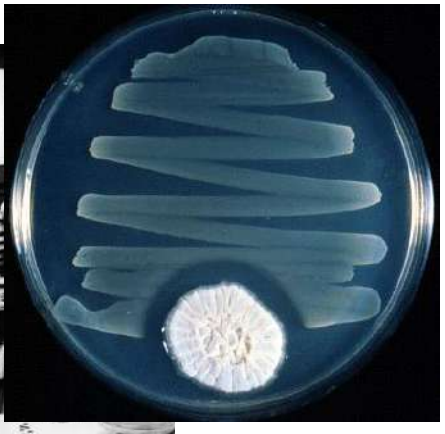
4

- In the late 1880s **Synthetic antibiotic chemotherapy** as a science and development of antibacterial began in Germany with **Paul Ehrlich**.
- Ehrlich noted that certain dyes would color human, animal, or bacterial cells, while others did not.
- He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host.
- After screening hundreds of dyes and chemicals against various organisms, he discovered that compound number 606 , **arsphenamine** , was active against syphilis spirochete .
- **Arsphenamine** was made available in 1910 under trade name **Salvarsan**. Ehrlich coined the phrase "**magic bullet**' to describe this new wonder drug.

5

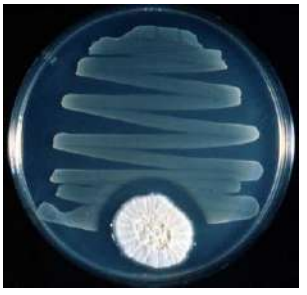
Fleming and Penicillin





6

- **Alexander Fleming** made a crucial discovery that led to the production of the “Wonder Drug”, **penicillin**.
- After leaving some used culture plates unattended for several weeks, he arrived back from his vacation to find fungus growing on them. On one plate, the Staphylococcus aureus bacteria that had been cultured there appeared to be inhibited by the fungus that had appeared. This fungus was found to be ***Penicillium notatum*** and everywhere it appeared on the plate, the bacterial growth was inhibited.



- Later, other scientists discovered that penicillin could cure certain infections in mice and rabbits. In turn, it did not harm the animals in any way.

7

Microbial Sources of Antibiotics

TABLE 20.1 **Representative Sources of Antibiotics**

Microorganism	Antibiotic
Gram-Positive Rods	
Bacillus subtilis	Bacitracin
Bacillus polymyxa	Polymyxin
Actinomycetes	
Streptomyces nodosus	Amphotericin B
Streptomyces venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Streptomyces erythraeus	Erythromycin
Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpureae	Gentamicin
Fungi	
Cephalosporium spp.	Cephalothin
Penicillium griseofulvum	Griseofulvin
Penicillium notatum	Penicillin

8

Characteristics of Antibiotics

Antibiotic are chemical substances (low-molecular weight substances) that can inhibit the growth of, and even destroy, harmful microorganisms.

They are derived from special microorganisms or other living systems.

Antibiotics are produced as secondary metabolites by certain groups of microorganisms, especially Streptomyces, Bacillus, and a few molds (Penicillium and Cephalosporium) that are inhabitants of soils on an industrial scale using a fermentation process.

Several hundreds of compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically-useful. The reason for this is that only compounds with selective toxicity can be used clinically. **The selective toxicity** of antibiotics means that they must be highly effective against the microbe but have minimal or no toxicity to humans. In practice, this is expressed by a **drug's therapeutic index (TI)** : - the ratio of the toxic dose {The dose at which the antibiotic becomes too toxic to the patient (host)} to the therapeutic dose (The dose required to eliminate the infection). The larger the index is the safer drug (antibiotic) for human use(the better) .

etter) .

$$\text{hemotherapeutic Index} = \frac{\text{Toxic Concentration (DTM)}}{\text{Effective Concentration (DCM)}}$$

DTM = dosis tolerata maxima (toxic)
DCM = dosis curativa minima (effective)

9

Antibiotics may have **a cidal (killing) effect** or **static(inhibitory) effect** on a range of microbes.

The range of bacteria or other microorganisms that is affected by a certain antibiotic is expressed as its **spectrum of action**.

Antibiotics effective against prokaryotes that kill or inhibit a wide range of Gram-positive and Gram-negative bacteria are said to **be broad spectrum.**

If effective mainly against Gram-positive or Gram-negative bacteria, they are **narrow spectrum**.

If effective against a single organism or disease, they are referred to as **limited spectrum**.

10

Antibiotic Spectrum of Activity

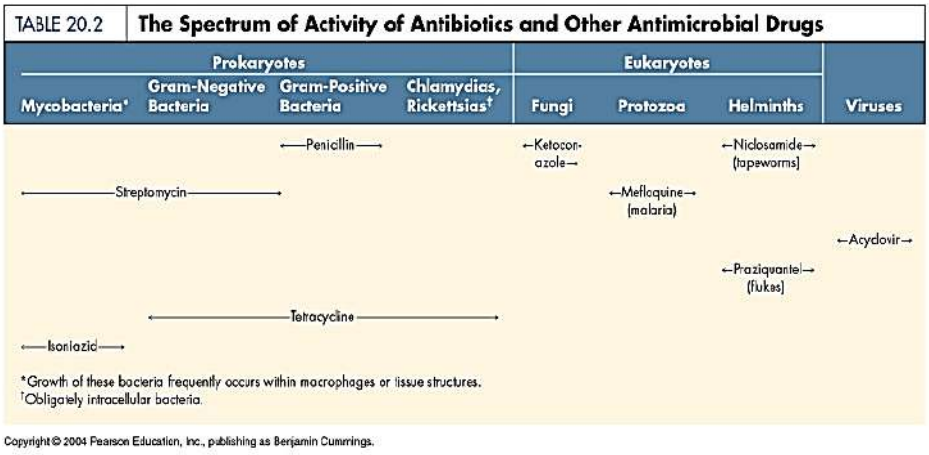


TABLE 20.2 The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs

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

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

- No antibiotic is effective against all microbes

11

A clinically-useful antibiotic should have as many of these characteristics as possible:

-It should have a wide spectrum of activity with the ability to destroy or inhibit many different species of pathogenic organisms.

-It should be nontoxic to the host and without undesirable side effects (selective toxicity with minimal side effects to host**).**

- bactericidal rather than bacteriostatic.

-It should be nonallergenic to the host.

-It should not eliminate the normal flora of the host.

-It should be able to reach the part of the human body where the infection is occurring.

-It should be inexpensive and easy to produce.

-It should be chemically-stable (have a long shelf-life).

-Microbial resistance is uncommon and unlikely to develop.

12

Words to Know

-Bactericidal drugs : Act by killing bacteria.

-Bacteriostatic drugs : Act to suppress or inhibit bacterial replication sufficiently until the immune system can eliminate the organisms

-Prophylactic drugs : Drugs taken to **prevent** a disease rather than treat an established infection

-Synergism : Certain drugs work better together in combination compared to being used individually.
-Antagonism : Certain drugs may decrease the effectiveness of others, or prove toxic when taken in combination.
-Mono therapy : Taking a single agent to treat an infection

-Combination therapy or **polytherapy** : Taking more than one drug to treat an infection. Conditions treated with combination therapy include tuberculosis, leprosy, cancer, malaria, and HIV/AIDS. One major benefit of combination therapies is that they reduce development of drug resistance, since a pathogen or tumor is less likely to have resistance to multiple drugs simultaneously.

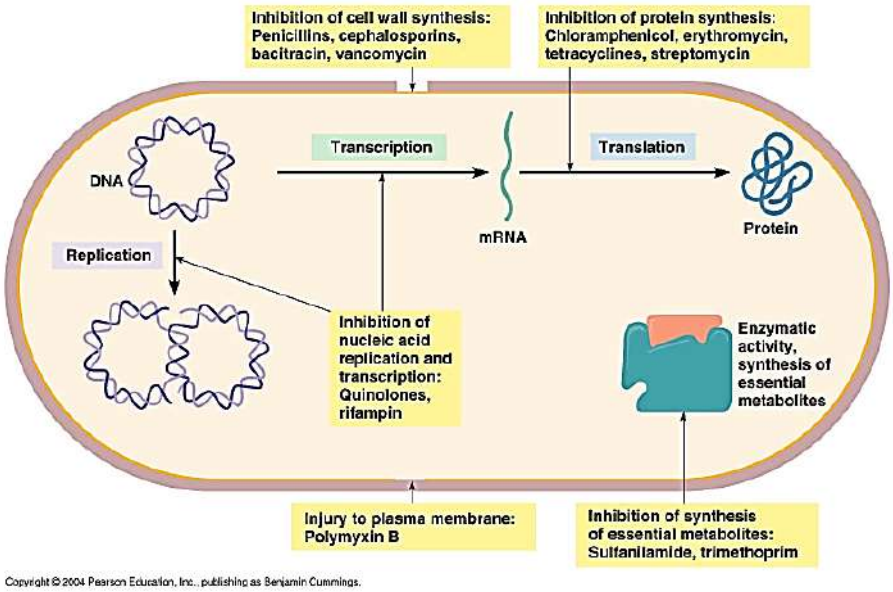
13

Mechanisms of Antimicrobial Action

- Bacteria have their own enzymes for
  - Cell wall formation
  - Protein synthesis
  - DNA replication
  - RNA synthesis
  - Synthesis of essential metabolites

14

Modes of Antimicrobial Action



15

Lecture #2

Antibiotics

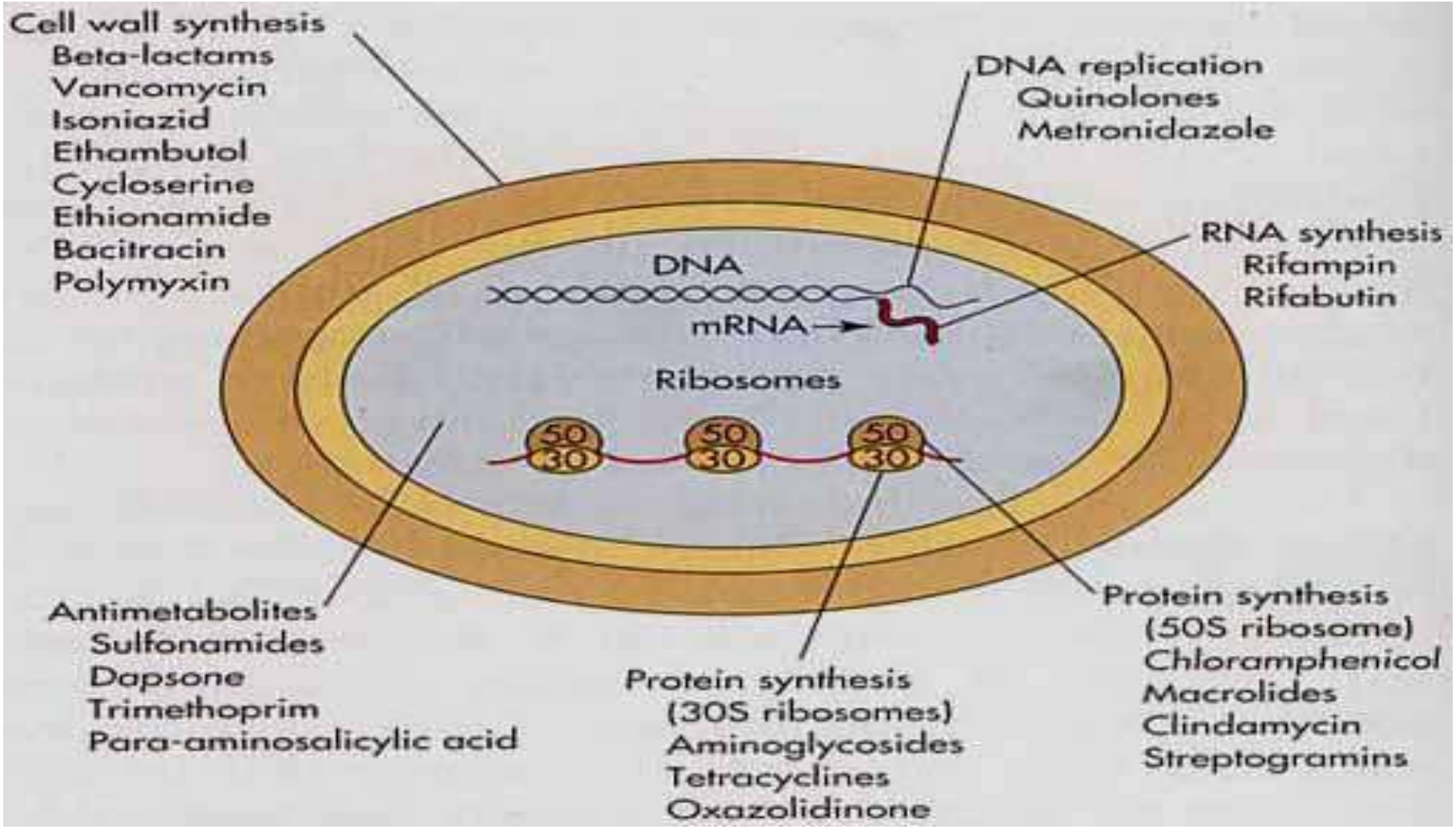
Antibiotic classes

An '**antibiotic class'** refers to a group of antibiotics with a very similar chemical structure. Because of their similar chemical structure, members of an antibiotic class have the same basic mechanism of action. Generally, within a class, there is the same core nucleus critical to function, while differing side chains modify the drug's toxicity, spectrum, pharmacokinetics, etc.

The main classes of antibiotics are:

- Beta-Lactams (Penicillins & Cephalosporins)
-Macrolides
-Quinolones
-Tetracyclines
-Aminoglycosides
- Glycopeptides
- Lincomycin

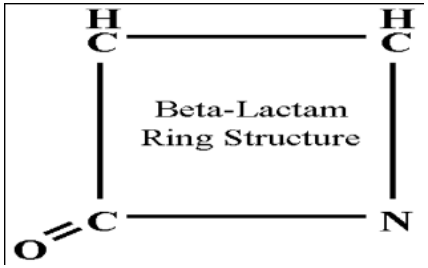
Below, the different antibiotic classes are grouped by their mechanism of action



Bacterial class
Inhibited of Cell Wall Synthesis

Beta-Lactam Antibiotics

Beta-Lactam Antibiotics : are a broad class of antibiotics, consisting of all antibiotic agents that contains a β-lactam nucleus in its molecular structure. β-lactam ring consisting of 3 carbon atoms and 1 nitrogen atom.



❖ Beta-lactam antibiotics are characterized by three fundamental structural requirements:

1. The beta-lactam structure
2. A free carboxyl acid group
3. One or more substituted amino acid side chains

These antibiotics contain a 4 membered beta lactam ring includes penicillin ,cephalosporins, monobactams, and carbapenems .They are the products of two groups of fungi, *Penicillium* and *Cephalosporium* molds .

Bacterial class
Inhibited of Cell Wall Synthesis

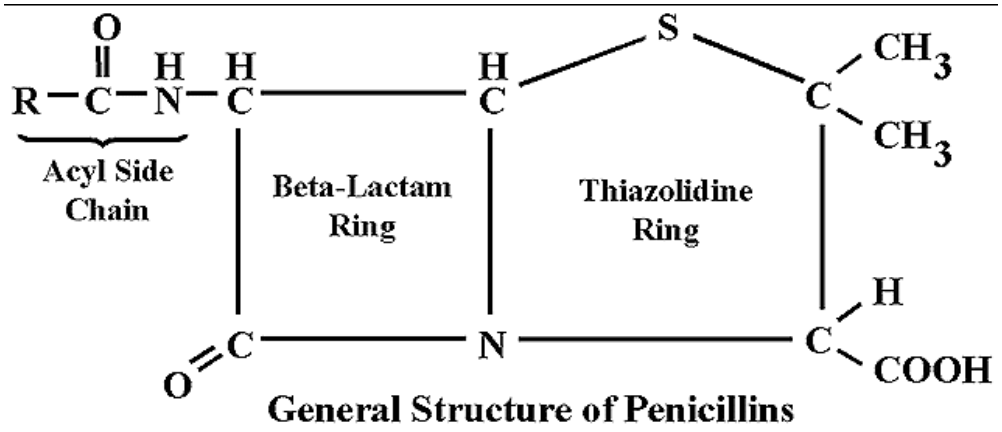
- Beta lactam antibiotics are normally bactericidal and require that cells be actively growing in order to exert their toxicity.

- Different beta lactams differ in their spectrum of activity and their effect on Gram-negative rods, as well as their toxicity, stability in the human body, rate of clearance from blood, whether they can be taken orally, ability to cross the blood-brain barrier, and susceptibility to bacterial beta-lactamases.

Bacterial class
Inhibited of Cell Wall Synthesis

The Penicillins

- The penicillins all share a beta-lactam ring attached to a thiazolidine ring.



General Structure of Penicillins

The ring is very strained and the bond between the carbonyl and the nitrogen in the β-lactam ring is very labile (site of cleavage by bacterial penicillinase or by acid) and responsible for the molecule reactivity.

Bacterial class
Inhibited of Cell Wall Synthesis

- The penicillin nucleus (6-aminopenicillanic acid) itself is the chief structural requirement for biological activity; metabolic transformation or chemical alteration of this portion of the molecule causes loss of all significant antibacterial activity. The nature of R-group determines the antibiotic's stability to enzymatic or acidic hydrolysis and affects its antibacterial spectrum so that the R - group substituent of the penicillin nucleus can be changed to give the molecule different antibacterial properties, change its pharmacokinetic properties, ability to get through **porins** of gram negatives, stability to beta-lactamases, etc.

Bacterial class
Inhibited of Cell Wall Synthesis

- **Mode of action**
- The targets of the penicillins are enzymes (transpeptidase) which called penicillin binding proteins (PBPs), the transpeptidase is involved in synthesis of the cell wall. Penicillin attacks bacterial cells by inactivating this enzyme which is essential for bacterial growth (peptidoglycan transpeptidase catalyses the cross-linking of the peptidoglycan, which forms the cell wall of the bacteria). The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms

Bacterial class
Inhibited of Cell Wall Synthesis

The peptidoglycan transpeptidase enzyme is not needed in animals as their cells do not have cell walls. Therefore, the penicillin can safely disrupt the bacterial cell wall biosynthesis without harming existing cells in the body. The penicillin stops the growth of the bacterial cell wall, causing the pressure inside the cell to rise considerably until the cell lyses and thus the cell is destroyed (in other words, the antibiotic causes cytolysis or death due to osmotic pressure). In addition, the build-up of peptidoglycan precursors triggers the activation of bacterial cell **wall hydrolases and autolysins**, which further digest the bacteria's existing peptidoglycan.

The activity of antibiotics depends on 3 things:

1-Affinity for the target (e.g. how well penicillin binds PBPs)

2-Permeability properties (e.g. through capsule, peptidoglycan)

3-Stability to bacterial enzymatic degradation (e.g. beta-lactamases)

Lecture #3

Bacterial class
Inhibited of Cell Wall Synthesis

Classification

The penicillins can be classified according to their antibacterial activity:

Natural penicillins, Antistaphylococcal penicillins, Aminopenicillins,
Extended spectrum penicillins: carboxypenicillins and ureidopenicillins

1- Natural penicillins (Narrow spectrum – penicillinase (lactamase) sensitive)

Natural penicillins, including **penicillin G** & **penicillin V**, are produced by fermentation of *Penicillium chrysogenum*.
They are active against non β-lactamase–producing gram-positive cocci (*Pneumococci*, *Staphylococci*, *Streptococci*), few gram-negative cocci (*meningococci* and *gonococci*), gram-positive bacilli (*Bacillus anthracis*, *Bacillus perfringens*, *Bacillus diphtheriae*), anaerobes (*Clostridum perfringens*, *C. tetani*), and spirochetes (*Treponema pallidum*, *T. pertenue* and *Leptospira*).
They are considered narrow spectrum since they are not effective against Gram-negative rods. The natural penicillins are very susceptible to inactivation by beta-lactamases.

Bacterial class
Inhibited of Cell Wall Synthesis

2- Narrow spectrum – penicillinase (lactamase) resistant (Anti-Staphylococcal Penicillins)

These drugs were created in response to the problem in the 1950's, staphylococcal infections in hospitals were resistant to penicillin due to production of beta-lactamases.

Anti-Staphylococcal Penicillins are ***semi-synthetic,*** and have big bulky side chains that provide **steric hindrance** to protect the beta-lactam from cleavage by the beta lactamases. This group of penicillin drugs includes:

a- Methicillin (Poor oral availability) **, b- Nafcillin, c. Isoxazolyl penicillins (**Good oral availability**) (Oxacillin, Cloxacillin , Dicloxacillin , Flucloxacillin).**

There are slight differences in each of these, i.e. administration, pharmacokinetics, etc.

Methicillin was the first member of this group, followed by oxacillin, nafcillin, cloxacillin and dicloxacillin.

Methicillin was the first penicillin developed through rational drug modification. Since then all bacteria which are resistant to methicillin are designated as methicillin resistant (e.g MRSA - methicillin-resistant S. aureus).

Due to the bulky side group, all of the antistaphylococcals have difficulty penetrating the cell membrane and have a poor range(less potent) of activity compared to other penicillins.

Spectrum*:* Antistaphylococcals have a very narrow spectrum as they were developed solely for killing β-lactamase producing staphylococci. Their major clinical indications are susceptible *S. aureus* and *S. epidermidis* infections.

Bacterial class
Inhibited of Cell Wall Synthesis

3- Broad spectrum – penicillinase (lactamase) sensitive(Aminopenicillins)

Ampicillin, amoxicillin, bacampicillin, Pivampicillin ,Talampicillin.

The aminopenicillins have a wider range of activity than natural or antistaphylococcal penicillins. However, they lack the bulky side groups and are susceptible to inactivation by beta-lactamases. Aminopenicillins have additional hydrophilic groups, allowing the drug to penetrate into Gram-negative bacteria via the porins. Advantages of aminopenicillins include higher oral absorption, higher serum levels, and longer half-lives. Aminopenicillins are resistant to gastric acids so can be administered orally.

Spectrum*:* Aminopenicillins are similar to penicillin G in the activity against Gram-positive organisms but are slightly weaker than the latter. - --- Aminopenicillins are more active against *enterococci* and *Listeria monocytogenes* compared to penicillin G. Gram-negative spectrum includes *Haemophilus influenzae*, *Salmonella*, *Shigella*, *Escherichia coli*, *Proteus mirabilis*, *N. gonorrhoeae*, *N. meningitidis*.

4- Extended spectrum – penicillinase (lactamase) sensitive (Anti-pseudomonal penicillins)

Extended-spectrum penicillins (also called antipseudomonals) include both **carboxypenicillins (carbenicillin and ticarcillin)** and **ureidopenicillins (piperacillin, azlocillin, and mezlocillin).** Antipseudomonal penicillins are similar to the aminopenicillins in structure but have either a carboxyl group or urea group instead of the amine.

In general, the antipseudomonal penicillins have greater activity than do other penicillins against gram-negative bacteria (especially *Pseudomonas* and *Proteus*) due to enhanced penetration through the cell wall of these bacteria.

The major advantage of carboxypenicillins is their activity against *Pseudomonas aeruginosa* (one of the major pathogens responsible for **nosocomial pneumonia**) and certain indole-positive *Proteus* species that are resistant to aminopenicillins. Ticarcillin is stronger against *P. aeruginosa* and *Enterobacter* species than carbenicillin.

Against anaerobes and Gram-positive organisms, carboxypenicillins generally have the same spectrum of activity as penicillin G. However, they are substantially weaker in comparison with penicillin G.

Ureidopenicillins have greater activity against *P. aeruginosa* compared to carbenicillin and ticarcillin. Piperacillin is the most potent of the extended-spectrum penicillins against *Pseudomonas*. The spectrum of piperacillin and mezlocillin is extended to include *Klebsiella*, *Enterobacter*, *Citrobacter*.

All antipseudomonals are destroyed by β-lactamases. The extended-spectrum penicillins are not used in the treatment of infections caused by Gram-positive bacteria because penicillin G and aminopenicillins are more potent against these organisms.

Adverse effects

The penicillins have minimal toxicity and are among the safest antibiotics. The most serious side effect of penicillins is allergy.

- **Penicillin Hypersensitivity**: penicillins are the most common cause of drug allergy. Allergic reactions occur in 0.7% – 8% of treatments ,10% of allergic reactions are life-threatening and 10% of these are fatal, manifestations of allergy to penicillins include rash, fever, bronchospasm, , serum sickness, exfoliative dermatitis.
- Common side effects: Many persons who take penicillins experience diarrhea, nausea, and vomiting.

Hepatotoxicity (cholestatic hepatitis) most commonly occurs with oxacillin, nafcillin, and flucloxacillin

- Other side effects are less common : Very high doses of penicillin G can cause kidney failure. Methicillin famous for interstitial nephritis

LECTURE 4

Antibiotics

The Cephalosporins

Cephalosporins are structurally and pharmacologically related to the penicillins. Cephalosporins are beta-lactam compounds in which the beta-lactam ring is fused to a 6-membered dihydrothiazine ring, thus forming the cephem nucleus. **Side chain modifications to the cephem nucleus confers 1) an improved spectrum of antibacterial activity, 2) pharmacokinetic advantages, and 3) additional side effects.**

Cephalosporin compounds were first isolated from cultures of *Cephalosporium* in 1948.They have a low toxicity and a somewhat broader spectrum than natural penicillins. They are often used as penicillin substitutes against Gram-negative bacteria and in surgical prophylaxis. They are subject to degradation by some bacterial beta-lactamases, but they tend to be resistant to beta-lactamases from *S. aureus.* Cephalosporin(**Bactericidal**) prevents cell wall synthesis by binding to enzymes called penicillin binding proteins (PBPs). These enzymes are essential for the synthesis of the bacterial cell wall.

Cephalosporins are derived from cephalosporin C(natural cephalosporins) which is an acid-stable molecule with antibacterial activity and is produced from *Cephalosporium acremonium*. All of them are semi- synthetic .The first agent cephalothin (cefalotin) was used in 1964.

Cephalosporins are grouped into "generations" based on their spectrum of antimicrobial activity.

Major differences between generations :increased activity against Gram-negative bacteria & increased resistance to class C β-lactamases = cephalosporinase. The first cephalosporins were designated first generation while later, more extended spectrum cephalosporins were classified as second generation cephalosporins. Each newer generation of cephalosporins has significantly greater gram-negative antimicrobial properties than the preceding generation, in most cases with decreased activity against gram-positive organisms. Fourth generation cephalosporins, however, have true broad spectrum activity. The newer agents have much longer half-lives resulting in the decrease of dosing frequency.

Classification

1- First Generation

First generation cephalosporins are moderate spectrum agents. They are effective alternatives for treating staphylococcal and streptococcal infections and therefore are alternatives for skin and soft-tissue infections, as well as for streptococcal pharyngitis. **Cefazolin, Cephalothin (parenteral) , Cephalexin, Cefadroxil, Cephradine (oral)**

- cephalothin, cefazolin, cephalexin. have good activity against most Gram positive cocci *(Streptococcus, pneumococcus* but not or methicillin-resistant *Staphylococcus).* They are more active against Gram negative organisms *(Escherichia co1i Kiebsiella pneumoniae,* and the **indole negative** *Proteus mirabilis)* than are the natural penicillins. They are effective against some anaerobic cocci *(Peptococcus* and *Peptosteptococcus,* but NOT *Bacteroides fragilis).*

*They are ineffective against *Pseudomonas aeruginosa, Enterobacter,* and indole-positive *Proteus* species.

*These drugs do not cross the blood-brain barrier.

cefazolin and cephalexin used for surgical prophylaxis, URIs, otitis media**.**

2- Second Generation

The second generation cephalosporins have a greater gram-negative spectrum while retaining some activity against gram-positive bacteria.

They are also more resistant to beta-lactamase. They are useful agents for treating upper and lower respiratory tract infections, sinusitis and otitis media. These agents are also active against *E. coli*, *Klebsiella and Proteus*, which makes them potential alternatives for treating urinary tract infections caused by these organisms.

Cefoxitin is a second generation cephalosporin with anaerobic activity .

• Cefaclor, Cefuroxime (Oral)

•Cefamandole, Cefonicid, Cefuroxime, Cefoxitin , Cefotetan , Ceforanide (Parenteral)

* **cefuroxime ,cefamandole, cefaclor** are effective against *Haemophilus influenza*

* **cefoxitin** is **effective against *Bacteroides fragilis***

*These drugs do not achieve adequate levels in the CSF. **Cefoxitin , cefuroxime** used prophylactically for Surgical prophylaxis abdominal or colorectal surgeries

3- Third Generation

Third generation cephalosporins have a broad spectrum of activity and further increased activity against gram-negative organisms. Some members of this group (particularly those available in an oral formulation) have decreased activity against gram-positive organisms. The spectrum is extended to include: *Enterobacter E.*

coli, *Proteus mirabilis*, *Klebsiella*, *Pseudomonas* (**ceftazidime and cefaperazone only**), *Serratia*, β **-**lactamase producing *Haemophillus influenza* and *Neisseria* species. **The parenteral third generation cephalosporins (ceftriaxone and cefotaxime)** have excellent activity against most strains of *Streptococcus pneumoniae*, including those with intermediate and high level resistance to penicillin.

- Only **cetizoxime and moxalactam** retain good **activity against *Bacteroides fragilis.***

-Ceftazidime IV and IM , excellent gram-negative coverage

*Used for difficult-to-treat organisms such as Pseudomonas spp.

- Cefotaxime

* Active against gram-negative bacteria , enterobacteria, gonococcus . Active against *Pseudomonas aeruginosa.*

* Penetrates the CNS => used for meningitis.

Ceftriaxone IV and IM, long half-life, once-a-day dosing, is effective as a **single dose therapy for uncomplicated Neisseria gonorrhea**

*Easily passes meninges and diffused into CSF to treat CNS infections.

- **Cefixime** Only oral third-generation agent(Tablet and suspension**)** *Best of available oral cephalosporins against gram-negative .

4- Fourth Generation
– cefpirome, cefepime

Fourth generation cephalosporins are extended spectrum agents with similar activity against gram-positive organisms as first generation cephalosporins. They also have a greater resistance to beta-lactamases than the third generation cephalosporins. Many can cross blood brain barrier and are effective in meningitis.

- Cefepime has broad gram-negative coverage with somewhat enhanced activity against pseudomonas but slightly lesser activity against pneumococci. - Cefpirome is more active against pneumococci and has somewhat lesser activity against pseudomonas. Cefepime and cefpirome are highly active against nosocomial pathogens such as Enterobacter and Acinetobacter and their use should therefore be restricted to the setting of nosocomial sepsis.
- Both antibiotics had good activity against *Staphylococcus aureus* and coagulase-negative staphylococci except for methicillin-resistant strains and *Staphylococcus haemolyticus* which were of borderline sensitivity.
- Both antibiotics had little useful activity against the *Bacteroides fragilis* group or *Bacteroides oralis* group but were active against most other anaerobes. *Clostridium difficile* and some other *Clostridium* species were resistant.

A cephalosporin Uses:

A cephalosporin with or without an aminoglycoside is first-line treatment of *Klebsiella*(Cephalosporins demonstrate synergistic activity when combined with an aminoglycoside to treat *Klebsiella*).

-First generation cephalosporins are used for surgical prophylaxis of wound infection.

-Third generation cephalosporins are used to treat meningitis due to *pneumococci, meningococci, and Haemophillus influenza.*

-Ceftriaxone is the drug of choice for treating beta-lactamase producing *Neisseria gonorrhea.*

Adverse effects

-Hypersensitivity reactions very similar to those that occur with penicillins may be seen.

-Nephrotoxicity has been reported. (cefamandole, cefotetan, moxalactam, cefoperazone). -Diarrhea may occur with oral forms. Many second and particularly third generation cephalosporins are ineffective against Gram-positive organisms, especially methicillin resistant Staphylococci and Enterococci.

-During treatment with such drugs, these resistant organisms as well as fungi, often proliferate and may induce **superinfection.** -Hyperprothrombinemia, Thrombocytopenia, Platelet dysfunction. Administration of vitamin K (10mg) twice a week can prevent this

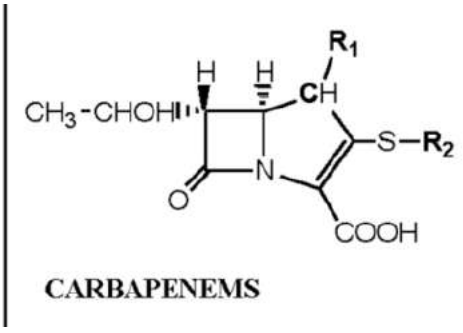
Lecture 5

Antibiotics

Other beta lactams

1-Carbepenems: are a new class of drugs which are structurallv similar to the penicillins. These drugs are derived from *Streptomyces* species and developed to deal with beta-lactamase producing Gram-negative organisms, which were resistant to broad spectrum and extended spectrum penicillins.

* The semisynthetic Carbapenems are **imipenem, meropenem, ertapenem** which act in the same way as the other beta-lactams. Widest spectrum, but may inactivated by class B β–lactamase = carbapenemase.



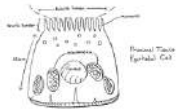
CARBAPENEMS

- Imipenem:

* Imipenem, like other β -lactams, binds to penicillin binding proteins , it is bactericidal. Imipenem differs from the penicillins in its antimicrobial spectrum. It

is a broad-spectrum antibiotic with excellent activity against a variety of gram positive and gram negative organism (both aerobic and anaerobic). It is resistant to most forms of b β -lactamase, including that produced by *Staphylococcus*. However, methicillin-resistant *Staphylococcus* is usually resistant to imipenem .Susceptible organisms include: *Streptococci*, *Enterococci. Staphylococci, Listeria*, *Enterobacteriaceae*, *Pseudomonas, Bacteroides*, and *Clostridium.*

***** Imipenem is rapidly hydrolyzed by the enzyme, dihydropeptidase, which is found in the brush border of the proximal renal tubule. It is always administered with **cilastatin**, an inhibitor of dipeptidase.



***Side efects:** Individuals who are allergic to the penicillins may demonstrate cross-reactivity with imipenem .Imipemem may produce nausea and vomiting.

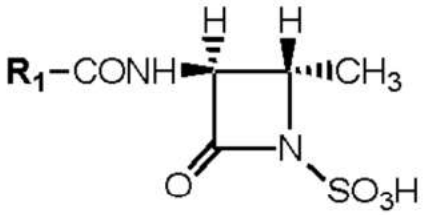
- Meropenem

*It is similar to imipenem. It is not degraded by dehydropeptidase, thus no cilastatin is needed. Excessive levels in kidney failure can cause seizures with imipenem but not with meropenem.

2- Monobactam (Aztreonam):

***Aztreonam:** This drug is a synthetic monocyclic beta-lactam (a monobactam) , originally isolated from the bacterium *Chromobacterium violaceum*. It's given IV/IM, Aztreonam interacts with penicillin binding proteins and induces the formation of long filamentous bacteria **, monobactams** are useful for the

treatment of allergic individuals. A person who becomes allergic to penicillin usually becomes allergic to the cephalosporins and the carbapenems as well. Such individuals can still be treated with the monobactams, which are structurally different so as not to induce allergy.



MONOBACTAMS

---

* The antimicrobial spectrum of aztreonam differs from that of other beta-lactams. It more closely resembles the spectrum of the aminoglycosides. Gram positive and anaerobic bacteria are resistant. Susceptible organisms include: (It has an unusual spectrum being active only against Gram-negative aerobic rods) Enterobacteriaceae, Pseudomonas, Hemophillus and Neisseria. Aztreonam is resistant to the beta-lactamase produced by gram negative organisms.

***Side effects:** Generally, the drug is well tolerated. Patients who are allergic to penicillins do not exhibit cross-reactions with aztreonam

Beta lactam resistance

Beta-lactamases

Beta-lactamases are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics. The lactamase enzyme breaks beta-lactam ring , deactivating the molecule's antibacterial properties.

*Penicillinase is a specific type of β-lactamase, showing specificity for penicillins.

*Cephalosporinases that can also hydrolyse cephalosporins.

*Broad-spectrum {beta}-lactamases, meaning that they are capable of inactivating penicillins and cephalosporins at the same rate.

*Extended spectrum of activity, represents the ESBLs, which are capable of inactivating third-generation cephalosporins (ceftazidime, cefotaxime, and cefpodoxime) as well as monobactams (aztreonam)

*carbenicillinase, these enzymes inactivate carbenicillin more than benzylpenicillin, with some effect on cloxacillin.

*cloxacillinases, enzymes inactivate cloxacillin more than benzylpenicillin, with some activity against carbenicillin. The correct term is "OXACILLINASE". These enzymes are able to inactivate the oxazolylpenicillins like oxacillin, cloxacillin, dicloxacillin.

*Carbapenemases, are able to hydrolyse carbapenems.

Beta-lactamase inhibitors

clavulanic acid, tazobactam, sulbactam

-poor antimicrobial activity on their own. They are potent inhibitors of many bacterial beta-lactamases and can protect hydrolyzable penicillins from inactivation by these enzymes, but poor activity for chromosomal cephalosporinases.

-They are beta-lactam structures , beta-lactamases inhibitor because they are β–lactam analogue.

Clavulanic acid is not an antibiotic. It is a beta-lactamase inhibitor sometimes combined with semisynthetic beta lactam antibiotics to overcome resistance in bacteria that produce beta-lactamase enzymes, which otherwise inactivate the antibiotic, clavulanic acid is an irreversible, "suicide" inhibitor of beta-lactamase.

Most commonly it is combined with amoxicillin is **clavamox** or **augmentin**. **(trade name)** .

They are available only in fixed combinations with specific penicillins:

Ampicillin + sulbactam= Unasyn

Amoxicillin + clavulanic acid= Augmentin

Ticarcillin + clavulanate potassium=Timentin

Piperacillin + tazobactam sodium= Tazocin (Zosyn)

Lecture 6

Antibiotics

Aminoglycosides

Aminoglycosides are a group of drugs sharing chemical, antimicrobial, pharmacologic, and toxic characteristics .They are potent bactericidal antibiotics include several natural and semisynthetic compounds that are used to treat bacterial diseases. They are particularly active against aerobic, gram negative bacteria and act synergistically against certain gram-positive organisms. The first aminoglycoside, streptomycin, was isolated from *Streptomyces griseus* in 1943. Neomycin, isolated from *Streptomyces fradiae*, had better activity than streptomycin against aerobic gram-negative bacilli but, because of its toxicity, could not safely be used systemically.

Gentamicin, isolated from Micromonospora in 1963, was a breakthrough in the treatment of gram-negative bacillary infections, including those caused by *Pseudomonas aeruginosa*. In the following decades, natural aminoglycosides, such as tobramycin, and semisynthetic aminoglycosides, such as netilmicin and amikacin, were identified and developed. At present, the group includes streptomycin, neomycin, kanamycin, amikacin, gentamicin, tobramycin, sisomicin, netilmicin, and others. Aminoglycosides that are derived from bacteria of the *Streptomyces* genus are named with the suffix *mycin*, whereas those that are derived from *Micromonospora* are named with the suffix *micin*.

Gentamicin is the most commonly used aminoglycoside, but amikacin may be particularly effective against resistant organisms. Aminoglycosides are used in the treatment of severe infections of the abdomen and urinary tract, as well as bacteremia and endocarditis. They are also used for prophylaxis, especially against endocarditis. All are potentially ototoxic and nephro toxic, though to different degrees. All can accumulate in renal failure. Single daily dosing of aminoglycosides is possible because of their rapid concentration-dependent killing and post-antibiotic effect in which bacterial cell killing continues for a brief period of time after the blood plasma concentration of the antibiotic has fallen below the so-called minimal inhibitory concentration .

Single daily dosing of aminoglycosides appears to be safe, efficacious and cost effective. In certain clinical situations, such as patients with endocarditis or pediatric patients, traditional multiple dosing is still usually recommended.

Initially aminoglycosides penetrate bacterial cell wall, to reach periplasmic space through porin channels (passive diffusion). **Further transport across cytoplasmic membrane takes place by active transport by proton pump; an oxygen-dependent process**.

That is why beta lactum antibiotics which weaken or inhibit bacterial cell wall synthesis facilitate passive diffusion of aminoglycosides if given together(synergistic action). Subsequently further transport of aminoglycosides across the cytoplasmic membrane takes place by energy dependent and oxygen dependent active transport .

As such transport cannot take place in anaerobic conditions and anaerobic bacteria have less energy available for aminoglycoside uptake into the bacterial cell, so aminoglycosides are inactive against anaerobic bacteria(Anaerobic bacteria are often resistant to aminoglycosides).

Under certain circumstances, aminoglycoside and β-lactam antibiotics exert a synergistic action in vivo against some bacterial strains when the two are administered jointly. For example, carbenicillin and gentamicin are synergistic against gentamicin-sensitive strains of P. aeruginosa and several other species of Gram-negative bacilli, and penicillin G and streptomycin (or gentamicin or kanamycin) tend to be more effective than either agent alone in the treatment of enterococcal endocarditis. The two antibiotic types should not be combined in the same solution because they are chemically incompatible. Damage to the cell wall caused by the β-lactam antibiotic interfere with cell wall syntheses so it increase penetration of the aminoglycoside into the bacterial cell. So we can decrease the dose due to synergistic effect.

Traditionally, the antibacterial properties of aminoglycosides were believed to result from inhibition of bacterial protein synthesis through irreversible binding to the 30S bacterial ribosome. This explanation, however, does not account for the potent bactericidal properties of these agents, since other antibiotics that inhibit the synthesis of proteins (such as tetracycline) are not bactericidal.

Recent experimental studies show that the initial site of action is the outer bacterial membrane. The cationic antibiotic molecules create fissures in the outer cell membrane, resulting in leakage of intracellular contents and enhanced antibiotic uptake. This rapid action at the outer membrane probably accounts for most of the bactericidal activity.

Structure Chemistry

Term aminoglycoside stems from there structure characterized by two amino sugars joined to a non sugar aminocyclitol by –o- glycosidic bond. In majority of aminoglycosides this aminocyclitol or non sugar moeity is 2- deoxystrepamine , however in streptomycin, the aminocyclitol is streptidine which is not placed centrally as in other aminoglycosides. Rather it is placed laterally to the amino sugar streptose which is joined by other aminosugar, (NMethyl L glucosamine, these 2 amino sugars are jointly called Streptobiosamine.

The aminoglycosides are thus strongly basic compounds that exist as polycations at physiological pH. Their inorganic acid salts are very soluble in water. All are available as sulfates. Solutions of the aminoglycoside salts are stable to autoclaving. The high water solubility of the aminoglycosides contributes to their pharmacokinetic properties. All aminoglycosides are more active at alkaline pH than at acid pH.

Aminoglycosides are poorly absorbed from the gastrointestinal tract. After parenteral administration, aminoglycosides are primarily distributed within the extracellular fluid. They distribute well into most body fluids but not into the central nervous system, bone, or fatty or connective tissues. They tend to concentrate in the kidneys and are excreted by glomerular filtration. Aminoglycosides are apparently not metabolized in vivo.

Mechanism of Action

The aminoglycosides act directly on the bacterial ribosome to inhibit the initiation of protein synthesis and to interfere with the fidelity of translation of the genetic message. They bind to the 30S ribosomal subunit to form a complex that cannot initiate proper amino acid polymerization. Difference in ribosomal units (Eukaryotes : 60S and 40 S subunit) is the basis of selectivity of antimicrobial

drugs against bacteria(this is why antibiotic drugs do not inhibit mammalian protein synthesis).

The binding of streptomycin and other aminoglycosides to ribosomes also causes misreading mutations of the genetic code ,apparently resulting from failure of specific aminoacyl RNAs to recognize the proper codons on messenger RNA (mRNA) and hence incorporation of improper amino acids into the peptide chain .All of the commercially available aminoglycoside antibiotics are bactericidal, except spectinomycin.

m

Mechanisms of action of aminoglycosides

Resistance to aminoglycosides is based on

(1) a deficiency of the ribosomal receptor (chromosomal mutant).

(2) enzymatic destruction of the drug (plasmid-mediated transmissible resistance of clinical importance).

(3) lack of permeability to the drug molecule and lack of active transport into the cell. The last can be chromosomal (eg, streptococci are relatively impermeable to aminoglycosides), or it can be plasmid-mediated (eg, in gramnegative enteric bacteria).

Lecture 7

Antibiotics

Streptomycin

Streptomycin was the first aminoglycoside—it was discovered in the 1940s as a product of *Streptomyces griseus.* It was studied in great detail and became the prototype of this class of drugs. After intramuscular injection, streptomycin is rapidly absorbed and widely distributed in tissues except the central nervous system. Only 5% of the extracellular concentration of streptomycin reaches the interior of the cell. Absorbed streptomycin is excreted by glomerular filtration into the urine. After oral administration, it is poorly absorbed from the gut; most of it is excreted in feces.

Streptomycin may be bactericidal for enterococci (eg, in endocarditis) when combined with a penicillin. In tularemia and plague, it may be given with a tetracycline. In tuberculosis, it is used in combination with other anti tuberculosis drugs (isoniazid, rifampin).

The therapeutic effectiveness of streptomycin is limited by the rapid emergence of resistant mutants. All microbial strains produce streptomycin-resistant chromosomal mutants with relatively high frequency. Chromosomal mutants have an alteration in the P 12 receptor on the 30S ribosomal subunit. Plasmid mediated resistance results in enzymatic destruction of the drug.

Fever, skin rashes, and other allergic manifestations may result from hypersensitivity to streptomycin. This occurs most frequently upon prolonged contact with the drug, in patients receiving a protracted course of treatment (eg, for tuberculosis), or in personnel preparing and handling the drug.
Streptomycin is ototoxic (particularly for the auditory portion of the eighth nerve) causing tinnitus, vertigo, and ataxia, which are often irreversible. It is moderately nephrotoxic.

Gentamicin

In concentrations of 0.5–5 μg/mL, gentamicin is bactericidal for many gram positive and gram-negative bacteria, including many strains of proteus, serratia, and pseudomonas. Gentamicin is ineffective against streptococci and bacteroides. Gentamicin has been used in serious infections caused by gram-negative bacteria resistant to other drugs. Penicillins may precipitate gentamicin in vitro (and thus must not be mixed), but in vivo they may facilitate the aminoglycoside entrance into streptococci and gram-negative rods and result in bactericidal synergism, beneficial in sepsis and endocarditis.
Gentamicin is toxic, particularly in the presence of impaired renal function. Gentamicin sulfate, 0.1%, has been used topically in creams or solutions for infected burns or skin lesions.

Tobramycin

This aminoglycoside closely resembles gentamicin, and there is some cross resistance between them. Separate susceptibility tests are desirable.

Tobramycin has slightly enhanced activity against *Pseudomonas aeruginosa* when compared with gentamicin.

The pharmacologic properties of tobramycin are virtually identical to those of gentamicin. Most of the drug is excreted by glomerular filtration. In renal failure, the drug dosage must be reduced, and monitoring of blood levels is desirable.

Like other aminoglycosides, tobramycin is ototoxic but perhaps less nephrotoxic than gentamicin. It should not be used concurrently with other drugs having similar adverse effects or with diuretics, which tend to enhance aminoglycoside tissue concentrations.

Sisomicin

Identical to gentamicin , more potent on pseudomonas and □-hemolytic streptococci (2-4 times more active against pseudomonas and proteus even those which are resistant to gentamicin) .

Netilmicin

Semisynthetic derivative of sisomicin , relatively resistant to aminoglycoside inactivating enzymes .More active against klebsiella, enterobacter & staphylococci , less active against pseudomonas aeruginosa .Doses and pharmacokinetics similar to gentamicin. Less ototoxic than gentamicin and tobramycin.

Neomycin wide spectrum active against Gm-ve bacilli and some gm+ve cocci **,**Pseudomonas and strep.pyogenes not sensitive .Too toxic for parenteral use , limited to topical use **.** Highly toxic for internal ear mainly auditory and also kidney **.**

Oral and topical administration does not cause systemic toxicity Topically used in skin, eye and external ear infections combined with bacitracin or polymyxin-B to widen antibacterial spectrum and to prevent emergence of resistant strains. Orally used for preparation of bowel before surgery and Hepatic coma(Supresses ammonia forming coliforms prevents encephalopathy) .

Kanamycin is a close relative of neomycin, with similar activity and complete cross-resistance. Paromomycin is also closely related and is used in amebiasis. These drugs are stable and poorly absorbed from the intestinal tract and other surfaces. Neither drug is used systemically because of ototoxicity and neurotoxicity. Oral doses of both neomycin and kanamycin are used for reduction of intestinal flora before large bowel surgery, often in combination with erythromycin. Otherwise, these drugs are mainly limited to topical application on infected surfaces (skin and wounds).

Framycetin

Very similar to neomycin .Too toxic for systemic administration .Used topically on skin, eye (Soframycin eye drops 0.5 % and cream 1 %) and ear .

Amikacin

Amikacin is a semisynthetic derivative of kanamycin. It is relatively resistant to several of the enzymes that inactivate gentamicin and tobramycin and therefore can be employed against some microorganisms resistant to the latter drugs. However, bacterial resistance due to impermeability to amikacin is slowly increasing. Many gram negative enteric bacteria are inhibited by amikacin in concentrations obtained after injection. Like all aminoglycosides, amikacin is

nephrotoxic and ototoxic . Its level should be monitored in patients with renal failure.

Spectinomycin

Spectinomycin is an aminocyclitol antibiotic (related to aminoglycosides) for intramuscular administration. Its sole application is in the single-dose treatment of gonorrhea caused by β-lactamase-producing gonococci or occurring in individuals hypersensitive to penicillin. About 5–10% of gonococci are probably resistant. There is usually pain at the injection site, and there may be nausea and fever.

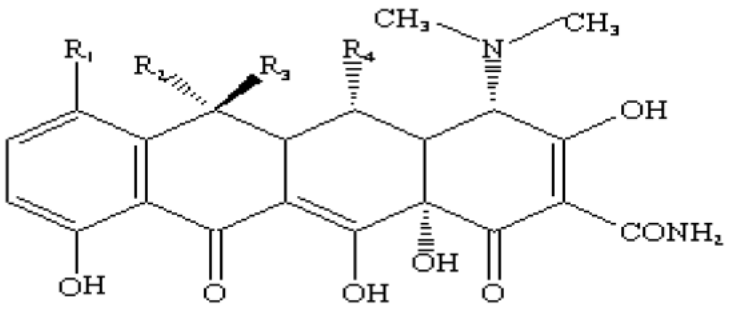
LECTURE 8

Antibiotics

Tetracyclines

The **tetracyclines** consist of eight related antibiotics which are all natural products of *Streptomyces*, although some can now be produced semisynthetically or synthetically.

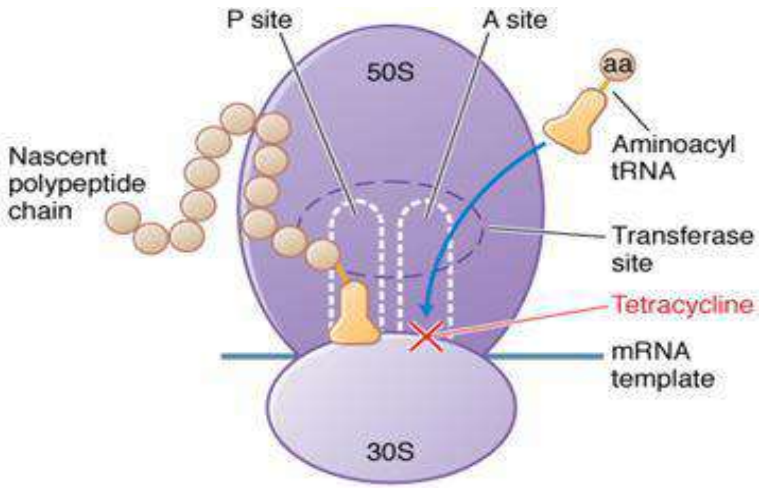
The basic tetracycline structure consists of four benzene rings with various constituents on each ring.The crystalline bases are faintly yellow, odorless, slightly bitter compounds. They are only slightly soluble in water at pH 7 but they can form soluble sodium salts and hydrochloride



General structure of tetracycline

The tetracycline core structure

The tetracyclines are broad-spectrum antibiotics with a wide range of activity against both Gram-positive and Gram-negative bacteria. *Pseudomonas aeruginosa* is less sensitive but is generally susceptible to tetracycline concentrations that are obtainable in the bladder. The tetracyclines are bacteriostatic compounds. They inhibit protein synthesis by blocking the binding of aminoacyl tRNA to the A site on the ribosome (act on 30S ribosomal subunit).



However, most bacteria possess an active transport system for tetracycline that will allow intracellular accumulation of the antibiotic at concentrations 50 times as great as that in the medium. This greatly enhances its antibacterial effectiveness and accounts for its specificity of action, since an effective concentration cannot be accumulated in animal cells (Penetration into cell requires an energy-dependent transport not present in mammals). Thus a blood level of tetracycline which is harmless to animal tissues can halt protein synthesis in invading bacteria.

The tetracyclines have a remarkably low toxicity and minimal side effects when takenby animals.

The combination of their broad spectrum and low toxicity has led to their overuse and misuse by the medical community and the wide-spread development of resistance has reduced their effectiveness.

The tetracyclines have activity against spirochetes and atypical bacteria, such as *Mycoplasma* and *Chlamydia* species, Rickettsia. It is first-line therapy for Chlamydia, Q fever and some protozoa. It is commonly used to treat acne today.
Chlortetracycline, the first tetracycline, was developed in 1948 as a product of*Streptomyces aureofaciens*. Followed by oxytetracycline and tetracyclines in 1950,1952 respectively . Chlortetracycline was altered to produce tetracycline.
Doxycycline and minocycline are semisynthetic derivatives.

Despite the success of the early tetracyclines, analogs were developed to improved water solubility either to allow parenteral administration or to enhance oral absorption.
These approaches resulted in the development of the semisynthetic compounds rolitetracycline and lymecycline.
Resistance – Common: Although tetracycline antibiotics have some roles in human nd veterinary medicine, the widespread emergence of microbial resistance due to efflux and ribosomal protection mechanisms has severely limited their effectiveness**,** bacterial resistance is typically the result of mutations that either prevent entrance of ntetracyclines into the cell or increase the export of tetracycline out of the cell.
Then resistance may be transmitted by plasmids. This plasmid mediates the production of an number of proteins that appear to affect transport of the drug into the cell, thereby preventing binding to the ribosomes.
The tetracyclines may be divided according to source into:
 Naturally occurring : Tetracycline, Chlortetracycline, Oxytetracycline
 Semi-synthetic : Doxycycline, Lymecycline, Meclocycline, Methacycline, Minocycline, Rolitetracycline
The tetracyclines may be divided into three groups based on their pharmacokinetic

traits. These groups are the short-acting group, intermediate-acting group, and longacting group. The varying half-lives are the result of different rates of renal excretion .

Short-Acting Tetracyclines

The short-acting **tetracyclines** (Half-life is 6-8 hrs) include **oxytetracycline** and tetracycline, the namesake of the class. Frequent dosing is needed because of the very short half-life of these agents. Oxytetracycline is no longer available in the

Unitedn States. Tetracycline has a broad spectrum of activity, with coverage of many aerobic gram-negative bacilli, atypical bacteria (such as *Chlamydia trachomatis*, *Chlamydia psittaci*, and *Mycoplasma pneumoniae*), and spirochetes, Tetracycline is also a secondline agent for *T. pallidum*. It is used for treatment of rickettsial infections, typhus, , trachoma, nongonococcal urethritis,. It is also commonly used for the treatment of acne.

Intermediate-Acting Tetracyclines (Half-life is ~12 hrs)

The only intermediate-acting agent available in the U.S. is meclocycline. Meclocycline is no longer used as an antibiotic.

Long-Acting Tetracyclines

The long-acting tetracycline agents (Half-life is 16 hrs or more), allowing to be used one or twice daily only, **doxycycline and minocycline**, are the more recently developed drugs. The main difference between these and the short-acting agents is thatthese may be dosed less frequently. Minocycline is usually the most active followed by doxycycline.

Doxycyclineis frequently used to treat chronic prostatitis, sinusitis, syphilis, chlamydia and acne. In addition, it is used in the treatment and prophylaxis of anthrax and in prophylaxis against malaria. It is also effective against *Yersinia*

pestis and is prescribed for the treatment of Lyme disease and Rocky Mountain spotted fever.

Because doxycycline is one of the few medications that is effective in treating Rocky Mountain spotted fever (with the next best alternative being chloramphenicol), it is indicated even for use in children for this illness. Doxycycline is also one (of many) recommended drugs for chemoprophylatic treatment of Malaria in travels to areas of the world where malaria is endemic.

Glycylcyclines(Tigecycline)

Glycylcycline antibiotics are a new generation of antibiotics derived from tetracyclines. They were developed to overcome the bacterial resistance to tetracyclines. They are the semisynthetic group e.g., 9-*(N,N*-dimethylglycylamido)-6- demethyl-6-deoxytetracycline, 9-(*N,N*-dimethylglycylamido)-minocycline, and 9-*t*- (butylglycylamido)-minocycline. These compounds possess a 9-glycylamido substituent.

Glycylcycline antibiotics long-acting tetracycline, inhibit bacterial reproduction by blocking bacterial protein synthesis. They have broad spectrum of activity against gram-negative and gram-positive bacteria, but are more potent against bacteria that is resistance to tetracyclines. Glycylcycline antibiotics are active against resistant organisms such as methicillin resistant staphylocci, penicillin-resistant streptococcus pneumoniae and vancomycin resistant enterococci. The drug is licenced for the treatment of skin and soft tissue infections as well as intra-abdominal infections.

Side effects

- Pregnant women are particularly sensitive to tetracycline -induced hepatic damage. Jaundice (increased UREA) , liver failure, kidney failure (In pregnant women experiencing pyelonephritis can be fatal).

-Children receiving long-or short term therapy with TET may develop brown discoloration of the teeth. The drug deposits in the teeth and bones probably due to its chelating property and the formation of a TET -calcium orthophosphate complex. This discoloration is permanent.

-Skeletal growth can be depressed when the drug is given to premature infants. Tetracycline crosses the placental barrier and can accumulate in fetal bones, thus delaying bone growth. They are also excreted in breast milk. Although bone and tooth defects are associated with the total dose of tetracycline given and occur more often after repeated courses so that must avoid giving to pregnant women and children under the age of 8.

- Allergic reactions and skin toxicity . Cause skin photosensitivity, so exposure to the Sun or intense light is not recommended, (Photoxicity) darkening of skin & sunburn when patient exposed to sunlight.

- Be inactivated by Ca^{2+} ion, so are not to be taken with milk, yogurt, and other dairy products.

-Drug-induced severe diarrhea, mucosal inflammation lupus, and hepatitis. Should not be given to patient with severe liver disease.

Lecture 9

Antibiotics

Quinolones Group

The quinolone antibiotics arose in the early 1960s, with the first examples possessing a narrow-spectrum of activity with unfavorable pharmacokinetic properties. Over time, the development of new quinolone antibiotics has led to improved analogues with an expanded spectrum and high efficacy. Nowadays, quinolones are widely used for treating a variety of infections. Quinolones are broad-spectrum antibiotics that are active against both Gram-positive and Gram-negative bacteria, including mycobacteria, and anaerobes. The quinolones are a family of antibiotics containing a bicyclic core structure related to the compound 4-quinolone (Fig. 1).1 Since their discovery in the early 1960s, they have gained increasing importance as key therapies to treat both community-acquired and severe hospital-acquired infections.2 The first quinolone antibiotic is generally considered to be nalidixic acid, which was reported in 1962 as part of a series of 1-alkyl-1,8-naphthyridines prepared at the Sterling-Winthrop Research Institute.

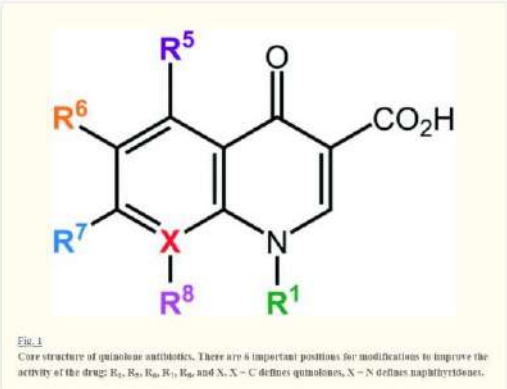


Fig. 1
Core structure of quinolone antibiotics. There are 6 important positions for modifications to improve the activity of the drug: R_5, R_6, R_7, R_8, R_1, R_8, and X. X = C defines quinolones, X = N defines naphthyridones.

The prototypical quinolone, nalidixic acid (technically a naphthyridone), was discovered in the 1960s as a by-product during the synthesis of anti-malarial quinine compounds.[3] It was soon found to act by inhibiting the activity of bacterial topoisomerase type II enzymes, inhibiting the bacterial replication.[13] In 1967, nalidixic acid was approved for clinical treatment for urinary tract infections (UTIs) caused by Gram-negative bacteria.[4] However, its use was limited because of the narrow spectrum of activity, low serum concentrations achieved, high inhibitory concentration required, and several adverse effects.[4] It was not until the 1980s that improved analogues were made, when the need for new treatments of diarrhea and UTIs caused by resistant *Shigella* and *Escherichia coli* led the attention of researchers to improve the activity and optimize the toxicity of the quinolones.

During the 1970s–1980s, the coverage of the quinolone class was expanded significantly by the breakthrough development of fluoroquinolones, which show a much broader spectrum of activity and improved pharmacokinetics compared to the first-generation quinolone.[5] Those fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against both Gram-negative and Gram-positive pathogens; importantly, they are also active against the causative agent of tuberculosis, *Mycobacterium tuberculosis*. Quinolones have been favoured as antibiotics for more than five decades because of their high potency, broad spectrum of activity, favorable bioavailability, convenient formulations, and high serum concentrations, as well as a comparatively low incidence of side effects.[6] Quinolones are widely prescribed for several different types of human infections,[7] with side effects including gastrointestinal reactions, CNS reactions, genotoxicity, phototoxicity, and some minor adverse effects

Many researchers have studied the structure–activity relationships of quinolone antibiotics. Fig. 1 presents the core structure of the basic quinolones with two major groups developed from it: quinolones and naphthyridones, which can be identified by the 'X' position. A carbon atom at the X position defines the quinolones, while a nitrogen atom at the X position defines the naphthyridones.14 Based on their spectrum of activity, quinolones are classified into four generations.15 The development of quinolones from generation to generation to obtain broader spectrum activity has proceeded by addition of different substituents into different position on the pharmacophore. Table 1 presents a summary of the quinolone development proces

Mode of action

They exert their actions by inhibiting bacterial nucleic acid synthesis through disrupting the enzymes topoisomerase IV and DNA gyrase, and by causing breakage of bacterial chromosomes, These two enzymes are critical bacterial enzymes that regulate the chromosomal supercoiling required for DNA synthesis.

Development of Quinolones

The first-generation quinolone activity was limited to only Gram-negative organisms, excluding *Pseudomonas* species. Shortly ,after the clinical introduction of nalidixic acid, it was found to cause rapid resistance development in a number of organisms, reducing its effectiveness and leading to investigations to discover analogues with improved properties.

Lecture 10

Antibiotics

The first second-generation quinolone, flumequine, exemplified the discovery that a key modification, adding a fluorine (F) atom at the R_6 position, could significantly improve the spectrum of activity. This change dramatically increased the quinolone activity, since almost all quinolone antibiotics have been designated as fluoroquinolones, with the exception of the most recent compounds from the fourth generation. Other fluoroquinolones from the second generation include enoxacin, norfloxacin, and ciprofloxacin, which were able to inhibit all Gram-negative organisms, including *Pseudomonas* species. In addition to the fluoro substituent, these drugs were further modified by addition of a piperazine ring to the R_7 position and addition of a cyclopropyl group to the R_1 position. The R_7 piperazine ring improved the Gram-negative potency, while the cyclopropyl group was found to improve the overall activity of the compounds. This combination made ciprofloxacin the most active compound among the early compounds of the second generation and made it the first choice used against *Pseudomonas aeruginosa* today. Subsequent development of the second generation produced analogues with activity against some Gram-positive bacteria, including *Staphylococcus aureus* but not *Streptococcus pneumoniae*, and some atypical organisms (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*). The presence of an alkylated piperazine group at the R_7 position, as in ofloxacin, marked the first modifications that help inhibit Gram-positive organisms.4 The addition of an $–OCH_3$ substituent to the R_8

position of the latter group also helped to improve Gram-positive activity

With the synthesis of fleroxacin, the quinolones **entered their third generation**. The improvements of this generation included addition of alkylated piperazine and pyrrolidinyl groups to the R_7 position, and $–NH_2$, –OH, and $–CH_3$ groups to the R_5 position to the pharmacophore. The cyclopropyl group at the R_1 position and the $–OCH_3$ group at position R_8 were kept unchanged from the second generation. The third generation also added new substituents, such as a chloro group (Cl) at the R_8 position; this was verified to improve the anti-Gram-positive activity of the drug.

Among all modifications at this position, 8-methoxyquinolone was shown to surpass other compounds in activity and spectrum. The improvement is best exemplified by comparing grepafloxacin and gatifloxacin; the MIC_{90} of gatifloxacin (8-MeO) improved significantly compared with that of grepafloxacin (8-H) . These modifications expanded the Gram-positive activity of the third generation, including penicillin-sensitive and penicillin-resistant *S. pneumoniae*, while the activity against atypical bacteria was also increased. While a piperazine group in the second generation improved the Gram-negative activity, the alkylated form of this group added to the Gram-positive activity of the fluoroquinolone compounds. A pyrrolidinyl group in this position showed the same improvement as the alkylated piperazine group. Manipulation of the group at the R_5 position was shown to increase the activity against Gram-positive organisms. The antibacterial potency improvement mediated by substitution at this position was found to increase in the order $–CH_3$, –OH, $–NH_2$, respectively. All the modifications (positions R_8, R_5, and R_7) presented in this third generation were designed to improve the activity against Gram-positive bacteria.

There are similarities between the structures of ciprofloxacin and sparfloxacin, but addition of $–NH_2$ at R_5 and alkylation of the piperazine group make the potency of sparfloxacin better than that of ciprofloxacin. It is similar in the case of grepafloxacin, with the $–CH_3$ substituted.

The spectrum of activity of fourth-generation compounds covers all the criteria of the third generation with the addition of activity against anaerobic organisms. The presence of nitrogen (N) at the R_8 position is responsible for the improved activity against anaerobes,31 while a 2,4-difluorophenyl group at the N position improves the overall potency of the drug. This modification can be seen from the structures of moxifloxacin, gemifloxacin, and trovafloxacin .

Other modifications are addition of an azabicyclic group and a bulky side chain on the pyrrolidine group at the R_7 position and addition of a difluoromethyl ether group at the R_8 position, which all improve the Gram-positive activity. The azabicyclic group at the R_7 position produced the highest potency against the Gram-positive bacteria, as demonstrated by comparing the potency and structure between moxifloxacin and gatifloxacin. These two compounds have an otherwise similar structure, differing only at the R_7 position. The azabicyclic group in moxifloxacin substantially improves Gram-positive potency compared with gatifloxacin

Quinolones resistance

However, bacteria have acquired resistance to quinolones, similar to other antibacterial agents, due to the overuse of these drugs. Mechanisms contributing to quinolone resistance are mediated by chromosomal mutations and/or plasmid gene uptake that alter the topoisomerase targets, modify the quinolone, and/or reduce drug accumulation by either decreased uptake or increased efflux