passion ACADEMIC team.

YU-Medicine

Sheet #15

Lec. Date : 29.10.2019

Lec. Title : principle of antibiotics

Written by : Zeenah Frihat

special thanks to ruaa ismael

if you come by any mistake , please kindly report it to shaghafbatch@gmail.com

Principle of Antimicrobial Cell Wall inhibitors

General Pharmacology

M212

Dr. Laila M. Matalqah

Ph.D. Pharmacology

Antimicrobial drugs

- Antimicrobial drugs (Antibiotics) are drugs used for infectious diseases caused by bacteria mainly
- Antimicrobial drugs (Antibiotics) are drugs effective in the treatment of infections because of their:
  - **selective toxicity**; that is, they have the ability to kill an invading microorganism without harming the cells of the host

in the past people used to name the antimicrobial as **chemotherapeutics**

Antimicrobial are drugs that can kill any microorganism NOT just bacteria

We can classify antimicrobial into:

- Antibacterial
- Antiviral
- Antifungal
- Anti parasites

Infectious disease is a disease caused by microorganisms.

What is the difference between our cell and bacterial cell ?

Why the drug is toxic to this microorganism, with selective toxicity without affecting our body even thought it act on mutations and on DNA..?

SHEET 2

- First: the difference in cell wall (human cells don't have cell wall)
- Bacteria posses a cell wall made of peptidoglycan cross linkage ,with 2 types of cells :
- **gram positive** posses a very thick cell wall.
- **Gram negative** posses a thin(narrow) cell wall & that's why it's the hardest to kill , because most drugs work in destroying the cell wall .
- Second : the difference in the ribosome structure (that's make drugs more selective)
- **In human** : 40s +60s
- **In bacteria** : 30s + 50 s
- Drugs works on the ribosome → inhibit it →prevent protein synthesis(protein synthesis inhibitor) →because protein important in growth→ no growth occur to bacterial cell , no multiplication nor reproduction .

SHEET 3

- So that's how antimicrobial have selective pathway (selective toxicity) that affect microorganism without affecting our human body ,and other than that enzymes .
- **Folate** or **Folic acid** is very imp in our body , its primary for synthesis of DNA , but its not synthesized mainly inside our cells , we take it from out side(Folate taken from the diet).
- *a small amount of Folate is synthesized inside our body*
- But bacterial cell can synthesize it inside cells ,present in nucleotides to synthesize DNA it self.
- so they have reductase enzymes , dihydrofolate reductase enzyme
- By inhibiting this pathway they can't synthesize folic acid which is the primary precursor for DNA and so on they cant synthesize DNA

SHEET 4

viruses

- The hardest microorganisms to be killed are viruses .
- Because they're **intracellular microorganism** which means they don't have any specific pathways.
- Virus is a non living microorganism consist of just a DNA coated by protein and doesn't have enzymes or specific pathways.
- It needs our enzymes to survive and for multiplication ,by uncoating inside our cell.
- **HIV** virus is a retrovirus causes AIDS & hepatitis B,C,D, which are viral diseases that are not totally cured , why?
- Because the antiviral MUST **face** the microorganism to kill it and so on MUST inter our cell to kill the virus which is harmful to our body.

Sites Of Antimicrobial Actions

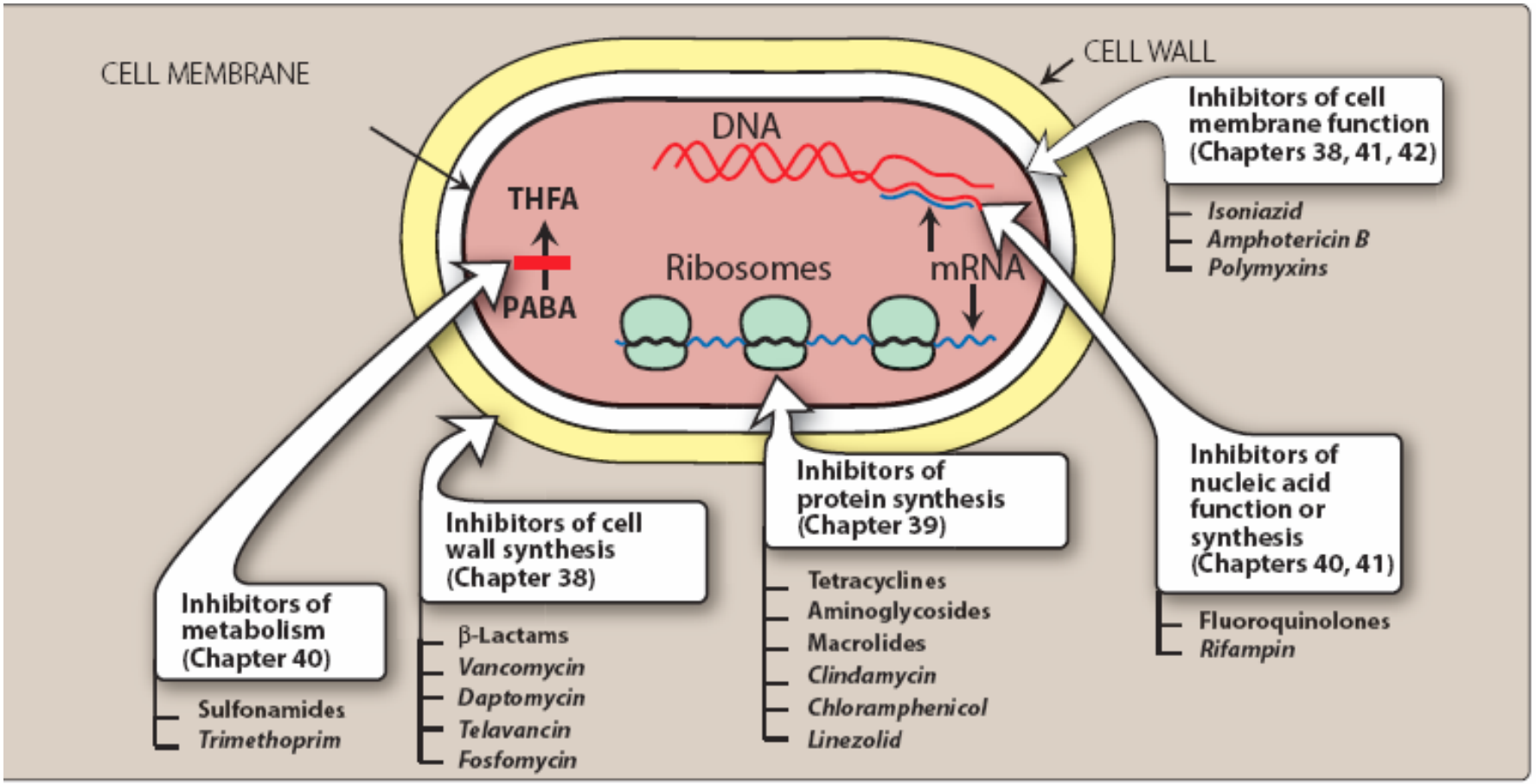


Figure 37.10
Classification of some antimicrobial agents by their sites of action. (THFA = tetrahydrofolic acid; PABA = *p*-aminobenzoic acid.)

Note that :

- Each ***mechanism of action*** has a group of drugs so we can classify the drugs according to site of action .

- fungus has a different cell wall , different plasma membrane (contain different substnaces)

Mechanisms of Action of antimicrobials

1. **Inhibition of bacterial cell wall synthesis** or activation of enzymes that disrupt cell walls (Penicillin, Cephalosporins, Vancomycin)
2. **Inhibition of protein synthesis** (tetracyclines, clindamycin, aminoglycosides)
3. **Inhibition of microbial cell membranes function** (anti-fungals)
4. **Inhibition of organism reproduction by interfering nucleic acid synthesis** (fluoroquinolones, -antivirals)
5. **Inhibition of cell metabolism and growth** (sulfonamides), *another Ex :tetrahydrofolate reductase enzyme which synthesize folic acid *

SHEET 6

Inhibition of bacterial cell wall synthesis

- Note that we have mother cells and generation cells , replicated by duplication to give 2 bacterial each time.
- The inhibitor of cell wall synthesis **can't kill the bacteria once its inside our body** because the cell wall already in the bacteria, THE TARGET is the new generation (the dividing cells) , when mother cell replicate next time the new generation will be cell wall less .
- So this mechanism inhibit the cell wall synthesis of the new generation NOT the mother cell wall.

Now , what about the mother cell , how can we kill it ?

- **Our immune system** can kill it and clarify out body .
- Conclusion: we need BOTH antibiotic and our immune system to kill the bacteria

SHEET 7

Inhibition of bacterial cell wall synthesis cont'd

- When new generation have **no cell wall** → that's mean hemolysis , water come in and out (affect isotonicity) , shrinkage,…

- Important drugs with this mechanism is **β -lactam** like cephalosporin and later on we will take the rest ..

SHEET 8

Inhibition of protein synthesis

- The drugs by this mechanism work on the ribosome

  (no transcription = no A.A = no protein)

- Protein is needed by bacterial cell for **growth** (either in size or other) without growing the bacteria will become weak and small so our IMMUNE SYSTEM can kill it , that's what we call **BACTERIOSTATIC DRUG**

- The inhibitor may work on large subunit of ribosome (50s) or small sub unit (30s)

SHEET 9

Inhibition of nucleic acid function or synthesis

- This mechanism inhibits DNA synthesis ,by inhibiting any enzyme that is involved in the pathway
- Ex : fluoroquinolones

Inhibition of cell membrane function

- Cell membrane is most imp in fungus (antifungal) like amphotericin B , polymyxins and isoniazid
- isoniazid is a drug for TB caused by **Mycobacterium tuberculosis**
- This bacteria differ than typical bacteria because the cell wall structure is different ,and they have cell membrane.
- That what's imp in it , we make **poring** (pores in cell memb.)→dysfunction of cell membrane →loss of permeability and selectivity .

Mechanisms of Action of antimicrobials

Mechanism of Action	Antimicrobial Agents
Inhibition of bacterial cell-wall synthesis	Penicillins, cephalosporins, imipenem/meropenem, aztreonam, vancomycin
Inhibition of bacterial protein synthesis	Aminoglycosides, chloramphenicol, macrolides, tetracyclines, streptogramins, linezolid
Inhibition of nucleic synthesis	Fluoroquinolones, rifampin
Inhibition of folic acid synthesis	Sulfonamides, trimethoprim, pyrimethamine

- So drugs are selective to bacteria in term of killing rate.
- Those drugs can make **side effects** , BUT these side effects have no relations with the mechanism of the drug .

- Like accumulation of the drug in the kidney → **nephrotoxicity**

- Allergies to certain substances , like allergy for **penicillin**

Drug Selection

Selection of the most appropriate antimicrobial agent requires knowing

1. **The organism's identity**: identifying the presence of microorganisms in body fluids that are normally sterile (blood, serum, cerebrospinal fluid [CSF], pleural fluid, synovial fluid, peritoneal fluid, and urine).
2. **Determining antimicrobial susceptibility of infective organisms to a particular agent**,

- After a pathogen is cultured, its susceptibility to specific antibiotics serves as a guide in choosing antimicrobial therapy.
- In contrast, most **gram-negative bacilli, enterococci, and staphylococcal species** often show unpredictable susceptibility patterns and require susceptibility testing to determine appropriate antimicrobial therapy.

SHEET 11

Drug Selection

- We have to choose the appropriate drug for each microorganism (selection)
- For ex , a drug against **gram+** will not be affective to **gram-**
- We have many consequences for selection of the right drug :
- **save patient's life :**

- By changing many drugs it will make more SIDE EFFECTS , more RESISTANCE to different types of bacteria and more COSTLY, and non effective.
- In some kind of microorganisms we have protocol like **tonsillitis** which is Sore throat , always caused by **gram positive bacteria** so we CAN use **penicillin**
- But sometimes we have to **identify** the correct pathogen.
- For ex : pneumonia (lung infection)happen by **gram positive** AND gram negative (if you chose the wrong drug it may lead to DEATH)

SHEET 12

Drug selection cont'd

*If the infection was found on some sterile of body fluid like:

- in lungs : we can take smear (sputum smears)mucus .
- Blood sample or serum for blood toxemia
- CSF for meningitis "السحايا"
- Pleural fluid around the lungs in case of congestive heart failure and result in infection
- Synovial fluid in the joints
- Peritoneal fluid after surgery
- Abdominal area or urine for urinary infection.

Another way is Determining antimicrobial susceptibility in hospitals but its not always available and not always can be done because of costs .

SHEET 13

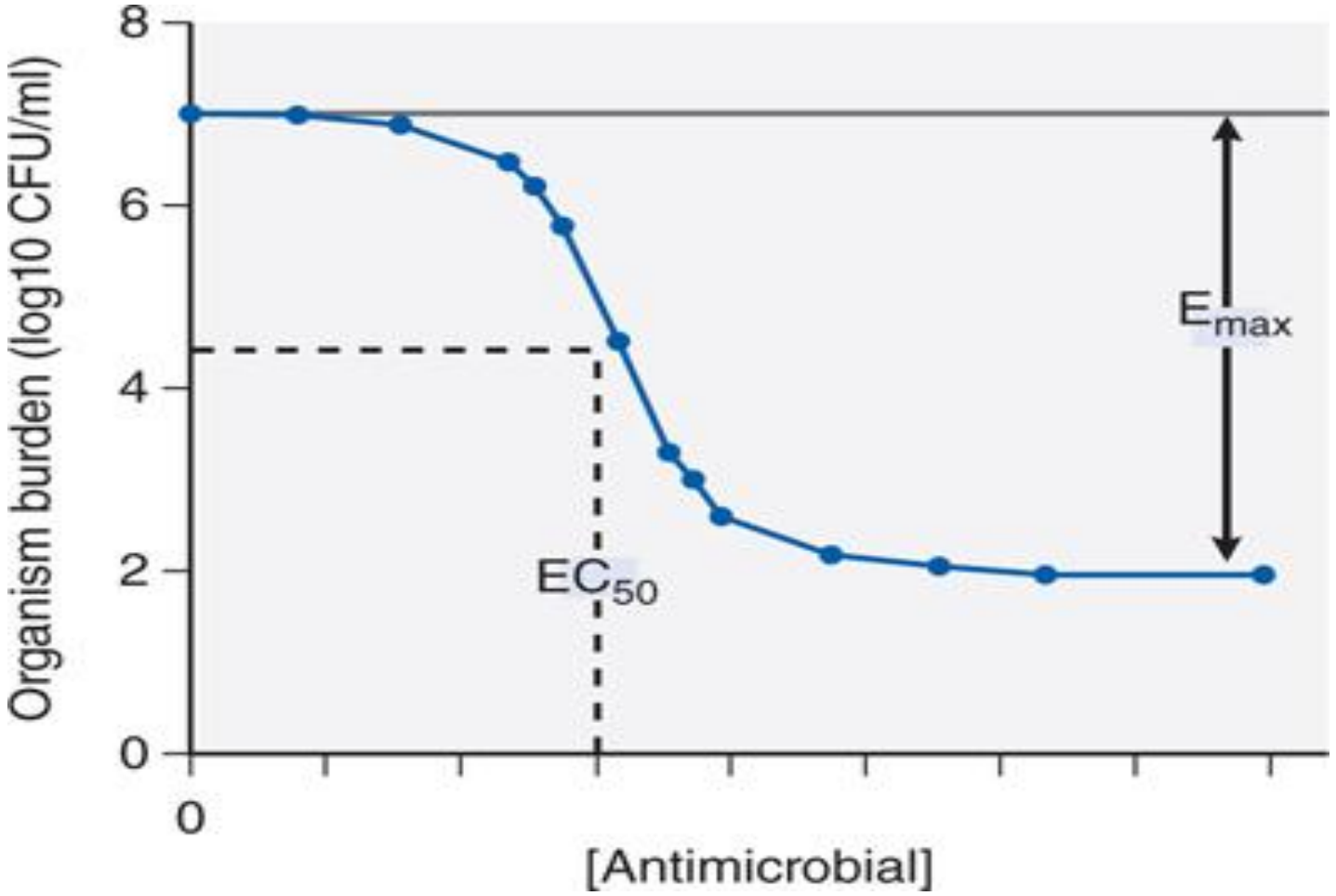
AN EXAMPLE IN LABORATORY

- If you took the sample and found its **staphylococcus aureus** , you can do sensitivity for 5 drugs for example.
- Bring culture media with disc of antimicrobial
- In the laboratory we culture the bacteria in Petri dish ,in this ex : **staphylococcus aureus**
- And add a disk of penicillin or cephalosporin for example
- Add one disk for each Petri dish
- Then we measure the zone of inhibition (the diameter) if diameter is 20 mm for example we compare it with standards (for Ex more than 10mm its susceptible or didn't kill any bacteria)
- **We use these letters in laboratory (S , R , I)**
- S=SENSITIVE , R=RESISTANCE , I=INTERMEDIATE

- BUT Not each Case of infection we make swap and take it to laboratory , its not protocol .

- The protocol mostly in gram negative like enterococcus and staphylococcus which are MRSA (Methicillin-resistant Staphylococcus aureus)

Susceptibility of the organism to antimicrobials



SHEET 15

FROM PREVIOUS DIAGRAM

- We measure **the Potency** of the antibiotic →by effective conc. of 50% (drug conc. that can kill 50% of microorganisms →this is the log of organism number)
- In the previous diagram the No. of cells that are killed increase

(no. of microorganisms decrease)

- BY THE LINE GOWING DOWN MEANS MORE KILLING BUT LESS MICROORGANISMS .

Antimicrobial characteristics

- **Bacteriostatic**: Inhibit the growth and replication of bacteria thus limiting the spread of infection until the body's immune system attacks, immobilizes, and eliminates the pathogen.
- **Bactericidal:** kill bacteria at drug serum levels achievable in the patient
- **Minimum inhibitory concentration (MIC):**is the lowest antimicrobial concentration that prevents visible growth of an organism after 24 hours of incubation.
- **Minimum bactericidal concentration (MBC) :** the minimum concentration of antibiotic that kills the bacteria
- OR is the lowest concentration of antimicrobial agent that results in a 99.9% decline in colony count after overnight broth dilution incubations

SHEET 16

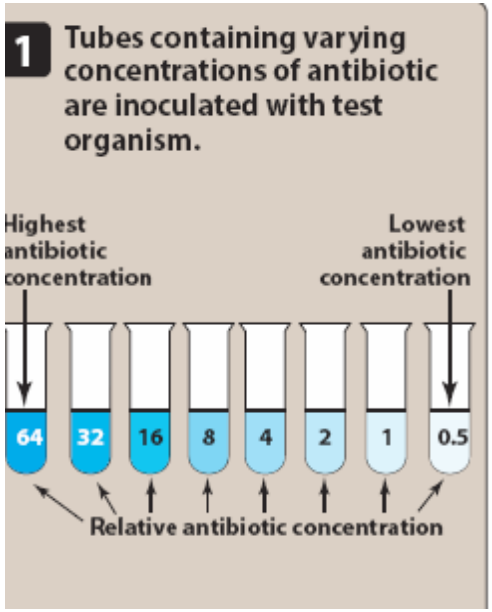
Antimicrobial characteristics

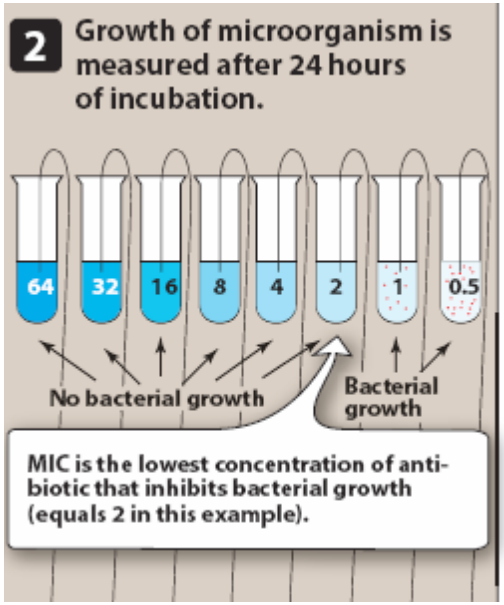
- **Bacteriostatic** : means it **inhibits** the growth microorganism without killing it , until our immune system can attack and clarify our body.
- Ex of a mechanism that can work as **Bacteriostatic** is inhibition of protein synthesis
- **Bactericidal** means killing , they are stronger and can kill them , so they don't depend on immune system
- Some doctors give antibiotic which is broad-spectrum and bactericidal as "they kill an ant with a rocket"

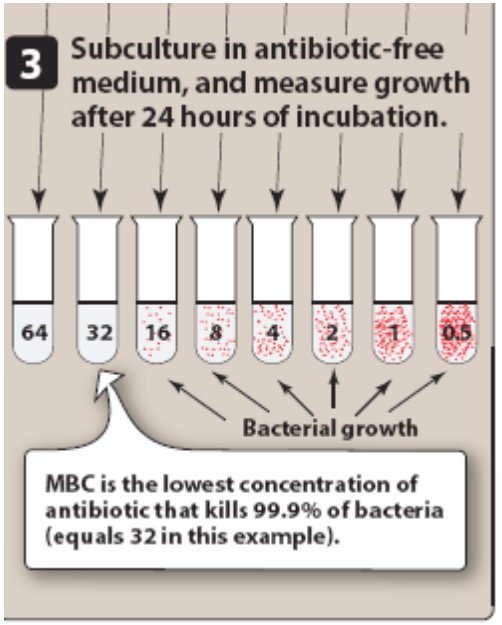
تشبيه : "كقتل نملة بصاروخ"

- And we don't need that , killing microbe that doesn't need bactericidal , so we should use **the safest** , the lowest spectrum

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of an antibiotic.







- We should know the conc. that inhibits the growth and that kill the microbe
- The dose of antibiotic depend on different things other than steady state.

From previous slide:

- We do cultivation of bacteria inside antibiotic , we put a certain conc. and then monitoring it for 24 hours..
- We monitor which one still have some growth & which not .
- So , which is the minimum inhibitory conc. in the previous diagram ?
- No. 2 because there isn't any growth , so this is the lowest conc. (inhibitory not killing)

SHEET 18

Cont'd

- If we want to know the minimum ***bactericidal*** conc. ..
- We make dilution and monitor them overnight (this trial is not common, not needy and time consuming) and the bactericidal will be the max.
- If the immune system is good , healthy , the MIC will be enough for treatment.
- The minimum bactericidal conc.(MBC) is number 32
- **MBC** that killed all microorganism is higher than **MIC** .

You can feel now you're watching a horror movie on **MBC2**

Drug Selection

3. Site of the infection

Drug's ability to penetrate infected tissues: Natural barriers to drug delivery such as the prostate, testes, placenta, the vitreous body of the eye, and the central nervous system (CNS through blood–brain barrier), depend:

A. The lipid solubility of a drug:

- For example, lipid-soluble drugs, such as *chloramphenicol and metronidazole,* have significant penetration into the CNS.
- β-lactam antibiotics, such as *penicillin,* are ionized at physiologic pH and have low solubility in lipids
  - However, In infections such as meningitis the barrier does not function as effectively, and local permeability is increased. Some β-lactam antibiotics can then enter the CSF in therapeutic amounts

SHEET 19

- Note : **Aminoglycoside** & **vancomycin** these drugs are very nephrotoxic they need IV infusion and admitted to the hospital.

  Another drug selection is **site of infection**
- BBB : penicillin is known very effective against staphylococcus and streptococcus (**gram positive**)
- Penicillin was only effective to gram positive but New generation of penicillin are more effective against **gram negative**.
- Gram positive Penicillin capable of killing staphylococcus which causes meningitis in LABORATORY
- The problem is that it can't cross the BBB, although its safe
- During meningitis inflammation , the BBB and the contact junction between capillaries will be open and inflamed ..
- During inflammation of the meningitis ,some drugs in normal patient (normal conditions) can't cross ,BUT after meningitis they can cross (because capillaries more widening)so it can cross BBB even if its ionized

SHEET 20

- B-lactam is an ionized drug and normally cant cross BBB , BUT in **meningitis** during inflammation , the capillaries will open and the junctions will be wider , so they can enter BBB

- During dental inflammation the absorption of the drug and distribution will be difficult , also difficult to anesthesia. Why ?
- because **the pus** inside the teeth will make acid media , and make ionization for most of the drugs .

- The antibiotic must inter site of infection with **active ingredient**
- Like urine , the effective drug of urinary infection must be eliminated mainly in the urine like penicillin , ciprofloxacin

Drug Selection

B. Molecular weight of the drug: a high molecular weight (vancomycin) penetrate poorly, even in the presence of meningeal inflammation.

C. Protein binding of the drug: A high degree of protein binding of a drug restricts its entry into the CSF.

- **Molecular wieght :**
- Vancomycin is very big even if there is inflammation in the CNS and having meningitis it CAN'T cross , so its stupid to use it for meningitis
- **Protein binding to the drug :**
- **Very imp because the drug bounded cant be distributed ,or do the action , they are null not available**

Drug Selection:

4. patients factors

1. **Immune system :** Alcoholism, diabetes, AIDS, malnutrition, autoimmune diseases, pregnancy **or advanced age. High doses of bactericidal agents or longer courses of treatment may be required**
2. **Renal dysfunction:** Serum creatinine levels are frequently used as an index of renal function for adjustment of drug regimens. However, direct monitoring of serum levels of some antibiotics (for example, **vancomycin, aminoglycosides**)
3. **Hepatic dysfunction:** Antibiotics that are concentrated or eliminated by the liver (for example, ***erythromycin and tetracycline***

SHEET 21

Immunesystem

- All patients with immunodeficiency have problems with antibiotics , including patients who take chemotherapy for cancer , radiation , pregnancy , elderly
- They have to ***use longer duration of the drug*** (ex. for 12 days instead of 4 days)
- Autoimmune disease like stevin john syndrome, nefritis, rheumatoid arthritis.
- Patient with glucocorticosteroids drugs are immunosuppressant
- so we have to use longer Course of treatment for them.

SHEET 22

Renal dysfunction

- Vancomycin and aminoglycosides (can't be taken by patients with renal problems) they causes auto toxicity and nephrotoxicity
- They are given **IV** to patients only who are resistance for many drugs (in emergency cases)
- We need them for lethal infection.

- How can we know that renal function is good ?
- By measuring Creatinine clearance if normal you can give the drug , and by monitor the function after giving the drug ..

SHEET 23

Hepatic dysfunction

- Can't be given drugs that are Mainly metabolized in the liver and affecting to liver .

- Note that :if we have problem with liver we give the patient a drug eliminated by the kidney (urine).
- ex. Give him penicillin instead of erythromycin

- if he has problem with renal we give him drug eliminated by the liver and so on ..

Drug Selection:

4. patients factors

1. **Age :.**
2. **Neonate: Renal or hepatic elimination processes are often poorly developed in newborns, making them more risky to toxic effects of chloramphenicol and sulfonamides.**
3. **Young children (12-18)* should not be treated with tetracyclines or quinolones, which affect bone growth and joints, respectively.** لانهم في مرحلة النمو
4. **Elderly patients may have decreased renal or liver function, which may alter the pharmacokinetics of certain antibiotics. (be careful ,must give in caution, giver the safest)**

5. Pregnancy and lactation

Many antibiotics cross the placental barrier or enter the nursing infant via the breast milk

CATE-GORY	DESCRIPTION	DRUG
A	No human fetal risk or remote possibility of fetal harm	
B	No controlled studies show human risk; animal studies suggest potential toxicity	β-Lactams β-Lactams with β-inhibitors Cephalosporins *Aztreonam* *Clindamycin* *Erythromycin* *Azithromycin* *Metronidazole* *Nitrofurantoin* Sulfonamides
C	Animal fetal toxicity demonstrated; human risk undefined	*Chloramphenicol* Fluoroquinolones *Clarithromycin* *Trimethoprim* *Vancomycin* *Gentamicin* *Trimethoprim-sulfa-methoxazole*
D	Human fetal risk present, but benefits mayoutweigh risks	Tetracyclines Aminoglycosides (except *genta-micin*)
X	Human fetal risk present but does not outweigh benefits; contraindicated in pregnancy	

sheet

Tetracycline is prevented because it binds with the tooth calcium of fetus and will result in a tooth deficit in the neonates
(اسنان لبنيه دائمة و ضعيفة)

6. Drug's toxicity and the risk-to-benefit ratio

7. Drug costs

SHEET 24

Pregnancy

- Lactation is breast feeding.
- First question : do the drug excreted by the milk ? Mostly yes more than 60%

FDA categorizes the drugs (not just antibiotic but all the drugs) according to pregnancy to 5 categories :

- ***Class A*** insulin and thyroxin (A has no human fatal harmful) , Given in the beginning of pregnancy
- ***Class B*** given in first trimester(from 1st month to 3rd) , but not controlled drugs (without studies , having some risk, 90% safe) .
- ***Class C*** studies on the animal shown certain toxicity but they don't have studies in humans.
- C can be given in third trimester(from 7th month its safe)
- ***Class D*** not safe , but they out weight the risk and the benefits (for example threaten life of mother so what do we want to save? mother or embryo?)
- ***Class X*** can't be given because its teratogenic can cause mutations 100%.

8. Antibiotic Combination Therapy

- Used when infection is caused by multiple microorganisms
- Serious infections in which a combination is synergistic (aminoglycoside and antipseudomonal penicillin)
  - β-lactam antibiotics are synergistic with the aminoglycosides.?? How?
- Likely emergence of drug resistant organisms
- In those who are immunosuppressed

- sometimes to give **less** conc. of the drug we can decrease it by combination with another drug to make synergism

Combination is very important in antimicrobial technique, why?

- First, so we don't built resistance (to be smarter than the microorganism)→by giving 2 drugs → if the bacteria could resist to one drug it won't do to the other (multidrug mechanism of action)

When we use this combination ?

1. immune system problem
2. TB (given drugs from 6 month minimum to 2 years) so combination is important
3. multi microorganism ,for example:

- In dental infection there is aerobic and anaerobic microbes so you must give him amoxicillin with metronidazole (one for aerobic bacteria & other for anaerobic)

”لو أن الناس كلما استصعبوا أمرا تركوه ماقام للناس دنيا ولادين“
-عمر بن عبد العزيز

THE END