Microbiology

number
8

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The last page includes definitions for the underlined asterisked words*

You can skip Extra notes, Enjoy!

❖ A Brief Introduction

The aim of antibiotics (antibacterial agents) usage is to target certain structures of bacteria, without harming human’s cells (or at least, having the least possible side effects) so the selectivity of antibiotics is really important, by targeting structures which are not found in human cells (or differ from human’s cells structures), as we mentioned in the previous lecture Antibiotics have many means to kill bacteria such as inhibition of cell wall synthesis, disrupting cell membrane functions, and this sheet will mention other means of killing bacteria such as inhibition of protein synthesis (by interfering with ribosomes), inhibition of nucleic acid synthesis, inhibition of certain metabolic pathways such as folate synthesis.

❖ Main Lecture’s Points

1) Killing Bacteria by antibiotics by:

A) Inhibition of protein synthesis (most of them are produced by Streptomyces and soil organisms).
   o antibiotics (tetracycline, macrolides, aminoglycosides, Clindamycin and Chloramphenicol)
   o Mechanisms of inhibition

B) Inhibition of certain metabolic pathways (folate synthesis)

C) Inhibition of nucleic acids synthesis.
   o Inhibitors of RNA synthesis and function
   o Inhibitors of DNA synthesis and function
   o DNA strand breakage

2) Anti-Tuberculosis drugs

3) Resistance

➢ Inhibition of microbial protein synthesis

Antibiotics act at sites of protein synthesis, which is the ribosome, BUT we, humans, have ribosomes too! Luckily, our ribosomes differ from bacteria’s, we have 80s (60s, 40s) while they have 70s (50s, 30s), this difference is medically significant, because antibiotics are selective, they can target 70s ribosomes but
cannot target ours (the 80s ribosomes), but have our ribosomes completely escaped?? it is true that our cytoplasmic ribosomes are 80s, but we should never forget that we have mitochondrial protein synthesis which is performed by 70s ribosomes (mitochondria are descendants of bacteria, bio101)

Remember: the aforementioned numbers relate to the sedimentation rate- 70s ribosomes consist of two subunits (30s and 50s), in terms of density. –

A) inhibitors:
  - Examples on Antibiotics that inhibit protein synthesis:
    - **Tetracyclines**
      - Effects on bacteria: bacteriostatic agent (static antibiotic)*
      - Mechanism: it inhibits protein synthesis at the ribosomal subunit 30S, it is an anti-30S ribosomal subunit.
    - **Macrolides** (such as erythromycin), **Clindamycin** and **Chloramphenicol**
      - Effects on bacteria: bacteriostatic agents, static antibiotics.
      - mechanism: they inhibit protein synthesis at the ribosomal subunit 50S, anti-50S ribosomal subunits
    - **Aminoglycosides** (such as gentamicin)
      - Effects on bacteria: bactericidal agent, (cidal antibiotic)*
      - mechanism: it inhibits protein synthesis at the 30S subunit, anti-30S ribosomal subunit.

You can find more details about the previous antibiotics in the following pages

B) Mechanisms of inhibition
Ribosomes are involved in the translation process, during the synthesis of proteins. In normal bacterial protein synthesis, the mRNA message is simultaneously “read” by several ribosomes, but before mentioning the mechanisms of protein synthesis inhibition, let’s have an extra note that might help,

A-Might-Be-Helpful EXTRA Note (you can skip)
1- Proteins are macromolecules composed of amino acids, one amino acid is connected to the other by a bond, this bond is called peptide bond.

2- tRNA molecule is transfer RNA that carries an amino acid to the ribosome. 
3- Ribosomes’ binding sites, as shown in the following model, ribosomes have three binding sites for tRNA which are:
   - P site: the site at which the tRNA carries the growing polypeptide chain
   - A site: the site where you can find the tRNA carrying the next amino acid to be added to the chain, (somehow like a waiting room)
   - E site: the site from which tRNAs leave

4- The protein synthesis (translation process) can be divided into three stages: initiation, elongation and termination
   - Initiation: the stage at which the initiation complex is formed, by bringing all the components together (the union of mRNA, small and large ribosomal subunits)
   - Elongation: the addition of amino acids one by one to the previous amino acid
   - Termination: no more elongation because of stop codons

Let’s answer our question, how do the antibiotics which inhibit protein synthesis work?

Those antibiotics try to disrupt one of the protein synthesis stages (the last point in the extra note :“”), by:

1) **Interfering with initiation**

Preventing the formation of initiation complex, The initiation complex composes of the mRNA and the two ribosomal subunits bound to each other.
2) **Interfering with elongation**
   
a- By Preventing the formation of a peptide chain, (either breaking the peptide bonds or inhibiting elongation factors)

   ![Diagram of peptide bond formation and inhibition](image1)

   b- Preventing the transfer/ translocation of amino acids from site A to P, and thus no more reading of the following codons occur.

   ![Diagram of amino acid translocation and inhibition](image2)

C) **More details about the inhibitors (antibiotics) of protein synthesis**

1- **Aminoglycosides**
   
   - **Examples**: streptomycine, gentamycin and amikacin
   - **Targets**: its activity on *Gram negative aerobics, tuberculosis and enterobacteria (enter- related to intestines)*
   - **Administration**: Aminoglycosides (specifically, gentamycin) can be given with beta lactam agents such as (penicillin) which leads to *synergism*, beta lactams work on cell wall inhibition and aminoglycans work on the translation process inhibition, this combination can be used in *sepsis* of unknown origin, this combination (beta lactam + aminoglycans) can be administered blindly because of their *broad-spectrum* + synergistic effect. And in the cases of immunocompromised patients.
• **Spectrum of activity**: broad spectrum.

• **Action**:
  o Binding to the ribosomal 30S subunit
  o preventing peptide chain initiation complex (no translation)
  o Also misreading for the messenger RNA.

• **Side effects**:
  o **ototoxicity** (may lead to deafness by affecting auditory - hearing- nerve, the eighth cranial nerve)
  o **nephroxicity** (affects kidney)

  *(to avoid these side effects, monitoring your patient’s drug levels is required)*

• **Clinical Significance**: before urologists insert catheter, they usually give a shot of gentamycin, because catheters and (certainly other instruments) increase the likelihood of Gram negative bacteria to enter the body, especially for the immunocompromised patients.

2- **Chloramphenicol**

• **Spectrum of activity**: broad- spectrum antibiotic

• **Mechanism**:
  o binding to the 50s subunit
  o preventing peptide formation

• **Cautions**: it is limited and rarely used because of its fatal side effects (they are rare though):
  o Grey baby syndrome
  o Aplastic (bone marrow suppression, no more production of all cellular parts of the blood (WBCs leukopenia, RBCs anemia, platelets thrombocytopenia))

*Not included but clinically important note*: Even the topical use of chloramphenicol is not recommended, some doctors prescribe phenicol to kids who suffer from conjunctivitis, but it can lead to aplastic anemia (even if it was topically* administered), USE ALTERNATIVES!

3- **Macrolides**
• **Examples:** Erythromycin, Clarithromycin, Telithromycin and Azithromycin

Azithromycin is a new generation, it has extra features:
  - It has more effective tissue penetration than the others
  - It has longer terminal half-life (reducing frequency (e.g. once daily), which leads to more compliance (the patient will not miss the dose, and will more likely follow the medical advice)

- if you haven’t understood, Go to Pharmacokinetics-

• **Targets:** Staphylococcus and Streptococcus (these are Gram positive), though some have wider applications, broader spectrums (such as Azithromycin which is anti-Gram negative too)

4- **Clindamycins,**
  - **Targets:** Gram Positive Bacteria
  - **Side Effects:** Pseudomembranous colitis

  To be honest, the doctor had said something which I didn’t understand, you can go back to the record.”D.

Here are some EXTRA book information:

1- That is better absorbed after oral administration and is more active against the organisms within its spectrum.
2- These include staphylococci, streptococci (these are Gram Positive) and most anaerobic bacteria, against which clindamycin exhibits outstanding activity.
3- Enthusiasm for the use of clindamycin has been tempered by an association with the occasional development of **severe diarrhea**, which sometimes progresses to a life-threatening pseudomembranous colitis.

5- **Tetracyclines**
  - **Examples:** Tetracycline, Doxycycline and Tigecycline (I don’t think this bit of info will add anything to your knowledge, but Tigecycline’s cost is about 70 dinars, it is used in urgent cases and associated with sudden death, why? because the tigecycline (glycylcycline) has activity against a lot of bacteria which they won’t get damage from other members of tetracycline family)
• **Targets**: (conventional gram positive and gram negative) mycoplasma, rickettsia, chlamydiae, and surprisingly malaria (microbiologists were surprised because malaria microorganism is not a bacterium but a parasite, but as we all know antibiotics were made to target bacteria)

• **Action**: inhibition of tRNA binding to ribosomes leading to failure of peptide chain elongation.

• **Side effects**: stain of developing teeth in pregnant women’s fetuses (permanent discolor -yellow & gray & brown) affected groups: fetuses (when pregnant ladies exposed to the drug), Infants, children to eight years old and adults,

• **Exceptions**: this antibiotic can be used if there was no other alternative.

6- **Fusidic acid**

• **Targets**: it has an unusual spectrum of activity that includes corynebacterial (which causes Diphtheria), Nocardia and M.tuberculosis, but it is usually regarded simply as an *antistaphylococcal agent*.

• **Mechanism**: it inhibits peptide elongation by blocking factor G which is involved in peptide elongation.

• **Usage**: it is used for the treatment of bone infections due to *Staphylococcus, because it penetrates well into bone*, we can bind it with the β lactam agents to kill the staphylococcal osteomyelitis.

7- **Mupirocin**

• **Uses**: It is a topical antibiotic and anti-MRSA
  
  o MRSA (methicillin-resistant *Staphylococcus aureus*) - a multi-resistant, fatal superbug, found in hospitals and generally in the community.
  
  o Mechanism: blocking incorporation of isoleucine into proteins.

• **Clinical advice** (mentioned in the lecture but *not included*): When a patient is caught with MRSA, you should think of decolonizing it (especially skin) to prevent its spread in the hospital (more often in ICU -intensive care unit-) , decolonization can be possible by using a chemical mixed with water, which is used for mouthwash and
patient bathing, in addition to using a nasal topical thing. (only respectable hospitals do it right!)

8- **Linezoid (oxazolidinone)**

- **Targets:** it is *narrow-spectrum* anti-Gram-positive agent, it is used exclusively against MRSA and other Gram-positive cocci resistant to older agents. Maybe to tuberculosis.
- **Spectrum of activity:** narrow spectrum
- **Possible Uses:** treatment of drug-resistant tuberculosis
- **Mechanism:** prevention of the formation of ribosomal initiation complex

➢ **Inhibitionn of folate**

- **Examples:**
  - Sulphonamides
  - Diaminopyrimidines (Trimethoprim)

  *Trimethoprim is less toxic (it seems that it has a wider therapeutic index).*

  - Co-trimoxazole is a combination of the above *sulphonamides* and *trimethoprim* (synergism)

- **Targets of Co-trimaxole:** Gram negative, Gram positive and aerobes

  - **Mechanism:** interfering with folic acid pathway synthesis (humans don’t have p-amniobenzoic acid, this is why it is only toxic to bacteria)
  - Sulphonamides inhibits folic synthesis by inhibiting (pteridine synthetase)
  - Trimethoprim is competitive with Dihydrofolate reductase

➢ **Inhibition of nucleic acid synthesis**
A) Inhibitors of RNA synthesis and function

a- Rifampicin (bactericidal)
   • Mode of action: These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of mRNA synthesis. (No transcription, no mRNA)
   • Spectrum of activity: They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis, leprosy and MRSA
   • Combination therapy: Since resistance is common, rifampicin is usually used in combination therapy and usually reserved for tuberculosis.

B) Inhibitors of DNA synthesis and function (Bactericidal)

   • Examples + Their Targets:
     ▪ Quinolones - nalidixic acid, (Gram negative)
     ▪ ciprofloxacin (Gram negative plus pseudomonas and G positive)
     ▪ gemifloxacin (Gram negative, pseudomonas, Gram positive plus some anaerobes)

   • effects on Bacteria: Bactericidal

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

   • Characteristics:
     • Absorbed well orally, good body distribution
     • Should be avoided in pregnant, lactating women (breastfeeding) and children (contraindicated* )

C) DNA strand breakage

   • Examples: Nitroimidazole e.g (metronidazole, tinidazole, nimorazole).
- **Mechanism**: At low redox values (no oxygen) they are reduced to a short intermediate form that damages the bacterial DNA, so, the expected bacterial activity is against Anaerobes*.

- **Anti-Tuberculosis drugs**
  - **Treatment period**: at least 6 months
  - **Examples on drugs**
    (A Combination of antibiotics to avoid resistance)
    - Isoniazid and Ethionamide
      - They are chemically related
      - They block mycolic acid (thick lipid, a component of cell wall) synthesis
    - Ethambutol - blocks arabinogalactan synthesis
    - Rifampicin

- **Resistance: Moqawameh (مقاومة)**

  **Drug-resistance Definition:**
  a state which describes the ability of bacteria and other microorganisms to withstand a drug that once killed them, meaning that antibiotics no longer work on bacteria, for example, if you stopped taking your antibiotic but you were partially treated, the bacteria would become resistant to it (it becomes a superbug) which cannot be killed using this drug (this is just a simple example, drug-resistance is one of the biggest universal problems nowadays, new generations of bacteria are developing because of drug abuse)

  **Culprits:**
  This Drug Resistance is Everyone’s responsibility, we cannot only blame healthcare workers, some people who work in the agricultural sector, use antibiotics for curing their animals and for other commercial reasons, how can this affect us as humans? (We are victims, too, because we could not escape the food chain sequence!)
Types of Drug Resistance

- **Intrinsic/ inherent drug resistance (no target site)**
  - The Bacteria which lack cell wall such as mycoplasma (lack target sites- peptidoglycans-) are intrinsically resistant
  - Cell wall is impermeable to antibiotics as in gram negative bacteria (vancomycin is too big to cross the cell wall) which make them intrinsically resistant

- **Acquired resistance**: Selection of resistant bacteria by antibiotics, this is common in areas of heavy antibiotic use (e.g hospitals) The resistance is initially emerged by genetic process then selected by antibiotics.

This figure which shows colored circles can simplify the concept:

- Yellow circles are very sensitive to antibiotic X
- Red is resistant (genetic transfer by plasmids, transposon)
- Antibiotic X will kill yellow, but will not kill red species.

(This is why we should carefully choose the right antibiotic)

**How to know the right/ appropriate antibiotic?**

By determining cross-resistant and multi-resistant bacteria, to avoid using the drugs which are resisted by bacteria:

- **Cross resistance**: Resistance to one member of a family will result in resistance or decreased susceptibility to other members within the same family

  *for example beta lactams family e.g Penicillins and Cephalosporins*, if certain bacteria is resistant to penicillin then it would be more likely resistant or less susceptible (less sensitive) to Cephalosporins,too.
- **Multi-resistance:** Resistance to more than one antibacterial agent (more than one family). Usually acquired by separate mechanisms.

**Genetics of resistance: (Genetics: Chromosomes and Plasmids)**

1. Intrinsic: No target
2. Acquired:
   A) **Chromosomal mutations:** Single step mutation in the antibiotic target leading to decreased antibiotic efficacy (needs high concentration of antibiotic, we need higher doses to have a response). Multistep mutations: changing the target which leads to complete resistance e.g: penicillin binding protein*
   B) **Transferable** via Genetic transfer (plasmids and transposons) – go back to previous lectures-

**Mechanisms of resistance:**

I. Decreased accumulation:
   - Decreased permeability because of porins mutations, so pores will not allow antibiotics to enter the bacterial cell (antibiotic inactivity)
   - Increasing antibiotic efflux (pumping it out of the bacteria using efflux pumps)

II. Modification of target
   - Mutation in target alter its shape, meaning Sequence mutation leads to target alteration, these targets will be unrecognizable to antibiotics
Pneumocooccus resistance to penicillins
Quinilones

- **Target bypass**: Supplementary enzymes will do the same target function but without binding to the antibacterial agent, meaning bacteria will try to find alternate pathways to survive (in which it can perform its functions), if one pathway is blocked by an antibiotic e.g Meticillin Resistant Staph aureus MRSA.
- **Target hyperproduction**: More drug is needed to inactivate the target

### III. Inactivation of the antibacterial agent:

- **β lactamase** (wide range of activity) is an enzyme produced by the bacteria. This enzyme will destroy the β- lactam ring (this is an essential ring in penicillin and cephalosporins) leading to inactivation of the antibacterial agent.
  > Some types of bacteria produce a β- lactamase with a wide range of activity (Extended-spectrum beta-lactamase ESBLs)

Acetylating, adenylating and phosphorylating enzymes: Produced by bacteria (gram negative bacteria) and cause resistance to aminoglycosides and chloramphenicol

### Some terms and their Definitions:

- **Broad-spectrum antibiotic**: wide-spectrum refers to an antibiotic that acts against a wide range of disease-causing bacteria.
- **Cidal Antibiotic/Bactericide**: An agent that kills bacteria. *Extra: Most such agents do not kill spores.*
- **Contraindication**: A condition which makes a particular treatment or procedure potentially inadvisable (can be either absolutely or relatively contraindicated)
- **DNA- gyrase**: an enzyme catalyzes the super-coiling DNA strands
- **Polysomes**: a group of several ribosomes
- **Penicillin-binding proteins**: proteins that catalyze the crosslinking of the two-peptide chains (of the cell wall) such as: transpeptidases and
carboxypeptidases, they are known as penicillin-binding proteins because they are targets for the beta-lactam antibiotics.

- **Sepsis**: poisoning of blood, which indicates bacterial infection in the bloodstream, -the presence of bacteria in blood (bacteremia) and its toxins, which may lead to organ failure, hay fever, tachycardia (increase heart rate), rise in WBC (white blood cells) which are inflammation markers

- **Static Antibiotic/Bacteriostatic agent**: An agent that inhibits the growth of bacteria. But doesn’t kill them

- **Synergism (synergistic effect)**: enhancement of activity. ie, (the combined action of two antibiotics is greater than the action of each one solely)

Examples *(they are mentioned above in the sheet but rephrased here)* are:
  - Two drugs may sequentially block a microbial metabolic pathway, such as *Sulfonamides* inhibit the step which leads to synthesis of folic acid. *Trimethoprim* inhibits the next metabolic step, the reduction of dihydro- to tetrahydrofolic acid
  - A drug such as a cell wall inhibitor may enhance the entry of another one into bacteria and thus produce synergistic effects. Such as beta lactamase that enhance the uptake of aminoglycosides (Penicillins enhance the uptake of gentamicin)

- **Topical administration**: medications that are applied to body surfaces such as skin or mucous membranes (including eye and nose drops)

Feedbacks are more than welcome :‘D

Good Luck!