

### Continuation of Antimicrobial Drugs :

- **Fusidic Acid**
  - **Steroid** antibiotic
    - Not actually a part of steroid drugs.
    - It has a similar structure to steroid drugs.
  - **Complex** structure.
  - Used in treatment of **topical infections** caused by **Staphylococcus aureus** or other **Gram-positive** bacteria.
    - *S. aureus* usually develops **resistance to Meticillin** drugs (*MRSA: Meticillin-Resistant Staphylococcus aureus*)
  - Used to **replace** Vancomycin in topical treatment but **not** systemic treatment.
    - Use of Fucidic acid in systemic treatment is usually associated with side effects.
  - Mechanism of action in relation to nucleic acid is by affecting **RNA synthesis**. It is also a **protein synthesis inhibitor**.
- **Sulfa Drugs (Sulfonamides)**
  - **Chemically-synthesized** drugs (*Not naturally excreted by living organisms*)
  - Have a very simple structure which includes **6 rings, Sulfur and carboxyl groups**.
  - Have **over 30** variations (*Types*) but all have the **same basic** structure and same reaction to bacteria.
  - Used against infections caused by **Group B Streptococci** (*Gram-positive*)
  - Mainly used against **Gram positive** bacteria (*And Gram-negative bacteria , to some extent*) causing **urinary tract infections**.
  - Have a **structural analogue** called **PABA**
    - Para-aminobenzoic acid
    - **Essential metabolite** in the end production of **Folic Acid** (*Which is important n protein synthesis*).
    - Sulfa Drugs compete for the same receptors as PABA enzymes which **inhibits Folic Acid synthesis**, thus **inhibiting protein synthesis**.
    - Humans don't have the necessary enzymes to convert PABA into Folate so their metabolism **isn't affected** by the drug.
  - They are **not** bacteriocidal drugs (*Do not kill bacteria*) but **Bacteriostatic** (*Inhibit reproduction*).
    - Dosage must always be **equal or higher** to PABA's concentration in order to ensure **complete inhibition** of folic acid synthesis.
  - They are usually **combined** with other drugs which work against Gram-positive and Gram-negative (*To a lesser extent*) bacteria such as **Trimethoprim**.
    - The Sulfamethoxazole-trimethoprim combination is called **Cotrimoxazole**.
    - Those two drugs have **synergetic** reactions meaning that they work together almost identically to achieve a **greater effect** than when working alone.
    - Produced in the **1960s**.
    - Usually used in **upper respiratory tract and urinary tract infections**.

- **Antituberculosis Drugs**
  - Used only to treat **tuberculosis and Brucellosis** (*Caused by Brucella*) according to **WHO**
  - Affect only one part of the cell wall of *Mycobacterium tuberculosis* (*And other species*) which is **Mycolic acid** (*Essential component of M. tuberculosis cell walls, usually only found in them*)
  - Tuberculosis is **not** easily treated due to the feature of the infection which is **granuloma** caused by the **aggregation of macrophages, monocytes and other cells** to keep the infection **local** and stop it from spreading.
  - At least **6 months** of treatment is required for recovery. This time range can go up to **2 years** in case of **resistant organisms** which will require the use of **more toxic** and expensive drugs.
    - Basic TB treatment for 6 months can cost 100 to 150 JOD.
    - In the case of resistant bacteria and longer treatment, the cost can go up to 10,000 JOD.
  - **Isoniazids (INH)** are **widely used** in treatment of tuberculosis but **not as a single drug** as to **not** allow the organism to **develop resistance** against them.
    - **Usually** combined with one of these drugs :
      - Ethambutol
      - Cycloserine
      - Rifampin
      - Streptomycin
- **Metronidazole**
  - Known commercially as **Flagyl**
  - **Best and only available** drug to use for **non-facultative anaerobic bacteria and protozoa** such as :
    - **Amoeba histolytica** : Causes gastrointestinal infections
    - **Giardia lamblia** : Causes infection of small intestines
    - **Trichomonas vaginalis** : Causes Vaginatis

### How to understand and evaluate laboratory results?

There are two important steps in lab tests to decide **which antibiotic to use** against a particular organism, especially in the cases of **repeated infections**.

- **Isolation**
  - Samples are taken from the patient's **blood and/or urine**.
  - Sample must be **fresh** (*Less than 24 hours old*) in order not to give **false** results because of **cell wall changes**.
  - Since susceptibility tests **cannot** be performed on **mixed colonies**, the pathogenic microorganism **must be isolated** in **pure** culture.
    - Selection of type of culture disc is **vital** because you must choose one that is compatible with the **type of bacteria** and the **infection's location** in the body.
    - Around **10 million cells** are placed in the plate and this can be measured by a **turbidity tube**.
- **Organism Recognition** (*Through susceptibility tests*)
  - Discs are **filter papers impregnated with an antibiotic against a certain type of bacteria**.
  - Most laboratories have discs which represent the **most common** types of antibiotics.
  - Every disc has a **definite amount** (*Concentration*) of antibiotic decided by **animal experiments** (*In vitro*) and the experimental results are later **converted** to human-appropriate doses.
    - Note that we used in vitro experiments to define elements of an **in vivo** experiment (*The susceptibility test*)
    - The **final concentrations** used for **human treatment** are decided by international scientific companies and experts who know the **end concentration** in the body after metabolism in order to **avoid toxicity**.
  - Most **common** type of disc used in susceptibility tests is **Diffusion Test Disc**
  - Susceptibility tests are used to determine if an organism is **Resistant (R)**, **Susceptible (S)** or **Intermediate susceptible (IS)**.
    - **Mueller-Hinton agars** (*Type of diffusion test disc*) are usually used for susceptibility tests.
    - This can be done through placing the sample in a disc and observing the **inhibition pattern** after **24 hours** in a **37°C temperature**.
    - If an antibiotic successfully inhibits the growth of bacteria in a dish, then the bacteria is susceptible.
      - The **inhibition zone** around the colony must be **no less** than a certain number (*18 mm for Cephoxime, 20 mm for Tetracycline, etc.*) defined by the antibiotic type in order to decide the susceptibility of a drug.

Patients with **kidney or liver function abnormalities** (*Cannot eliminate drugs properly*) or **chronic infections** usually require more **accurate** tests in order to give them the **least** amount of drug possible as to not cause toxicity.

- **Minimal Inhibitory Concentration Test**

- To measure **the lowest concentration** of drug which can **inhibit the visible growth** of bacteria and still remain safe for the patient.
- **Similar** to disk diffusion tests but uses **different** concentrations (*Gradient*) in order to be specific about the exact concentration needed.
- MIC can be determined by noticing the concentration at which inhibition **begins** and this is the concentration given to the patient.

Remember that choosing the correct type of antibiotic is **vital** because any misuse, increase or decrease of **dose or duration** or mistake in administration can help **develop resistance** against drug.

- This requires the **hospital infection expert** to find a **new** drug which can be quite difficult, especially in **sensitive cases** where **toxicity** is expected.
- It can also cause **side effects** related to gastrointestines and kidney functions.
- This is one of the reasons why it's important to know the percentage of **resistance to common E. coli and other common pathogenic microorganism** in a community.
  - **Amoxicillin and Amicillin cannot** be used anymore for **urinary tract infections in Jordan** due to resistance.

In **healthy** patients, increasing the duration for one or two days or changing the dose can be done without expecting too much complications.

### **Quick Review :**

Streptococci groups are classified according to :

- **Hemolytic properties**
  - $\alpha$ -hemolytic
  - $\beta$ -hemolytic
  - Non-hemolytic
- **Lancefield Antigens**
  - Antigenic characteristics of C carbohydrate (*Found on cell wall*)
    - Given letter names from A till S
      - $\beta$ -hemolytic Streptococci only go as far as F, and from those, only A and B are of any clinical importance.

A **mixture** of the above-mentioned classifications is used in modern day microbiology.

Remember that **all** Streptococci are **Catalase-negative**, whereas Staphylococci and Micrococci are **Catalase-positive**

## $\beta$ -hemolytic Streptococci

- Cause **complete** destruction (*Hemolysis*) of red blood cells when incubated in **blood agar**, hence the **clear zone of hemolysis** around the colony.
- Represented by **Group A Streptococci**
  - Also known as *Streptococcus pyogenes* (*Pus-producing*)
  - Has more than **80 subtypes**, which means each type will stimulate the generation of a **different** antibody in humans.
    - This is the reason producing potent vaccines is **almost impossible** (*There are no vaccines*).
    - Also the reason it's difficult to understand why one type predominates in a community when an infection is spread.
  - In any community, there are people with **immunity** for this group but they still carry it and can easily transmit it to other people and that's why there's a **relatively constant** percentage of people infected with this group and another percentage of **healthy carriers**.
  - **Penicillin** and **Erythromycin** can be used to treat infections caused by this group (*Can also be used for Group B*).
  - Produce **severe inflammatory reactions** in infected tissues.
  - Associated with **excretion of a variety of extracellular enzymes and toxins**.
    - The **mixture** of enzymes and toxins is responsible for the feature of inflammation in the **upper respiratory tract**.
  - The antigenic structure of the organism following lysis may spread into the bloodstream and cause what is known as **Post-streptococcal disease**.
    - This disease can be recognized in the forms of **Rheumatic Heart Fever** or **complications of Rheumatism**.



- This group produces many important **toxins** :
  - **Pyrogenic exotoxin** (*Erythrogenic*)
    - Associated with **throat and skin infections**
    - Produces **Scarlet Fever** (الحمى القرمزية)
      - Causes **skin rashes**, especially in **children**.
      - The human body produces **specific antibodies** (*Develops Immunity*) against the disease after the first contraction so it can only be observed **once** during a person's lifetime.
  - **Superficial Skin Toxin**
    - More **dangerous**
    - Associated with **wound infections** and **Toxic Shock Syndrome**.
      - Toxic Shock Syndrome is a multisystem inflammatory response to bacterial exotoxins which can be **fatal**. Common symptoms :
        - Circulatory system abnormalities
        - Kidney failure
        - Severe damage to infected tissues in the form of **putrefaction** (*Decomposition of proteins in the cell*).
  - **Hemolysin Toxins**
    - Can be used in **laboratory detection** for this specific group
    - Contributes to **inflammatory reaction** and **diffusion of infection**.
- In the case of **recurring** upper respiratory tract infections in **children**
  - These cases are usually presented as **sore throats**.
  - They manifest in the form of **tonsillitis** (*Inflammation of tonsils*) and **pharyngitis**.
  - Can cause **Rheumatic Fever**.
  - If not treated with antimicrobial drugs, it is safe to assume that the patient will slowly develop and **immune response** to the group's antigens which might cause **Post-Streptococcal Disease** (*Glomerulonephritis*) on the long run.
  - Can be **treated and its recurrences prevented** with **monthly long-acting Penicillin** which can be taken for years, depending on the **clinical manifestations** of the disease.

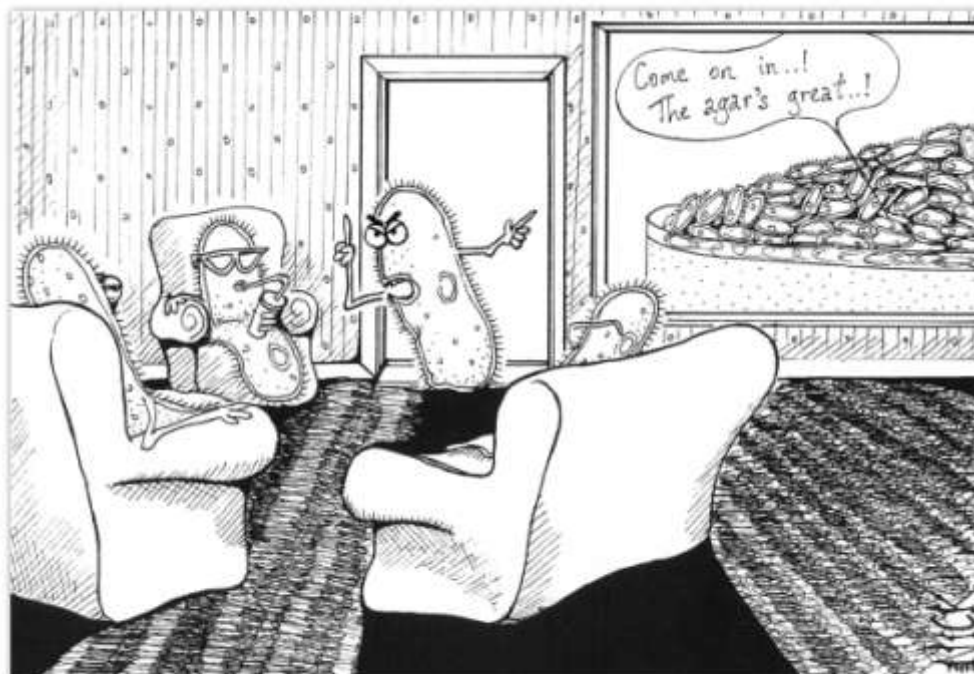
- Another group to represent this classification is **Group B Streptococci**
  - **Sulfonamides** were the **first** drugs used to treat infections associated with this group (*Even though at the time, they didn't know the difference between groups A and B*).
  - **Rarely** associated with upper respiratory tract infections.
  - Found mostly in the **intestines**.
  - Found in the **vagina** but to a lesser extent.
    - Under normal conditions, this group is **part of the vaginal flora** which is **not associated** with the development of any type of disease.
    - **Opportunistic Pathogens** which can cause infections during :
      - **Pregnancy**
        - **Urinary tract** infections.
        - In the case of **injured** uteruses, the organism can invade **subcutaneous tissue** of it and disseminate through the abdomen and into the blood to produce **Puerperal Fever** (*Childbed Fever*).
      - **Delivery**
        - **Contamination** of the **amniotic fluid** which can be **swallowed** by the newborn.
        - This can cause **Lung Infection Sepsis**, or as it is more commonly known, **Neonatal Sepsis**.
          - Sepsis is a potentially **fatal** whole-body inflammation caused by severe infection.
          - Neonatal Sepsis is often associated with **Blood Sepsis and Meningitis**.
- **Groups C, D, E & F Streptococci**
  - Can be associated with upper respiratory tract infections.
  - **Cannot** be associated with Post-Streptococcal Disease for two reasons :
    - Cell walls of these Streptococci **aren't** complete.
    - They do **not** excrete specific enzymes and toxins.

### $\alpha$ -hemolytic Streptococci

- **Partial** destruction (*Lyse*) of red blood cells
- **Less virulent** and less associated with infection
- More common in **upper respiratory tract infections**, especially in the **oral cavity** with diseases such as :
  - Oral abscesses
  - Dental caries (*Decay or cavity*)
- Can **disseminate** (*Spread*) into the **bloodstream** and cause **Endocarditis** (*Inflammation of the inner layer of the heart*) if the patient has congenital heart defects or heart muscle injuries.
- Produce **green** pigmentation in blood agar
  - The green color indicated the presence of **unlysed RBCs** and a green-colored **metabolite of Hemoglobin** (*Remnant of the destroyed RBCs*)
  - **Viridians Streptococci** also produce green pigmentation and partially lyses RBCs, so the need to distinguish between two types of Streptococci arises.
    - Distinguishing between **S. Pneumoniae** and **V. Streptococci** (*Usually found in upper respiratory tracts*) can be done through a test called **Optician Disc Test**.
      - **An antibiotic-like substance** is placed in the center of a patient-sample-inoculated plate. If there is an **inhibition zone** around the disc, then it is Streptococcus pneumoniae, if not, then it's V. Streptococcus.
    - To distinguish between **Streptococcus pneumoniae** and  **$\beta$ -hemolytic Streptococci**, you must use a **Bacitracin** disc .
      - A type of **antibiotic** which can **inhibit** Gram-positive bacteria and it **only** inhibits Group A Streptococci.
    - Both above-mentioned distinguishing methods are only **98% accurate** so in order to confirm the result **100%**, especially in cases like **Meningitis**, we must do **serological tests** where we use an **antiserum** (*Plural is antisera*), which is a **blood serum containing antibodies**, against **antigens** of Streptococcus pneumoniae or Group A Streptococci.
- An example of this group is **Streptococcus pneumoniae**
  - Very **virulent**.
  - Highly **invasive**.
  - Has **vaccines** because most of them have **polysaccharide-capsules** which can be inhibited.
  - Getting vaccinated is **highly recommended** for patients who are **very young** (*Less than one year old*), **very old** or have **immunity deficiency** because they are susceptible to develop pneumonia following bacterial pneumonia, especially due to S. pneumoniae, after a viral infection like Influenza. ???
  - Some physicians forsake the patient by giving antimicrobial drugs to make sure they don't develop pneumonia.



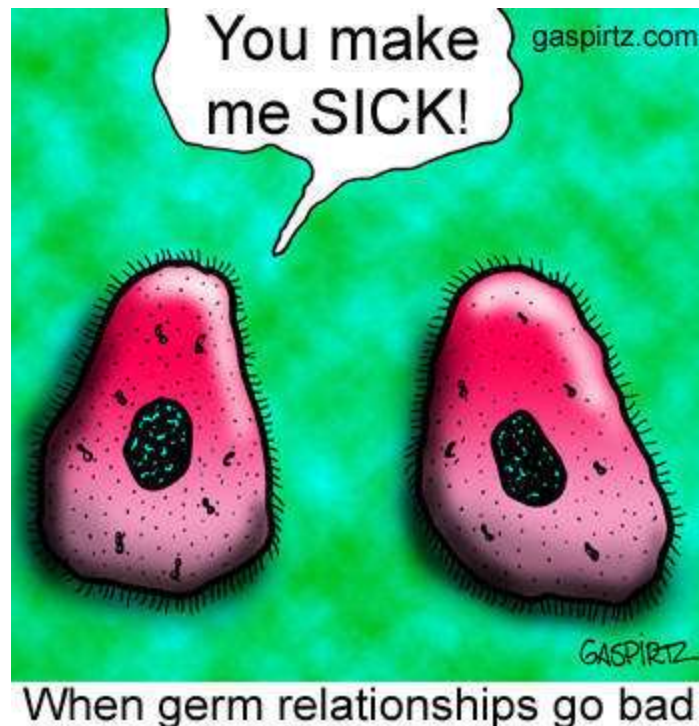
- **Widespread resistance** to antimicrobial drugs, especially to  **$\beta$ -lactam drugs** such as:
  - **Penicillin**
    - Resistance is developed against **first and second generation** drugs
  - **Cephalosporins**
    - **First-generation** drugs are **effective**, especially if there is **sepsis or meningitis** involved. *You do not have to memorize these drugs' names*
      - Cefotaxine
      - Cefotaxime
      - Ceftriaxone
    - **Second-generation** drugs are **relatively useless**
  - Up to **90%** of *S. Pneumoniae* in Jordan are resistant to **Penicillin G, Ampicillin and Amoxicillin**.
- Patients with **deficient immune responses**, such as patients with **Leukemia, Lymphoma or other bacterial or viral infections** (*Which typically damage the superficial layers of mucosa*) can be susceptible to an **above-normal invasiveness** of *S. pneumoniae* in the upper respiratory tract which can **spread** to the lungs in feature of pneumonia. Within a short period, there will be:
  - Intensive scarring in lung tissue
  - Accumulation of fluid
  - Feature of pneumonia which is **fatal**
- Referring to slide 10, you will notice the presence of **diplococci** associated with large capsules composed of polysaccharides, so we can conclude that invasive *S. pneumoniae* are usually **encapsulated**.
- Non-capsulated *S. pneumoniae* are usually found in the **upper respiratory tract of healthy individuals**.
- Remember that during **transduction**, at the presence of **specific macrophages**, non-capsulated bacteria **can** become capsulated.



"I wish you'd learn to put the lid on your Petri Dish, Harry! We came here today with just four kids but now it looks like we've got several million...!!"

### Non-hemolytic Streptococci ( $\gamma$ -hemolytic)

- Weren't mentioned by the professor specifically but **Enterococci** are considered to be non-hemolytic Streptococci (*The professor said that Enterococci are **not** really part of the Streptococci*)
  - Part of the **Micrococcaceae** family.
  - Composed mainly of single cocci, diplococci and short cocci.
  - **Catalase-negative**, just like Streptococci.
  - Began to appear in human intestinal tracts in the last 50 to 60 years due to the **close contact** between humans and animals.
  - Enterococci are important in the intestines of animals but are of less importance in humans.
  - **Increase** in number with **wide use of Cephalosporin** (Short or long periods) because they are not effective against Enterococci (*Using Cephalosporins will most likely increase number of Enterococci*).
  - As indicated by its most common location, this group usually causes **intestinal tract infections**.
  - Can also cause **urinary tract infections**.
    - Usually observed in **hospitalized patients** (*Hospital-acquired infection*) rather than community patients (*Patients who get infected at home, not the hospital and get admitted into an outpatient clinic*).
    - Caused by **urinary catheters** and other invasive techniques.



## Gram-Positive Bacilli

A large group of bacilli which has **various morphological structures**, they can be :

- Rods
- Rods with bulging at one end
- Pleomorphic (*As seen on slide 3*)
  - They **are difficult to identify through Gram** staining for inexperienced individuals.

On the left side of slide 3, you can see a different kind of structure known as **Pleomorphic** (*Different shapes of bacilli*) which appear as cocci or short bacilli or long bacilli. These are known as **diphtheroids** .

On the right side, you can see larger rods which usually have **clear gram positive color**. But depending on **age of culture**, Gram -positive slides can show Gram-negative coloring. The **empty spaces** within the bacteria indicate presence of **capsules** (*In both aerobic and anaerobic spore-forming bacilli*).

### Types :

- **Corynebacterium diphtheriae**
  - The word diphtheria originated from the **clinical feature** of the disease which is associated with the **production of potent, dangerous and highly necrotic** (*Cause severe inflammatory reaction which inhibits protein synthesis*) **toxins** which are **excreted** from growing cells in vegetative forms.
  - Associated with **pleomorphic bacilli** in Gram stain
  - Part of a group of **corynebacteria** which is divided into :
    - **Toxigenic**
      - corynebacterium diphtheriae
    - **Non-toxigenic**
      - The ones present in the **normal flora** of our upper **respiratory tract**, mucosa of the throat specifically.
      - Can also be found in the **oral cavity** in smaller numbers.
      - Do **not** exist in the intestines.
      - Certain diphtheroids can **survive on the skin**.
  - Show **localized skin infection** called Skin Diphtheriae
  - Can be confused with **Group A Streptococci**
  - Requires a **special** blood agar culture medium to be identified in the lab.
    - **Rarely** tested for anymore.
    - When tested, first thing they do is a **toxigenicity test** to see how much damage It has done to the patient.
  - **Highly susceptible** to **penicillin and erythromycin** (*Show no resistance*)
  - The diphtheria toxin gene is **encoded by a bacteriophage found in toxigenic strains**.
  - Infection starts in upper respiratory tract with a group of Streptococcus, other bacteria or viruses.

- Cause a very **intense** inflammation marked by:
  - WBC accumulation and
  - Damage to the mucosa
    - Can be recognized by the appearance of **grey pseudomembrane colitis** on the throat.
- In less than 48 hours, diphtheriae **replicate and produces** potent toxins that will be **absorbed from the lymph and bloodstream** then carried to the rest of the body, some of the major damages are :
  - Liver necrosis
  - Heart muscle necrosis
  - Myocarditis
  - Death in less than 3 days
- In the mid-1950's, before vaccines, diphtheriae took the lives of many children.
- **Vaccines** are now given to prevent the spread of this disease
  - The vaccine includes **diphtheriae toxoid** which is the **formaline- inactivated** version of the toxins
  - Could be given alone but is usually given as part of the **Triple Vaccine** (*Two more vaccines can be added to the triple one*)
    - Diphtheriae (الخنق)
    - Tetanus (الكزاز)
    - Bordetella Pertussis (السعال الديكي)
- Patients are treated with **antimicrobial drugs and anti diphtherotoxins**
- Last two cases recorded in Jordan were in **1992** after two women came back from abroad carrying the disease.
- There are cases of **pseudodiphtheriae** which give the impression of the disease but are actually different in diagnosis.

Don't forget to read the slides for extra details required from you.

The professor said he won't ask about treatment of specific infections in the midterm but you must know basics, classifications and common knowledge.

I apologize for any lacking in this sheet.

MRS is very helpful if you still haven't quite grasped some information

