MICROBIOLOGY I

Pharmacy college

2nd stage

Lec 1 + 2

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What is microbiology?

- Microbiology is the study of microscopic organisms (bacteria, viruses, fungi, protozoa, and algae) that are generally too small to be seen with the naked eye.
- **Pharmaceutical Microbiology** is an applied branch focused on:

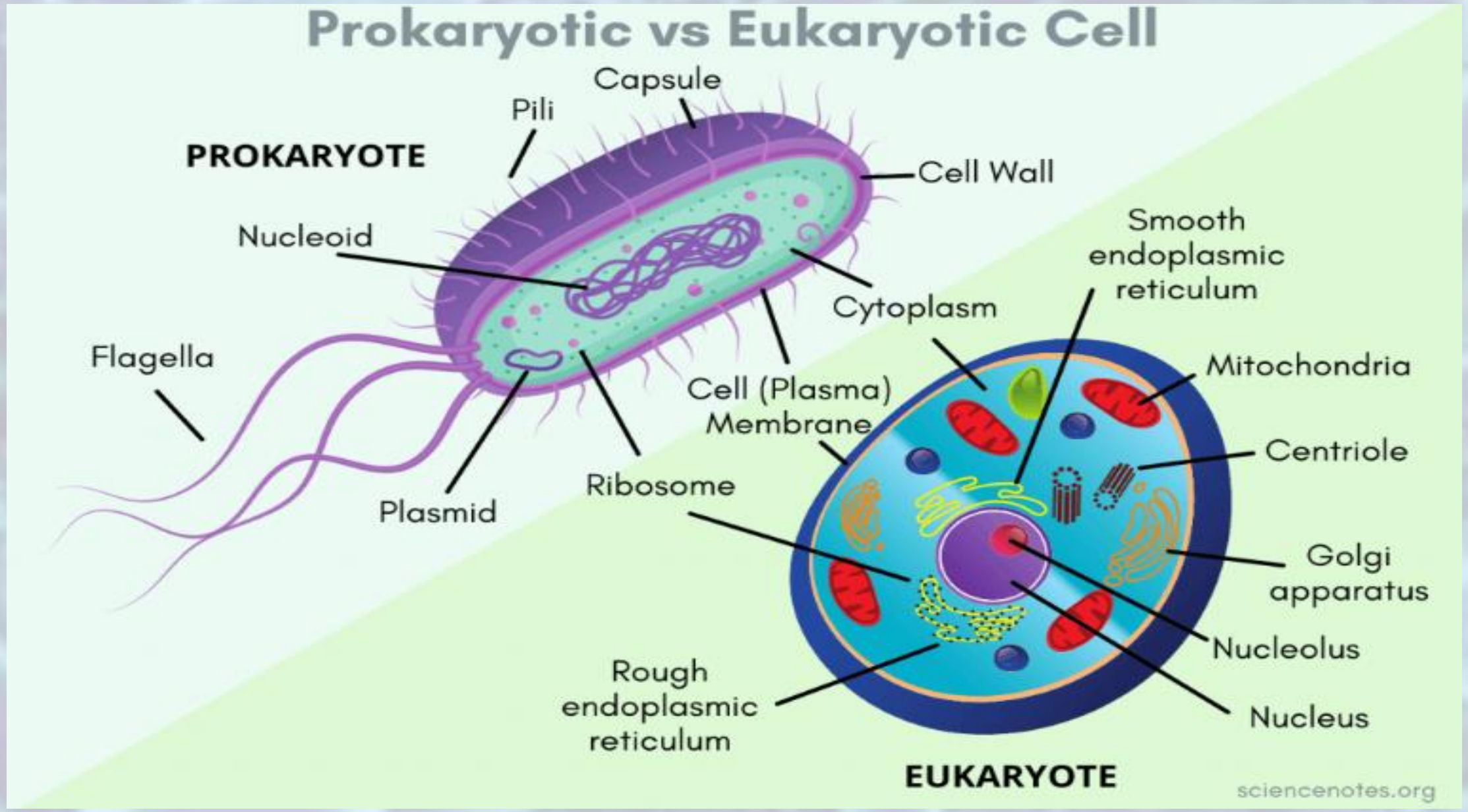
1- The use of microorganisms to produce **life-saving drugs** (e.g., antibiotics, vitamins, and vaccines).

2- The exclusion, control, and elimination of microorganisms to ensure **drug safety and sterility**.

Prokaryotes vs. Eukaryotes

- Cells that have a well-defined nucleus are called eukaryotic, whereas cells that lack a nucleus are called prokaryotic.
- **All bacteria are prokaryotes.**

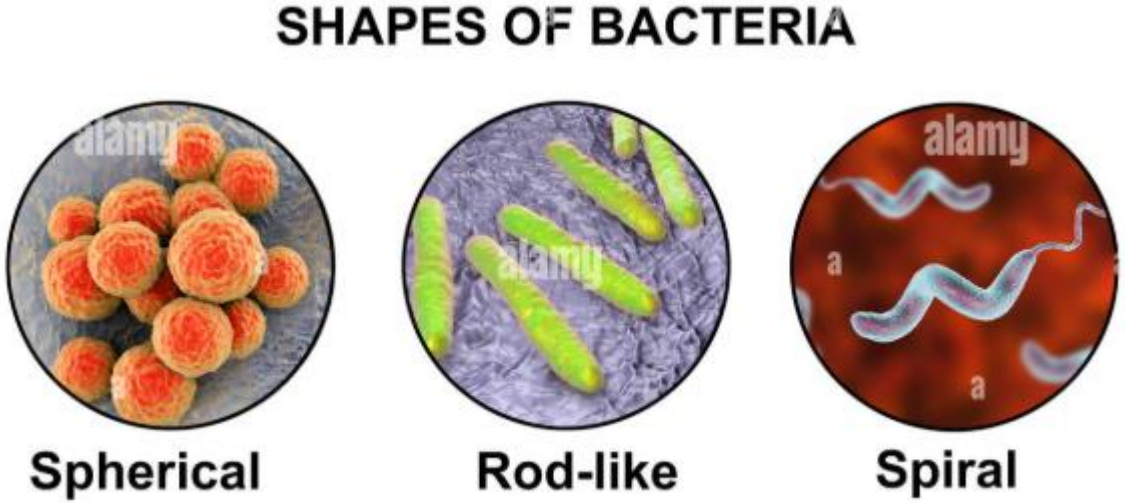
CHARACTERISTIC	PROKARYOTIC CELLS	EUKARYOTIC CELLS
Chromosome	Usually single, circular[1]	Multiple
Nucleus	No nuclear envelope or nucleoli	Membrane bound, nucleoli present
Membrane-bound organelles	Not present	Present (examples include mitochondria and endoplasmic reticulum)
Cell wall	Usually present, many contain peptidoglycan	Present in plant cells, no peptidoglycan
Plasma membrane	No carbohydrates, most lack sterols	Sterols and carbohydrates present
Ribosome	70S	80S (70S in organelles)
Average size	0.2–2 mm in diameter	10–100 mm in diameter


Prokaryotic vs Eukaryotic Cell
PROKARYOTE
Capsule
Pili
Cell Wall
Nucleoid
Cytoplasm
Flagella
Cell (Plasma) Membrane
Ribosome
Plasmid
Smooth endoplasmic reticulum
Mitochondria
Centriole
Golgi apparatus
Nucleolus
Nucleus
Rough endoplasmic reticulum
EUKARYOTE
sciencenotes.org

Bacterial classification and structure

1. Shape and Arrangement (Morphology)

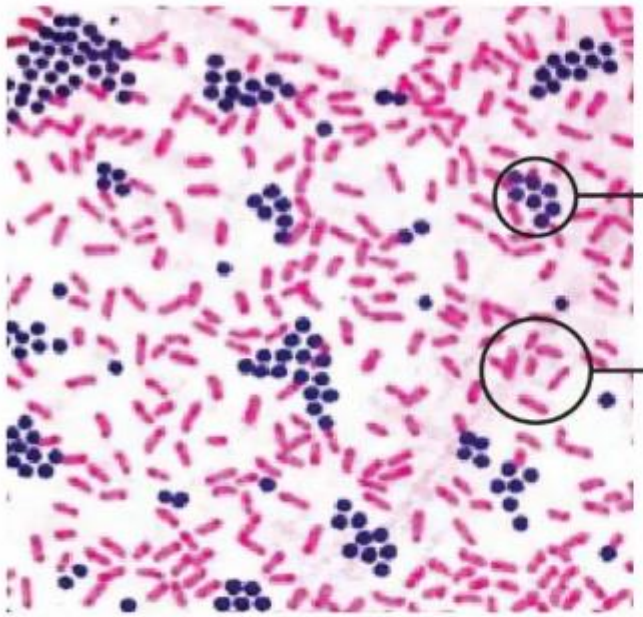
- Bacteria are primarily identified by three basic shapes:
- **Cocci (Spherical):**
  - *Clusters:* **Staphylococci** (e.g., *Staphylococcus aureus*, causing skin and soft tissue infections).
  - *Chains:* **Streptococci** (e.g., *Streptococcus pneumoniae*, causing pneumonia).
  - *Pairs:* **Diplococci** (e.g., *Neisseria meningitidis*).
- **Bacilli (Rod-shaped):**
  - Examples include *Escherichia coli* (E. coli) and *Pseudomonas aeruginosa*.
- **Spirals (Spirochetes/Spirilla):**
  - Examples include *Treponema pallidum* (the causative agent of syphilis).



2. The Gram Stain Strategy

- Developed by Hans Christian Gram, this differential staining technique divides bacteria into two massive pharmacological categories based on cell wall anatomy:
- **Gram-Positive (G+):** Retain the primary crystal violet stain and appear purple under the microscope e.g., *Staphylococcus aureus*.
- **Gram-Negative (G−):** Lose the crystal violet stain, accept the safranin counterstain, and appear pink/red.

e.g., *Escherichia coli* .



3. Oxygen Requirements

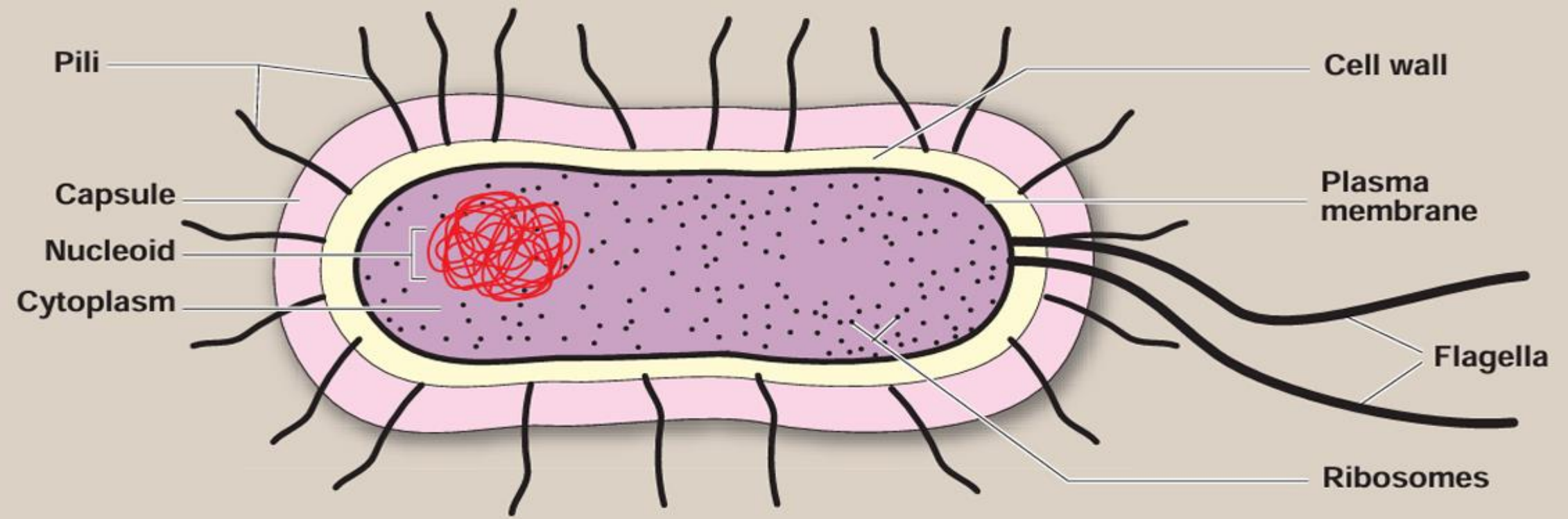
- Understanding metabolic oxygen requirements helps pharmacists understand where infections thrive in the body:
- **Strict (Obligate) Aerobes:** Require oxygen to survive

(e.g., *Mycobacterium tuberculosis* in the lungs).

- **Strict (Obligate) Anaerobes:** Killed by oxygen; thrive in deep tissues or the gut (e.g., *Clostridium difficile*).
- **Facultative Anaerobes:** Can grow with or without oxygen, but prefer oxygen (e.g., E. coli).

Bacterial Structure & Pharmacological Targets

- Bacterial structures are divided into essential components (which all bacteria have to survive) and accessory components (which only some bacteria possess to enhance survival or virulence).

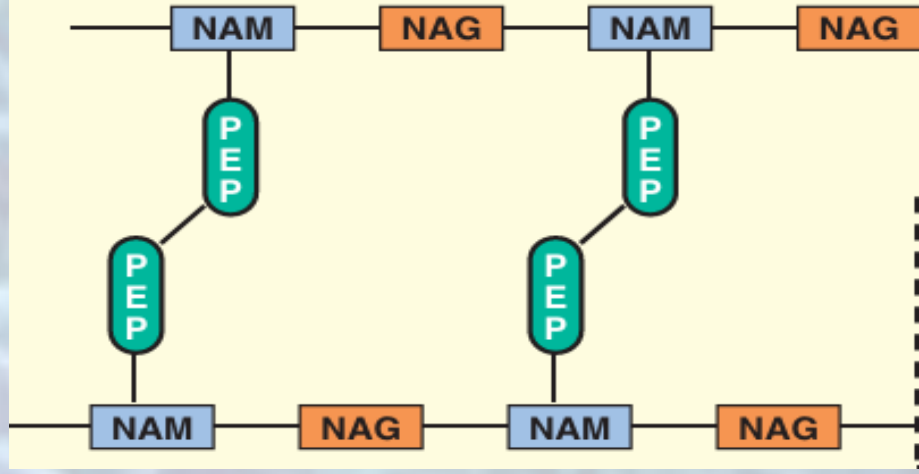


1. The Essential Components

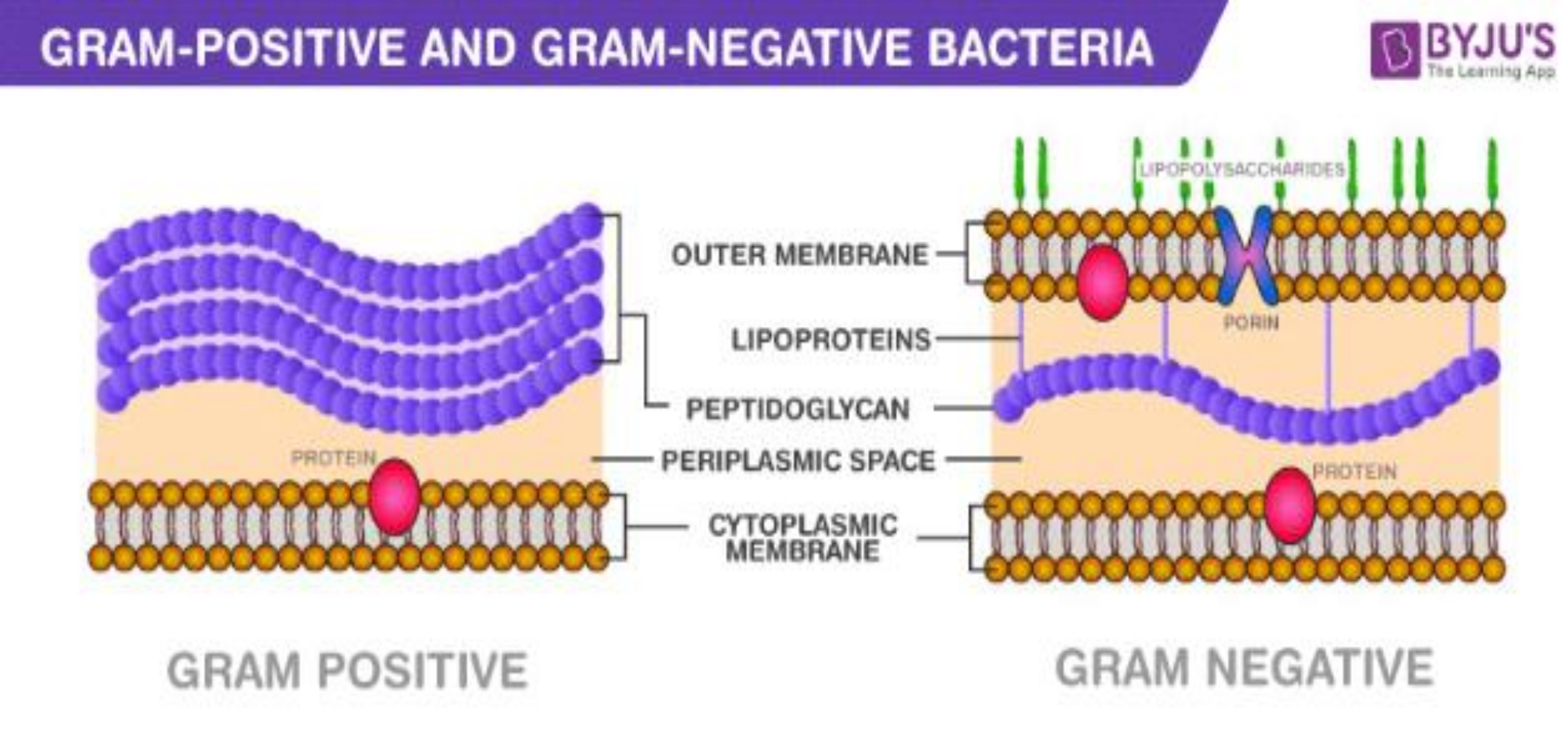
A. The Cell Wall (The Peptidoglycan Net)

- The cell wall prevents osmotic lysis (bursting) of the bacterium. It is made of a unique polymer called peptidoglycan.
- **The Gram-Positive Cell Wall:** Features a very thick, multi-layered peptidoglycan mesh containing teichoic acids. Because it lacks an outer protective barrier, it is highly susceptible to Beta-lactam antibiotics (like penicillins and cephalosporins) which inhibit cell wall synthesis.
- **The Gram-Negative Cell Wall:** Features a very thin peptidoglycan layer surrounded by a complex Outer Membrane. This outer membrane contains Lipopolysaccharide (LPS)—also known as endotoxin, which can trigger deadly septic shock in patients. The outer membrane acts as a barrier, making Gram-negative bacteria naturally more resistant to many standard antibiotics.

NAM	=	N-acetylmuramic acid
NAG	=	N-acetylglucosamine
BPP	=	Bactoprenol phosphate
PEP	=	Cross-linking peptide
DAP	=	Diaminopimelic acid



peptidoglycan



Comparison of gram-positive and gram-negative bacterial cell walls

B. The Cytoplasmic Membrane

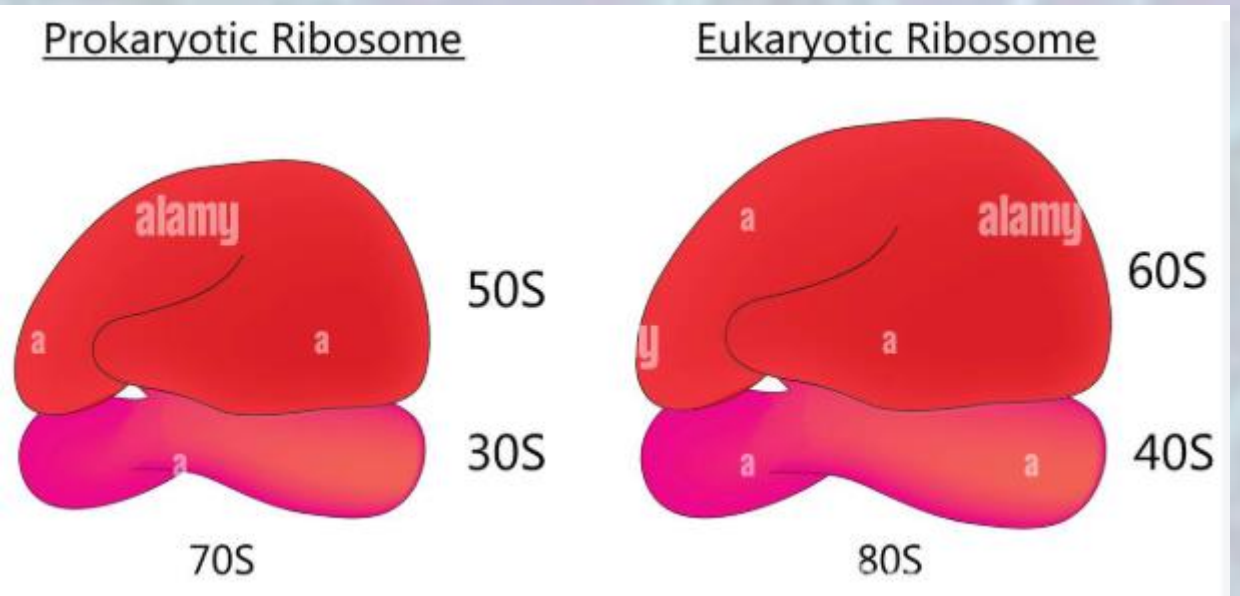
- A phospholipid bilayer lacking sterols (unlike human cells).
- It acts as a selective permeability barrier and handles energy (ATP) generation since bacteria lack mitochondria.
- **Pharmacy Target:** Drugs like Polymyxins and Daptomycin disrupt this membrane, causing cellular leakage.

C. The Nucleoid (Bacterial Chromosome)

- A single, circular, tightly coiled double-stranded DNA molecule that floats freely in the cytoplasm (no nuclear envelope).
- **Pharmacy Target:** Fluoroquinolones (like ciprofloxacin) target DNA gyrase/topoisomerase enzymes to halt bacterial DNA replication.

D. Ribosomes (The 70S Machinery)

- Bacterial ribosomes are responsible for protein synthesis and are structurally smaller (**70S**, split into 50S and 30S subunits) than human ribosomes (**80S**).
- **Pharmacy Target:** This structural difference permits selective toxicity.
- 30S Inhibitors: Aminoglycosides, Tetracyclines.
- 50S Inhibitors: Macrolides (Erythromycin, Azithromycin), Chloramphenicol.



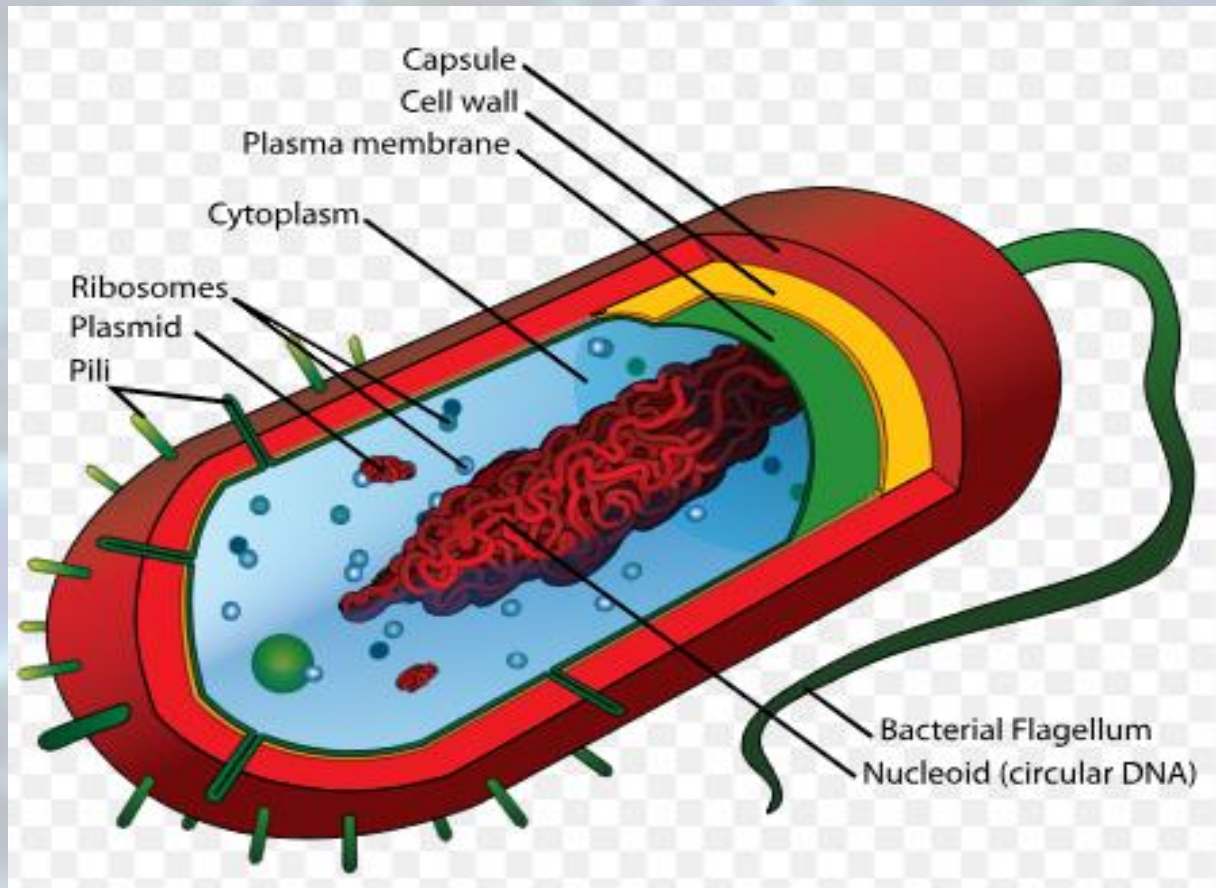
2. The Accessory Components (Virulence Factors)

A. Capsule

- A slimy, polysaccharide outer layer secreted outside the cell wall. It acts as an invisibility coat, preventing human white blood cells from engulfing the bacteria (phagocytosis). Capsulated strains are highly virulent.

B. Plasmids

- Small, circular, extrachromosomal DNA molecules that replicate independently of the main chromosome. They frequently carry genes for antibiotic resistance (R-plasmids) and can be passed horizontally between different bacteria.



C. Pili and Fimbriae

- **Fimbriae:** Hair-like projections used for adhering to host tissues (e.g., E. coli using fimbriae to stick to the urinary tract, causing UTIs).
- **Pili (Sex Pili):** Longer tubes used to physically connect two bacteria to transfer plasmids—a major mechanism of spreading drug resistance.

D. Flagella

- Long, whip-like appendages used for locomotion (motility), allowing pathogens to swim toward nutrients or spread through host tissues.

E. Endospores

- Dehydrated, dormant structures formed inside certain Gram-positive rods (like Bacillus and Clostridium species) during harsh environmental conditions. Endospores are extremely resistant to heat, radiation, desiccation, and standard disinfectants.
- **Pharmacy Impact**: Autoclaving (steam under pressure at 121°C) is required to destroy endospores to achieve true sterility in pharmaceutical preparations.

Bacterial nomenclature

- Bacterial nomenclature uses a binomial system where scientific names consist of a capitalized genus followed by a lowercase species epithet, both written in italic.
- Examples:

Escherichia coli (*E. Coli)*

Staphylococcus aureus

Clostridium difficile

Mycobacterium tuberculosis

Bacterial pathogenicity

- **Pathogenicity:**

The qualitative ability of an infectious agent to cause disease.

- **Virulence:**

The quantitative degree or measure of pathogenicity. It indicates how harmful a pathogen is. **A highly virulent organism requires a lower infecting dose.**

- **Opportunistic Pathogens:** Microbes that ordinarily do not cause disease in healthy individuals but exploit predisposing factors (e.g., immunocompromised status, diabetes, broken skin barriers) to initiate infection.

The Chain of Bacterial Pathogenesis:

[Transmission] ➔ [Adherence] ➔ [Colonization/Invasion] ➔ [Immune Evasion] ➔ [Tissue Damage]

1. Entry into the host: The first step of the infectious process is the entry of the microorganism into the host by one of several ports: via the respiratory, GI, or urogenital tract or through skin that has been cut, punctured, or burned. Once entry is achieved, the pathogen must overcome diverse host defenses before it can establish itself.

2. Adherence to host cells: Some bacteria (for example, *Escherichia coli*) use pili to adhere to the surface of host cells.

- Adherence enhances virulence by preventing the bacteria from being carried away by mucus or washed from organs with significant fluid flow, such as the urinary and the GI tracts.

3. Invasiveness: Invasive bacteria are those that can enter host cells or penetrate mucosal surfaces, spreading from the initial site of infection. Invasiveness is facilitated by several bacterial enzymes, the most notable of which are collagenase and hyaluronidase.

4. Iron sequestering: Iron is an essential nutrient for most bacteria. To obtain the iron required for growth, bacteria produce iron-binding compounds, called siderophores. These compounds capture iron from the host by chelation.

5. Virulence factors that inhibit phagocytosis: The most important antiphagocytic structure is the capsule external to the cell wall, such as in S. pneumonia.

6. Bacterial toxins: Some bacteria cause disease by producing toxic substances, of which there are two general types: exotoxins and endotoxin.

- **Exotoxins**, which are proteins, are secreted by both gram-positive and gram-negative bacteria.
- In contrast, **endotoxin**, which is synonymous with lipopolysaccharide (LPS), is not secreted but instead is an integral component of the cell walls of gram-negative bacteria.

Pathogenicity Depends on:

a. Bacterial virulence factors

- Virulence factors are those characteristics of a bacterium that enhance its pathogenicity.
- ***Pharmacological Note:*** *These factors represent prime targets for anti-virulence drugs and vaccines.*

b. Host-mediated pathogenesis

- The pathogenesis of many bacterial infections is caused by the host response rather than by bacterial factors.
- Classic examples of host response–mediated pathogenesis are seen in diseases such as gram-negative bacterial sepsis. The tissue damage in these infections is caused by various cytokines released from the lymphocytes, macrophages, and polymorphonuclear leukocytes at the site of infection or in the blood stream. Often the host response is so intense that host tissues are destroyed, allowing remaining bacteria to proliferate.

c. Antigenic variation pathogenesis

- A successful pathogen must evade the host's immune system that recognizes bacterial surface antigens.
- One important evasive strategy for the pathogen is to change its surface antigens. This is accomplished by several mechanisms.
- One mechanism, called phase variation, which is the reversible ability of certain bacteria to turn off and turn on the expression of genes coding for surface antigens.
- A second mechanism, called antigenic variation, where the expressed surface antigen can assume many different antigenic structures.

Lec 2

Bacterial Genetics

- **Pharmacological Importance:** Bacterial genetic mechanisms explain how a single sensitive bacterium can acquire resistance genes and rapidly transform an entire infection into a multi-drug resistant (MDR) clinical challenge.

Structure of the Bacterial Genome

- Bacteria are prokaryotes, meaning their genetic material is not enclosed in a nuclear membrane. Their genome consists of two main components:

A. The Bacterial Chromosome: A single, circular, double-stranded DNA molecule containing all essential housekeeping genes. It is tightly packed in a region called the nucleoid via supercoiling (managed by enzymes like DNA gyrase—the primary target of fluoroquinolone antibiotics).

B. Plasmids: Small, circular, self-replicating, extrachromosomal DNA molecules.

- While not essential for survival under normal conditions, they carry specialized genes **R-Plasmids.**
- **Resistance Plasmids (R-Plasmids) :** Carry genes that encode resistance to antimicrobials (e.g., enzymes like beta-lactamases).
- **Virulence Plasmids:** Carry genes for toxins or colonization factors.

Horizontal Gene Transfer (HGT)

- Unlike eukaryotes, which inherit genes vertically (from parent to offspring), bacteria can share genes laterally with neighboring cells of the same or different species. This is the primary driver of rapid resistance spread.

Mechanism	Description / Requirement	Pharmacological & Clinical Significance
1. **Transformation**	Uptake of **naked DNA** fragments from the surrounding environment by a competent cell.	Dead, resistant bacteria in a biofilm can lyse and release their resistance genes, which are then absorbed by live bacteria.
2. Transduction	Transfer of bacterial DNA from one cell to another via a bacterial virus called a **bacteriophage**.	Phages can accidentally package virulence genes (e.g., Shiga toxin or Diphtheria toxin genes) and inject them into harmless strains.
3. Conjugation	One-way transfer of DNA (usually plasmids) through direct cell-to-cell contact via a **sex pilus**.	The most efficient method of spreading multi-drug resistance. A single donor can rapidly pass an R-plasmid across an entire bacterial population.

Mobile Genetic Elements ("Jumping Genes")

- Transposons: Small pieces of DNA that can move (transpose) from one location to another within a genome (e.g., from a chromosome to a plasmid, or vice versa).
- **Clinical Relevance:** They often carry antibiotic resistance genes. If a transposon jumps from a chromosome onto a conjugative plasmid, that resistance trait instantly becomes highly transmissible to other bacteria.

Genetic Mutations and Antibiotic Selection

- Mutations are permanent, inheritable changes in the DNA sequence.
- They can happen spontaneously during DNA replication errors or be induced by mutagens.
- This change can completely prevent an antibiotic from binding to its target site.

- **The Role of Antibiotics (Selection Pressure):**
- Antibiotics do not cause mutations. Instead, they act as a selective filter.
- When an antibiotic is administered, it kills off the susceptible population. The rare, mutated, resistant individual survives, multiplies, and becomes the dominant strain.

Bacterial Growth Physiology

- Bacterial growth refers to an increase in the number of cellular individuals (population growth) rather than the size of an individual cell.
- Bacteria reproduce primarily via binary fission, a process where a single cell duplicates its genetic material and divides into two identical daughter cells.
- **Generation Time (Doubling Time):** The time required for a bacterial population to double in number.
- This varies significantly by species and environmental conditions (e.g., ~20 minutes for *Escherichia coli* in rich media vs. ~24 hours for *Mycobacterium tuberculosis*).

The Bacterial Growth Curve (Batch Culture)

- When inoculated into a closed system with limited nutrients, bacteria follow a predictable four-phase growth pattern:

1- Lag Phase: Intense metabolic activity, enzyme synthesis, and adaptation to the new environment. No immediate increase in cell number.

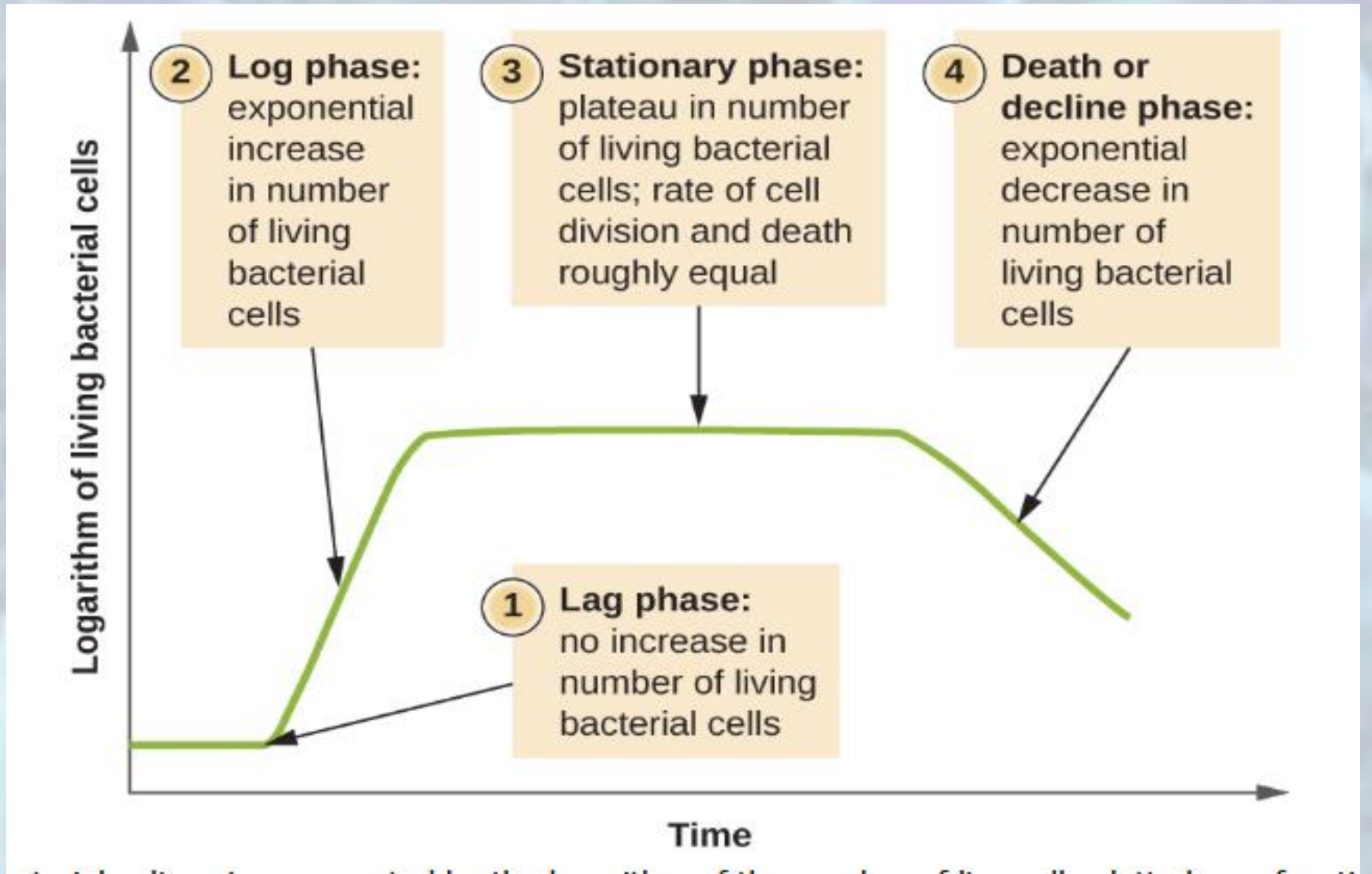
2- Exponential (Log) Phase: Cellular reproduction is most active. Generation time is constant.

- **Pharmacological note:** This is the phase where bacteria are most susceptible to beta-lactam antibiotics (e.g., penicillins) because cell wall synthesis is maximal.

3- Stationary Phase: Growth rate slows as nutrients deplete and toxic metabolic byproducts accumulate. Total number of viable cells remains constant (Rate of cell division = Rate of cell death).

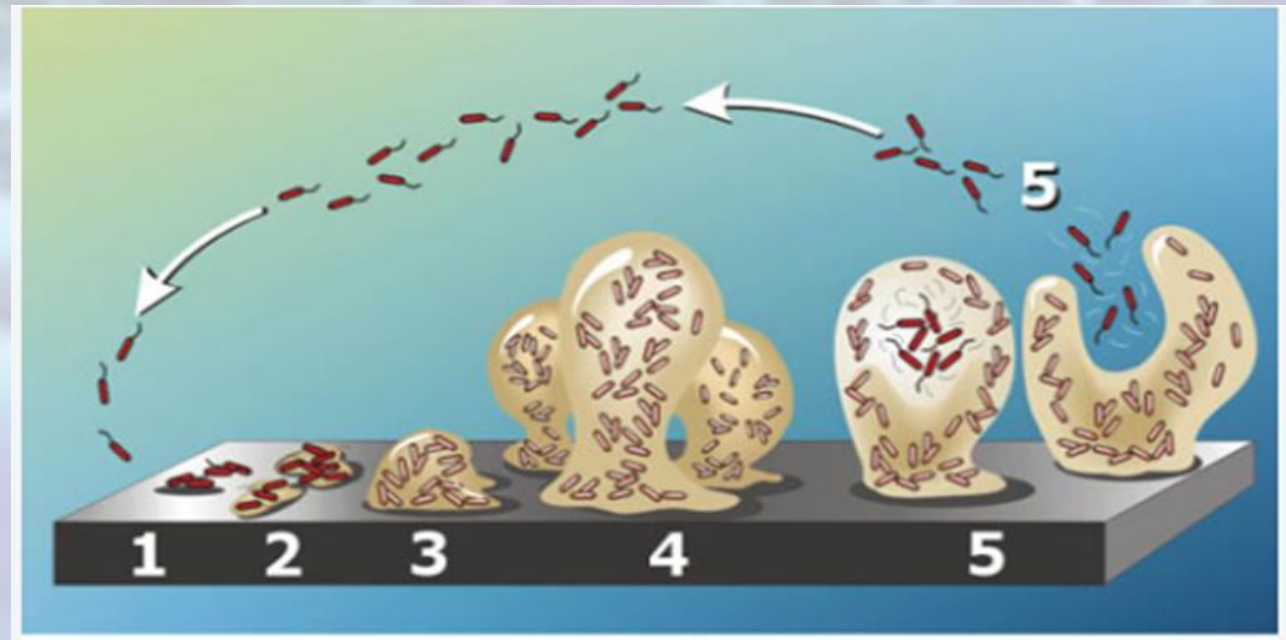
4- Death (Decline) Phase: Logarithmic decline in the number of viable cells due to severe nutrient deprivation and toxic buildup.

Bacterial growth curve



Biofilms

- A biofilm is a structured community of bacterial cells irreversibly attached to a surface and enclosed within a self-produced matrix of Extracellular Polymeric Substances (EPS).
- EPS is composed of polysaccharides, structural proteins, lipids, and extracellular DNA (eDNA).
- Biofilms represent a fundamental shift from free-floating (planktonic) life to a sedentary, protected lifestyle, altering gene expression up to 70%.



Clinical and Pharmaceutical Relevance:

- Biofilms present severe challenge in clinical pharmacy and healthcare setups due to tolerance and resistance mechanisms, which are:
- Diffusion Barriers: The dense EPS matrix slows down the penetration of positively charged antibiotics (e.g., aminoglycosides).
- Altered Microenvironment: Low oxygen and acidic pockets within the biofilm drive bacteria into a slow-growing or metabolic dormant state, neutralizing drugs that target active metabolism.
- Persister Cells: A subpopulation of phenotypic variants that tolerate lethal doses of antibiotics without carrying genetic resistance mutations. They regenerate the biofilm once treatment stops.
- Medical Devices: Biofilms routinely form on indwelling catheters, prosthetic joints, cardiac valves, and contact lenses, often necessitating surgical removal.

Normal Flora

- **Microbiota:** A diverse **population** of microorganisms (mostly bacteria and fungi) that permanently or temporarily colonize the skin and mucous membranes of healthy individuals without causing disease under normal conditions.
- **Microbiome:** The collective **genome** of all microorganisms residing in and on the human body.
- **Sterile Sites:** Internal organs and body fluids (e.g., blood, brain, cerebrospinal fluid [CSF], muscles, and the lower respiratory tract) must remain completely sterile in a healthy individual. The presence of any microbes in these areas is a direct indicator of infection.

Anatomical Distribution and Key Species

1- Skin: A dry, acidic, and saline environment. Dominated by desiccation-resistant bacteria such as *Staphylococcus epidermidis* and *Propionibacterium acnes* (associated with acne vulgaris).

2- Oral Cavity & Upper Respiratory Tract: A moist environment rich in nutrients. Notably colonized by *Streptococcus mutans*, which is responsible for dental caries via carbohydrate metabolism.

3- Colon (Gastrointestinal Tract): Houses the largest population and highest diversity of microbes in the body. Anaerobic bacteria make up 96% to 99% of the total biomass, outnumbering facultative anaerobes by 1000-fold. Key species include *Bacteroides fragilis* and *Escherichia coli*.

4- Vagina: Heavily dominated by Lactobacillus species. These play a critical pharmaceutical role by producing lactic acid to maintain a low, acidic pH, thereby preventing the overgrowth of vaginal pathogens like *Candida albicans.*

Physiological and Clinical Benefits of Normal Flora

- Bacterial Interference (Colonization Resistance): The normal flora prevents the colonization of pathogenic microbes by physically blocking cell receptors and severely competing for essential nutrients.
- Nutritional and Metabolic Support: Intestinal bacteria (such as E. coli) synthesize and secrete Vitamin K and B-complex vitamins, which are absorbed by the host.
- Immune System Maturation: The normal flora primes the immune system from birth, training immune cells to differentiate between symbiotic and pathogenic microbes, and stimulating the production of natural cross-reactive antibodies.

Pharmaceutical Insights and Clinical Alerts

A- Opportunistic Pathogens:

- Friendly flora can turn into life-threatening pathogens under two specific scenarios:

1. Tissue Displacement:

- If Viridans Streptococci are displaced from the oral cavity into the bloodstream during dental surgery, they can colonize damaged heart valves, causing subacute bacterial endocarditis.
- Similarly, if E. coli is displaced from the colon to the urethra, it causes urinary tract infections (UTIs).

2. Host Immunosuppression: In patients undergoing chemotherapy, burn victims, or those with advanced HIV, their own normal flora can break through mucosal barriers, causing severe bacteremia and sepsis.

B- Antibiotic-Associated Dysbiosis

- When **broad-spectrum antibiotics** are overprescribed, they eradicate protective resident gut flora along with the target pathogen. This microbial clearance leaves an empty place, allowing the opportunistic and highly resistant bacterium ***Clostridioides difficile*** to proliferate. This overgrowth leads to pseudomembranous colitis, characterized by severe, watery diarrhea.
- **The Pharmacist's Role:** Clinically evaluate antibiotic prescriptions to prevent unnecessary broad-spectrum usage. Counsel patients on the rationale behind **probiotic supplementation** to help restore microbial homeostasis during or after completing an antimicrobial course.

Antimicrobial Resistance (AMR)

- **Antimicrobial Resistance (AMR):** Occurs when microorganisms (bacteria, viruses, fungi, and parasites) evolve mechanisms to resist the drugs designed to kill them.
- **Antibiotic Resistance vs. AMR:** Antibiotic resistance applies specifically to bacteria targeting antibacterial drugs.

Molecular Mechanisms of Bacterial Resistance

1- Enzymatic Inactivation: Production of bacterial enzymes that neutralize drugs. *Example:* **Beta-lactamases** hydrolyze the beta-lactam ring of penicillins.

2- Efflux Pumps: Active transport proteins that vacuum antibiotics straight out of the bacterial cell before they reach their intracellular targets.

3- Target Site Modification: Structural mutations in bacterial components (like ribosomes or penicillin-binding proteins) that prevent the drug from binding.

4- Decreased Permeability: Downregulation or structural modification of outer membrane channels (**porins**), blocking drug entry entirely.

Genetics of Resistance

- **Intrinsic Resistance:** Natural, inherent properties of a bacterial species (e.g., Gram-negative bacteria are naturally resistant to Vancomycin due to their outer membrane barrier).
- **Acquired Resistance:** Gained via random chromosome mutations or acquired from another organism via **Horizontal Gene Transfer (HGT)**.

Thank You