مقدم من فريق أفق الطبي

General Microbiology

Structure of Bacterial Cell

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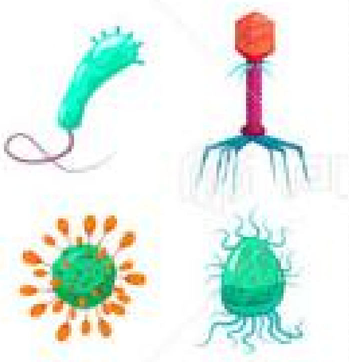


Shape & size of bacteria

- Bacteria are classified by shape into three basic groups:
- cocci,
- certain cocci occur in pairs (diplococci), some in chains (streptococci), and others in grapelike clusters (staphylococci).
- bacilli,
- حلزوني Spirochetes
- The arrangement of rods and spirochetes is medically less
- important

Shape & size of bacteria

- Bacteria not only vary in size but also in shape, the more typical forms are:
- Cocci (spherical shape):
- a. Diplococci b. Streptococci C. Staphylococci
- Bacilli (rod shaped):

  a- Gram negative bacilli (e.g. *Salmonella* and *Shigella*)

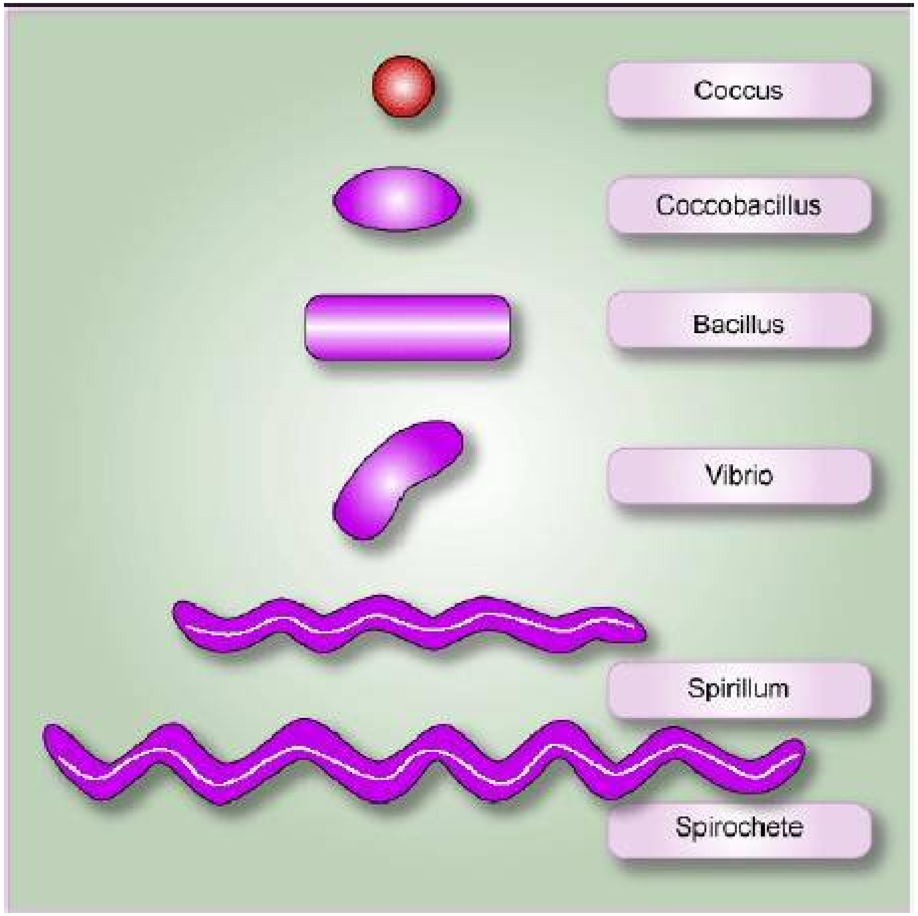
  b- Gram positive bacilli

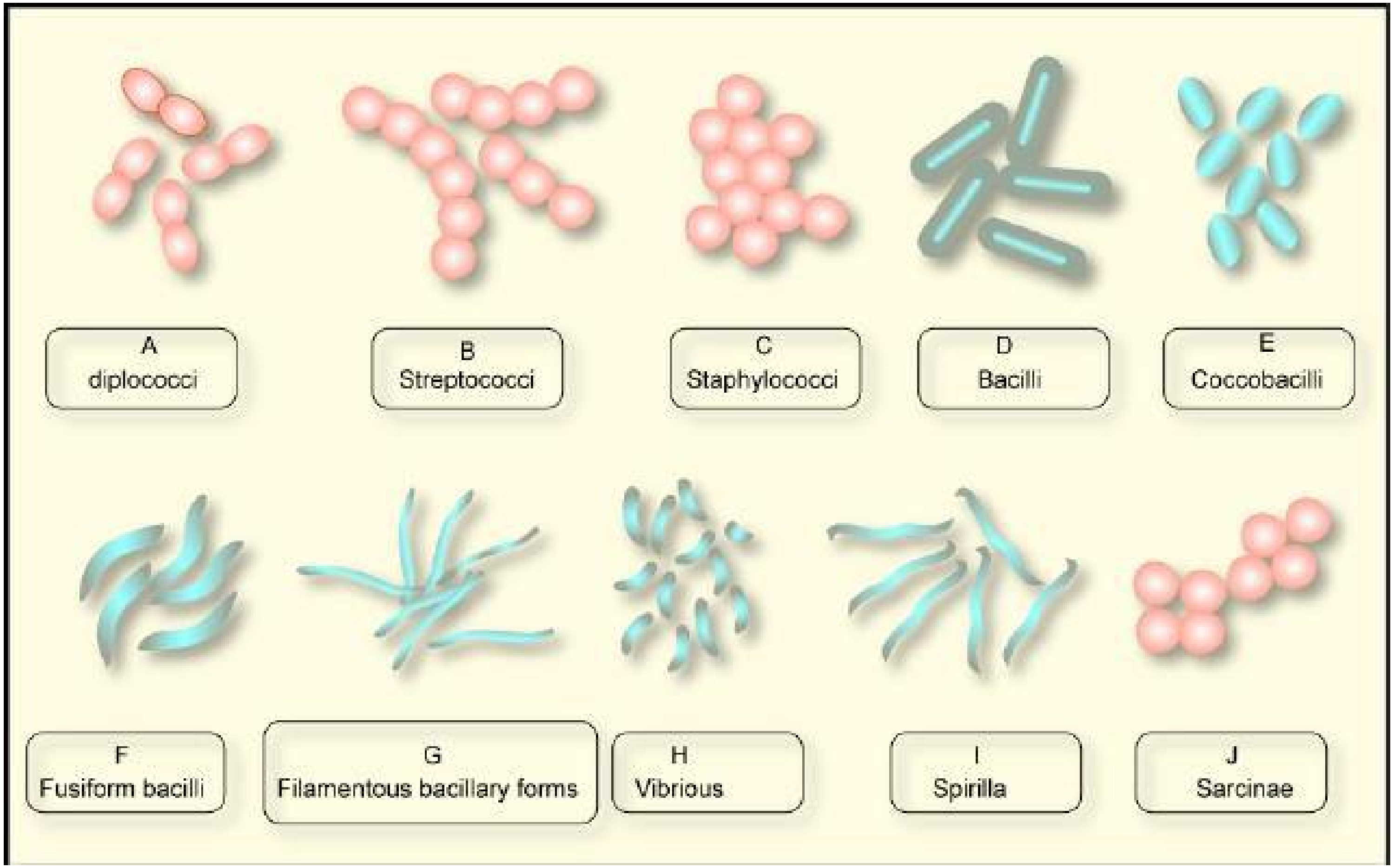
  - Sporing (aerobic and anaerobic)
  - Non sporing (e.g. *Corynebacterium)*

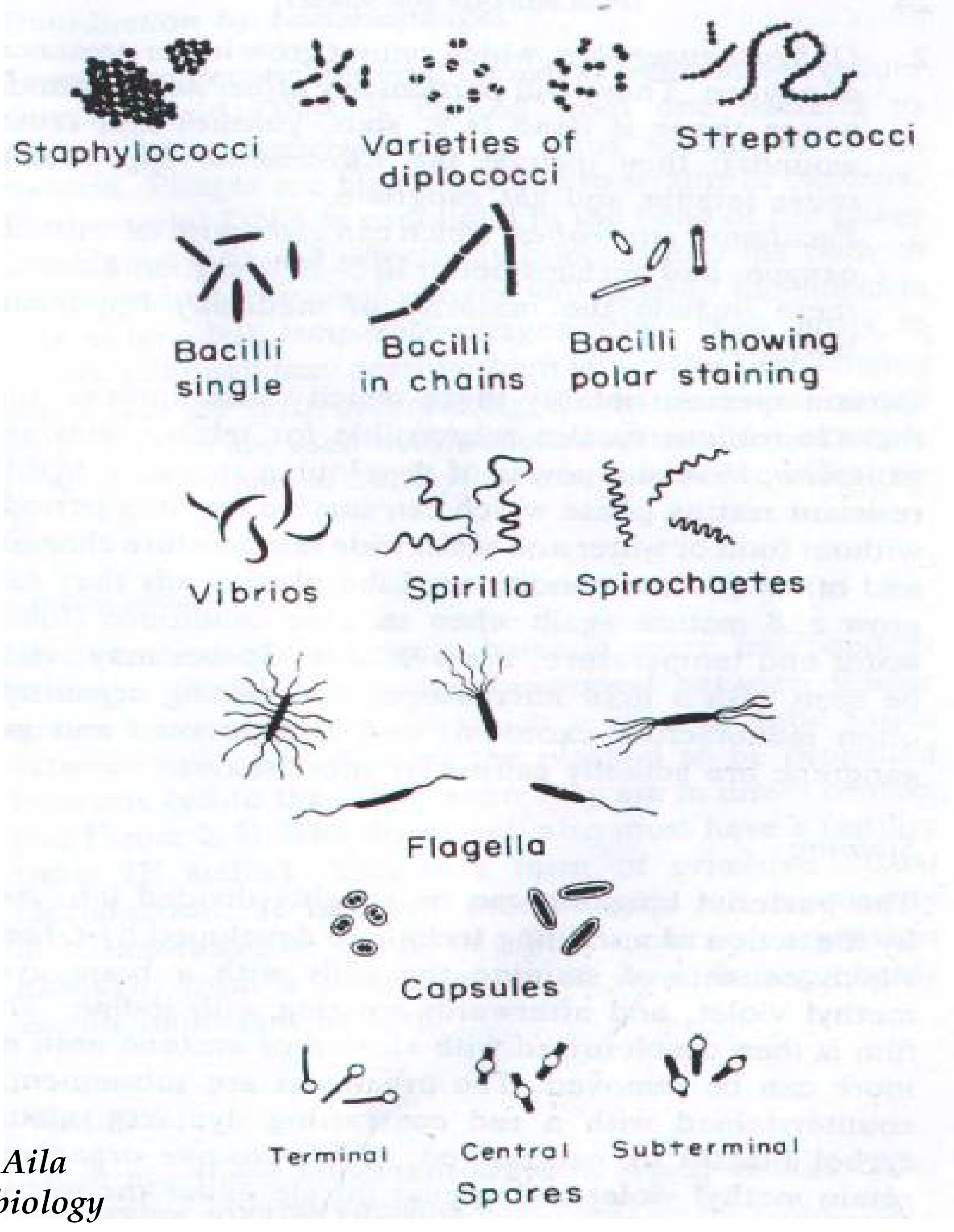
  c- Acid fast bacilli (e.g. *Mycobacterium*) → تسبب السل الرئوي.

Gross Morphology of Bacteria

- Vibrios (comma shaped) e.g. *Vibrio cholera*
- Spiral e.g. Spirochetes. حلزوني
- خيطي Filamentous e.g. Actinomyces.
- Fusiforms (spindle shaped).


Coccus
Coccobacillus
Bacillus
Vibrio
Spirillum
Spirochete


A
diplococci
B
Streptococci
C
Staphylococci
D
Bacilli
E
Coccobacilli
F
Fusiform bacilli
G
Filamentous bacillary forms
H
Vibrious
I
Spirilla
J
Sarcinae


Staphylococci
Varieties of diplococci
Streptococci
Bacilli single
Bacilli in chains
Bacilli showing polar staining
Vibrios
Spirilla
Spirochaetes
Flagella
Capsules
Terminal
Central
Subterminal
Spores

Shape & size of bacteria

- Bacteria range in size from about 0.2 to 5 µm
- The smallest bacteria (Mycoplasma) are about the same size as the largest viruses (poxviruses)
- The longest bacteria rods are the size of some yeasts and human red blood cells (7 µm).

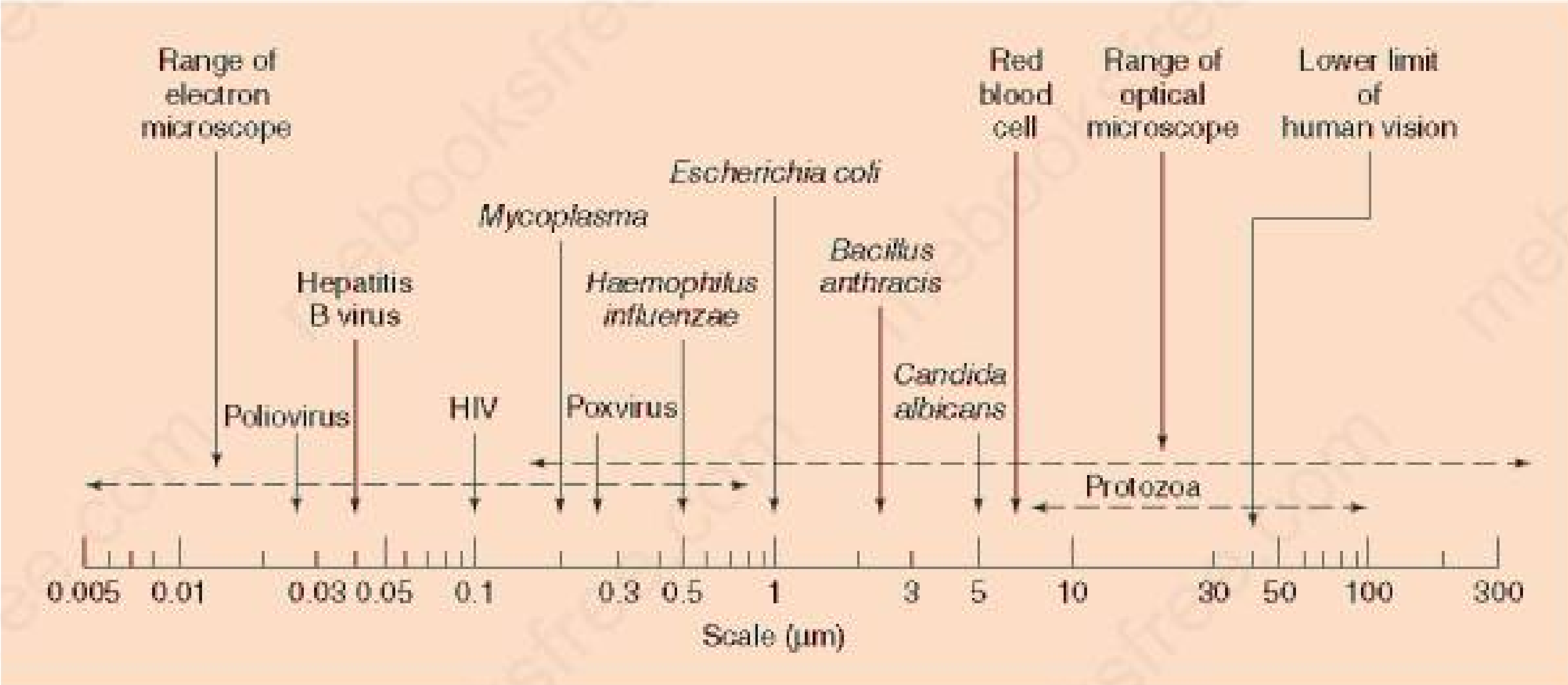


FIGURE 2–2 Sizes of representative bacteria, viruses, yeasts, protozoa, and human red cells. The bacteria range in size from *Mycoplasma*,

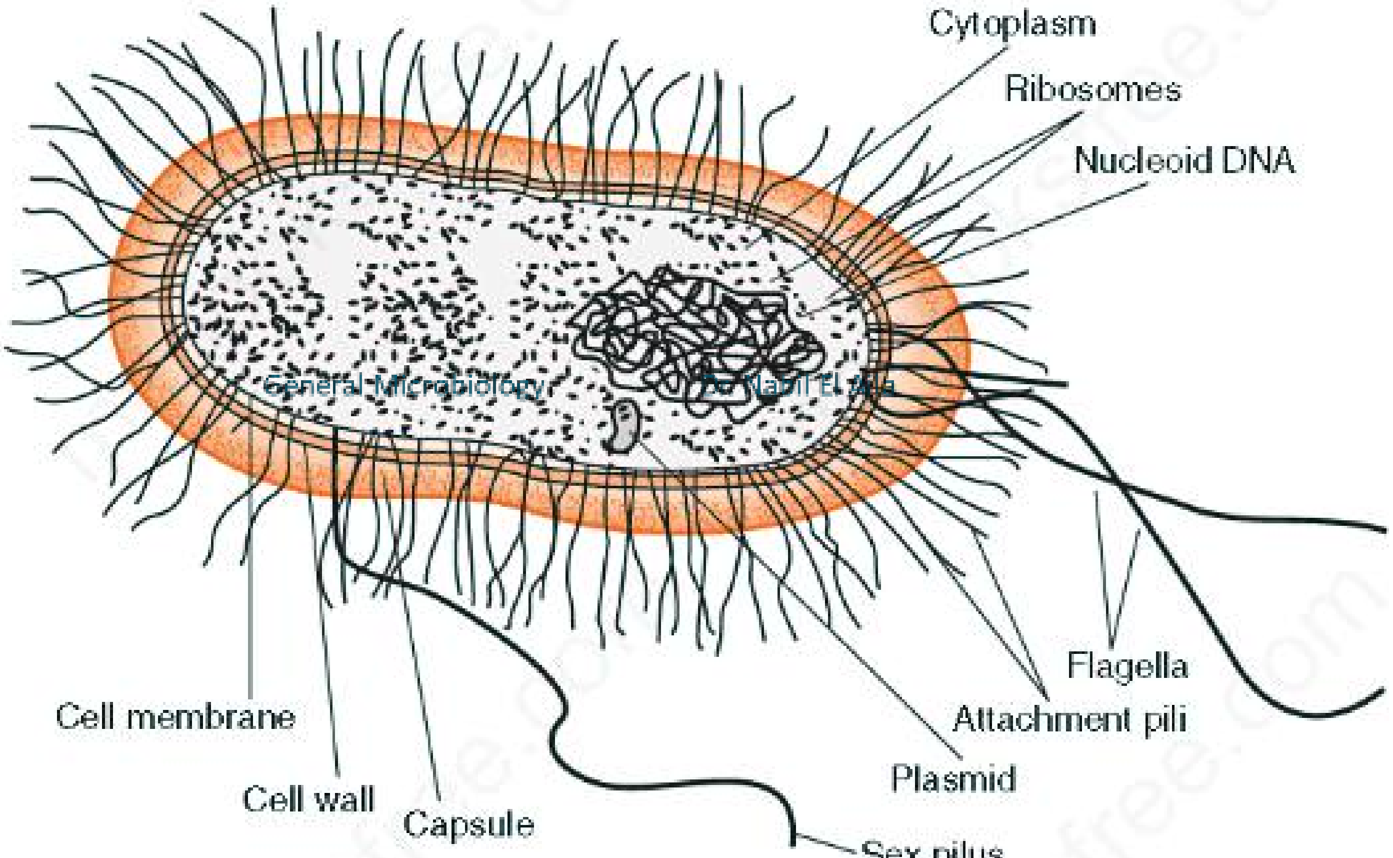


FIGURE 2–3 Bacterial structure. (Reproduced with permission from Ryan K et al. *Sherris Medical Microbiology*. 4th ed. Copyright 2004, McGraw-Hill.)

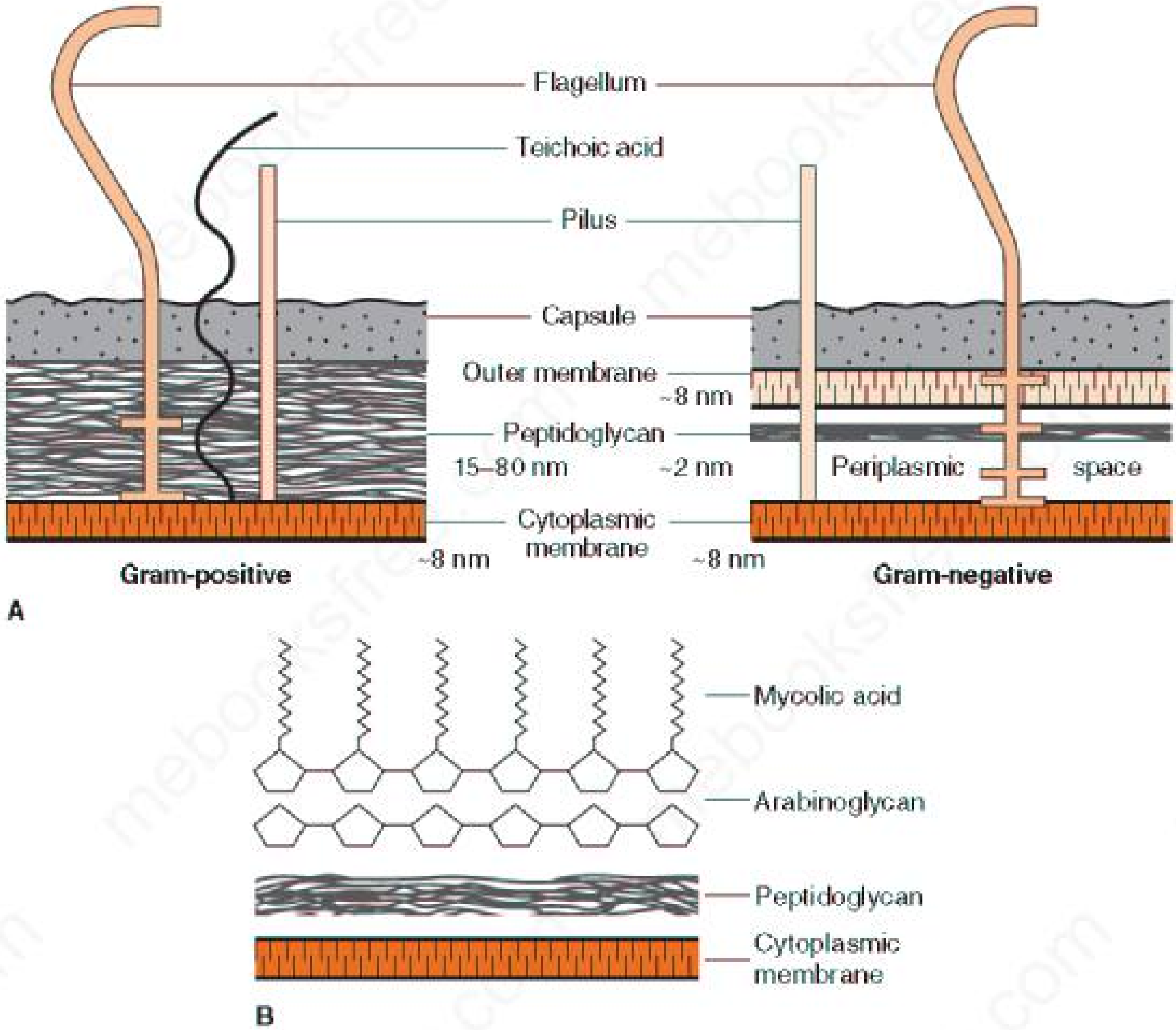
STRUCTURE OF BACTERIA

Cell Wall

- The cell wall is the outermost component common to all bacteria (except Mycoplasma species, which are bounded by a cell membrane, not a cell wall).
- Some bacteria have surface features external to the cell wall, such as a capsule, flagella, and pili

Cell Walls of Gram-Positive and Gram-Negative Bacteria

- The peptidoglycan layer is much thicker in gram positive than in gram-negative bacteria.
- Many gram-positive bacteria also have fibers of teichoic acid that protrude outside the peptidoglycan, whereas <u>gram-negative bacteria do not have teichoic acids.</u>
- Gram-negative bacteria have a complex outer layer consisting of lipopolysaccharide, lipoprotein, and phospholipid.
- Lying between the outer-membrane layer and the cytoplasmic membrane in gram-negative bacteria is the periplasmic space, which is the site, in some species, of enzymes <u>called β-lactamases that degrade penicillins and other β-lactam drugs</u>


Flagellum
Teichoic acid
Pilus
Capsule
Outer membrane
~8 nm
Peptidoglycan
15–80 nm
~2 nm
Periplasmic
space
Cytoplasmic
membrane
~8 nm
~8 nm
Gram-positive
Gram-negative
A
Mycolic acid
Arabinoglycan
Peptidoglycan
Cytoplasmic
membrane
B

Cell walls of bacteria

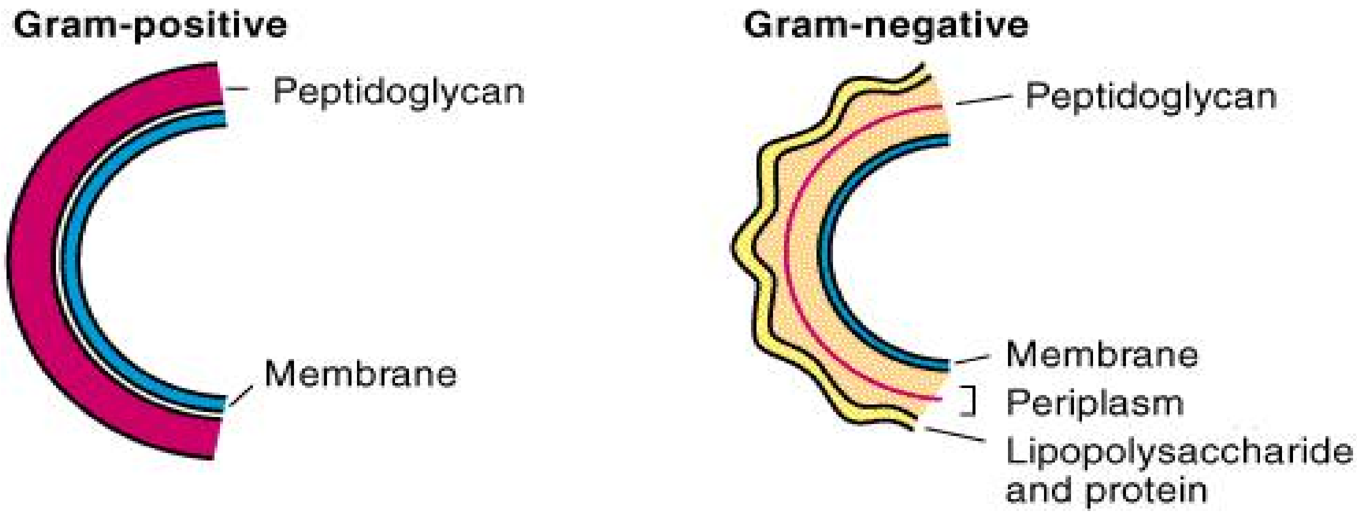


TABLE 2–2 Comparison of Cell Walls of Gram-Positive and Gram-Negative Bacteria

Component	Gram-Positive Cells	Gram-Negative Cells
Peptidoglycan	Thicker; multilayer	Thinner; single layer
Teichoic acids	Yes	No
Lipopolysaccharide (endotoxin)	No	Yes

⇐ البكتيريا التي تسبب التهابات في جسم الإنسان ، مش كلها بنقدر نزرعها في الوسط البيئي في المختبر

مهمة جداً في الطب ، وذلك لمعرفة نوع البكتيريا ، وبالتالي إعطاء التشخيص والعلاج المناسب .

TABLE 2–3 Medically Important Bacteria That Cannot Be Seen in the Gram Stain

Name	Reason	Alternative Microscopic Approach
Mycobacteria, including *M. tuberculosis* * slow growing Bacteria → تنمو في مختبرات خاصة .	Too much lipid in cell wall so dye cannot penetrate	Acid-fast stain → بقدرش أستخدملها gram stain
Treponema pallidum	Too thin to see	Dark-field microscopy or fluorescent antibody
Mycoplasma pneumoniae	No cell wall; very small	None
Legionella pneumophila	Poor uptake of red counterstain	Prolong time of counterstain
Chlamydiae, including *C. trachomatis*	Intracellular; very small	Inclusion bodies in cytoplasm
Rickettsiae	Intracellular; very small	Giemsa or other tissue stains

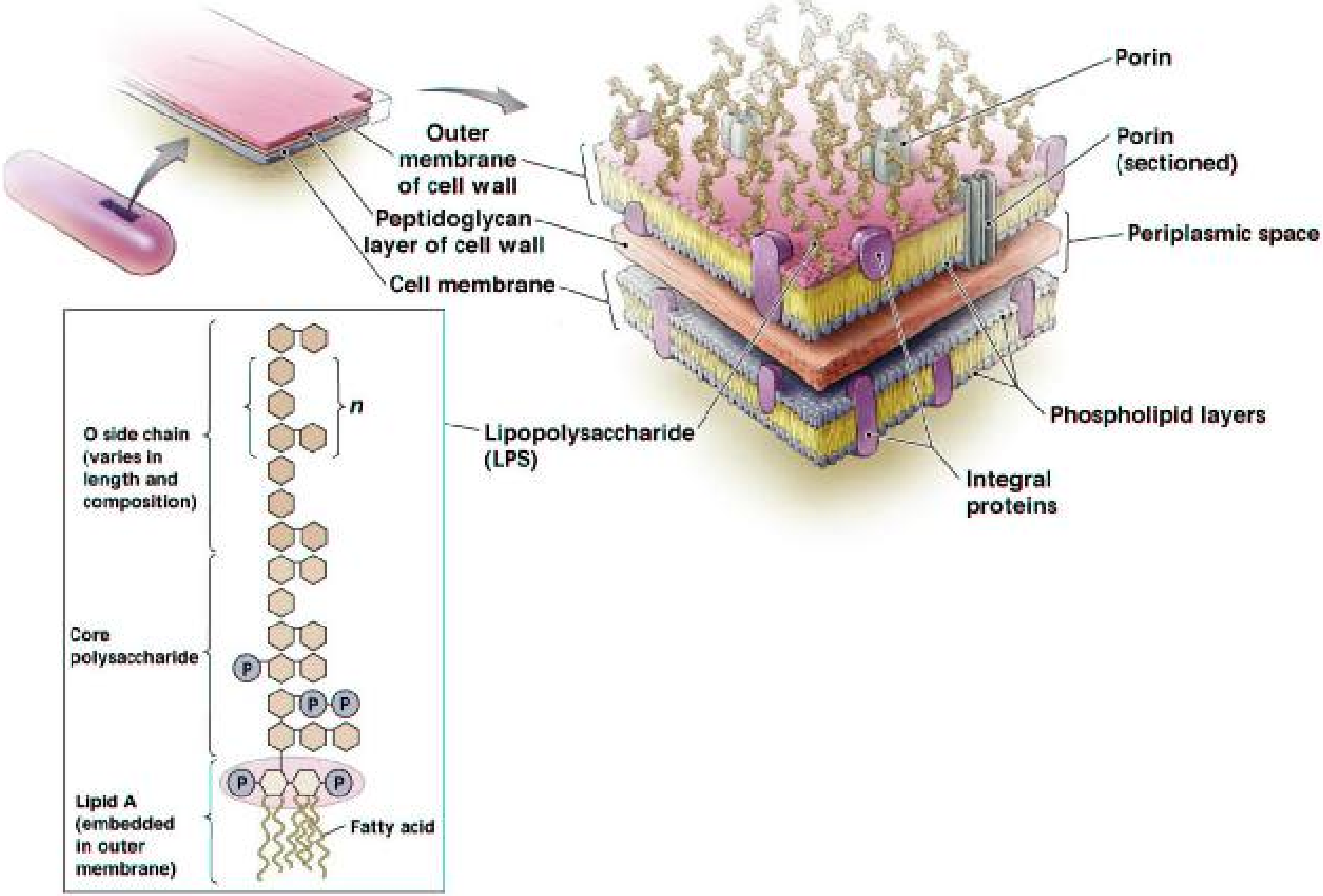
Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Glycan (sugar) backbone with peptide side chains that are cross-linked	Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme
Outer membrane of gram-negative bacteria	Lipid A	Toxic component of endotoxin
	Polysaccharide	Major surface antigen used frequently in laboratory diagnosis
Surface fibers of gram-positive bacteria	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis
Plasma membrane	Lipoprotein bilayer without sterols	Site of oxidative and transport enzymes
Ribosome	RNA and protein in 50S and 30S subunits	Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol
Nucleoid	DNA	Genetic material
Mesosome	Invagination of plasma membrane	Participates in cell division and secretion
Periplasm	Space between plasma membrane and outer membrane	Contains many hydrolytic enzymes, including β-lactamases
Nonessential components		
Capsule	Polysaccharide[1]	Protects against phagocytosis
Pilus or fimbria	Glycoprotein	Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation
Flagellum	Protein	Motility
Spore	Keratinlike coat, dipicolinic acid	Provides resistance to dehydration, heat, and chemicals
Plasmid	DNA	Contains a variety of genes for antibiotic resistance and toxins
Granule	Glycogen, lipids, polyphosphates	Site of nutrients in cytoplasm
Glycocalyx	Polysaccharide	Mediates adherence to surfaces

Cell Wall

- The cell wall has several other important properties:
- (1) In Gram-negative bacteria, it contains endotoxin, a lipopolysaccharide → variolance factor تُعتبر → تستغله البكتيريا لما تهاجم جسم الإنسان.
- (2) Its polysaccharides and proteins are antigens that are useful in laboratory identification.
- (3) Its porin proteins play a role in facilitating the passage of small, hydrophilic molecules into the cell.

Porin proteins in the outer membrane of gram-negative bacteria act as a channel to allow the entry of essential substances

Bacterial Cell Wall



(b) Gram-negative cell wall

Cell Walls of Acid-Fast Bacteria

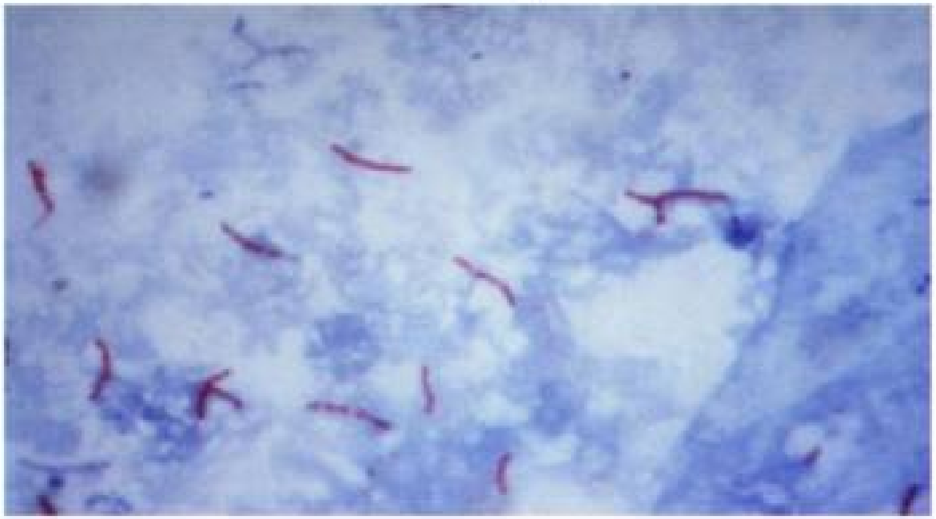
- Mycobacteria (e.g., Mycobacterium tuberculosis) have an unusual cell wall, resulting in their inability to be Gram-stained
- These bacteria are said to be acid-fast (stain) because they resist decolorization with acid–alcohol after being stained with carbolfuchsin.

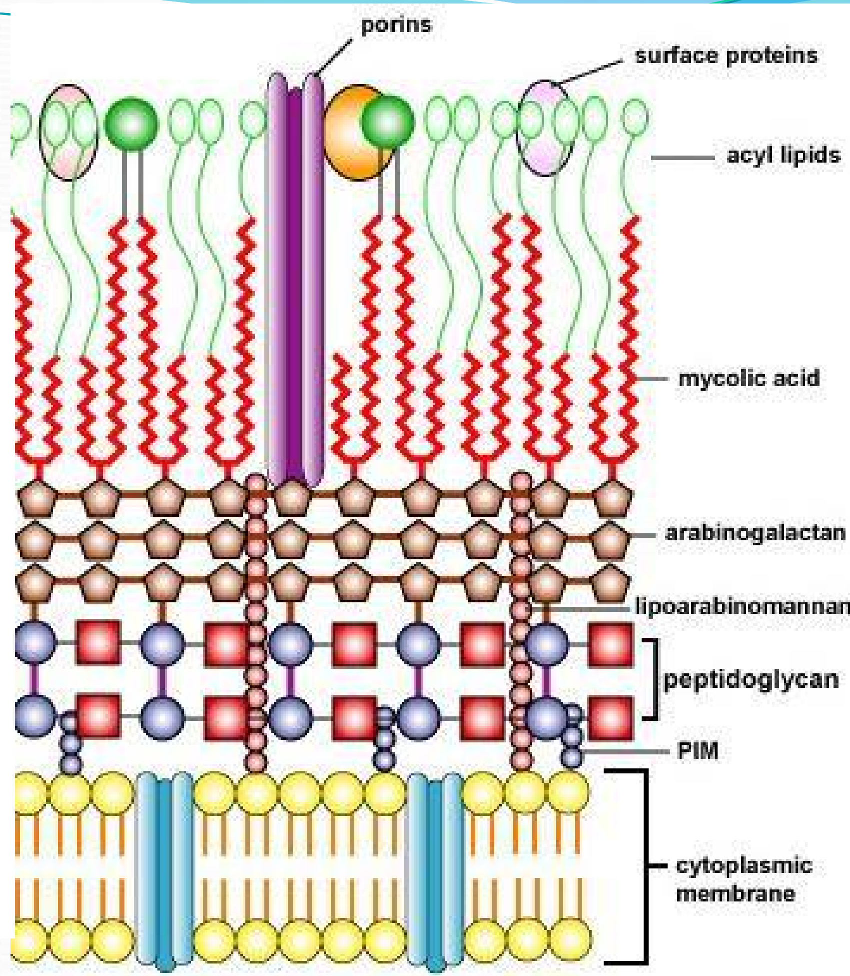
يصبح ال gram possitive ← colorless

ولكنه ال acid fast هو الذي يقاوم acid alcohol ← منبقى لونها أحمر وهو لونه ال

- This property is related to the high concentration of lipids, called mycolic acids, in the cell wall of mycobacteria.
- Nocardia asteroides is weakly acid-fast.

Mycobacterium tuberculosis - Ziehl-Neelsen Staining





Structure of an Acid-Fast Cell Wall.

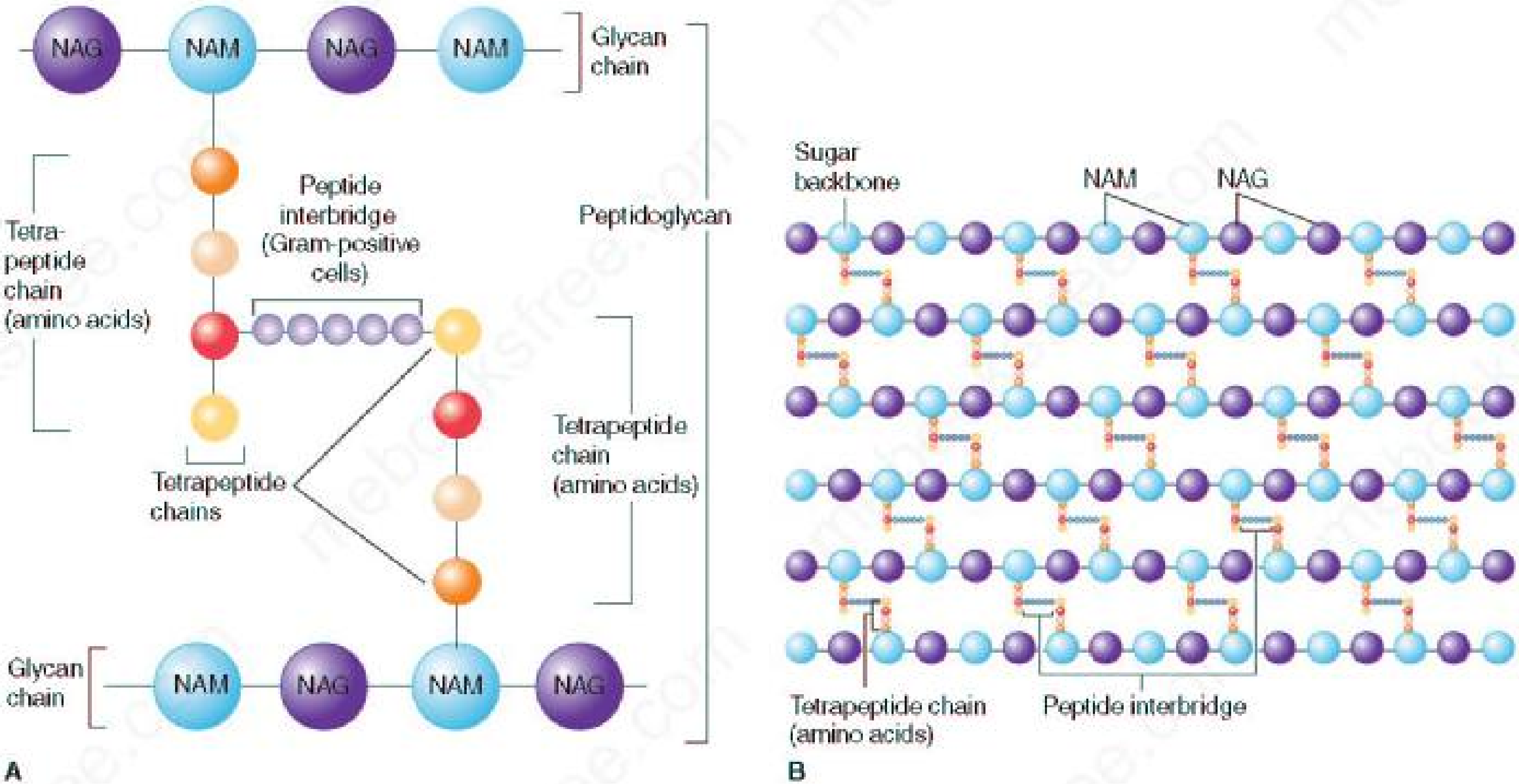
Thick layer of peptidoglycan ← gram possitive في ال

Thin layer of peptido... ← gram negative في ال

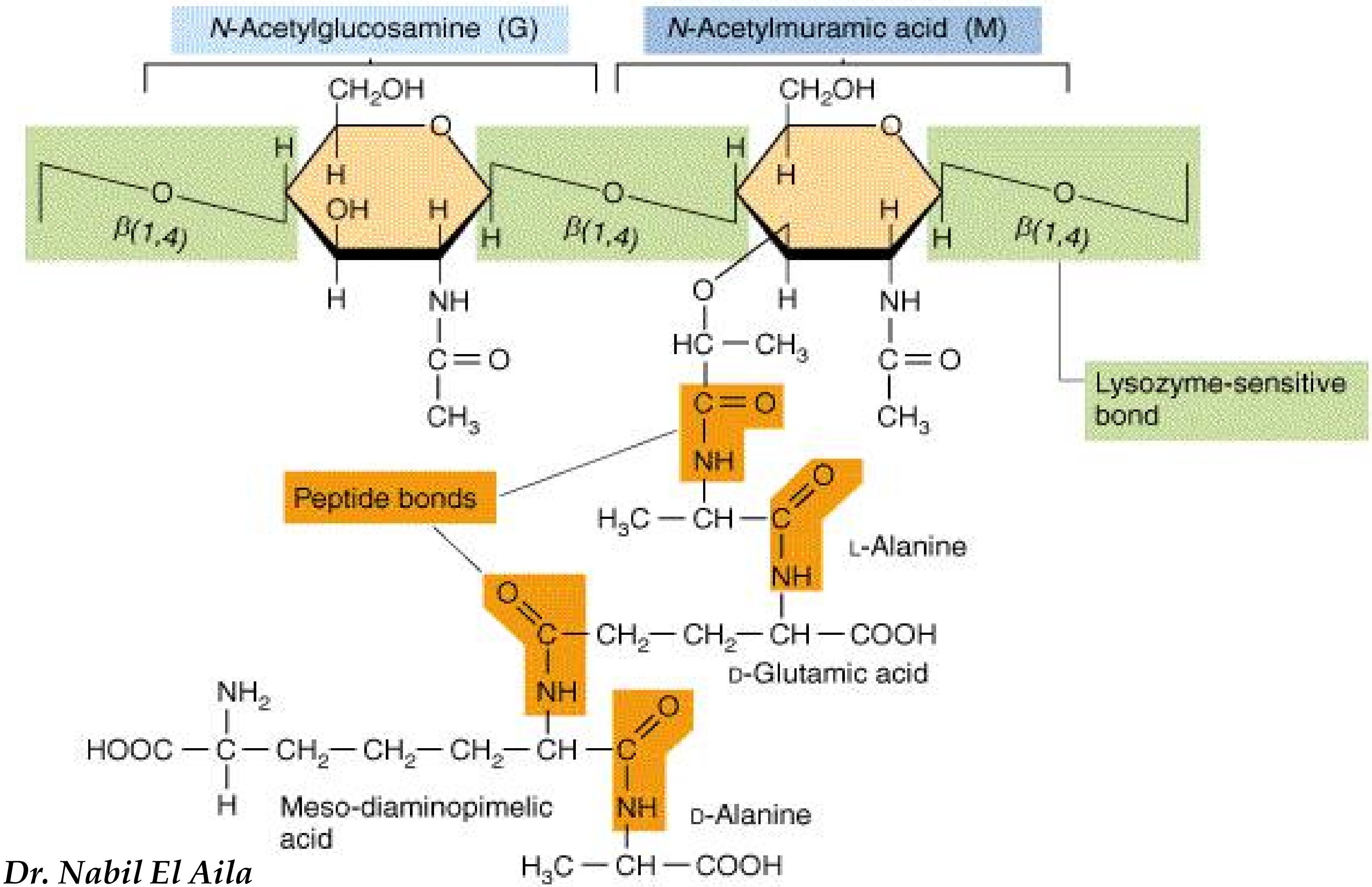
Peptidoglycan

- Peptidoglycan is a complex, interwoven network that surrounds the entire cell and is composed of a single covalently linked macromolecule.
- It is found only in bacterial cell walls. It provides rigid support for the cell, is important in maintaining the characteristic shape of the cell, and allows the cell to withstand media of low osmotic pressure, such as water.
- The term peptidoglycan is derived from the peptides and the sugars (glycan) that make up the molecule. Synonyms for peptidoglycan are murein and mucopeptide.

- The carbohydrate backbone, which is composed of alternating N-acetylmuramic acid and N-acetylglucosamine molecules.
- Attached to each of the muramic acid molecules is a tetrapeptide consisting of both D- and L-amino acids, the precise composition of which differs from one bacterium to another.
- Two of these amino acids are : diaminopimelic acid, which is unique to bacterial cell walls, and d-alanine, which is involved in the cross-links between the tetrapeptides and in the action of penicillin.
- This tetrapeptide contains the rare d-isomers of amino acids, most proteins contain the l-isomer
- The other important component in this network is the peptide cross-link between the two tetrapeptides.
- Peptidoglycan is a good target for antibacterial drugs inhibit the synthesis of peptidoglycan <u>by inhibiting the transpeptidase that makes the cross-links between the two adjacent tetrapeptides</u>


NAG
NAM
NAG
NAM
Glycan chain
Peptidoglycan
Tetra-peptide chain (amino acids)
Peptide interbridge (Gram-positive cells)
Tetrapeptide chains
Tetrapeptide chain (amino acids)
Glycan chain
NAM
NAG
NAM
NAG
A
Sugar backbone
NAM
NAG
Tetrapeptide chain (amino acids)
Peptide interbridge
B

Structure of peptidoglycan



Lysozyme

- An enzyme present in human tears, mucus, and saliva, can cleave the peptidoglycan backbone by breaking its glycosyl bonds
- Lysozyme contributing to the natural resistance of the host to microbial infection.
- Lysozyme- treated bacteria may swell and rupture as a result of the entry of water into the cells, which have a high internal osmotic pressure.
- If the lysozyme-treated cells are in a solution with the same osmotic pressure as that of the bacterial interior, they will survive as spherical forms, called protoplasts, surrounded only by a cytoplasmic membrane.

Lipopolysaccharide

- The lipopolysaccharide (LPS) of the outer membrane of the cell wall of gram-negative bacteria is endotoxin
- It is responsible for many of the features of disease, such as fever and shock (especially hypotension), caused by these organisms
- It is called endotoxin because it is an integral part of the cell wall, in contrast to exotoxins, which are actively secreted from the bacteria

The LPS is composed of three distinct units

- (1) A phospholipid called lipid A, which is responsible for the toxic effects.
- (2) A core polysaccharide of five sugars linked through ketodeoxyoctulonate (KDO) to lipid A.
- (3) An outer polysaccharide consisting of up to 25 repeating units of three to five sugars.

ملاحظة ا في ال cell wall يوجد 3 antigens : somatic, flagella, capsular

- This outer polymer is the important somatic, or O, antigen of several gram negative bacteria that is used to identify certain organisms in the clinical laboratory.

مهم في تعريف البكتيريا

- Some bacteria, notably members of the genus Neisseria, have an outer lipooligosaccharide (LOS) containing very few repeating units of sugars.

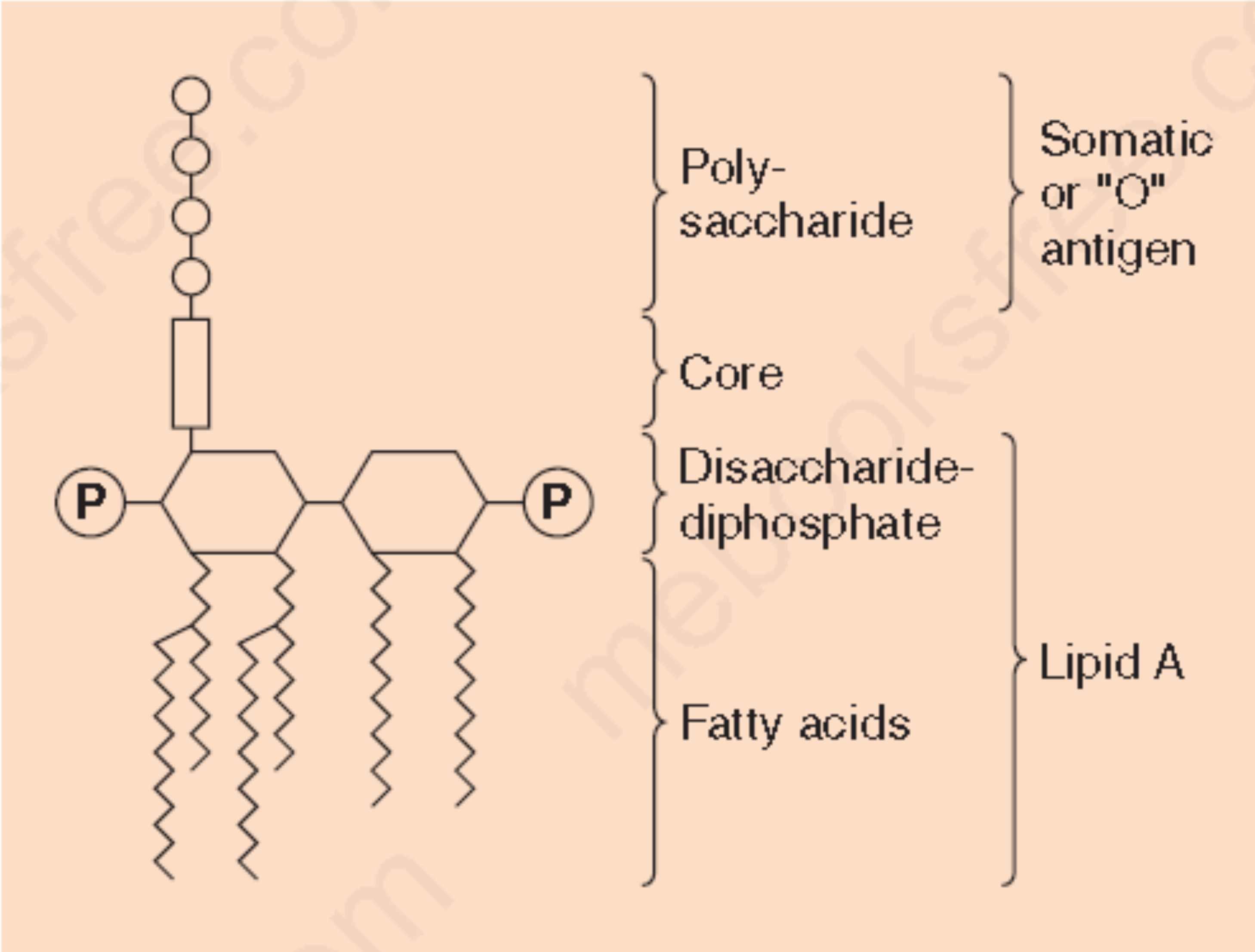


FIGURE 2–6 Endotoxin (lipopolysaccharide [LPS]) structure. Th
O-antigen polysaccharide is exposed on the exterior of the cell,

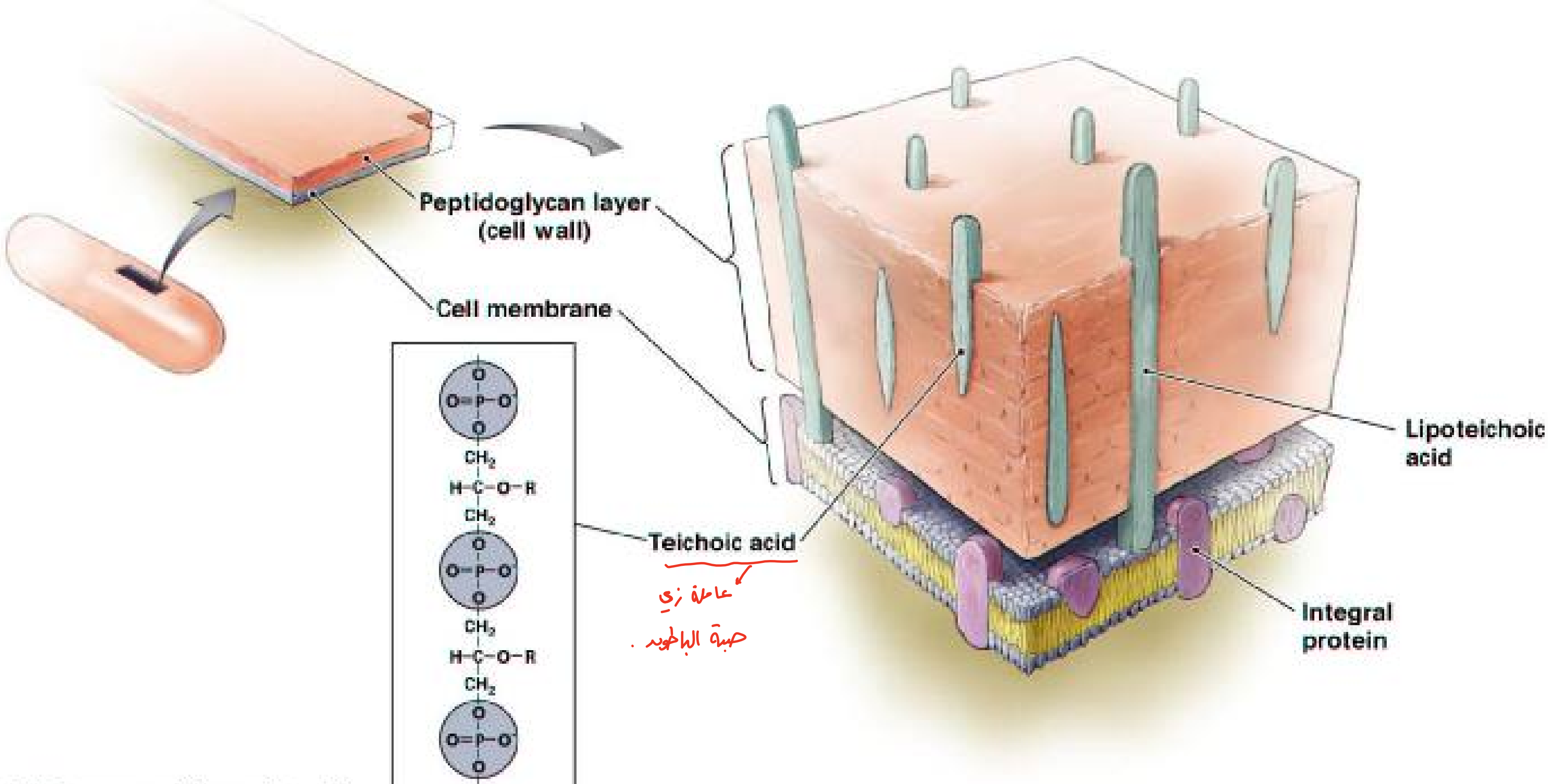
موجود في gram (+)

ومش موجود في gram (-)

Teichoic Acid

- Teichoic acids are fibers located in the outer layer of the gram-positive cell wall and extend from it.
- They are composed of polymers of either glycerol phosphate or ribitol phosphate.
- Some polymers of glycerol teichoic acid penetrate the peptidoglycan layer and are covalently linked to the lipid in the cytoplasmic membrane, in which case the are called lipoteichoic acid
- The medical importance of teichoic acids lies in their ability to induce inflammation and septic shock when caused by certain gram-positive bacteria
- Teichoic acids also mediate the attachment of staphylococci to mucosal cells

Bacterial Cell Walls



(a) Gram-positive cell wall

Gram stain

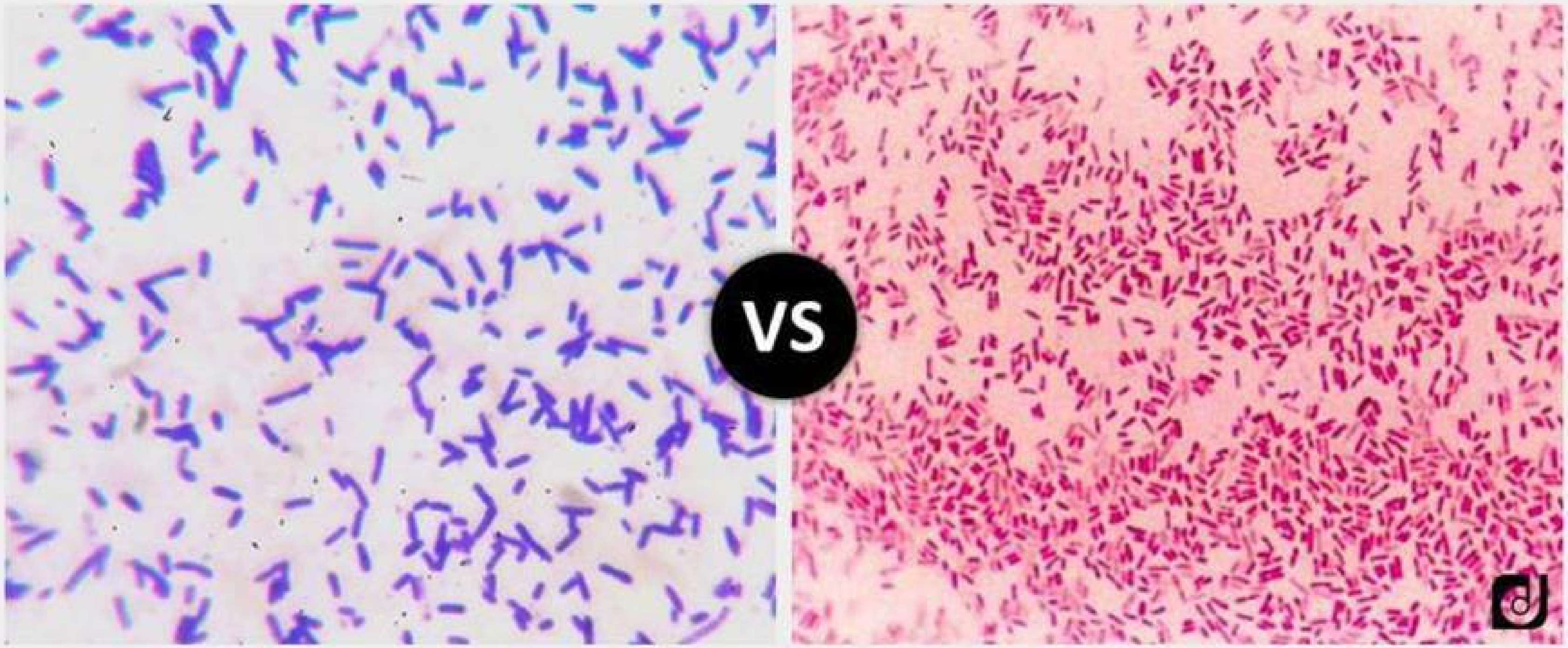
- It separates most bacteria into two groups:
- the gram-positive bacteria, which stain blue, and the gram negative bacteria, which stain red.

The Gram stain involves the following four-step procedure:

- (1) The crystal violet dye stains all cells blue/purple.
- (2) The iodine solution (a mordant) is added to form a crystal violet–iodine complex; all cells continue to appear blue.
- (3) The organic solvent, such as acetone or ethanol, extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled gram-positive bacteria.

Lipoprotien, phospholipid, Lipopolysacchrid : تحتوي على

- The gram-negative organisms appear colorless; the gram-positive bacteria remain blue.
- (4) The red dye safranin stains the decolorized gram negative cells red/pink; the gram-positive bacteria remain blue.



Gram Positive Bacteria vs. Gram Negative Bacteria

Cytoplasmic Membrane

- It is composed of a phospholipid bilayer
- Eukaryotic membranes contain sterols, whereas prokaryotes generally do not. The only prokaryotes that have sterols in their membranes are members of the genus Mycoplasma

The membrane has four important functions:

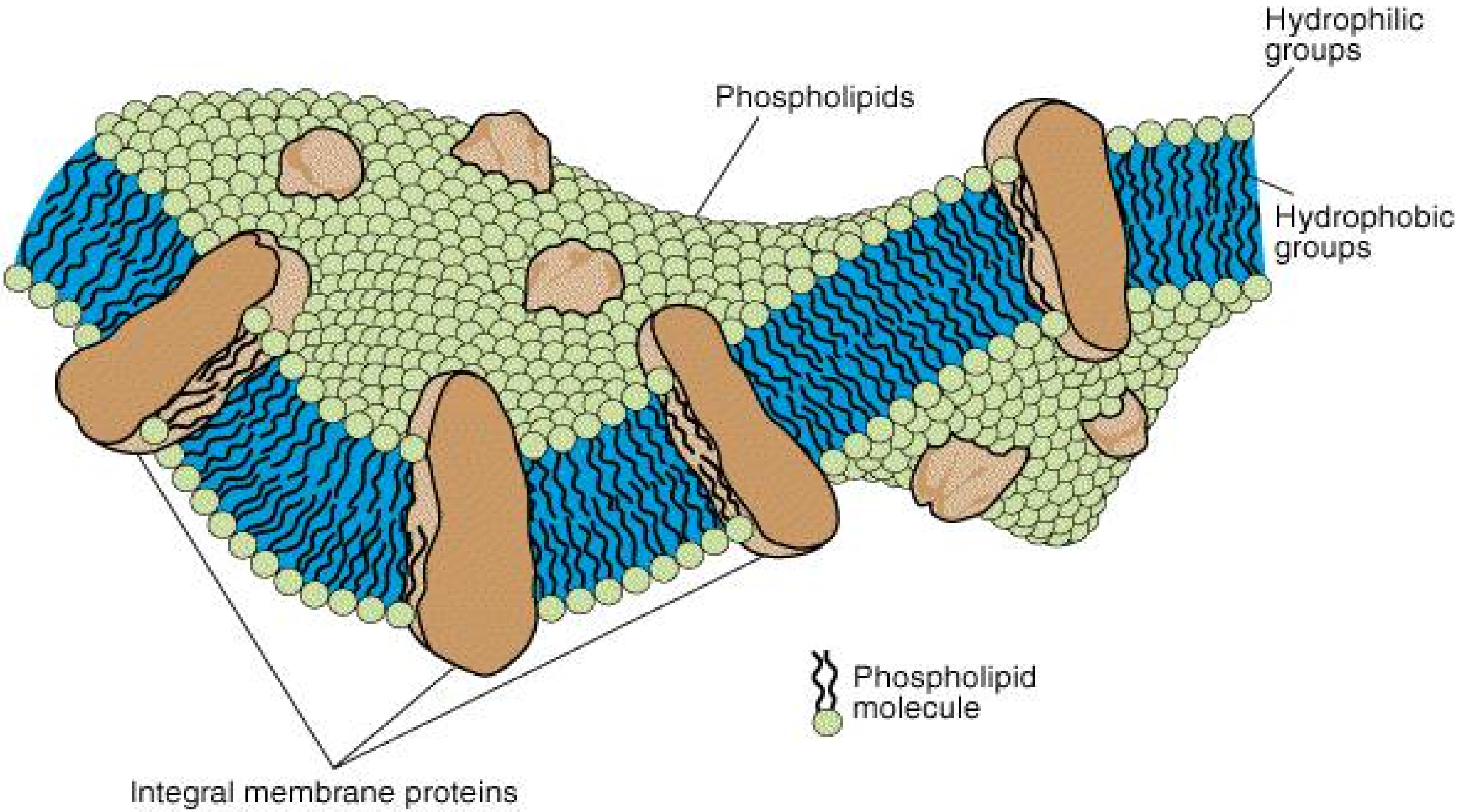
(1) active transport of molecules into the cell,

(2) energy generation by oxidative phosphorylation,

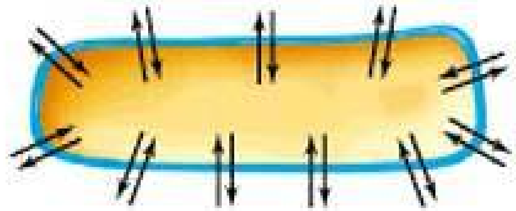
(3) synthesis of precursors of the cell wall,

(4) secretion of enzymes and toxins.

The cytoplasmic membrane

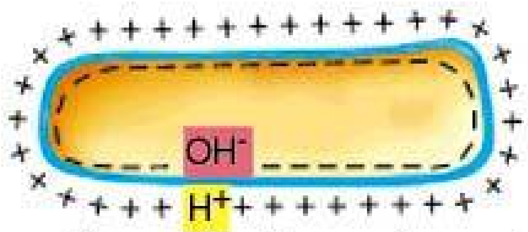


Functions of the cytoplasmic membrane(1)



Permeability Barrier — Prevents leakage and functions as a gateway for transport of nutrients into and out of the cell

Protein Anchor — Site of many proteins involved in transport, bioenergetics, and chemotaxis



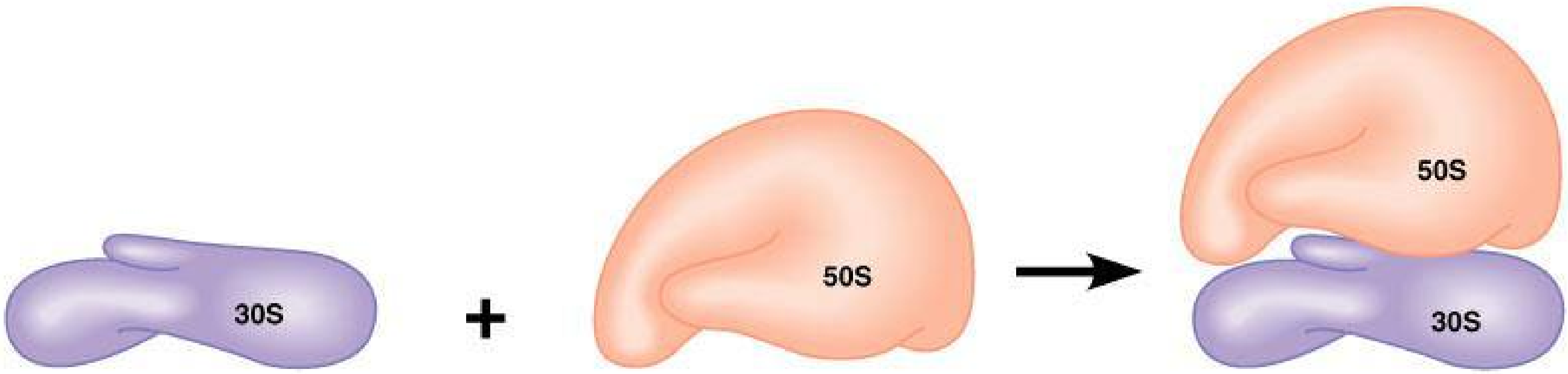
Energy Conservation — Site of generation and use of the proton motive force

Cytoplasm

- The cytoplasm has two distinct areas when seen in the electron microscope:
- (1) An amorphous matrix that contains <u>ribosomes, nutrient granules, metabolites, and plasmids.</u>
- (2) An inner, nucleoid region composed of DNA.

Ribosomes

- Bacterial ribosomes are the site of protein synthesis as in eukaryotic cells, but they differ from eukaryotic ribosomes in size and chemical composition.
- Bacterial ribosomes are 70S in size, with 50S and 30S subunits, whereas eukaryotic ribosomes are 80S in size, with 60S and 40S subunits.
- The differences in both the ribosomal RNAs and proteins constitute the basis of the selective action of several antibiotics that inhibit bacterial, but not human, protein synthesis



(a) Small subunit **(b)** Large subunit **(c)** Complete 70S ribosome

Granules

- The cytoplasm contains several different types of granules that serve as storage areas for nutrients and stain characteristically with certain dyes.
- For example, volutin is a reserve of high energy stored in the form of polymerized metaphosphate.
- It appears as a "metachromatic" granule since it stains red with methylene blue dye instead of blue as one would expect.
- Metachromatic granules are a characteristic feature of Corynebacterium diphtheriae, the cause of diphtheria.

Nucleoid

- **The nucleoid** is the area of the cytoplasm in which DNA is located.
- The DNA of prokaryotes is a single, circular molecule that has a molecular weight (MW) of approximately 2×10^9 and contains about 2000 genes.(By contrast, human DNA has approximately 100,000 genes.)
- The nucleoid contains no nuclear membrane, no nucleolus, no mitotic spindle, and no histones
- One major difference between bacterial DNA and eukaryotic DNA is that bacterial DNA has no introns, whereas eukaryotic DNA does.

Plasmids

- Plasmids are extrachromosomal, double-stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome.
- Plasmids occur in both gram-positive and gram-negative bacteria

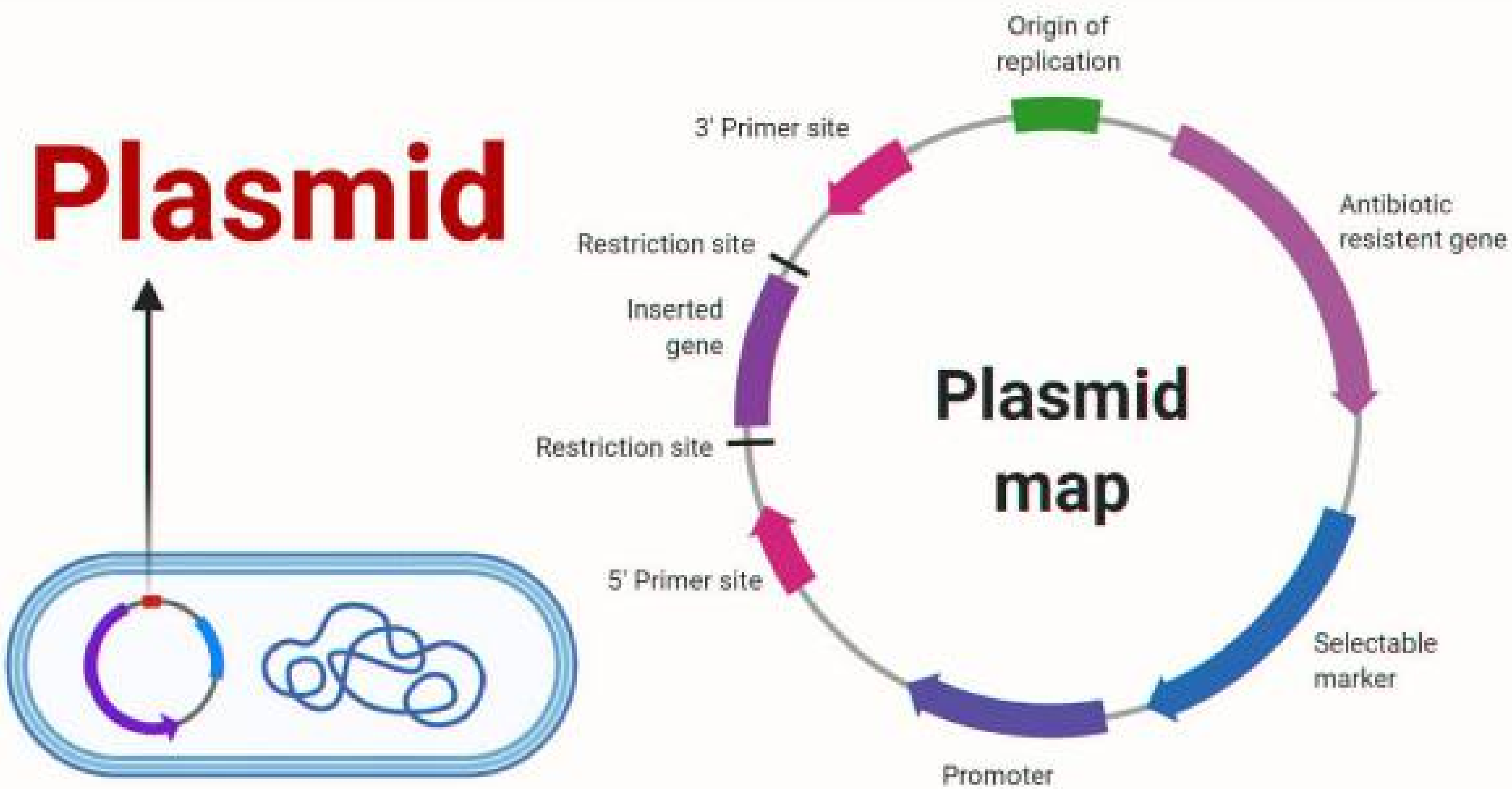
(1) Transmissible plasmids:

- Can be transferred from cell to cell by conjugation
- They are large (MW 40–100 million), since they contain about a dozen genes responsible for the synthesis of the sex pilus and for the enzymes required for transfer.
- They are usually present in a few (1–3) copies per cell.

molecular wight

- Non transmissible plasmids are small (MW 3–20 million), since they do not contain the transfer genes; they are frequently present in many (10–60) copies per cell.
- Plasmids carry the genes for the following functions;
- (1) Antibiotic resistance, which is mediated by a variety of enzymes, such as the beta-lactamase of S. aureus, Escherichia coli, and Klebsiella pneumoniae.
- (2) Exotoxins, such as the enterotoxins of E. coli, anthrax toxin of Bacillus anthracis, exfoliative toxin of S. aureus and tetanus toxin of Clostridium tetani.
- (3) Pili (fimbriae), which mediate the adherence of bacteria to epithelial cells.

- (4) Resistance to heavy metals, such as mercury, the active component of some antiseptics (e.g., merthiolate and mercurochrome), and silver, which is mediated by a reductase enzyme.
- (5) Resistance to ultraviolet light, which is mediated by DNA repair enzymes.



Plasmid
Origin of replication
3' Primer site
Antibiotic resistent gene
Restriction site
Inserted gene
Plasmid map
Restriction site
5' Primer site
Selectable marker
Promoter

- **Other plasmid-encoded products of interest are as follows:**
- (1) Bacteriocins are <u>toxic proteins produced by certain bacteria</u> that are lethal for other bacteria.

Two common mechanisms of action of bacteriocins are

- (i) degradation of bacterial cell membranes by producing pores in the membrane and
- (ii) degradation of bacterial DNA by DNAse.

colicins made by E. coli and pyocins made by Pseudomonas aeruginosa.

- (2) Nitrogen fixation enzymes in Rhizobium in the root nodules of legumes.
- (3) Tumors caused by Agrobacterium in plants.
- (4) Several antibiotics produced by Streptomyces.
- (5) A variety of degradative enzymes that are produced by Pseudomonas

(Jumping genes)

Transposons

- Transposons are pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages.

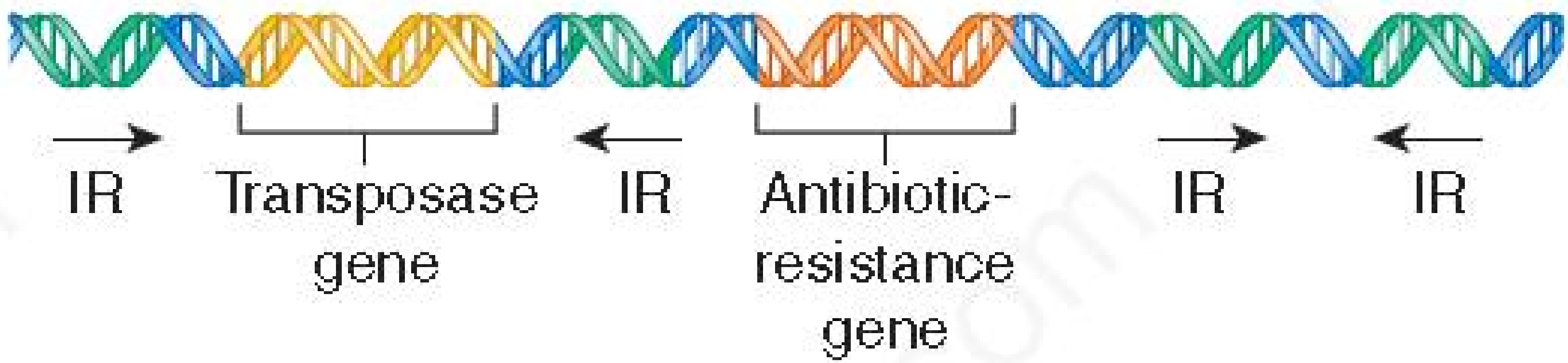
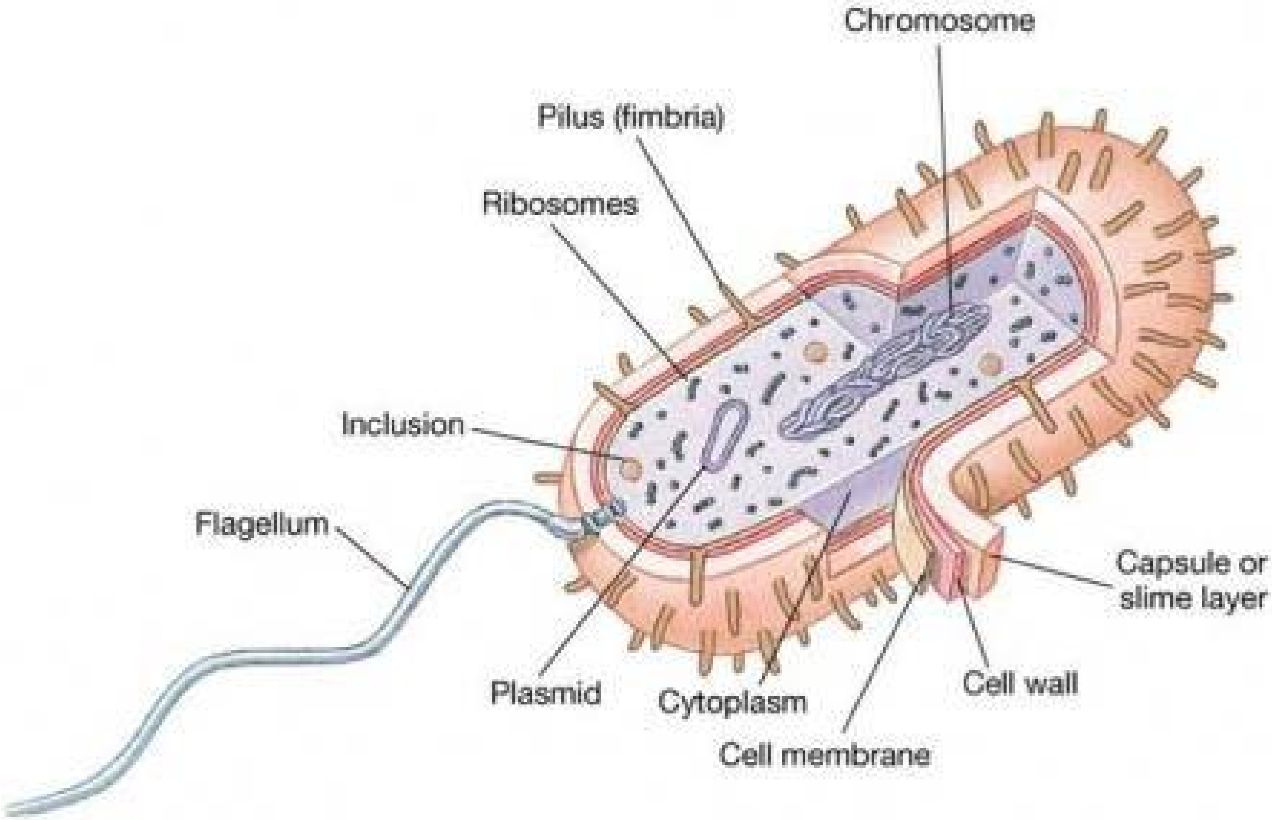


FIGURE 2–7 Transposon genes. This transposon is carrying a drug-resistance gene. IR, inverted repeat. (Reproduced with permission


Chromosome
Pilus (fimbria)
Ribosomes
Inclusion
Flagellum
Capsule or slime layer
Plasmid
Cytoplasm
Cell wall
Cell membrane

Structures Outside the Cell Wall

Capsule:

- The capsule is a gelatinous layer covering the entire bacterium.
- It is composed of polysaccharide, except in the anthrax bacillus, which has a capsule of polymerized d-glutamic acid.
- **The capsule is important for four reasons:**
- It is a determinant of virulence of many bacteria since it limits the ability of phagocytes to engulf the bacteria.
- Specific identification of an organism can be made by using antiserum against the capsular polysaccharide.
- Capsular polysaccharides are used as the antigens in certain vaccines because they are capable of eliciting protective antibodies.
- The capsule may play a role in the adherence of bacteria to human tissues,

Flagella

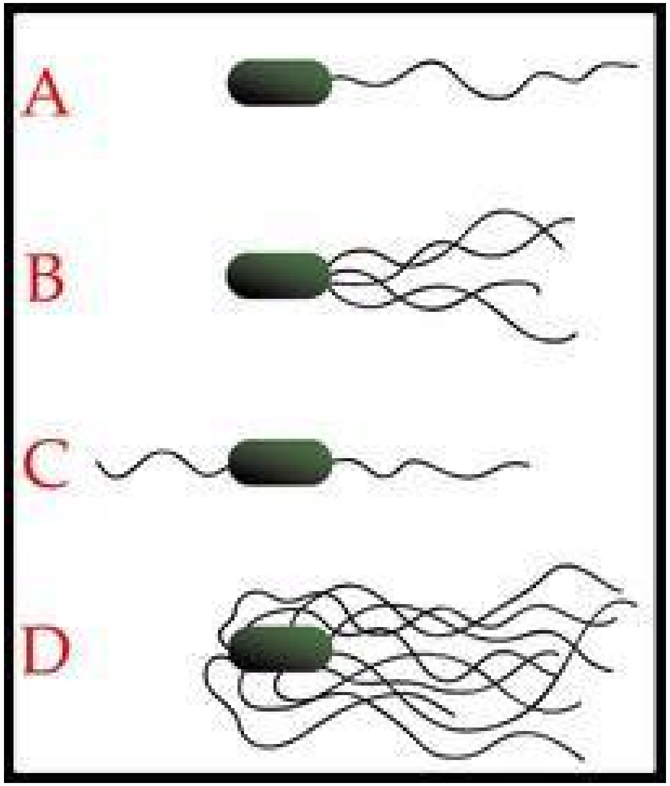
- Flagella are long, whiplike appendages that move the bacteria toward nutrients and other attractants, a process called chemotaxis.
- The long filament, which acts as a propeller, is composed of many subunits of a single protein, flagellin, arranged in several intertwined chains.
- The energy for movement, the proton motive force, is provided by adenosine triphosphate (ATP), derived from the passage of ions across the membrane.

Flagella are medically important for two reasons:

(1) Some species of motile bacteria (e.g., E. coli and Proteus species) are common causes of urinary tract infections.

- Flagella may play a role in pathogenesis by propelling the bacteria up the urethra into the bladder.

2) Some species of bacteria (e.g., Salmonella species) are identified in the clinical laboratory by the use of specific antibodies against flagellar proteins.



A. Monotrichous polar
B. Lophotrichous polar
C. Amphitrichous polar
D. Petrichous

Pili (Fimbriae)

- Pili are hairlike filaments that extend from the cell surface.
- Composed of subunits of pilin, a protein arranged in helical strands.
- They are found mainly on gram-negative organisms.

Pili have two important roles:

- (1) They mediate the attachment of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection for some organisms.
- Mutants of Neisseria gonorrhoeae that do not form pili are nonpathogens.
- (2) A specialized kind of pilus, the sex pilus, forms the attachment between the male (donor) and the female (recipient) bacteria during conjugation

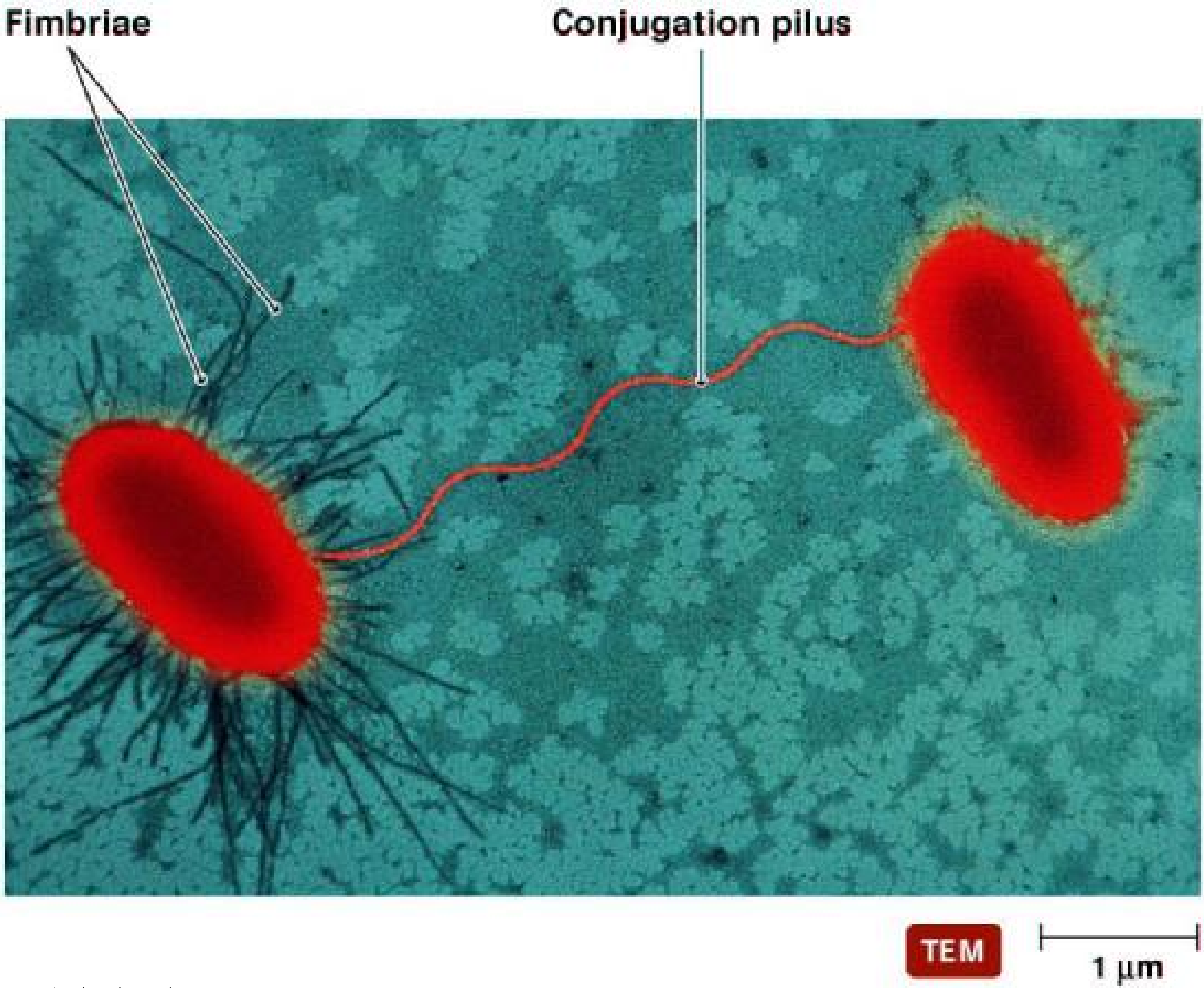
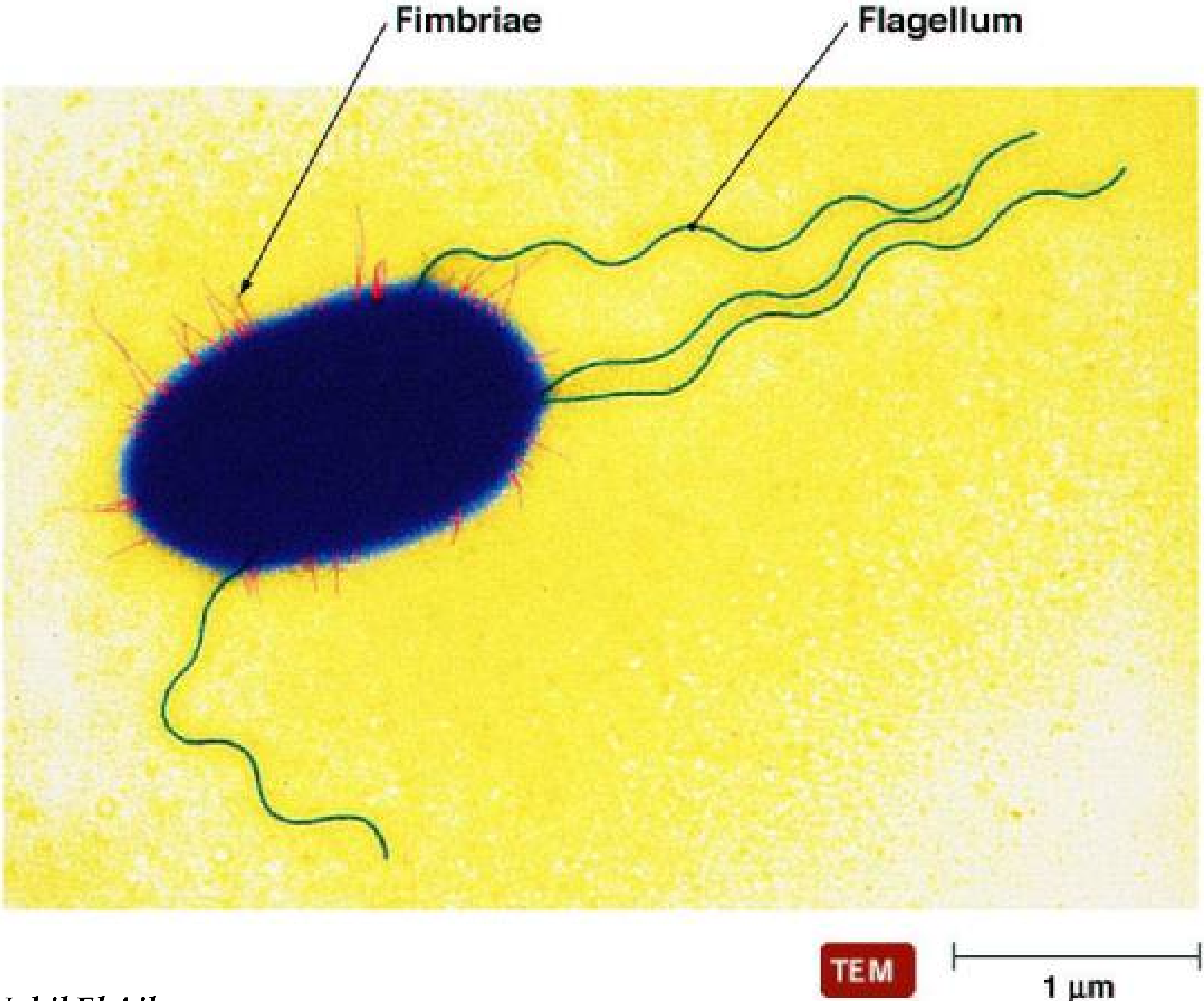


Figure 3.10



Dr. Nabil El Aila
General Microbiology

Figure 3.9

Glycocalyx (Slime Layer)

- The glycocalyx is a polysaccharide coating that is secreted by many bacteria.
- It covers surfaces like a film and allows the bacteria to adhere firmly to various structures (e.g., skin, heart valves, prosthetic joints, and catheters).

هي مستعمرات بكتيرية تتراكم فوق بعضها → هذا يصعب استجابة الإنسان للمضاد الحيوي.

- The glycocalyxm is an important component of biofilms .
- It is the glycocalyx-producing strains of P. aeruginosa that cause respiratory tract infections in cystic fibrosis patients,
- It is the glycocalyx-producing strains of Staphylococcus epidermidis and viridans streptococci that cause endocarditis.
- The glycocalyx also mediates adherence of certain bacteria, such as Streptococcus mutans, to the surface of teeth. This plays an important role in the formation of plaque, the precursor of dental caries.

Bacterial Spores

- These highly resistant structures are formed in response to adverse conditions by two genera of medically important gram-positive rods:
- the genus Bacillus, which includes the agent of anthrax, and the genus Clostridium, which includes the agents of tetanus and botulism.
- Spore formation (sporulation) occurs when nutrients, such as sources of carbon and nitrogen, are depleted inside the cell
- It contains bacterial DNA, a small amount of cytoplasm, cell membrane, peptidoglycan, very little water, and most importantly, a thick, keratinlike coat that is responsible for the remarkable resistance of the spore to heat, dehydration, radiation, and chemicals.

- This resistance may be mediated by dipicolinic acid, a calcium ion chelator found only in spores.
- The medical importance of spores lies in their extraordinary resistance to heat and chemicals.
- As a result of their resistance to heat, sterilization cannot be achieved by
- boiling. Steam heating under pressure (autoclaving) at 121°C, for at least 15 minutes, is required to ensure the sterility of products for medical use.

Bacterial Spores Structure, Importance and examples

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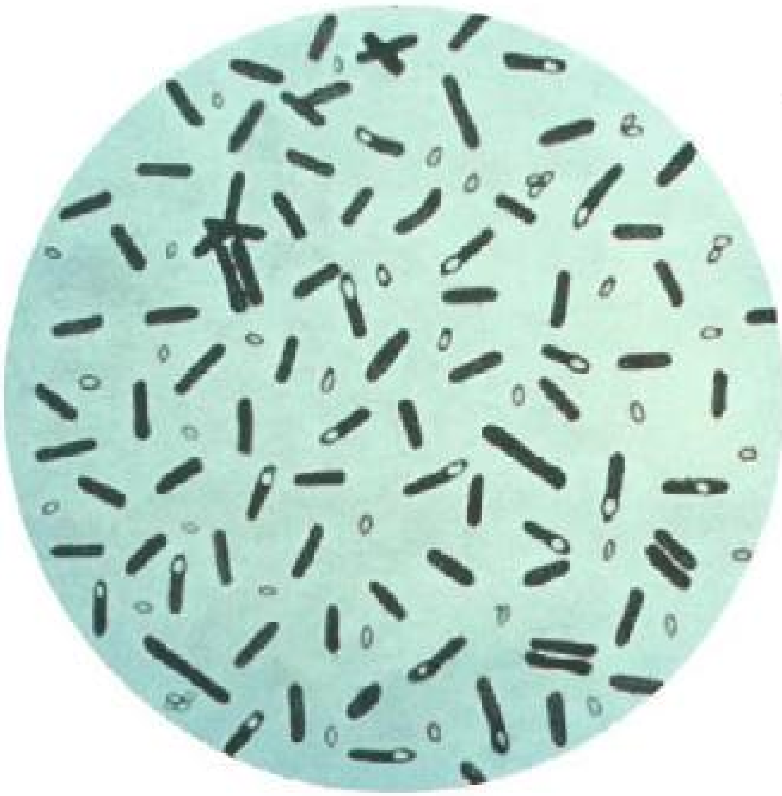

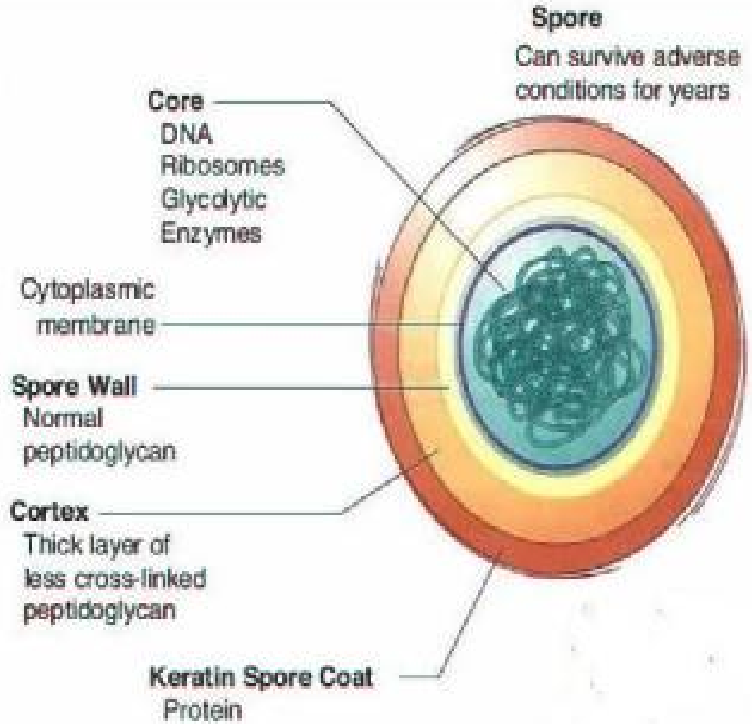

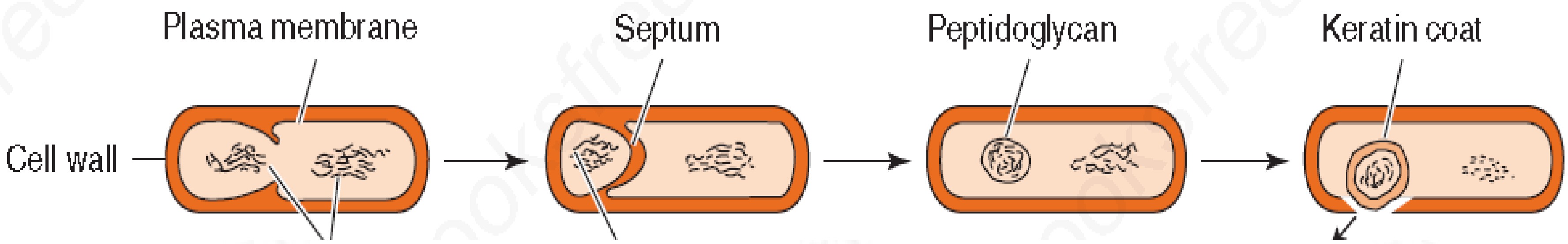

TABLE 2–4 Important Features of Spores and Their Medical Implications

Important Features of Spores	Medical Implications
Highly resistant to heating; spores are not killed by boiling (100°C), but are killed at 121°C.	Medical supplies must be heated to 121°C for at least 15 minutes to be sterilized.
Highly resistant to many chemicals, including most disinfectants, due to the thick, keratinlike coat of the spore.	Only solutions designated as sporicidal will kill spores.
They can survive for many years, especially in the soil.	Wounds contaminated with soil can be infected with spores and cause diseases such as tetanus (*C. tetani*) and gas gangrene (*C. perfringens*).
They exhibit no measurable metabolic activity.	Antibiotics are ineffective against spores because antibiotics act by inhibiting certain metabolic pathways of bacteria. Also, spore coat is impermeable to antibiotics.
Spores form when nutrients are insufficient but then germinate to form bacteria when nutrients become available.	Spores are not often found at the site of infections because nutrients are not limiting. Bacteria rather than spores are usually seen in Gram-stained smears.
Spores are produced by members of only two genera of bacteria of medical importance, *Bacillus* and *Clostridium*, both of which are gram-positive rods.	Infections transmitted by spores are caused by species of either *Bacillus* or *Clostridium*.

End of Lecture