GRAM POSITIVE & GRAM NEGATIVE BACTERIA

(Foundation Block, Microbiology)

Lecturer name: *Dr. Khalifa Binkhamis & Dr. Fawzia Alotaibi*

Department of Pathology, Microbiology Unit

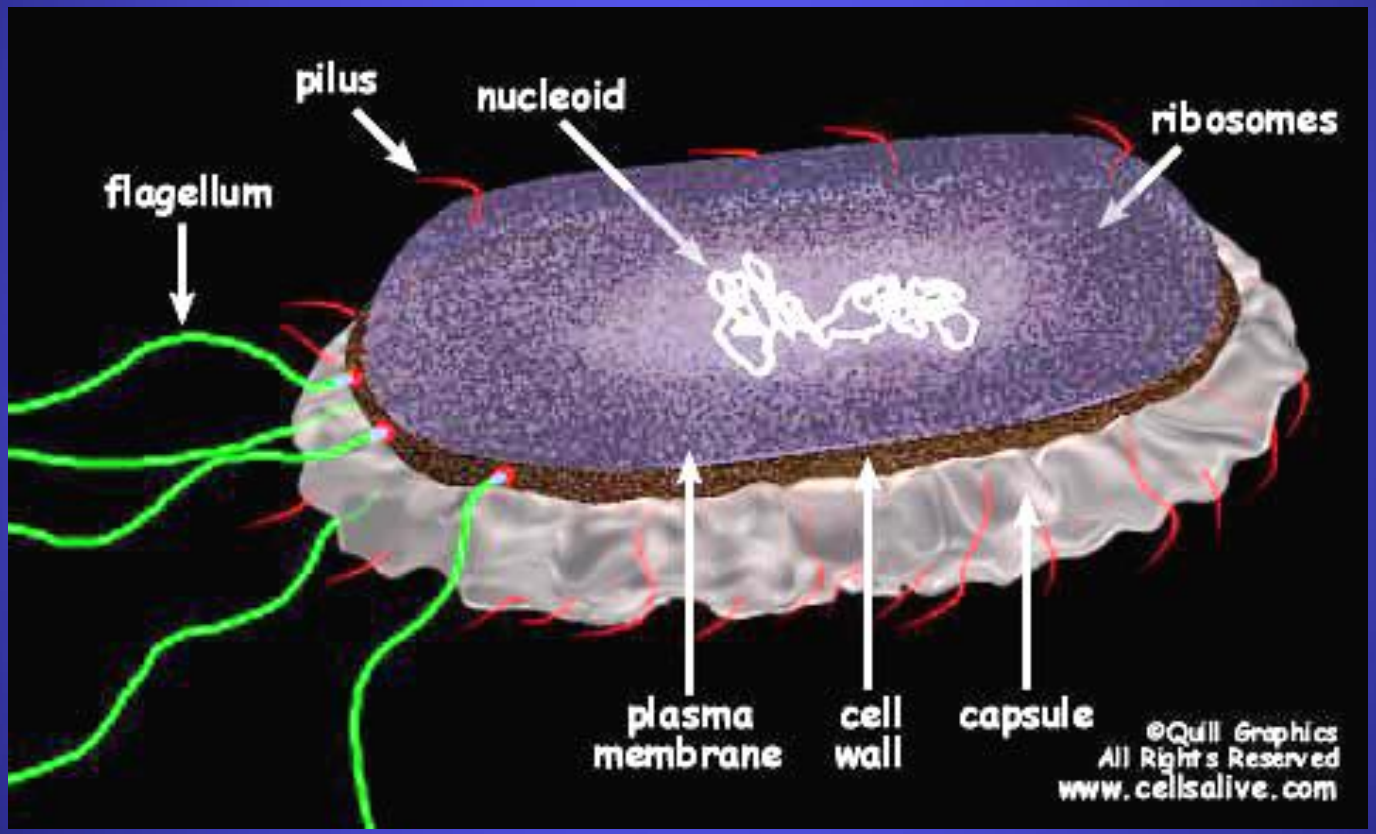
Objectives:

By the end of this lecture, the student should able to:

- Recall the general basic characteristics of bacteria
- Differentiate between gram positive and gram negative bacteria.
- Recall the classes and groups of gram positive bacteria, cocci and bacilli (rods)
- Recall the classes and groups of gram negative bacteria, cocci and bacilli (rods)

- Recall the common infections and diseases caused by these organisms
- Recall the common identification characteristics of these groups

Bacterial cells

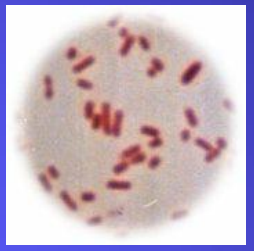


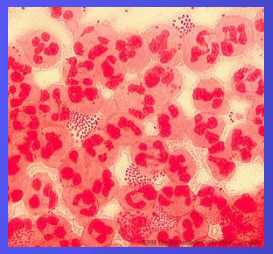
GRAM STAIN

- Developed in 1884 by the Danish physician Hans Christian Gram

- An important tool in bacterial taxonomy, distinguishing so-called **Gram-positive bacteria**, which remain coloured after the staining procedure, from **Gram-negative bacteria**, which do not retain dye and need to be counter-stained.

- Can be applied to **pure cultures** of bacteria or to **clinical specimens**





Top: Pure culture of *E. coli (Gram-negative rods)*
Bottom: *Neisseria gonorrhoeae* in a smear of urethral pus
(*Gram-negative cocci, with pus cells*)

CELL WALL

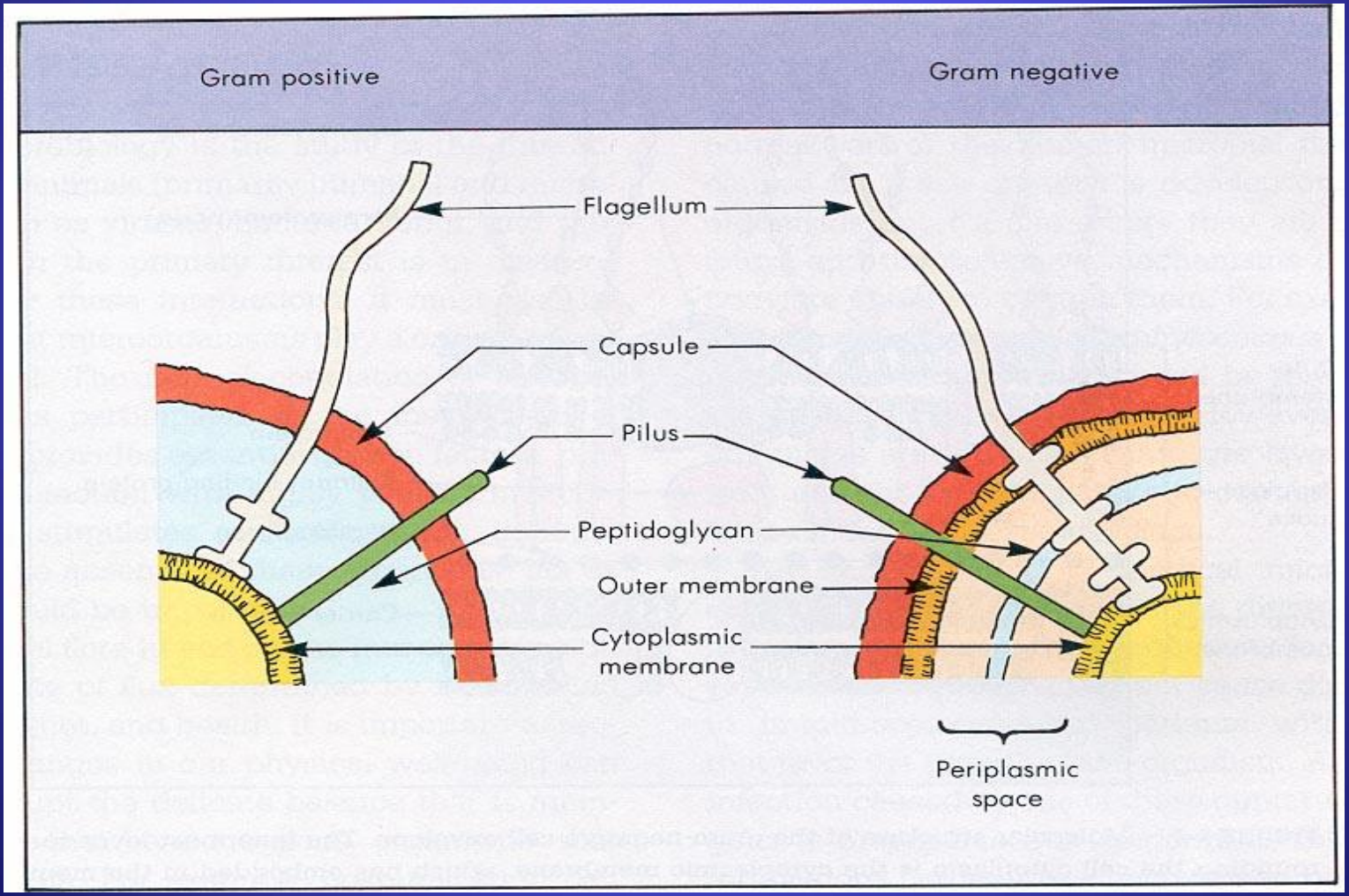
Gram positive cell wall

- Consists of
  - a thick, homogenous sheath of peptidoglycan 20-80 nm thick
  - tightly bound acidic polysaccharides, including teichoic acid and lipoteichoic acid
  - cell membrane
- Retain crystal violet and stain purple

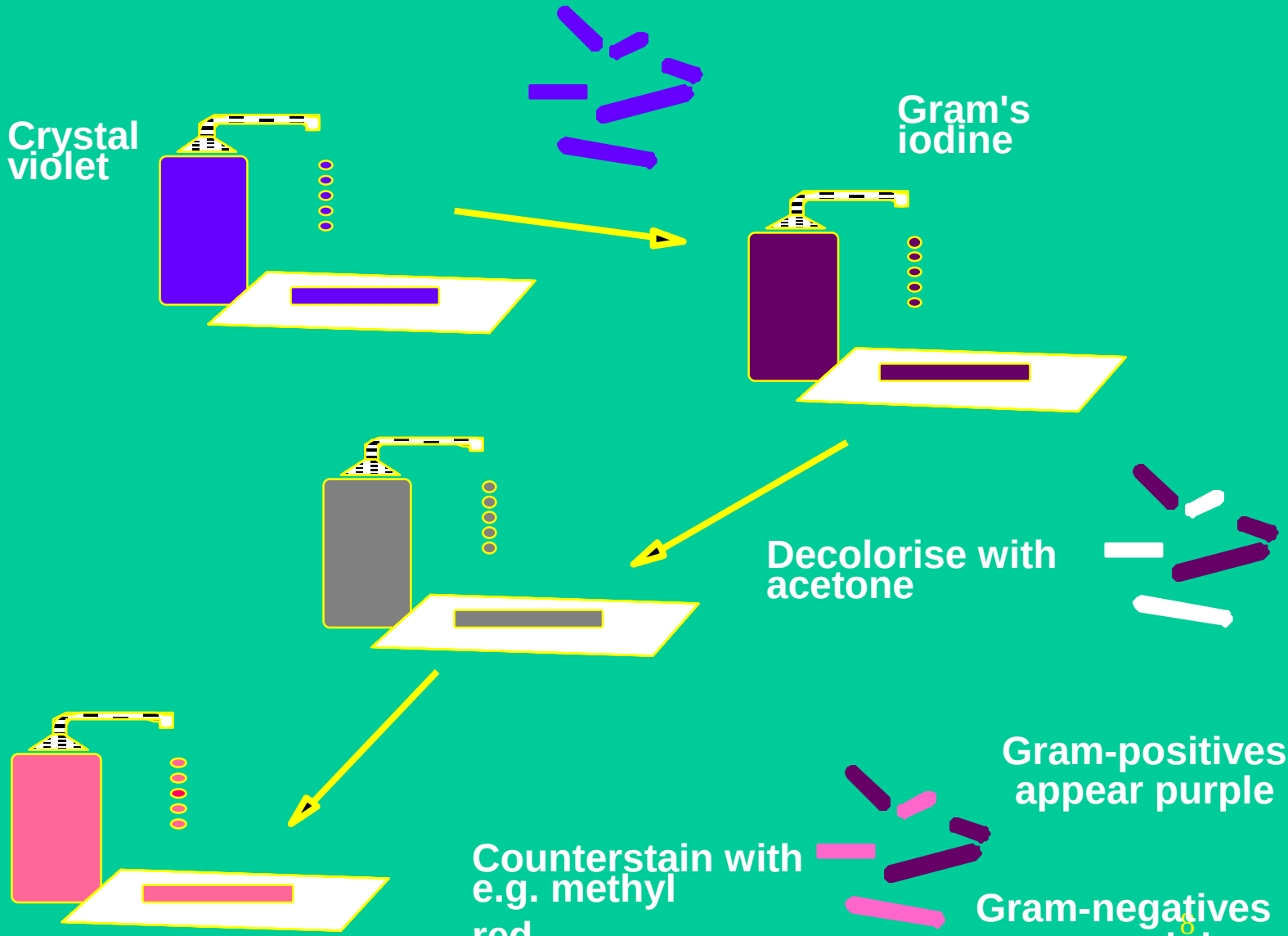
Gram negative cell wall

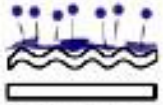
- Consists of
  - an outer membrane containing lipopolysaccharide (LPS)
  - thin shell of peptidoglycan
  - periplasmic space
  - inner membrane
- Lose crystal violet and stain pink from safranin counterstain

Gram Positive Gram Negative

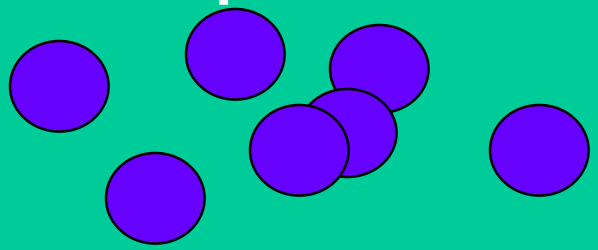


The Gram Stain



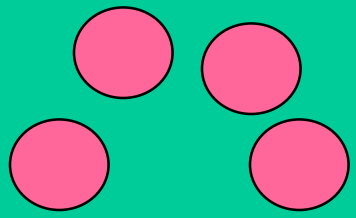
	Microscopic Appearance of Cell		Chemical Reaction in Cell Wall (very magnified view)	
Step	**Gram (+)**	**Gram (–)**	**Gram (+)**	**Gram (–)**
1. Crystal violet				
			Both cell walls affix the dye	
2. Gram's iodine				
			Dye crystals trapped in wall	No effect of iodine
3. Alcohol				
			Crystals remain in cell wall	Cell wall partially dissolved, loses dye
4. Safranin (red dye)				
			Red dye has no effect	Red dye stains the colorless cell

Gram-positive cocci

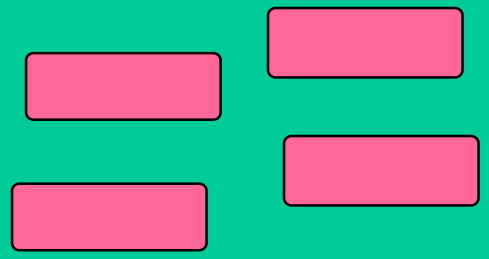


Gram-positive rods

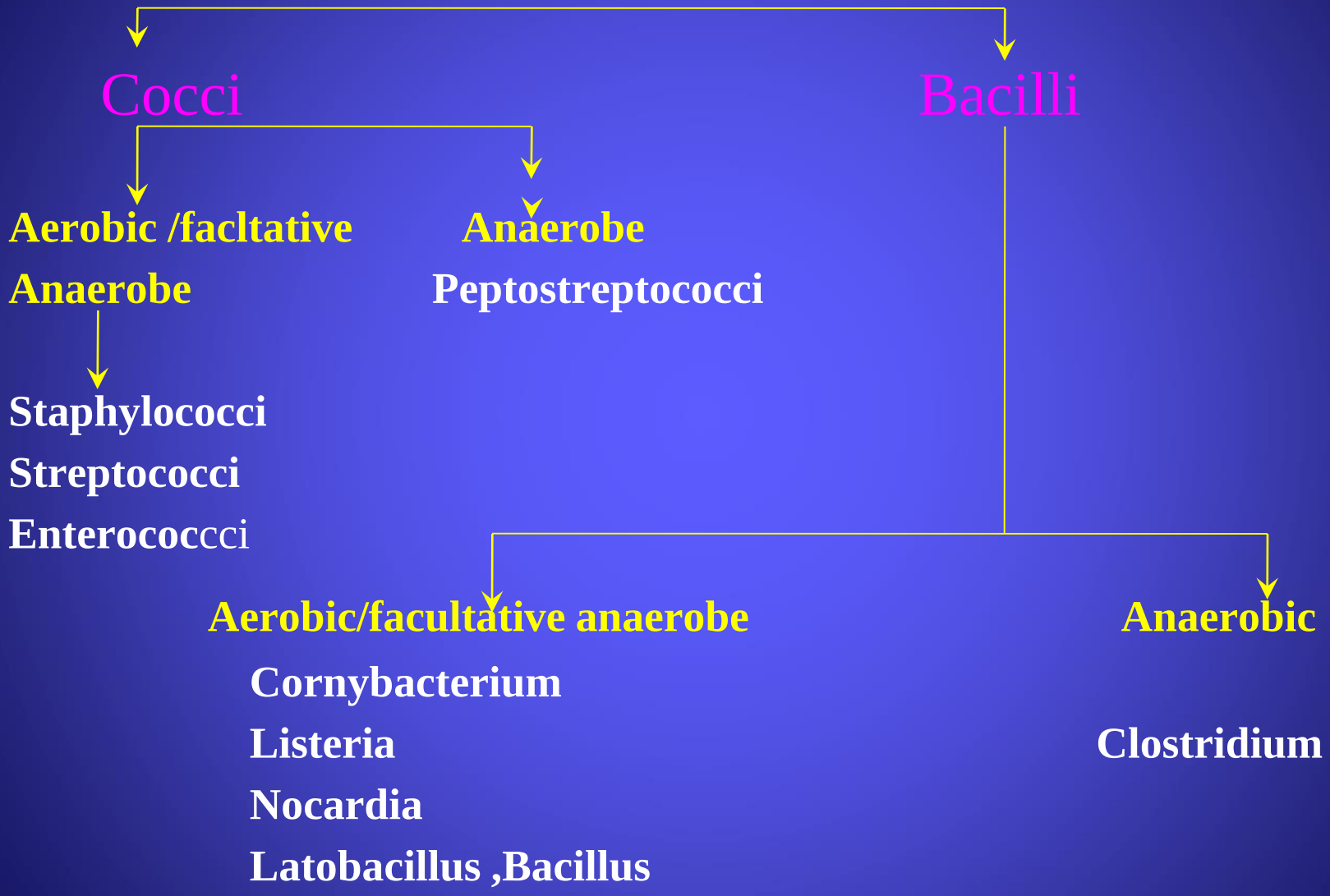
Gram-negative cocci

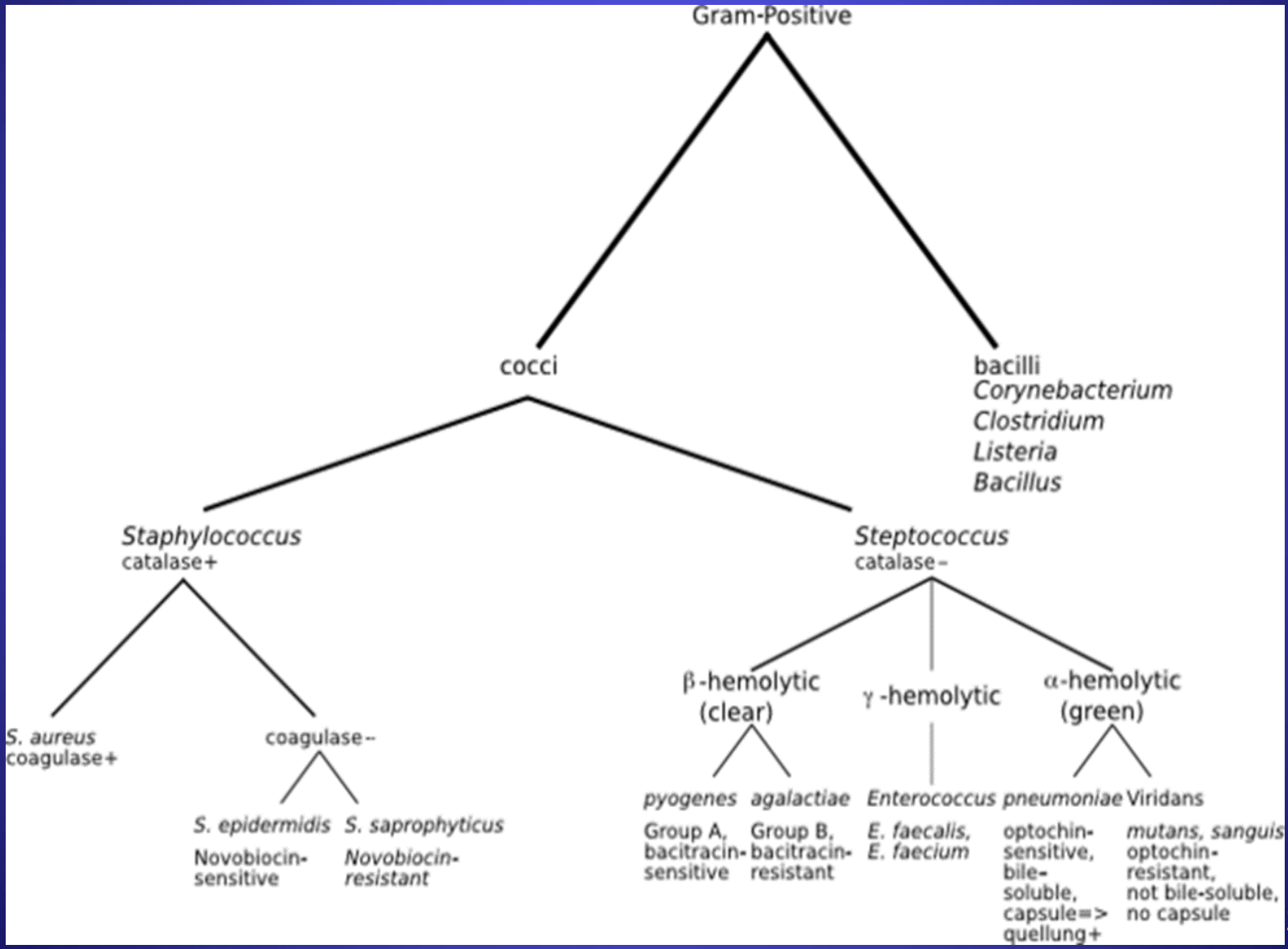


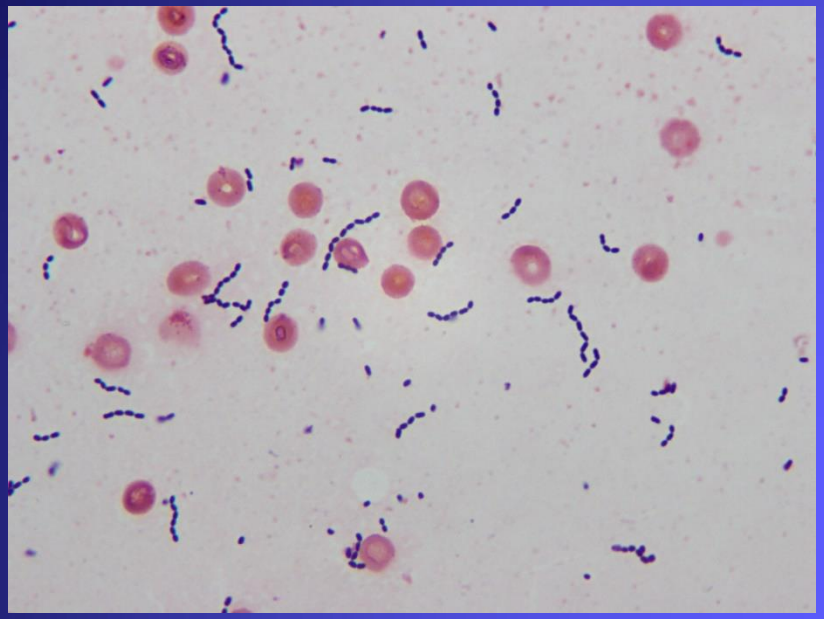
Gram-negative rods



Gram positive bacteria

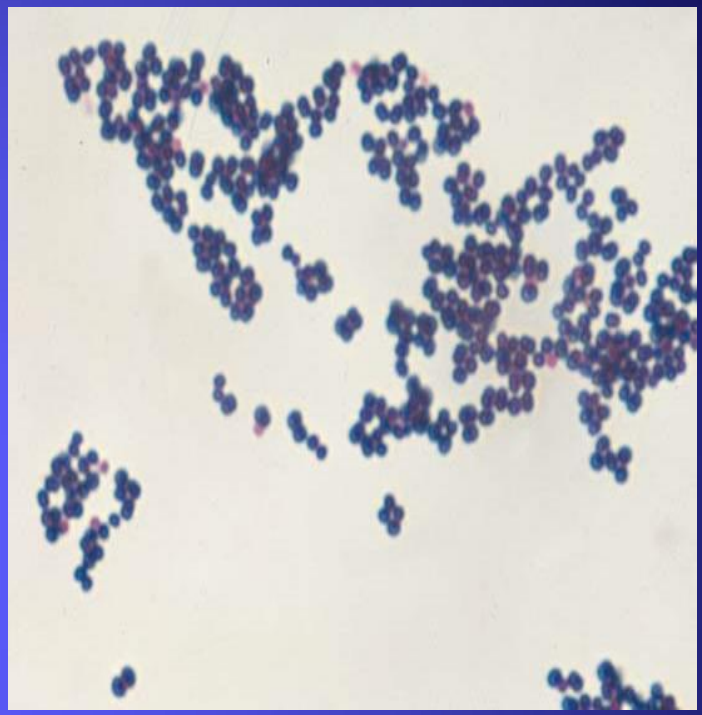



Gram-Positive
cocci
bacilli
Corynebacterium
Clostridium
Listeria
Bacillus
Staphylococcus
catalase+
Steptococcus
catalase–
S. aureus
coagulase+
coagulase–
S. epidermidis
Novobiocin-sensitive
S. saprophyticus
Novobiocin-resistant
β-hemolytic (clear)
γ-hemolytic
α-hemolytic (green)
pyogenes
Group A, bacitracin-sensitive
agalactiae
Group B, bacitracin-resistant
Enterococcus
E. faecalis, E. faecium
pneumoniae
optochin-sensitive, bile-soluble, capsule=> quellung+
Viridans
mutans, sanguis
optochin-resistant, not bile-soluble, no capsule



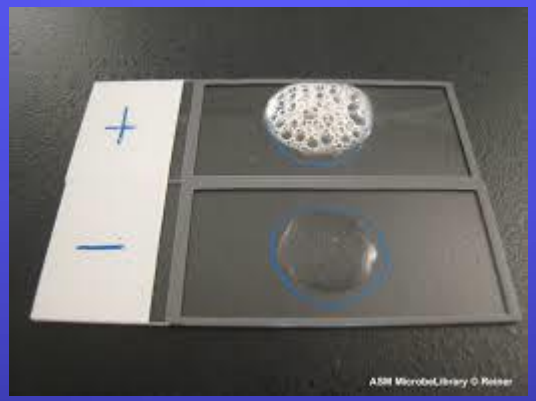
Gram positive cocci in chain

Streptococci



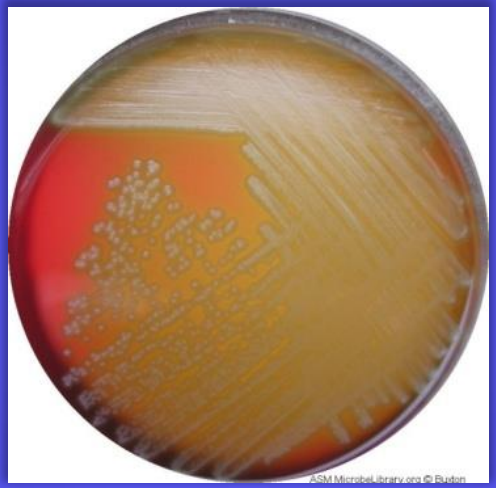
Gram positive cocci in clusters

Staphylococci

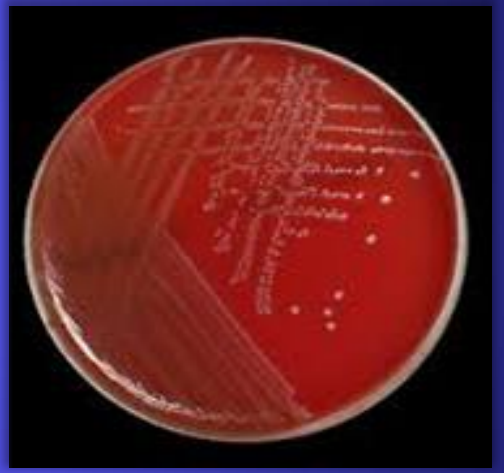


Catalase

Beta-hemolytic

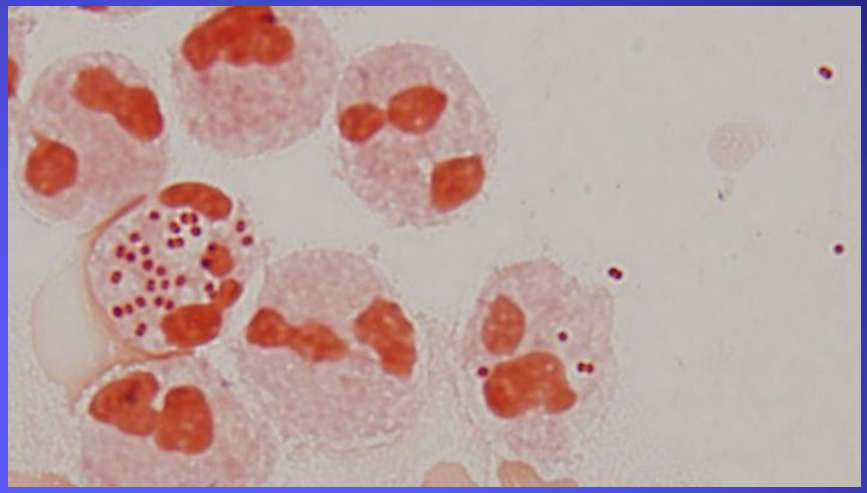


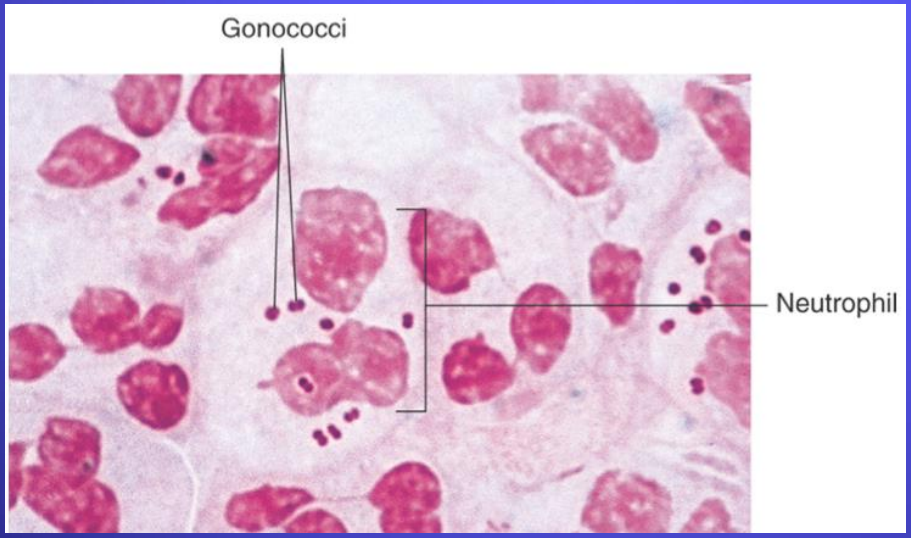
Alpha-hemolytic



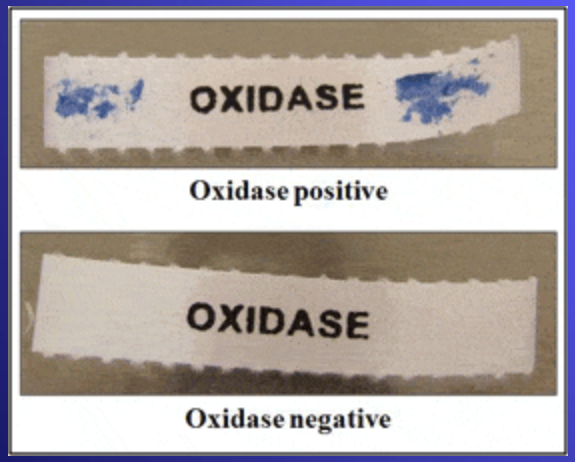
Non-hemolytic

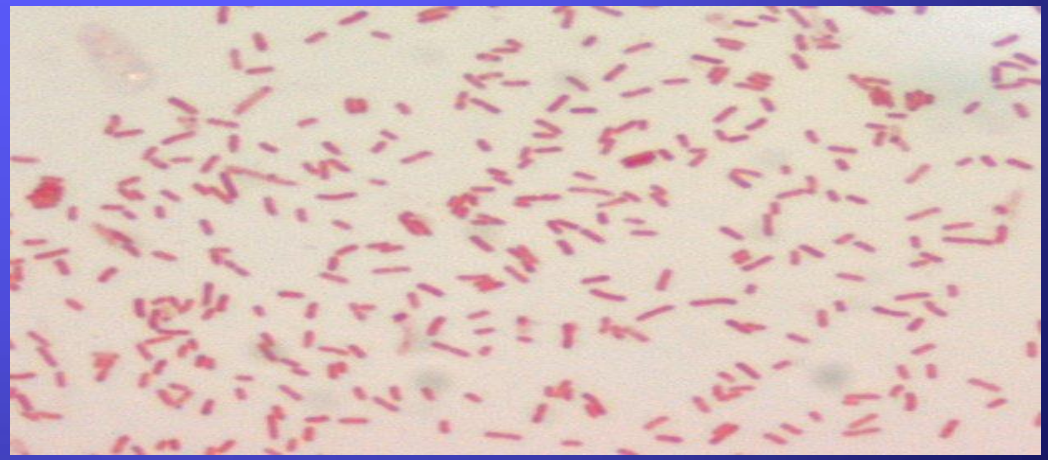
Gram	O2	Arrangment	Example	Diseases
Gram Positive Cocci		Gram + cocci in chains	Strep.pneumoniae Alpha hemolysis	Pneumonia meningitis
			Group A strept Beta hemolysis	Pharyngitis (Sore throat) Rheumatic fever
	Aerobic	Catalase negative	Group B strept Beta hemolysis	Neonatal infection
			Enterococcus Non hemolysis	Urine, blood infection
		Gram + cocci in clusters Catalase +	Staph aureus	Soft tissue bone joint blood and heart
			Coagulase – staph Staph epidermidis	Catheter related infections
	Anaerobic	Gram + cocci in chains	Peptostreptococcus	Brain abscess
Gram Negative cocci	Aerobic	Gram – cocci in pairs	Neisseria and Moraxella	Genital , Meninges and respiratory
	Anaerobic	Gram – cocci	Veillonella	Rare cause infection



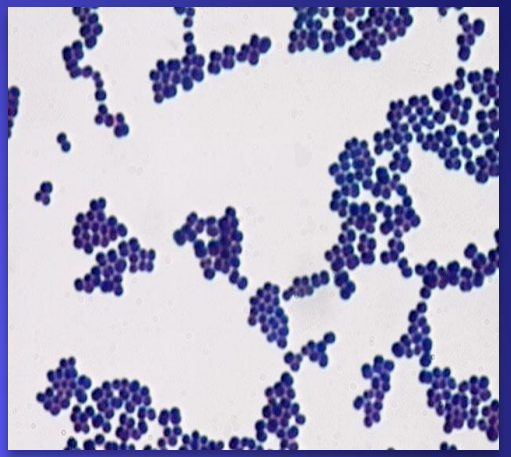

Gonococci
Neutrophil

Gram	O2	Characteristics	Examples
Gram Positive Bacilli	Aerobic	Spore forming	Bacillus antherasis
		Non-Spore forming	Listeria Corynebacterium
	Anaerobic	Spore forming	Clostridia
		Non-Spore forming	Eubacterium
Gram negative Bacilli	Aerobic	Sugar fermenter Enteric Bacteria Oxidase -	E. coli
		Sugar fermenter Oxidase +	Vibrio cholerae
		Non fermenter Oxidase +	Pseudomonas
		Non fermenter Oxidase -	Acinetobacter
		fastidious	Haemophilus influenzae
	Anaerobic		Bacteroides
Non Gram Stainable			Spirochetes
			Mycoplasma

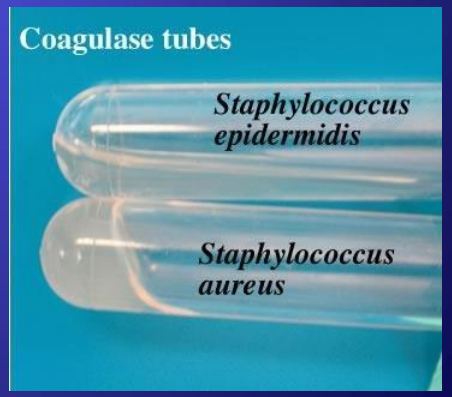

OXIDASE
Oxidase positive
OXIDASE
Oxidase negative



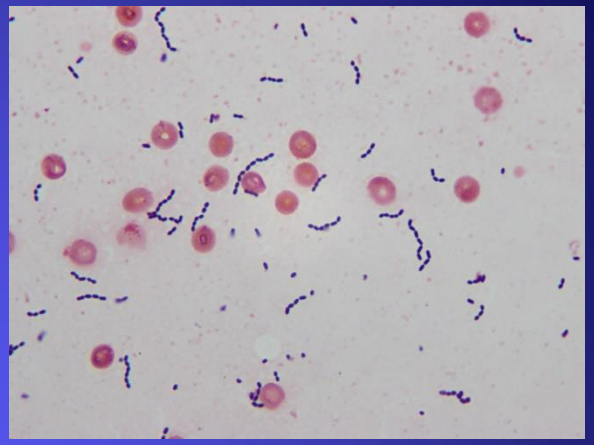
Gram-positive Cocci



- **Staphylococci**
  - **Catalase-positive**
  - **Gram-positive cocci in clusters**
- ***Staphylococcus aureus***
  - **coagulase-positive, most important pathogen**
- ***Staph. epidermidis***
  - **and other coagulase negative staphylococci e.g. S saprophiticus**



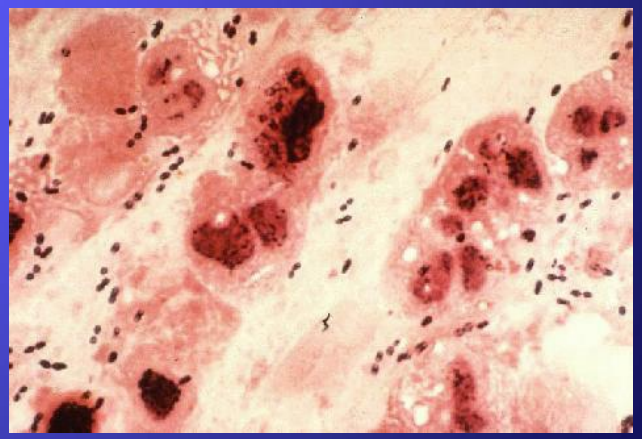
Streptococci



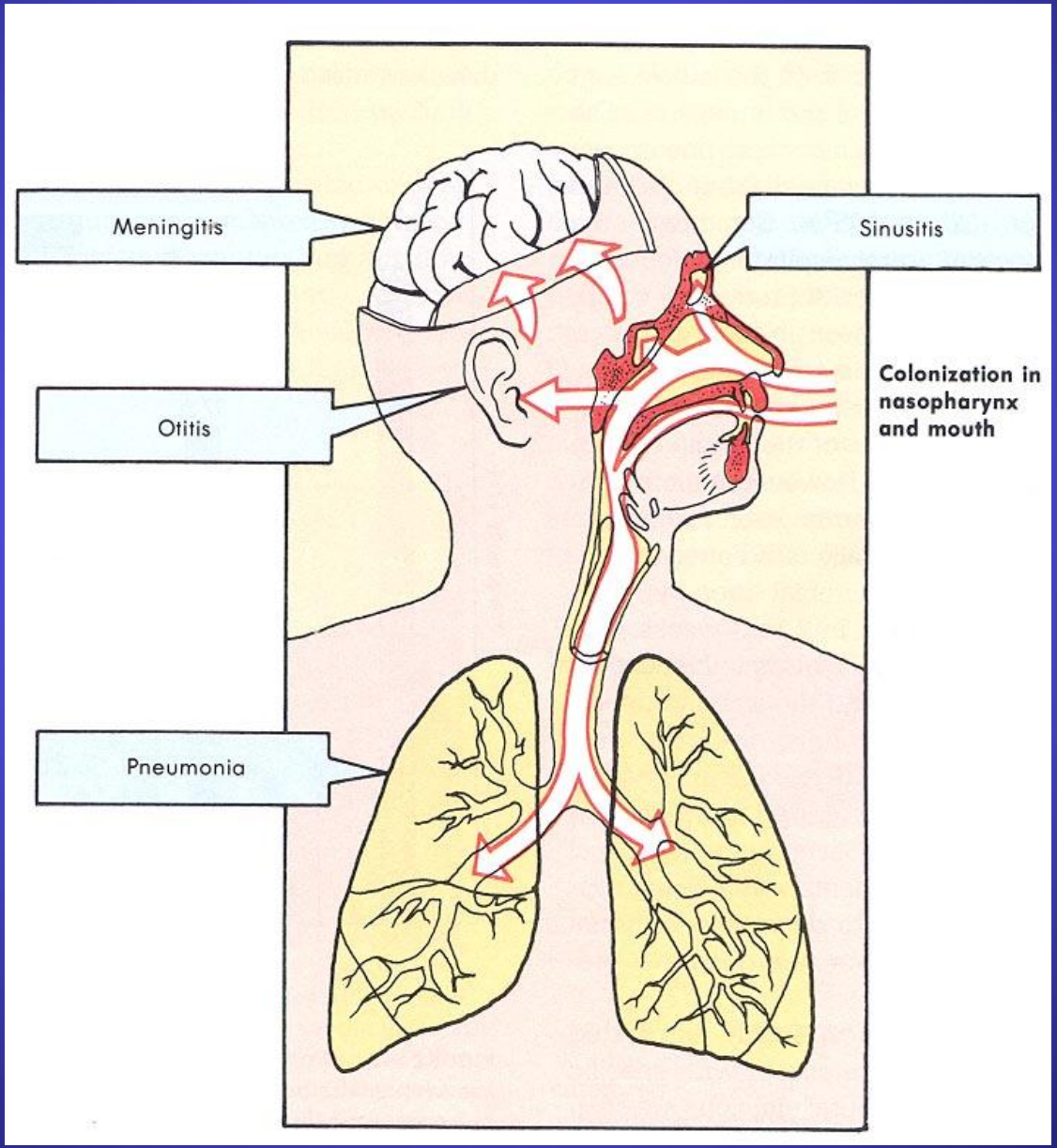
- **Catalase-negative**
- **Gram-positive cocci in chains or pairs**
- Divided by type of hemolysis.

- Alpha hemolytic:
  - *S. viridans*- oral flora - infective endocarditis
  - *S. pneumoniae*-important cause of community acquired pneumonia

- Beta hemolytic:
  - *S. pyogenes*, group A streptococcus
    - Important cause of pharyngitis and cellulitis



S. pneumoniae



GRAM POSITIVE BACILLI

- A-Spore forming
- B-Non spore forming

Spore forming are divided into:-

Aerobic spore forming most important is *Bacillus spp. (e.g. anthracis, that causes anthracis)*

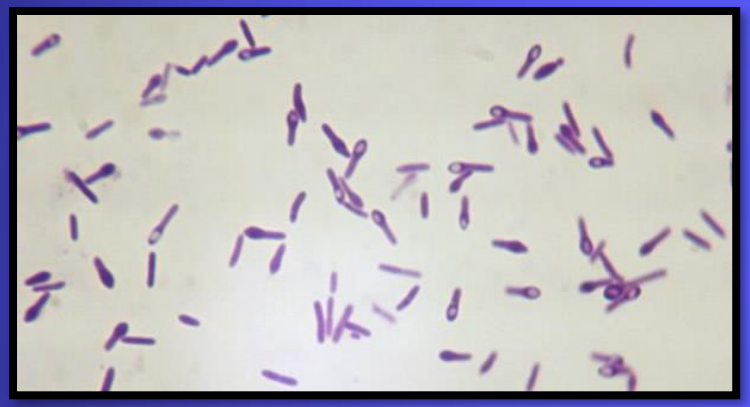
Anaerobic spore forming

Clostridium spp.

GRAM POSITIVE BACILLI

Anaerobic gram positive bacilli

- C. tetani - **Tetanus**



- C. perfringens

Gas gangrene

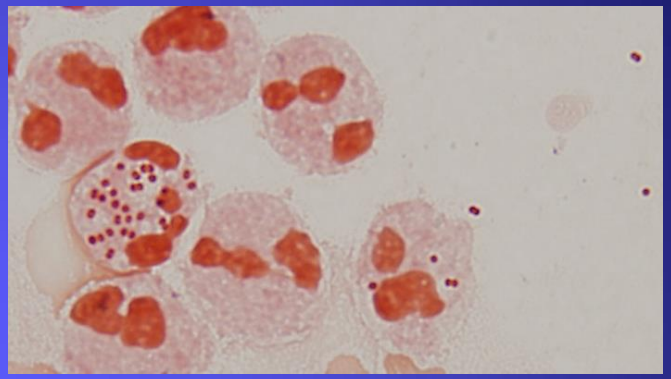
- **C. botulinum - botulism**
  - **Descending weakness-->paralysis**
  - **diplopia, dysphagia-->respiratory failure**

GRAM POSITIVE BACILLI

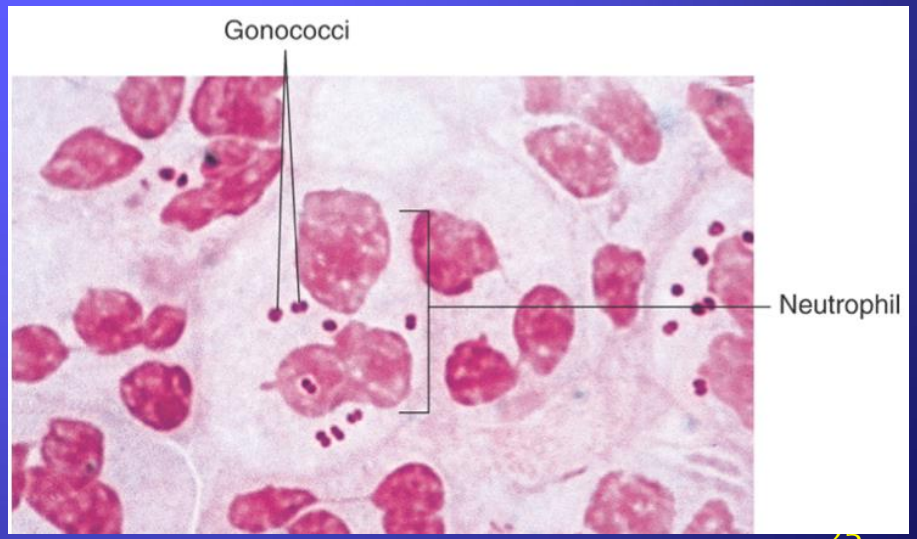
Aerobic gram positive bacilli

- **Corynebacterium diphtheriae**
  - **Fever, pharyngitis, cervical LAD**
  - **thick, gray, adherent membrane**
  - **sequelae-->airway obstruction, myocarditis**

Gram-Negative Cocci

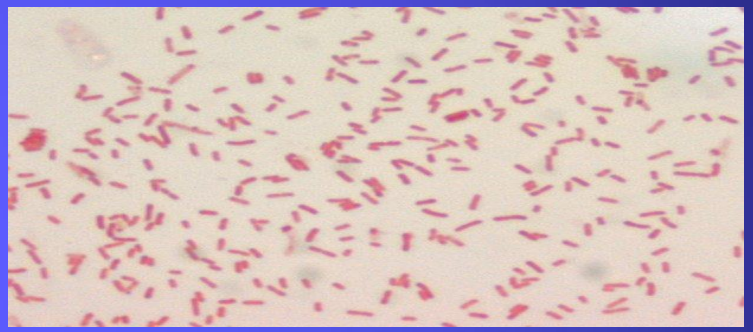


- *Neisseria gonorrhoeae*
  - *The Gonococcus*
- *Neisseria meningitidis*
  - *The Meningococcus*
- Both Gram-negative intracellular diplococci
- Moraxella catarrhalis



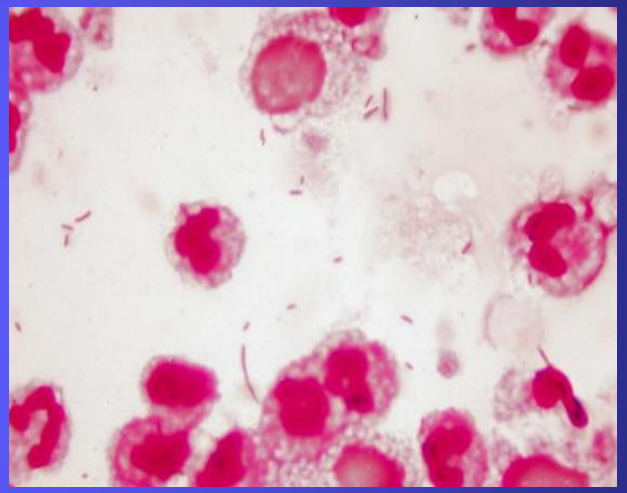
Gram-Negative Rods

- Enteric Bacteria they ferment sugars most important are;
  - *E. coli*
  - *Salmonella*
  - *Shigella*
  - *Yersinia and Klebsiella pneumoniae*
  - *Proteus*



Gram-Negative Rods

- Fastidious GNRs
  - *Bordetella pertussis*
  - *Haemophilus influenzae*
  - *Campylobacter jejuni*
  - *Helicobacter pylori*
  - *Legionella pneumophila*
- Anaerobic GNRs
  - *Bacteroides fragilis*
  - *Fusobacterium*



Non fermentative gram negative rods i.e. they do not ferment sugars e.g.

- Oxidase positive: Pseudomonas, causes infection in immunocompromised patients
- Oxidise negative non fermentative e.g. Acinobacter spp.

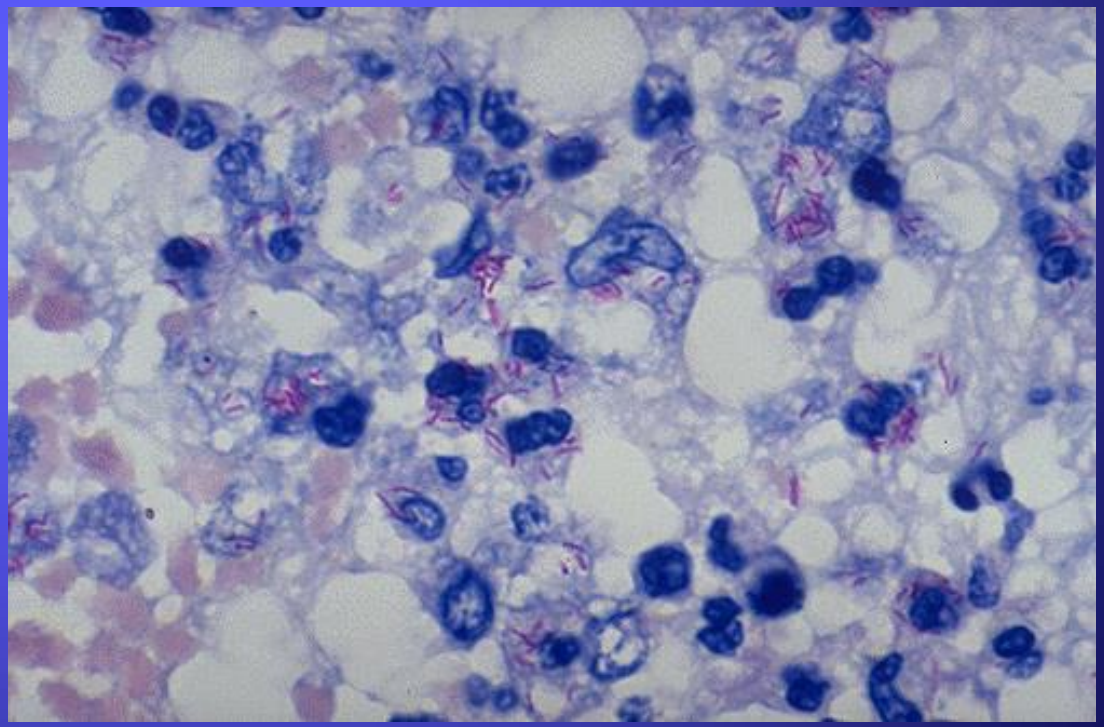
Oxidise positive comma shaped and also fermentative most important is *Vibrio cholerae* that causes cholera which is a disease characterized by severe diarrhea and dehydration

Non-Gram-stainable bacteria

- Unusual gram-positives
- Some spirochaetes (e.g Treponema pallidum (cause of syphilis))
- Bacteria with no cell wall
- Obligate intra-cellular bacteria

Unusual Gram-positives

- Mycobacteria
  - Contain mycolic acid in cell wall



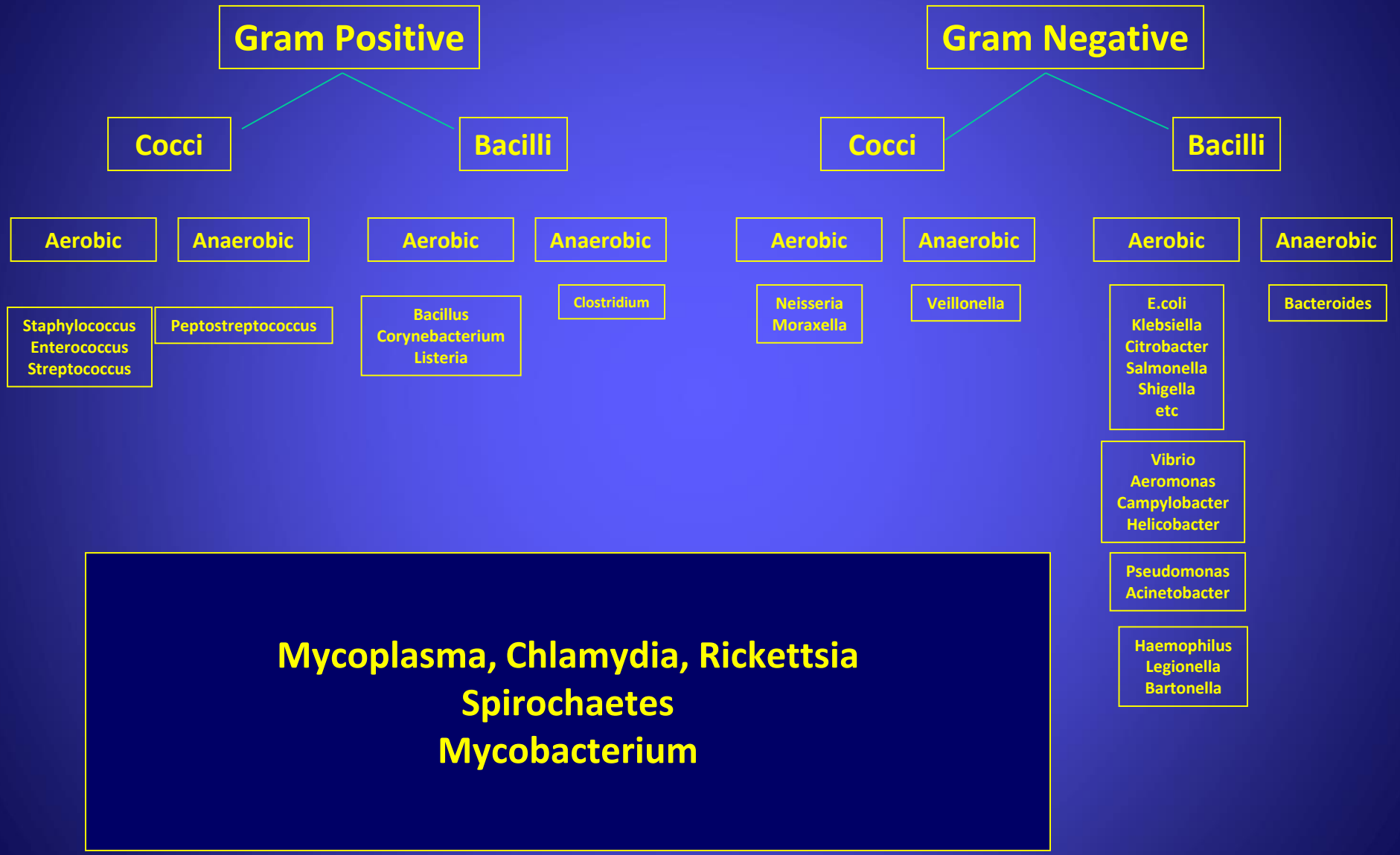
Non-Gram-stainable bacteria

No cell wall

- Mycoplasmas
  - Smallest free-living organisms
  - No cell wall
  - *M. pneumonia, M. genitalium*

Obligate intra-cellular

- Chlamydia
  - *C. pneumoniae, C. trachomatis*
- Rickettsia


Gram Positive
Cocci
Bacilli
Aerobic
Anaerobic
Aerobic
Anaerobic
Staphylococcus
Enterococcus
Streptococcus
Peptostreptococcus
Bacillus
Corynebacterium
Listeria
Clostridium
Gram Negative
Cocci
Bacilli
Aerobic
Anaerobic
Aerobic
Anaerobic
Neisseria
Moraxella
Veillonella
E.coli
Klebsiella
Citrobacter
Salmonella
Shigella
etc
Bacteroides
Vibrio
Aeromonas
Campylobacter
Helicobacter
Pseudomonas
Acinetobacter
Haemophilus
Legionella
Bartonella
Mycoplasma, Chlamydia, Rickettsia
Spirochaetes
Mycobacterium