

GRAM POSITIVE & GRAM NEGATIVE BACTERIA

(Foundation Block, Microbiology)

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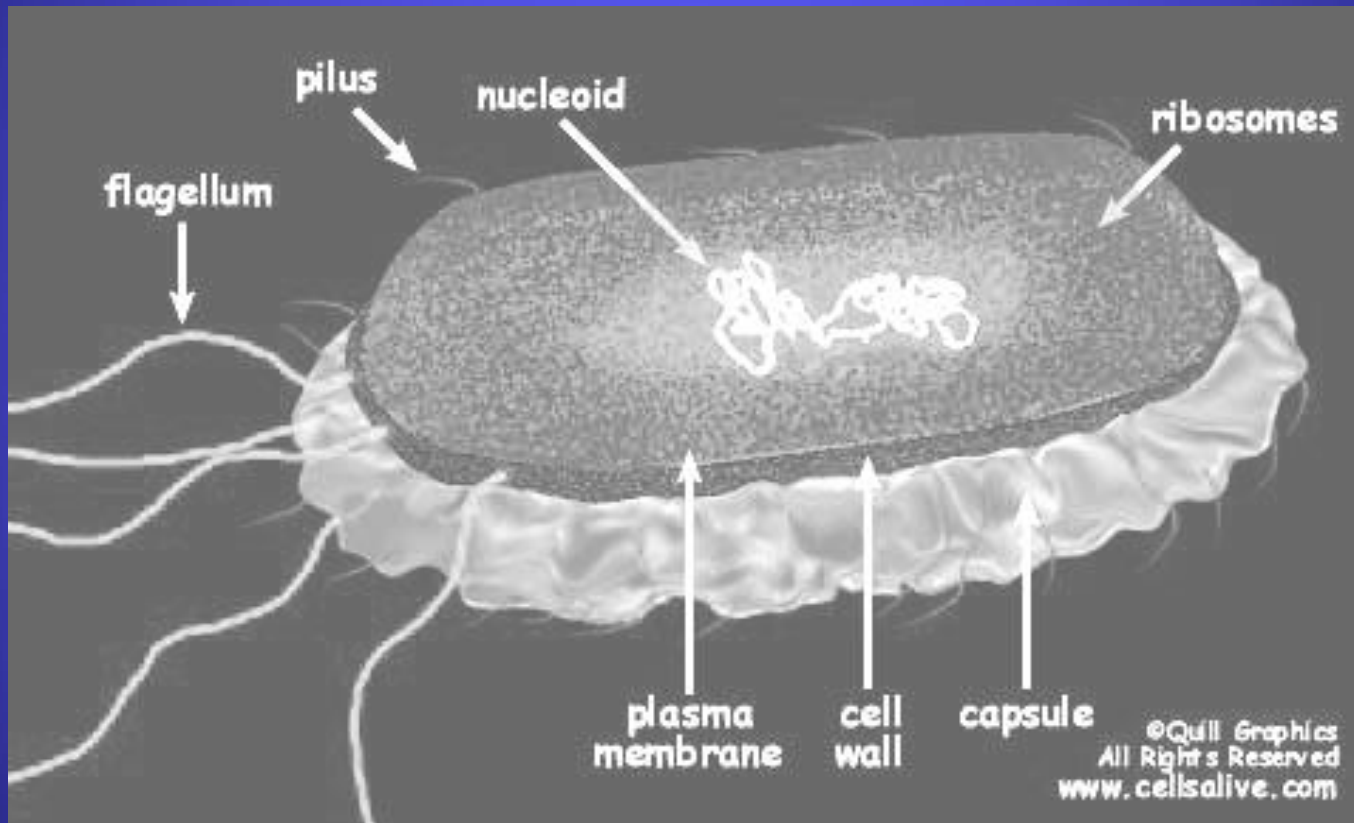
Objectives:

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

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

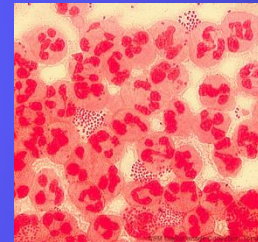
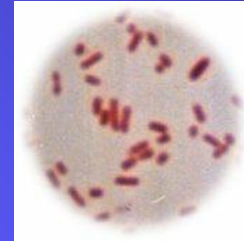
- Recall the different groups, genera and species of gram negative bacteria (cocci and bacilli (rods))
- Recall the common infections and diseases caused by these organisms
- Recall the common identification characteristics of these groups and organisms
- Recall the different non gram sustainable bacteria

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

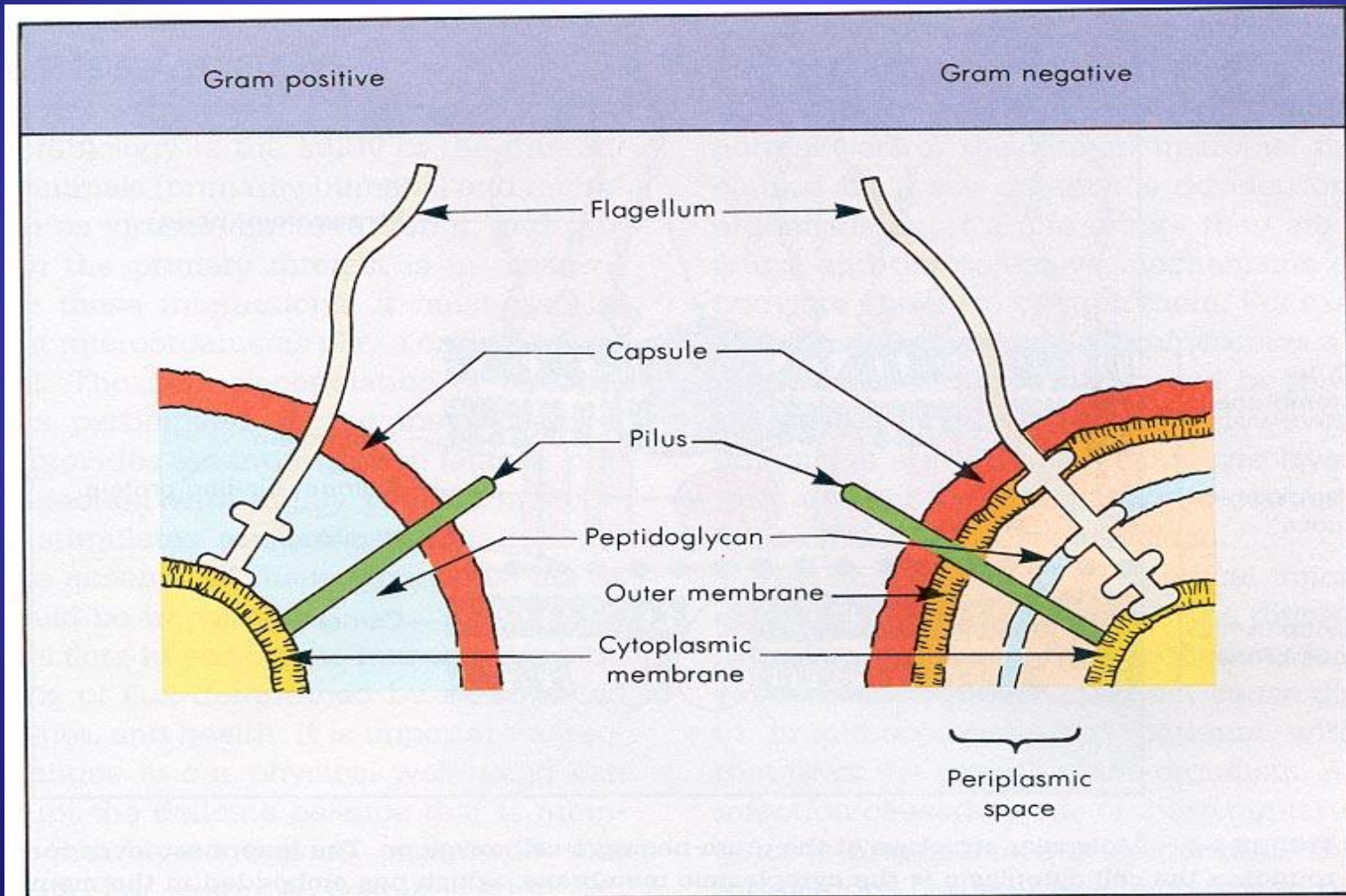
- Consists of
 - a thick, homogenous sheath of peptidoglycan 20-80 nm **thick**
 - tightly bound acidic polysaccharides, including **teichoic acid and lipoteichoic acid**
 - cell membrane
- **Retain crystal violet and stain purple**

Gram negative cell wall

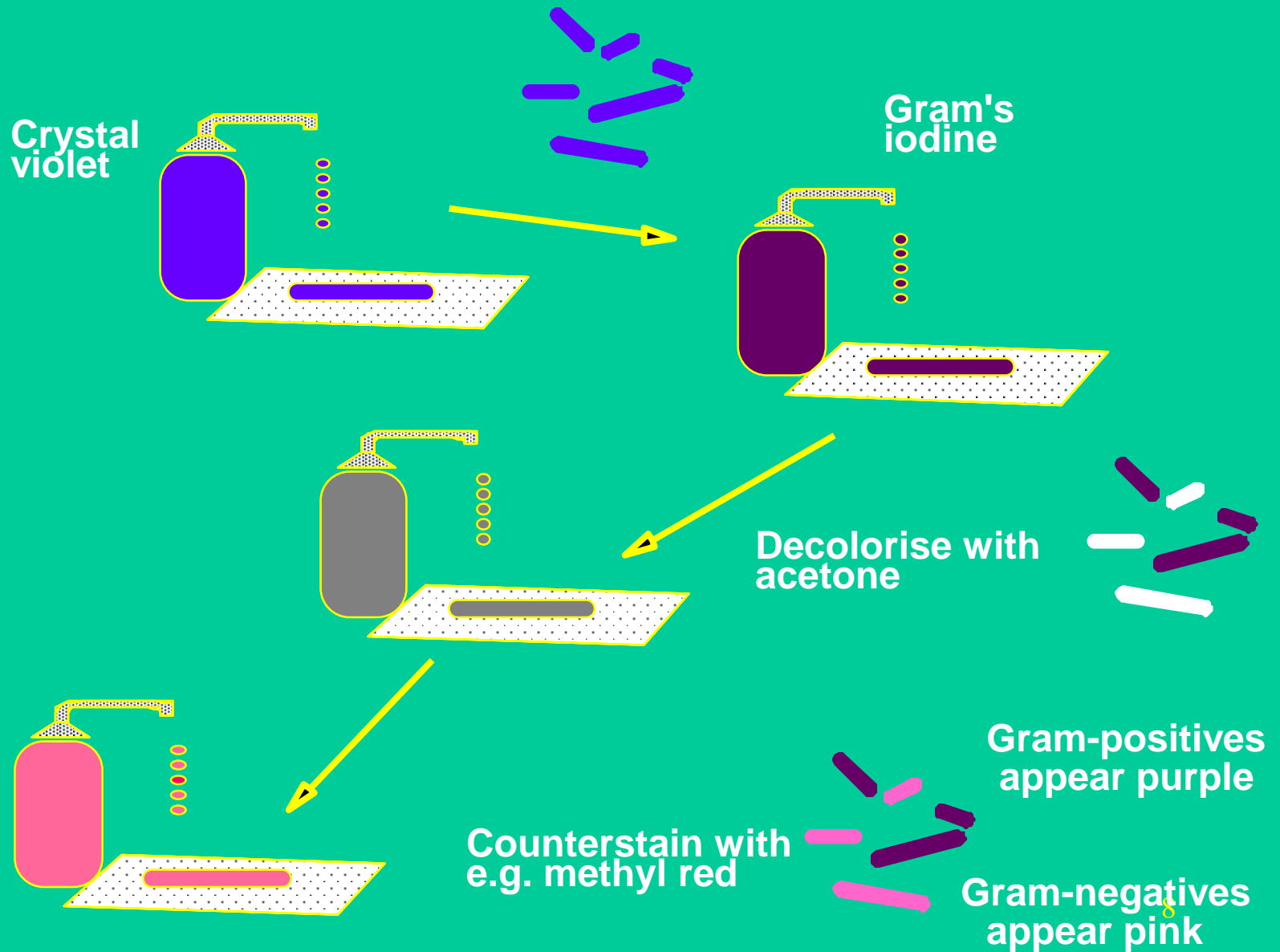
- Consists of
 - an outer membrane containing lipopolysaccharide (LPS)
 - **thin** shell of peptidoglycan
 - **periplasmic space**
 - inner membrane
- **Lose crystal violet and stain pink from safranin counterstain**










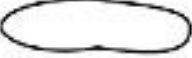






Gram Positive

Gram Negative



The Gram Stain



Step	Microscopic Appearance of Cell		Chemical Reaction in Cell Wall (very magnified view)	
	Gram (+)	Gram (-)	Gram (+)	Gram (-)
1. Crystal violet				
2. Gram's iodine				
3. Alcohol				
4. Safranin (red dye)				

Both cell walls affix the dye

Dye crystals trapped in wall

No effect of iodine

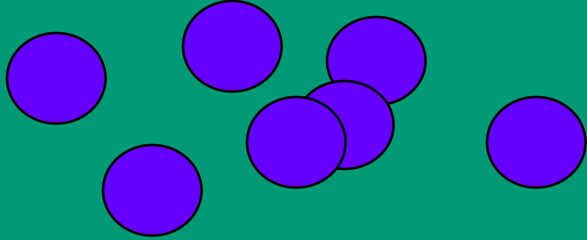
Crystals remain in cell wall

Cell wall partially dissolved, loses dye

Red dye has no effect

Red dye stains the colorless cell

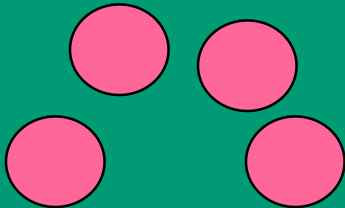
Gram-positive cocci



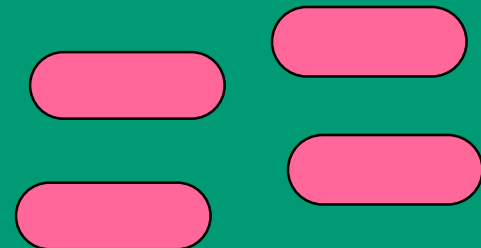
Gram-positive rods



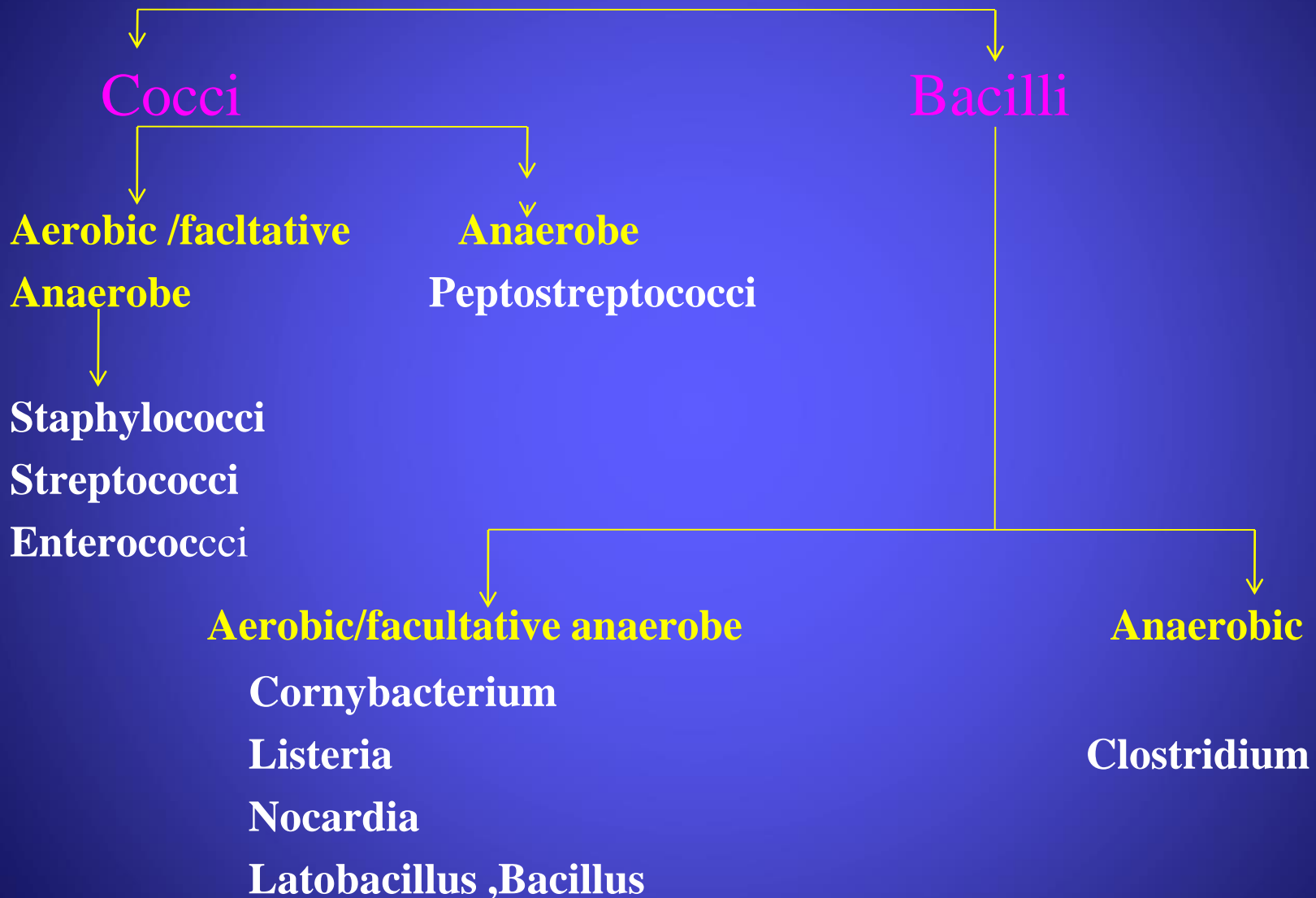
Gram-negative cocci

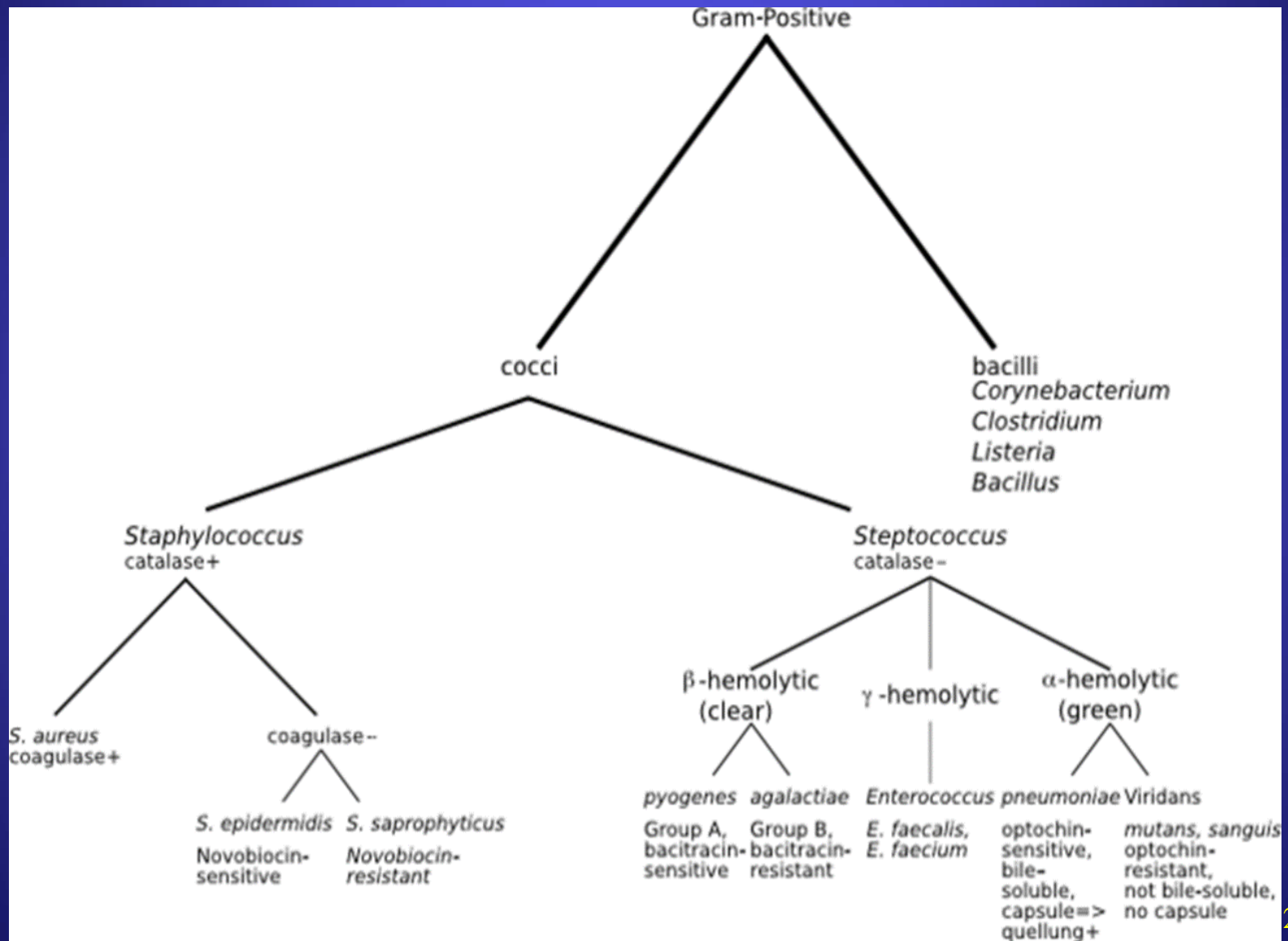


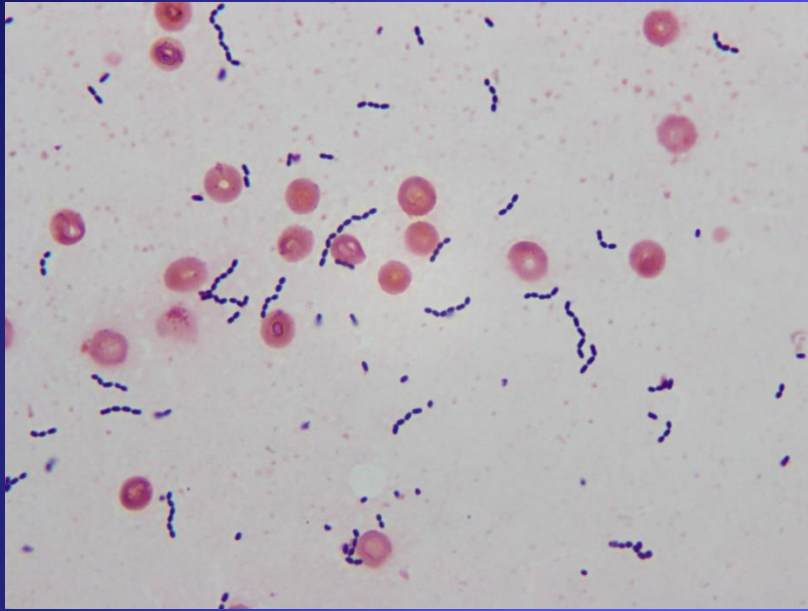
Gram-negative rods



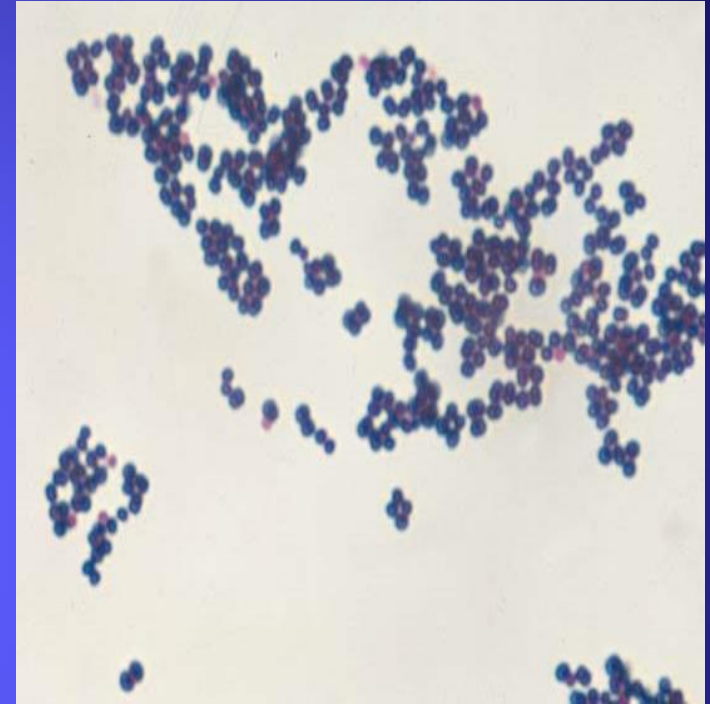
Gram positive bacteria



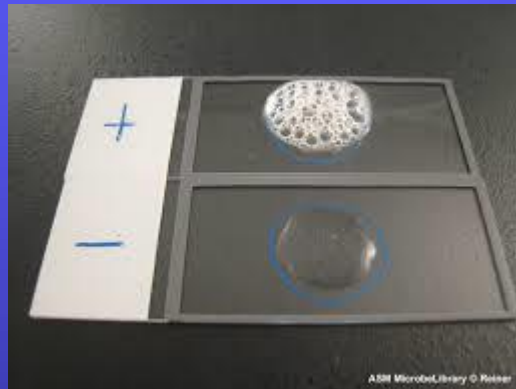




Gram positive cocci in chain
Streptococci



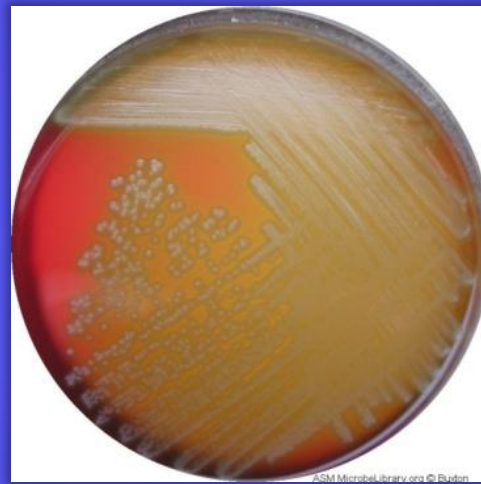
Gram positive cocci in clusters
Staphylococci



Catalase



Beta-
hemolyt
ic

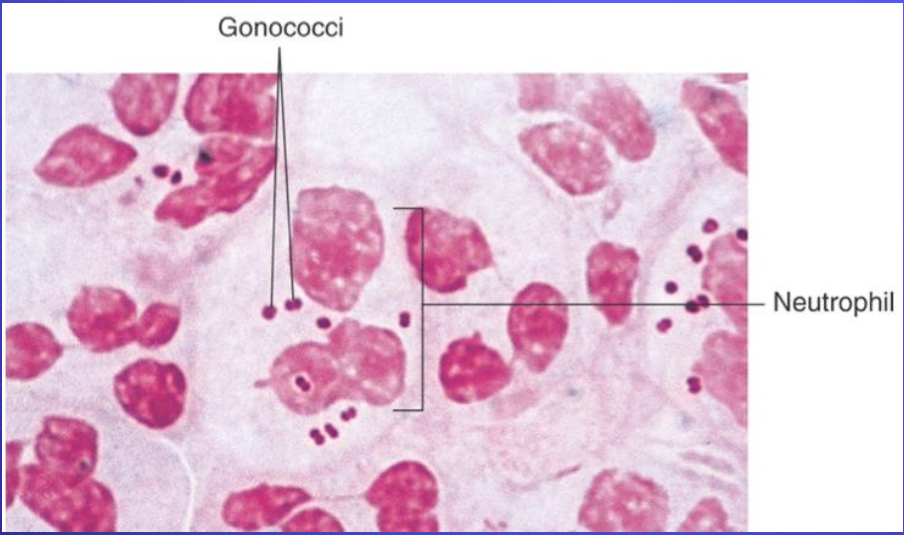
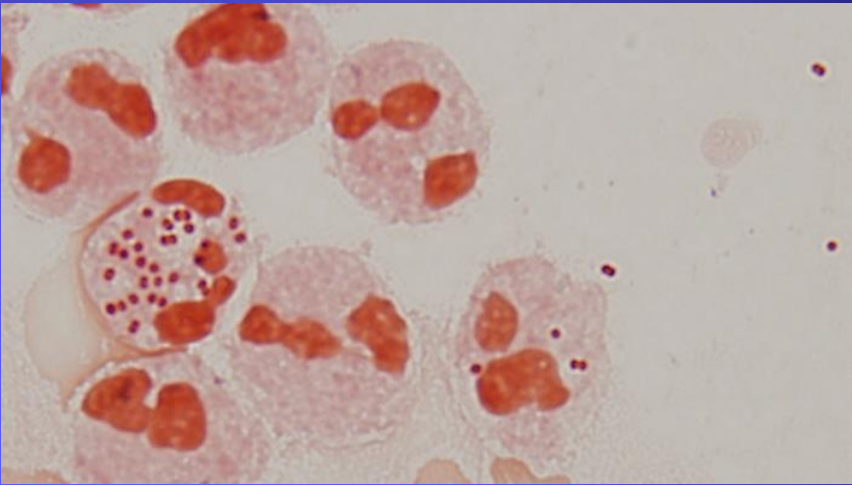


Alpha-
hemolyt
ic

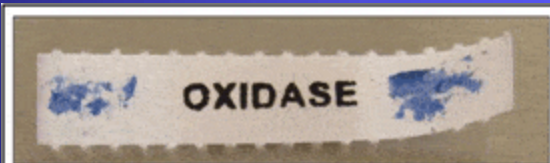


Non-
hemolyt
ic

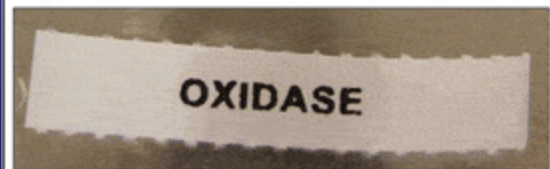
Gram	O2	Arrangement	Example	Diseases
Gram Positive Cocci		Gram + cocci in chains	Strep.pneumoniae Alpha hemolysis	Pneumonia meningitis
		Catalase negative	Group A strept Beta hemolysis	Pharyngitis (Sore throat) Rheumatic fever
	Aerobic		Group B strept Beta hemolysis	Neonatal infection
			Enterococcus Non hemolysis	Urine, blood infection
			Gram + cocci in clusters Catalase +	Staph aureus
			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
Anaerobic		Gram – cocci	Veillonella	Rare cause infection



Gram	O2	Characteristics	Examples
Gram Positive Bacilli	Aerobic	Spore forming	Bacillus anthracis
		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 positive



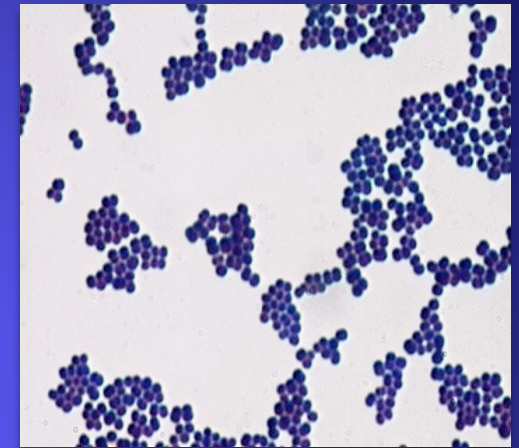
Oxidase negative



Gram-positive Cocci

- **Staphylococci**

- Catalase-positive
- Gram-positive cocci in **clusters**



- *Staphylococcus aureus*

- coagulase-positive, most important pathogen

- *Staph. epidermidis*

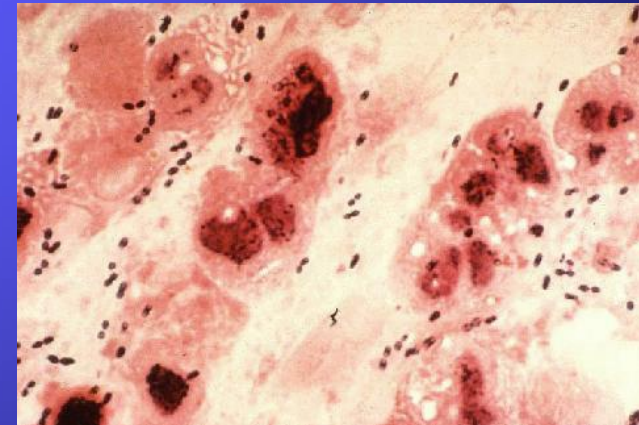
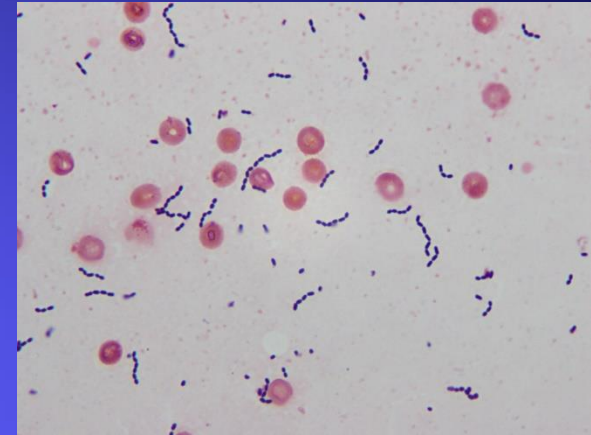
- and other coagulase negative staphylococci e.g. *S. saprophiticus*

Coagulase tubes

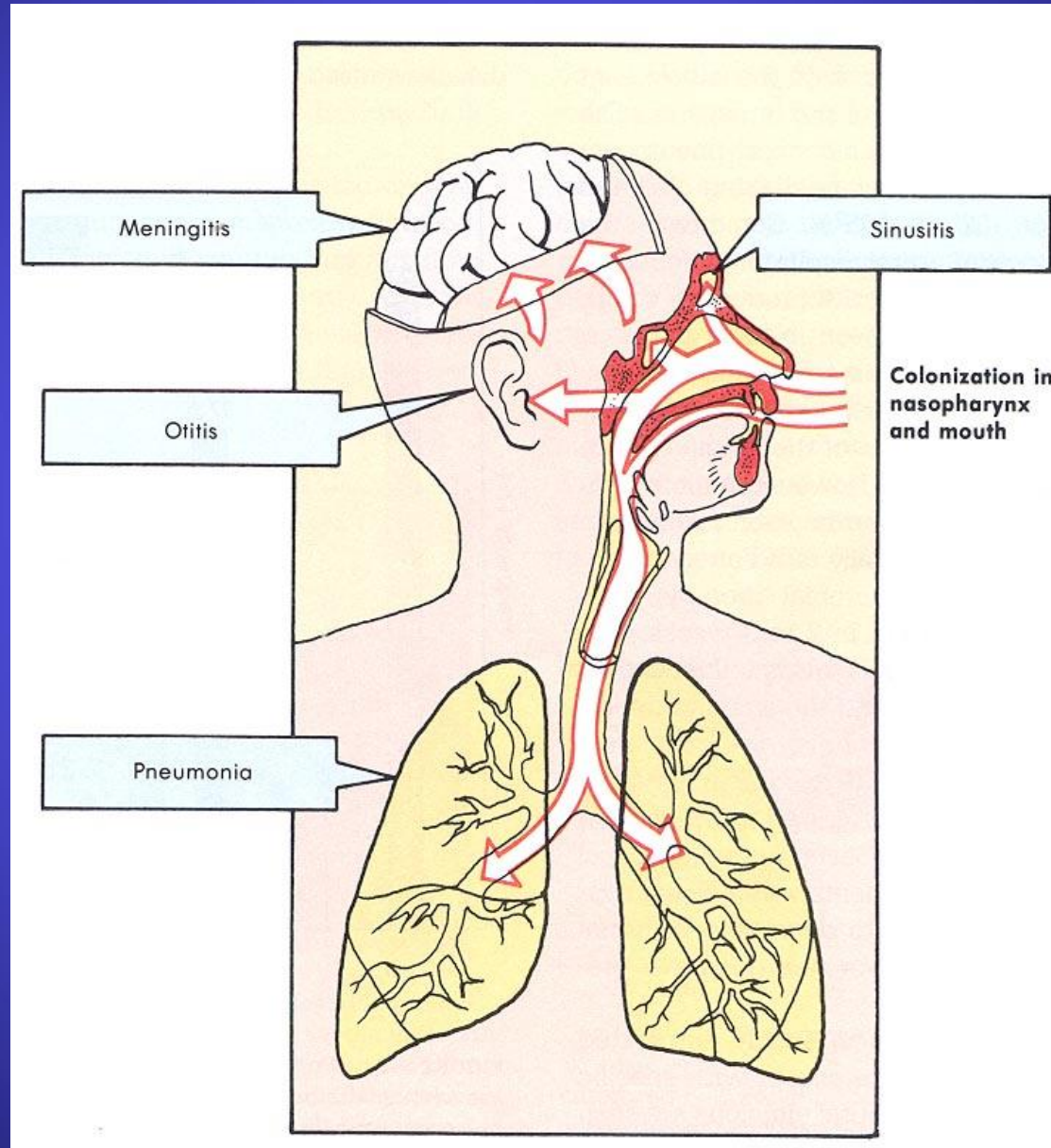


Streptococci

- 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

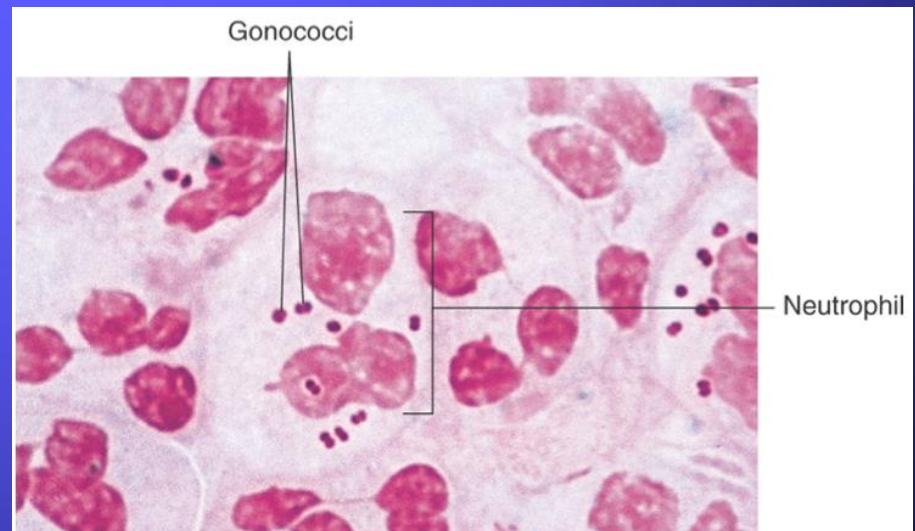
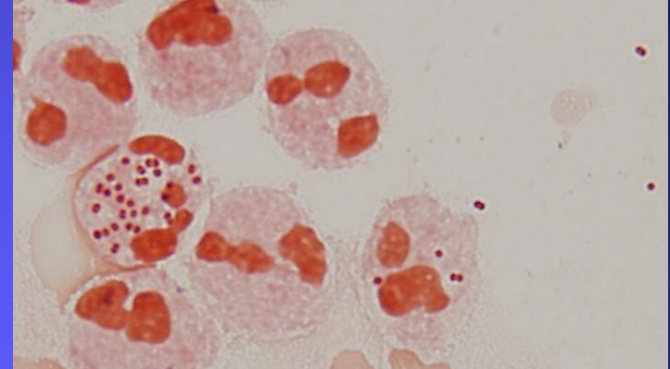
Aerobic gram positive bacilli

- **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*



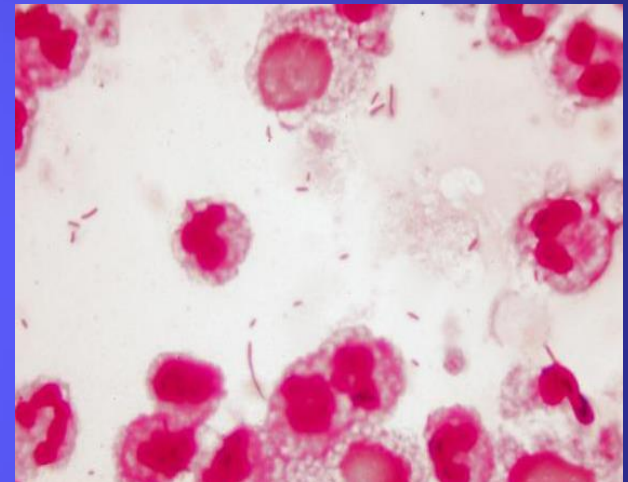
Gram-Negative Rods

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



Gram-Negative Rods

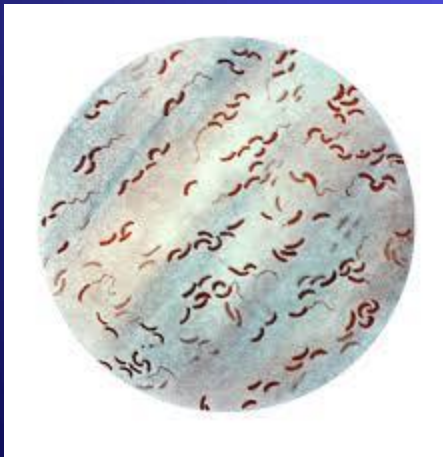
- Fastidious GNRs
 - *Bordetella pertussis*
 - *Haemophilus influenzae*
 - *Campylobacter jejuni*
 - *Helicobacter pylori*
 - *Legionella pneumophila*
- Anaerobic GNRs
 - *Bacteroides fragilis*
 - *Fusobacterium*



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

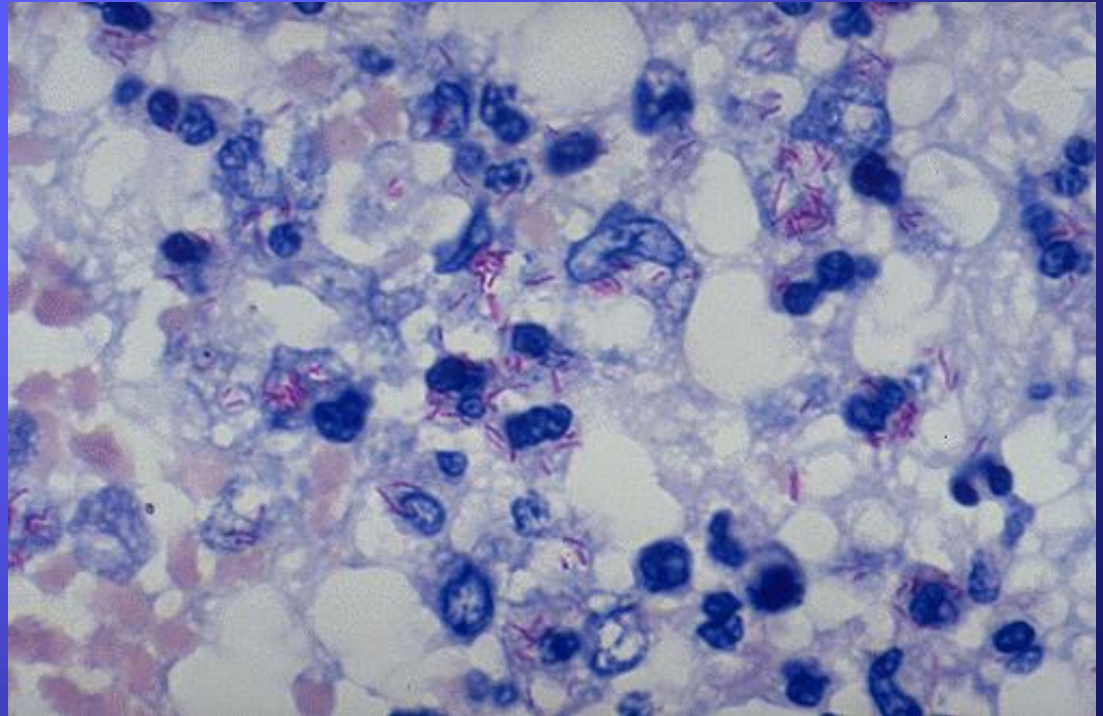


Non-Gram-stainable bacteria

- 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. pneumoniae*, *M. genitalium*

Obligate intra-cellular

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

Gram Positive

Cocci

Aerobic

Staphylococcus
Enterococcus
Streptococcus

Anaerobic

Peptostreptococcus

Bacilli

Aerobic

Bacillus
Corynebacterium
Listeria

Anaerobic

Clostridium

Gram Negative

Cocci

Aerobic

Neisseria
Moraxella

Anaerobic

Veillonella

Bacilli

Aerobic

E.coli
Klebsiella
Citrobacter
Salmonella
Shigella
etc

Vibrio
Aeromonas
Campylobacter
Helicobacter

Pseudomonas
Acinetobacter

Haemophilus
Legionella
Bartonella

Anaerobic

Bacteroides

**Mycoplasma, Chlamydia, Rickettsia
Spirochaetes
Mycobacterium**