Microbiology Team
Med441

GRAM POSITIVE & GRAM NEGATIVE BACTERIA

MED441
KING SAUD UNIVERSITY

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Editing File

Color Index:

- Main text
- Boys slides only
- Girls slides only
- Doctor's notes
- Extra information
- ***Important***

Objectives

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

Bacterial cells

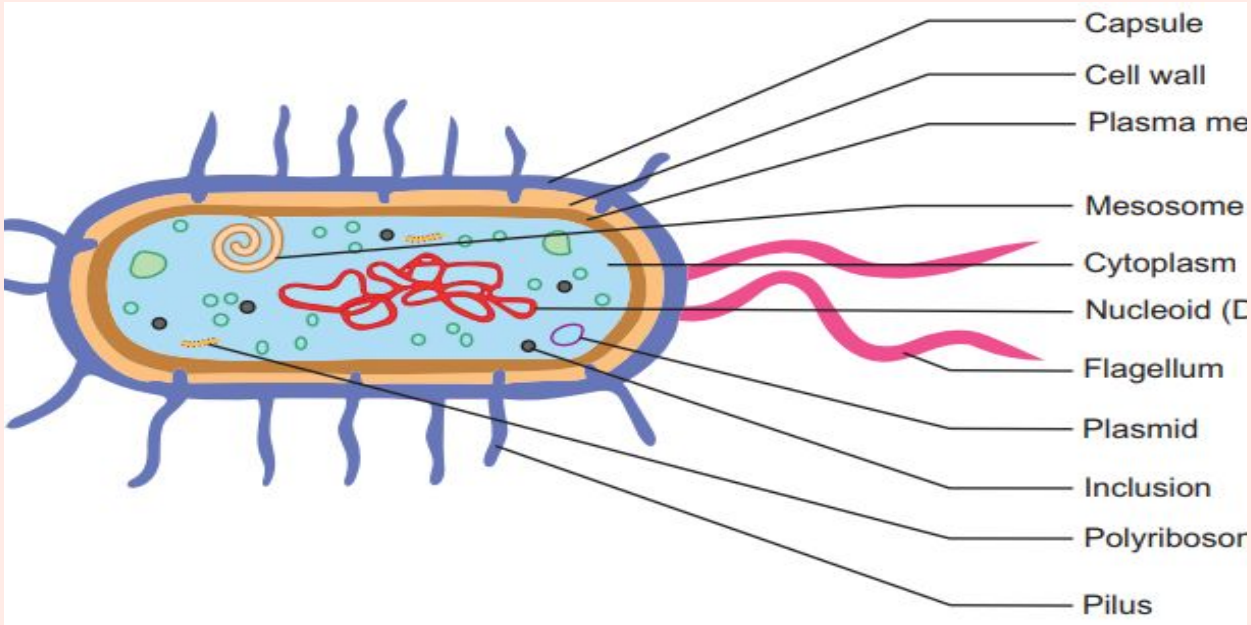
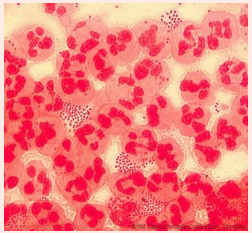


Figure 1.9: Ultrastructure of a bacterial cell

Gram Stain

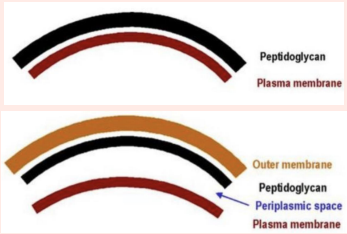
- Developed in 1884 by the Danish physician Hans Christian Gram
- An important tool in bacterial taxonomy (the branch of science concerned with classification).
  * It is used to distinguish **Gram-positive** bacteria, which **remain** coloured after the staining procedure, from **Gram- negative** bacteria, which **do not retain** dye and need to be **counter-stained.**
- Can be applied to pure cultures of bacteria or to clinical specimens.

Pure culture of E. coli
(Gram-negative rods)



Neisseria gonorrhoeae in a smear of urethral pus
(Gram-negative cocci, with pus cells)

Cell wall



Gram positive

1- Inner cytoplasmic membrane.
2- Outer thick homogenous peptidoglycan layer (20-80 nm).

The cell wall contains tightly bound acidic polysaccharides:
1- Teichoic acid
2- Lipoteichoic acid

Retain crystal violet and stain purple

Gram negative

1- Inner cytoplasmic membrane.
2- Thin shell of peptidoglycan
3- Outer membrane composed of lipopolysaccharide (LPS) .(endotoxin)

Have periplasmic space (between plasma membrane and the thin peptidoglycan layer)

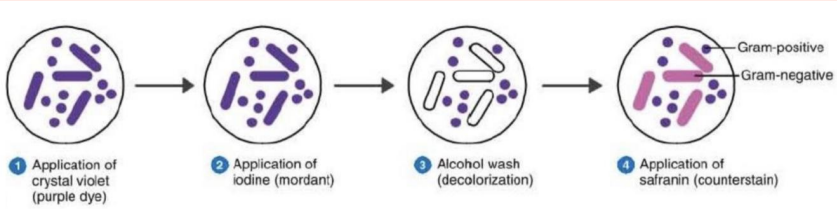
Lose crystal violet and stain pink from safranin counterstain.

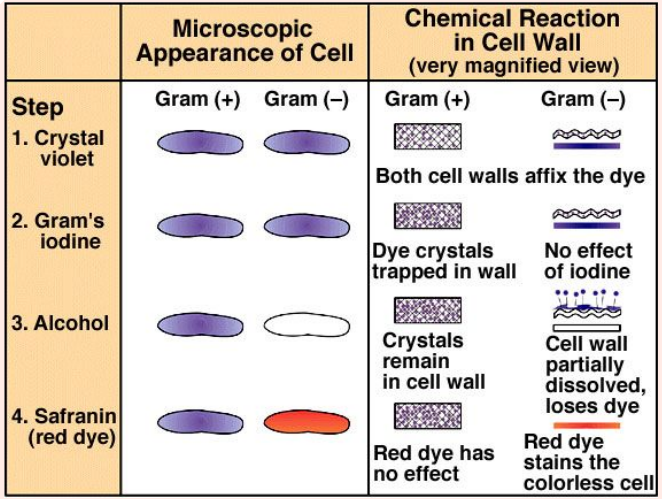
Gram Stain Process

Helpful video

There are 4 steps to the Gram stain :

1. Pour the **crystal violet stain**.
2. Wash off with water and flood with **iodine solution**.
3. Wash off with water and then decolorize with **acetone**.
4. **Counterstain** with ***safranin/methyl red***, and wash with water.





(From 439)
Bacteria can be classified based on their need for O2:
1. **Obligate aerobes**: require oxygen for survival & growth
2. **Facultative anaerobes**: they are anaerobic but can grow in aerobic environment if necessary
3. **Obligate anaerobes**: only grow under anaerobic conditions, die in the presence of oxygen

Gram positive *cocci*
Aerobic

Streptococci

Chain

Catalase-

“Subdivision in next slide”

Alpha **Beta** **Gamma**

Staphylococci

Catalase+

Clusters

Staphylococci can also be further classified based on present of ***Coagulase*** . (coagulase test)

Coagulase -
Other staphylococci (E.g Staphylococcus epidermidis)

Coagulase +
Staphylococcus Aureus
Most important pathogen

1-Gram Positive Cocci

All staphylococci have the enzyme catalase, Streptococci Do not

- Aerobic Gram + cocci can be divided based on 2 characteristics:

1. **Orientation (aerobic):**
   -Cluster (***Staphylococci***)
   -Chain (***Streptococci***)
2. **Presence of catalase enzymes (aerobic):**
   -Catalase +ve (***Staphylococci***)
   -Catalase -ve (***Streptococci***)

Dr.Note: Hydrogen peroxide is used to test the presence of catalase enzyme. If bubbles appear than it is staphylococci (catalase +ve)

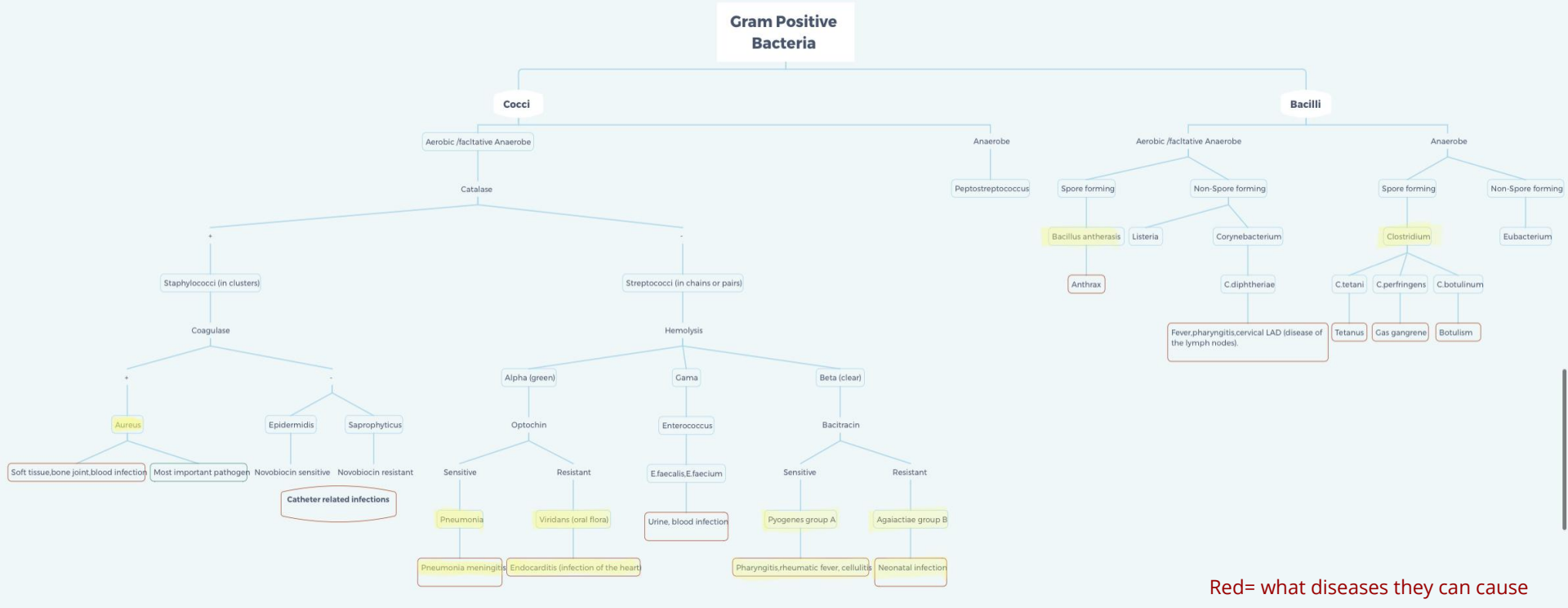
1.Streptocci: can be further divided into 3 groups based on their ***specific hemolytic*** ability:

Beta-hemolytic	**Alpha-hemolytic**	**Non-hemolytic** (a.k.a Gamma-hemolytic)
Completely lyse the RBCs, leaving clear zone of hemolysis around the colony	Partially lyse the RBCs, leaving a greenish zone of hemolysis around the colony	Unable to llyse the RBCs.
Group A:***Streptococcus pyogenes*** Group B: causes neonatal infection	***Streptococcus viridans***(oral flora) ***Streptococcus pneumonia*** (community aquired pneumonea)	E.g Enterococcus

2-Gram Positive bacilli

	Aerobic facultative anaerobe	Anaerobic
Spore forming	**Bacillus anthracis** Cause: Anthrax	**Clostridium** There are 3 types: -C.tetani Cause: Tetanus -C.perfringens Cause: Gas gangrene -C.botulinum Cause: Botulism
Nonspore forming	1-**Listeria** 2-**Corynebacterium**: C.diphtheriae that causes: Fever,pharyngitis,cervical LAD (disease of the lymph nodes). thick gray adherent membrane, subsequale airway obstruction, myocarditis.	**Eubacterium**

Gram Positive Bacteria Overview



Red= what diseases they can cause

Doctor's notes:
Gram positive bacilli are usually normal flora.

1-Gram Negative cocci

Aerobic / Facultative anaerobic	***Neisseria gonorrhoeae*** (Transmitted asexually)	***Neisseria meningitidis*** (Affects CNS)	***Moraxella catarrhalis***
Disease causes:	***Gonorrhea***	Meningitis	—

Both are gram -ve ***Intracellular diplocooci***

Anaerobic	Vellonella	———	———

2-Gram Negative Bacilli

Fastidious Aerobic

1) Bordetella pertussis
2) ***Haemophilus influenzae***
3) Campylobacter jejuni
4) Helicobacter pylori
5) Legionella pneumophila

Anaerobic

Bacteroides fragilis | Fusobacterium

Aerobic/Facultative anaerobic

Sugar Fermentation

Oxidase +

Pseudomonas

Causes:
infection (in immunocompromised patients)

Oxidase -

Acinetobacter spp .

Oxidase +ve

Vibrio cholerae
Comma shaped

Cause:
Cholera

Oxidase -ve

Also classified as ***enteric bacteria***

1) ***E.coli***
2) ***Salmonella***
3) Proteus
4) ***Shigella***
5) Yersinia & klebsiellosis pneumoniae

Diseases and Definitions

- **Clostridium botulinum** causes **botulism**, symptoms include:

  1- Paralysis

  2- Diplopia (double vision)

  3- Dysphagia (difficulty swallowing)

  4- Respiratory failure

- **Corynebacterium diphtheriae** could cause fever and **pharyngitis** which is characterized as thick, grey, adherent membrane at the back of the throat.

  - sequelae-->airway obstruction, myocarditis

- **Vibrio cholerae** could cause **cholera** which is characterized by severe diarrhea and dehydration.

Enterics : gram negative bacteria that are part of the GIT normal flora or cases GIT disease e.g ***E.coli*** , ***Salmonela*** .

Fastidious: have ***special nutritional*** requirements.

Non-Staining Gram Bacteria

01

Unusual Gram positive

Mycobacteria
Contain mycolic acid in cell wall

02

No cell wall (MYCOPLASMA)

Smallest free-living organisms
E.g
Mycoplasma pneumonia
Mycoplasma genitalium

03

Obligate intracellular bacteria

Chlamydia pneumoniae
Chlamydia trachomatis
Rickettsia

04

Some spirochaetes

Treponema
pallidum (cause of syphilis)

Summaries :

Cocci

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

Bacilli

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

[Gram positive bacteria summary]

[Gram negative bacteria summary]

Thank you Abdulaziz Alqahtani

[Gram +,- bacteria summary]

Thank you Yara Almufleh

Questions & Answers

Q:1 Which of the following bacteria is catalase +ve			
A) Staphylococci	B) Streptococci	C) Both	D) None
Q:2 the first reagent used in gram stain is:			
A) iodine	B) crystal violet	C) methyl red	D) acetone
Q:3 a patient presented to the hospital with severe diarrhea and dehydration, he most likely has:			
A) mycobacteria infection	B)peptostreptococcus infection	C) vibrio cholera infection	D) neisseria infection
Q:4 Which of the following considered as Gram Negative Fastidious Bacilli:			
A) haemophilus influenzae	B) Pseudomonas	C) E. Coli	D)Vellonella
A:5 Which of the following considered as Gram Negative Aerobic Cocci :			
A) Neisseria gonorrhoeae	B) Vibrio cholerae	C) botulinum	D) E.coli

1: A
2: B
3: C
4: A
5: A

Questions & Answers

Q:1 list 3 aerobic gram positive cocci :	A: staphylococcus , enterococcus , and streptococcus
Q:2 what is/are the gram Positive, bacilli ,aerobic and non spore forming bacteria:	A:listeria and corynebacterium
Q:3 list 2 Anaerobic gram negative Bacilli	Bacteroides fragilis , Fusobacterium
Q:4 What is the name of bacteria that causes Gonorrhea	Neisseria gonorrhoeae

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