

Gram Positive & Gram Negative Bacteria



Objectives

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

Colour index:

- **Red: Important.**
- Grey: Extra info & explanation.
- **Purple: only in girl's slides.**
- **Green: Only in boy's slides.**
-

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Gram Stain

Extra: The G in Gram stain must always be upper case because it refers to a name

- ❖ Developed in 1884 by the Danish physician Hans Christian Gram.
- ❖ An important tool in bacterial taxonomy (the branch of science concerned with classification).
 - Helps in determining the bacteria's shape (cocci or bacilli).
 - It is used to distinguish Gram-positive bacteria from Gram-negative bacteria.

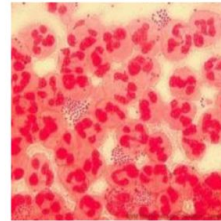
Retain stain color

Does not retain stain color, and must be counter stained

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



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



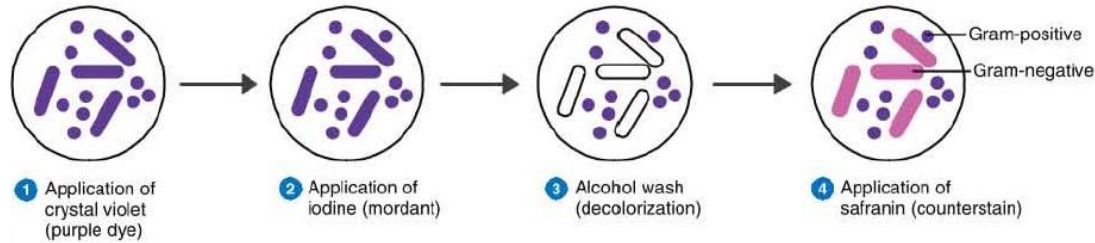
Neisseria gonorrhoeae in a smear of urethral pus (Gram-negative cocci, with pus cells)

Gram Stain Process:

Note: not all bacteria stain well with Gram stain

There are 4 steps to the Gram stain (ignore the time):

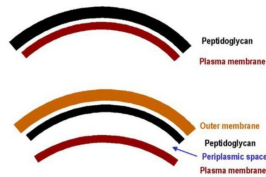
1. Pour the **crystal violet stain** and wait 60 sec
2. Wash off with water and flood with **iodine solution**, wait 60 sec
3. Wash off with water and then decolorize with **95% alcohol**
4. **Counterstain** with **safranin**, wait 30 sec and wash with water



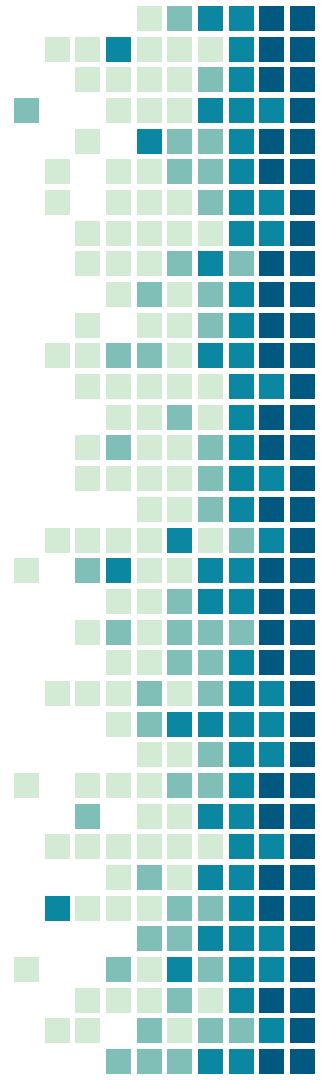
Bacteria can be classified based on their need for O_2 :

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

Cell Wall



Gram positive cell	Gram negative cell
<p>Cell wall is made of 2 layers:</p> <ol style="list-style-type: none">1- Inner cytoplasmic membrane.2- Outer thick peptidoglycan layer (20-80 nm).	<p>Cell wall is made of 3 layers:</p> <ol style="list-style-type: none">1- Inner cytoplasmic membrane.2- Thin peptidoglycan layer.3- Outer membrane composed of endotoxin lipopolysaccharide (LPS) and porins.
<p>The cell wall contains tightly bound acidic polysaccharides:</p> <ol style="list-style-type: none">1- Teichoic acid2- Lipoteichoic acid	<p>NO teichoic acid</p>
<p>NO periplasmic space</p>	<p>Have periplasmic space (between plasma membrane and the thin peptidoglycan layer) ★ It can trap antibiotics (E.g. penicillin), thus Gram negative can be more resistant than Gram positive.</p>
<p>Retain crystal violet and stain purple</p>	<p>Lose crystal violet and stain pink from safranin counterstain.</p>



Gram Positive Cocci

All staphylococci have the enzyme catalase , Streptococci DO NOT

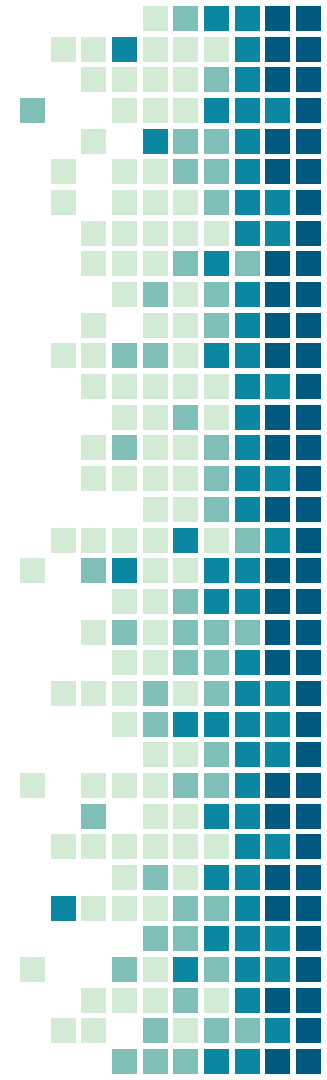
- Gram (+) cocci can be divided by 2 categories:
 1. **Orientation:** Either clusters (staphylococci) or chain (streptococci)
 2. **Presence of catalase enzyme:** Either catalase positive (staphylococcus) or catalase negative (streptococcus)

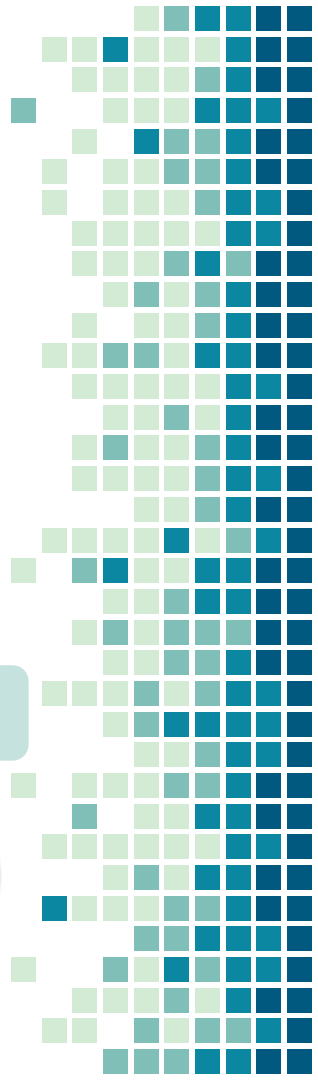
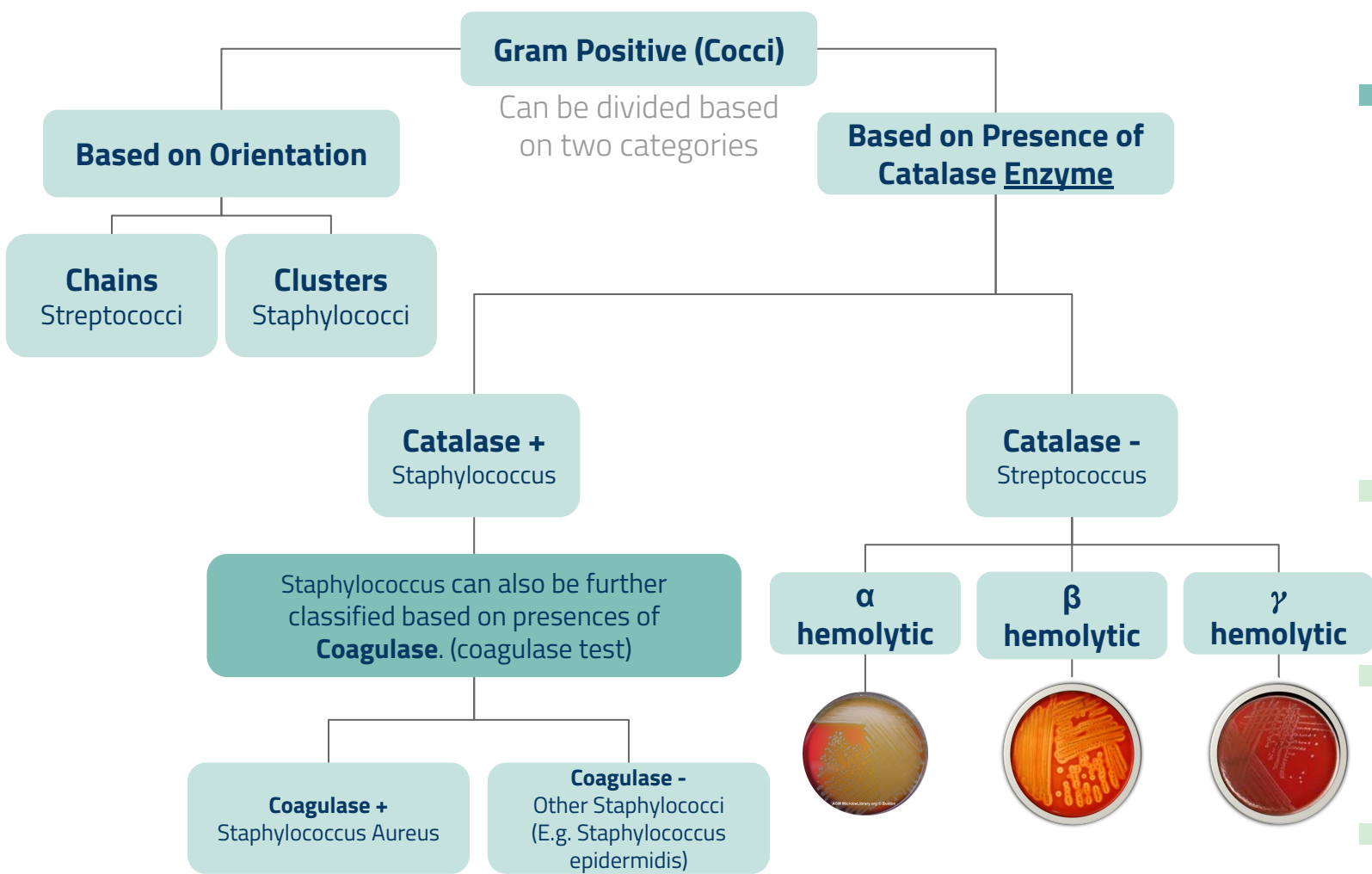
Extra: Hydrogen peroxide is used to test for the presence of catalase, if bubbles appear then its staphylococci if no bubbles appear then it is streptococci

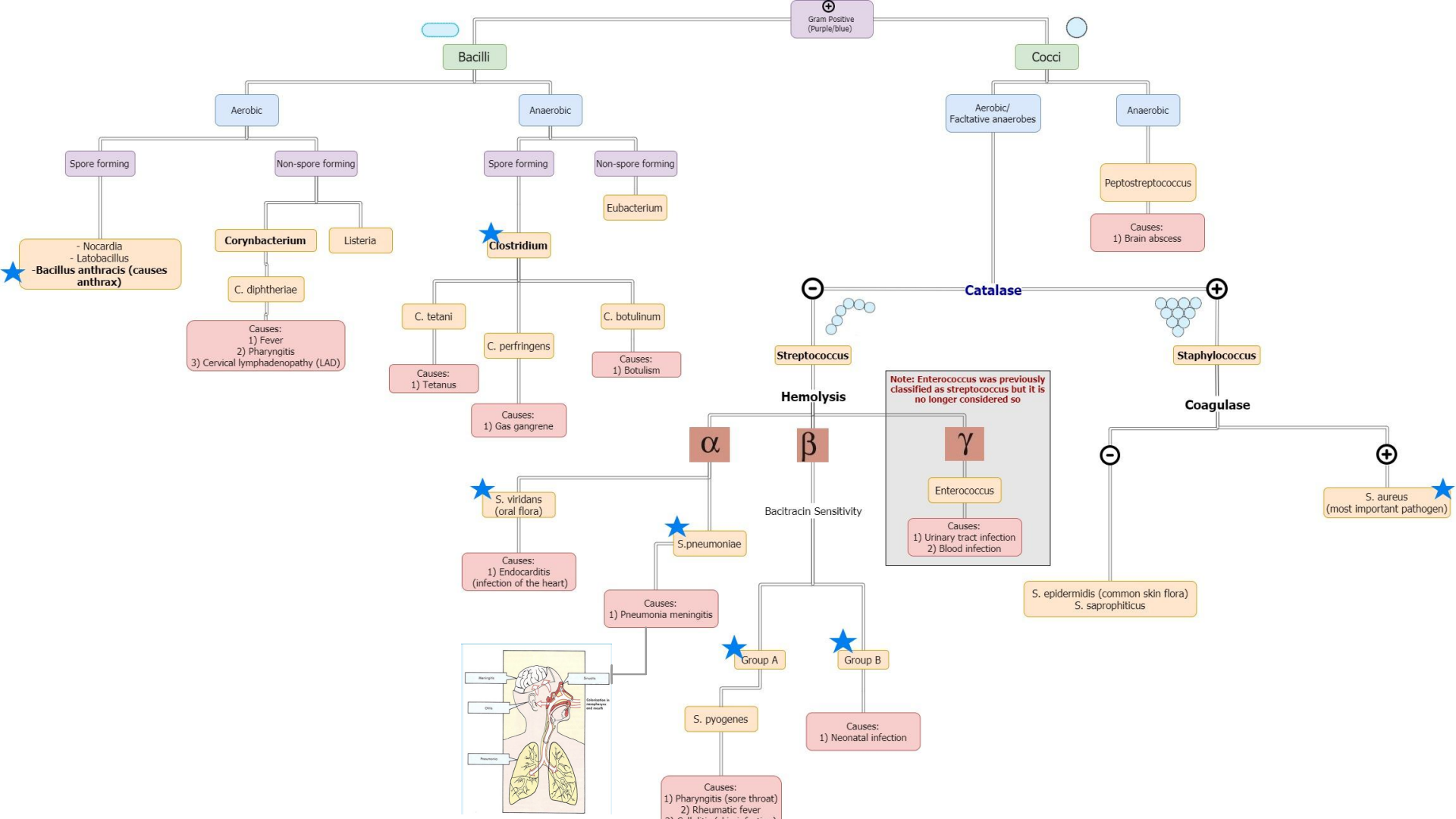
- Streptococci can be further divided into 3 groups based on their specific hemolytic ability:

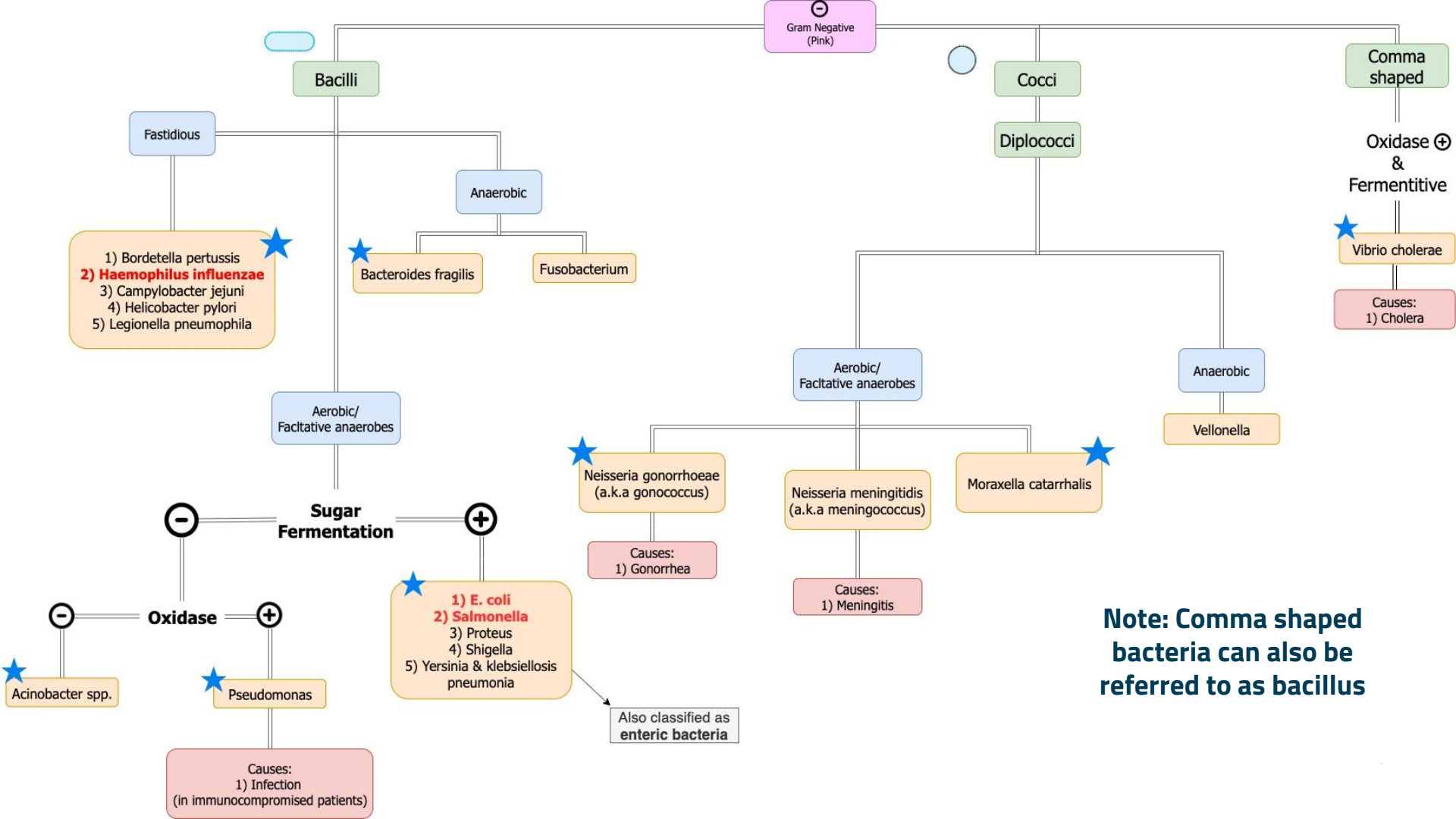
1. Alpha-hemolytic	2. Beta-hemolytic	3. Non-hemolytic (a.k.a Gamma-hemolytic)
Partially lyse the RBCs, leaving a greenish discoloration of the culture around the colony	Completely lyse the RBCs, leaving a clear zone (space) of hemolysis around the colony.	Unable to hemolyze RBCs
E.g <i>Streptococcus viridans</i> , <i>Streptococcus pneumoniae</i>	E.g <i>Streptococcus pyogenes</i>	E.g Enterococcus

Hemolysis: the destruction of RBCs which leads to the release of hemoglobin from within the RBC









Diseases and Definitions

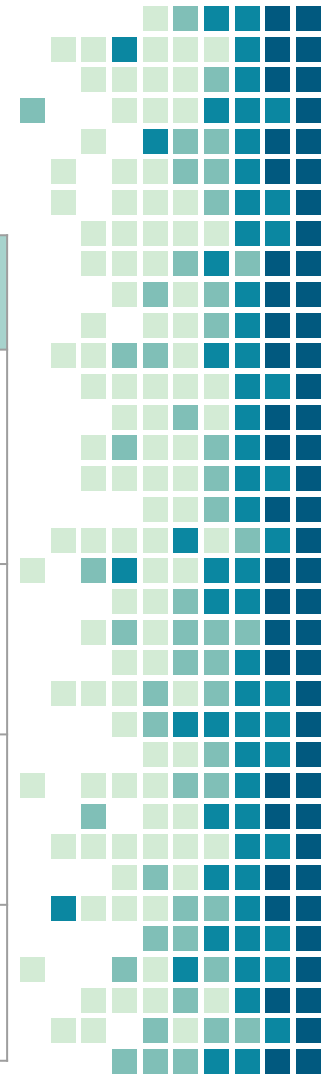
- **Clostridium botulinum** causes **botulism**, symptoms include:
 - 1- Paralysis
 - 2- Diplopia (double vision)
 - 3- Dysphagia (difficulty swallowing)
 - 4- Respiratory failure
- **Corynebacterium diphtheriae** could cause **pharyngitis** which is characterized as thick, grey, adherent membrane at 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 cause GIT diseases
e.g **E.coli**, **Salmonella**

Fastidious: have special nutritional requirements.

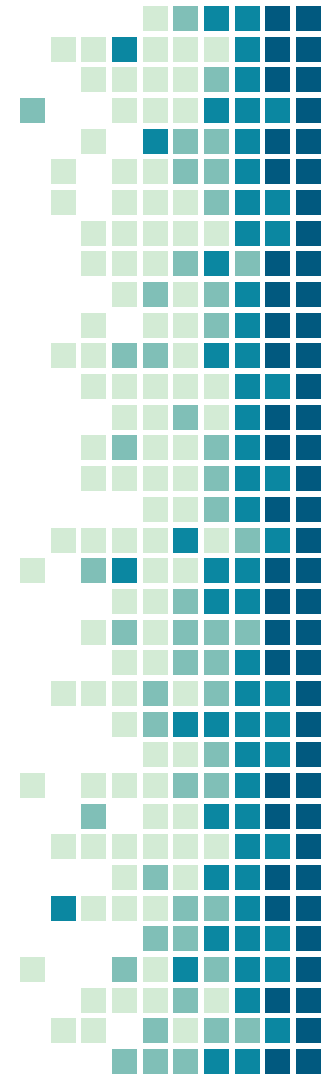
Non-Staining Gram Bacteria, cannot be stained.



Cause	Example
Unusual Gram positive (unusual cell wall)	Mycobacteria TB (cell wall contain mycolic acid which prevents stain retention) To avoid this problem acid-fast stain is used.
No cell wall (MYCOPLASMA)	-Mycoplasma pneumonia -Mycoplasma genitalium
Obligate intracellular bacteria (must live inside organism)	- Chlamydia pneumoniae - Chlamydia trachomatis - Rickettsia
Some spirochaetes	Treponema pallidum (cause syphilis) مرض الزهري

Summary

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MCQs

1-Which of the following is not a disease caused by clostridium spp:

- a) Tetanus
- b) Meningitis
- c) Gas gangrene
- d) Botulism

2-Which of the following statements is not true for E.coli:

- a) It is gram negative
- b) It is oxidase positive
- c) It is capable of fermenting sugar
- d) It is Bacillus

3-Give an example of fastidious bacteria

- a) Vibrio cholerae
- b) Listeria
- c) Acinetobacter
- d) Haemophilus influenzae

4-Which of the following bacteria has periplasmic space?

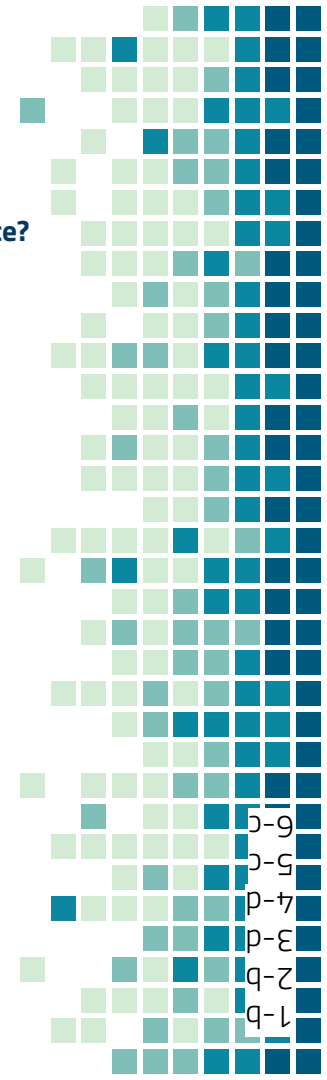
- a) Peptostreptococcus
- b) Listeria
- c) Corynebacterium
- d) Escherichia coli

5-Pharyngitis is a disease usually caused by:

- a) Group A alpha hemolysis
- b) Group B alpha hemolysis
- c) Group A beta hemolysis
- d) Group B beta hemolysis

6-What is the word used to describe microorganisms with a specific nutritional requirement?

- a) Spore-forming
- b) Facultative
- c) Fastidious
- d) none of the above



MCQs

7- A patient enters the hospital and complains with breathing problems, double vision and numbness in his lower limbs. He is later diagnosed with botulism, which of the following bacteria is responsible for this?

- A- Staphylococcus aureus
- B- Streptococcus pneumoniae
- C- Clostridium tetani
- D- Clostridium botulinum

8- Which of the following enteric bacteria can ferment sugars?

- A- Salmonella
- B- Vibrio cholera
- C- Streptococcus viridans
- D- Bordetella pertussis

9- Give an example of a fastidious Gram-negative rod.

- A- Helicobacter pylori
- B- Shigella
- C- Moraxella
- D- Corynebacterium

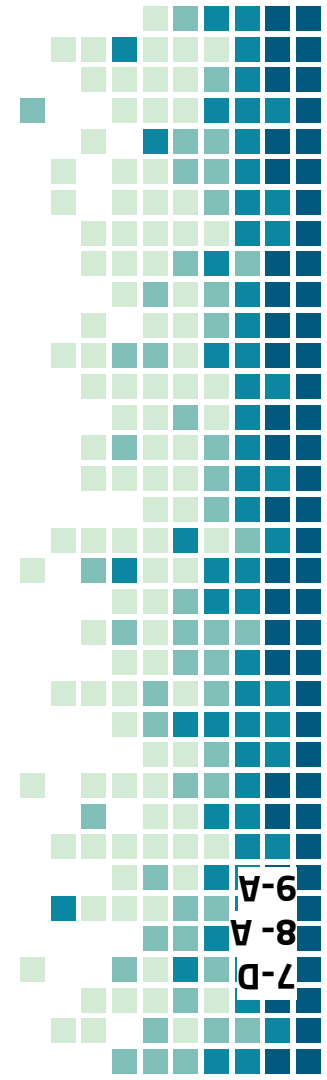
SAQ

Q1- State 2 methods that can be used to identify a bacteria as streptococcus or staphylococcus.

Gram stain & catalase test

Q2- Name an oxidase positive, non fermentative bacteria.

Acinobacter



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