MED437
KING SAUD UNIVERSITY

MICROBIOLOGY
437

Microbiology – Lecture 6
Gram Positive and Gram Negative Bacteria

TEAM 437

Red: important
Green : doctor notes
Black : original slides
Grey: extra information

In this link, you will find any corrections or notes unmentioned in the team's work. Please check the link below frequently.

https://docs.google.com/presentation/d/1yIQt3G8UDFG6xYMRhXkTk-dS54NeTfhJaPe_y0M-kjk/edit?usp=sharing

Objectives

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437

1. Know the general basic characteristics of bacteria
2. Differentiate between gram positive and gram negative bacteria characteristics.
3. Know the classes and groups of gram positive bacteria, cocci and bacilli (rods)
4. Know the common identification characteristic of these groups
5. Know the common infections and diseases caused by these organisms and the antibiotics used for their treatment
6. Know the classes and groups of gram negative bacteria, cocci and bacilli (rods)
7. Know the common identification methods for These organisms
8. Know the commonest infectious and diseases caused by these bacteria and the antibiotics used for their treatment

Bacterial cells general characteristics

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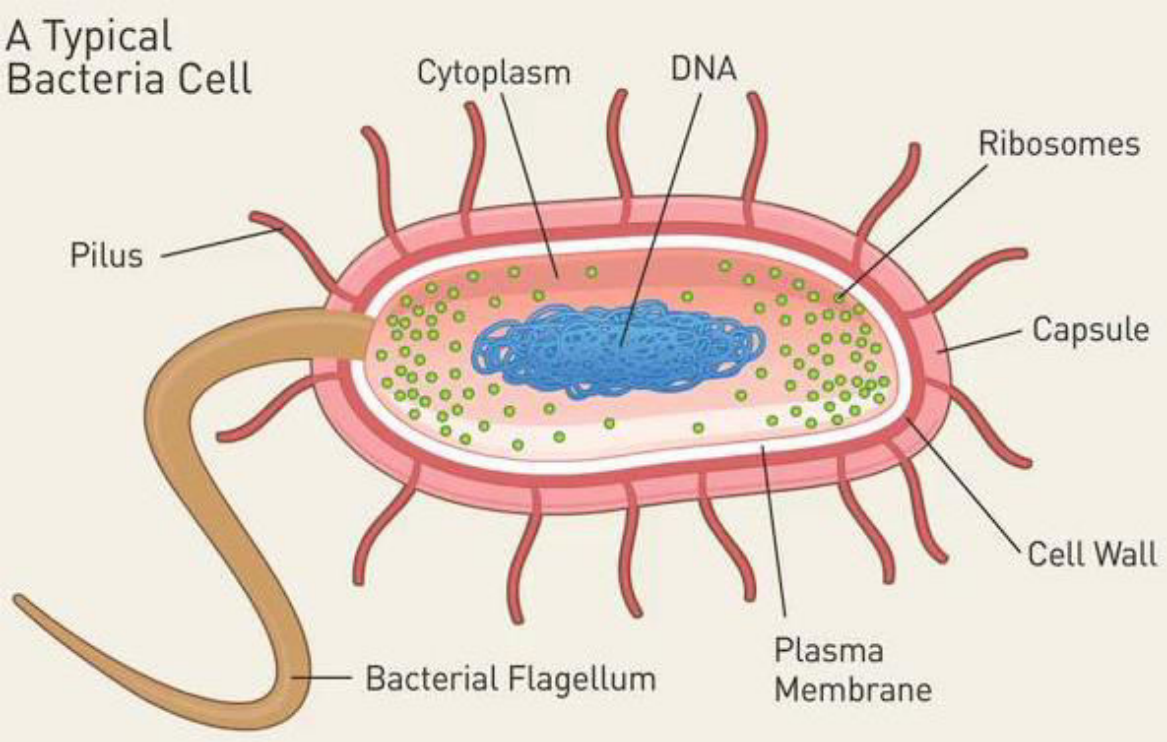

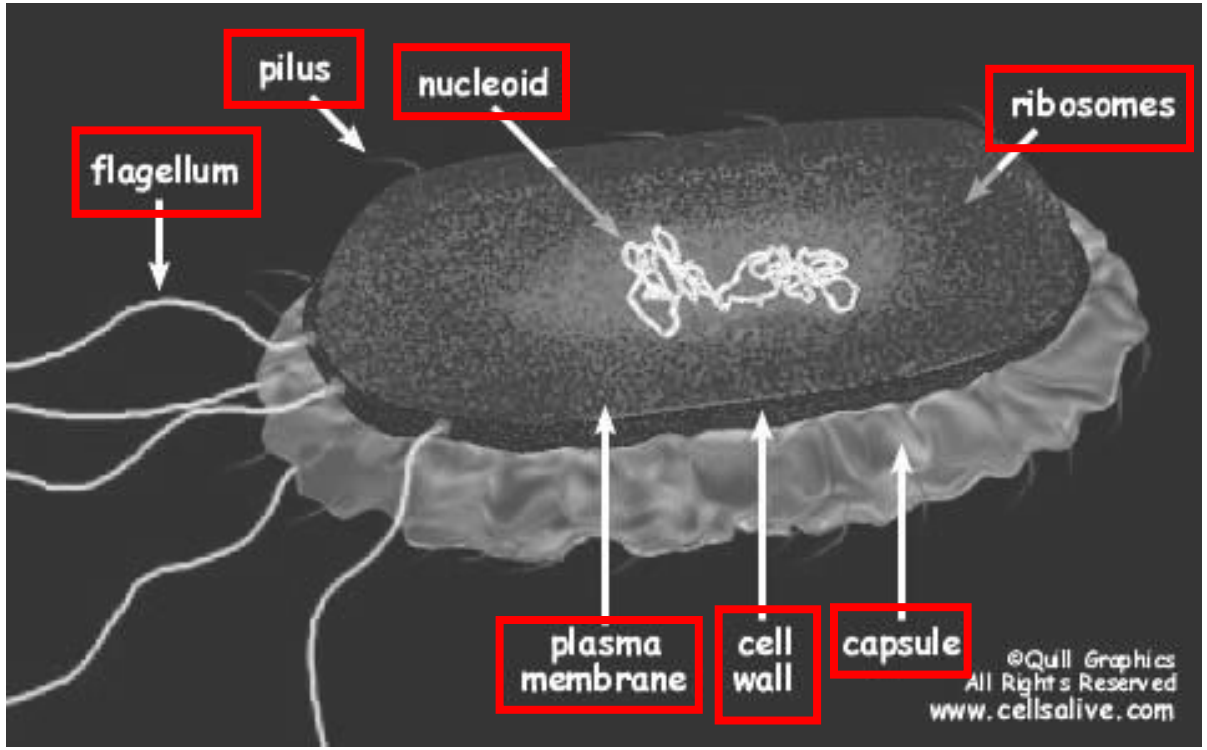

webpage with more info

Video with more info, skip to 1:27

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), distinguishing so-called **Gram-positive bacteria**, which remain colored 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**

Video for more info

Cell wall

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Gram positive cell wall

–Consists of:

1- Thick, homogenous sheath of peptidoglycan 20-80 nm thick

2- Tightly bound **acidic polysaccharides**, including **teichoic acid** and **lipoteichoic acid**

3- cell membrane

•Retain crystal violet and stain PURPLE or BLUE

Gram negative cell wall

–Consists of:

–1- An outer membrane containing lipopolysaccharide (LPS)

–2- Thin shell of peptidoglycan

–3- Periplasmic space (between the cell wall & plasma membrane)

–4- Inner membrane

•Lose crystal violet and stain PINK or RED from safranin counterstain

The Gram Stain

Video for more info

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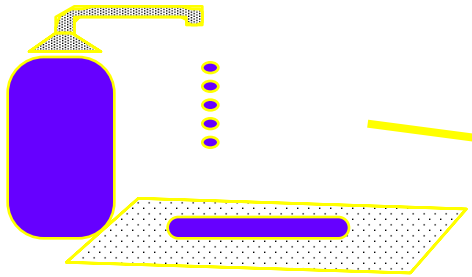
1

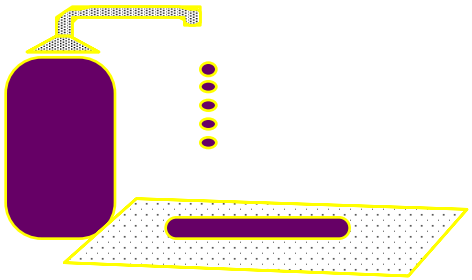
Crystal violet

Stain the Peptidoglycan in BLUE color. ALL bacteria will be stained with the crystal violet.

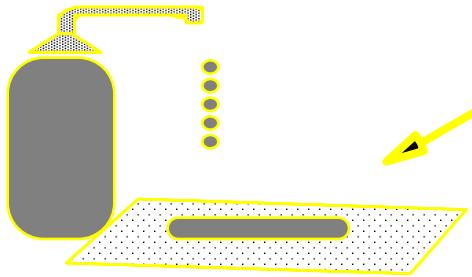


يثبت الصبغة

2 **Gram's iodine**



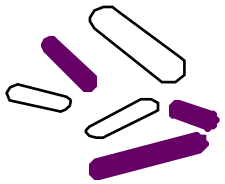
تحتفظ هذه البكتيريا بلونها بعد أن تصبغت بصبغة جرام مهما تعرضت لأي مادة كيميائية، فهي كالكرستال قوية وصلبة Team 435



3

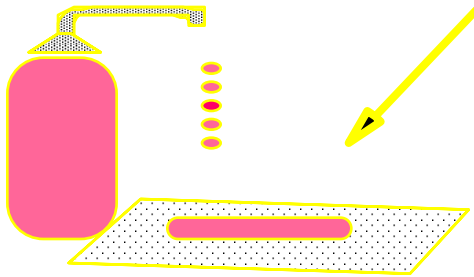
Decolorise with acetone

نخفف الصبغه بكحول



Alcohol: to extract the Crystal Violet stain from the peptidoglycan.
Gram -ve: extracted easily.
Gram +ve: hard to extract.

Red Stain

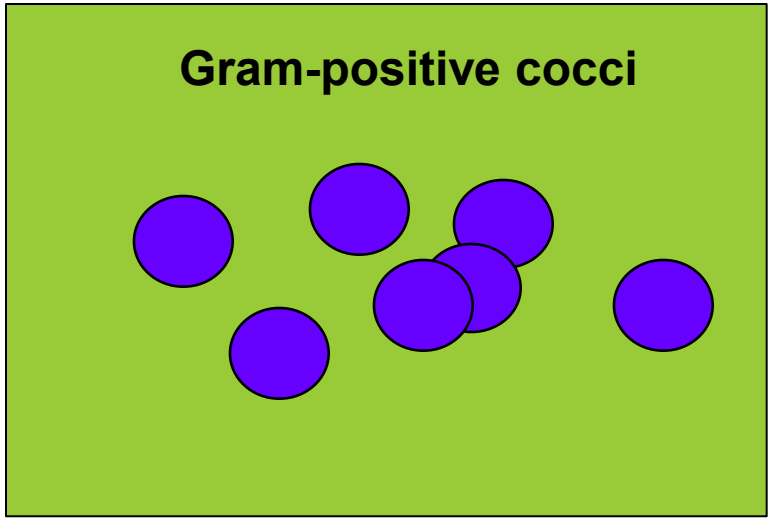


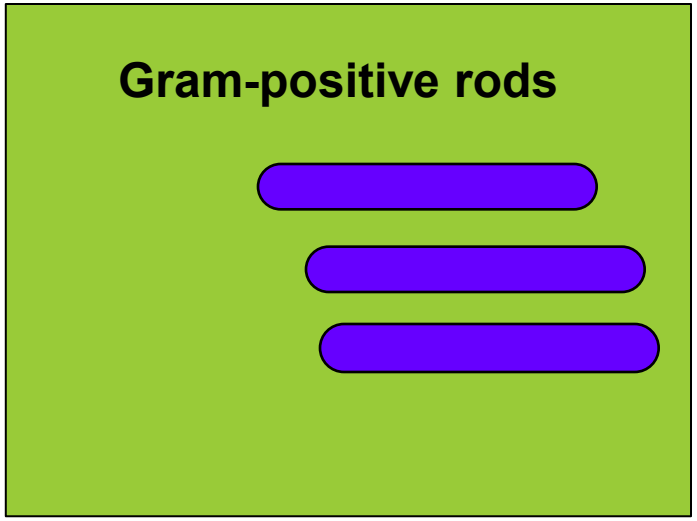
4

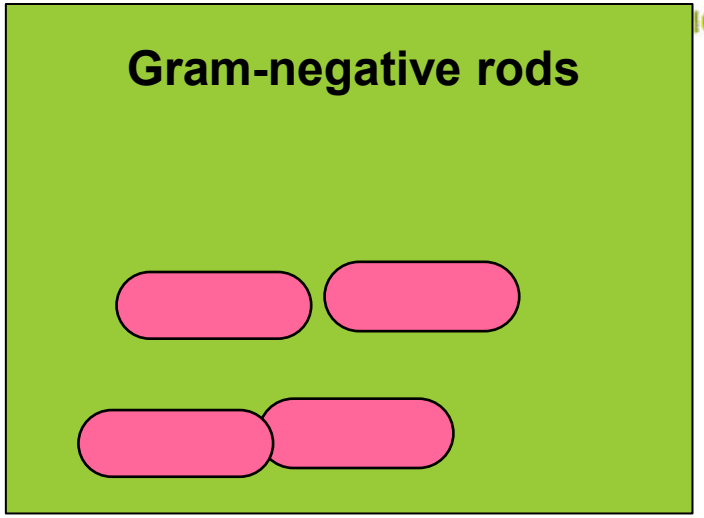
Counterstain (only for gram -) with e.g. methyl red

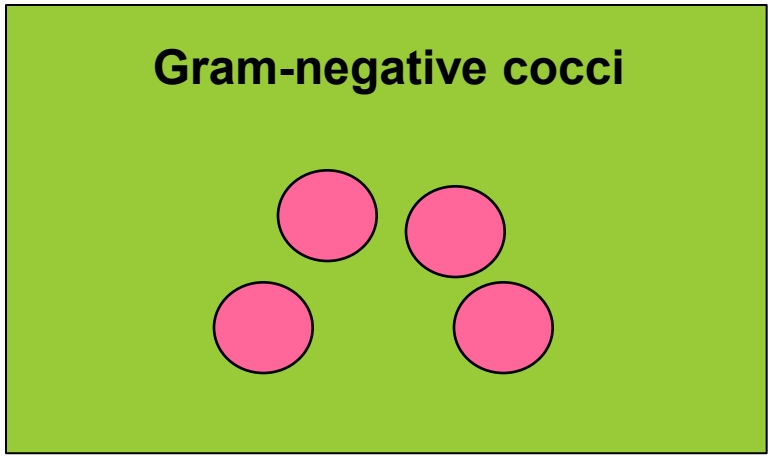
Safranin counterstain

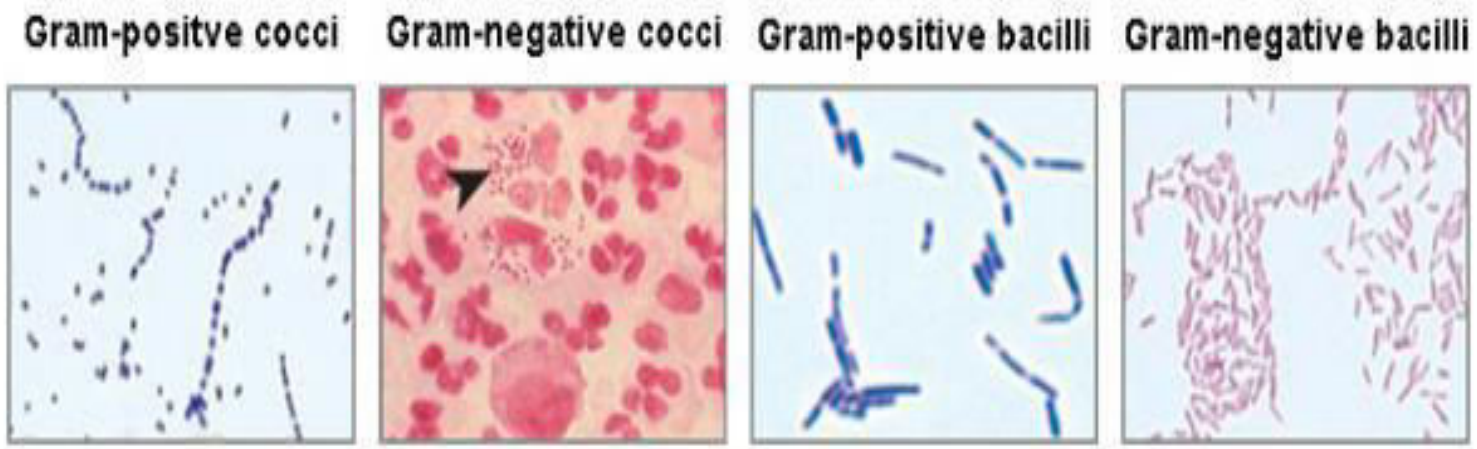
حتى لا تنسى تذكر أن:
الزعفران لونه أحمر و نوع هذه البكتيريا تتصبغ باللون الأحمر











Color and shape of the cells help classify which type of bacteria are present.

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The Gram Stain

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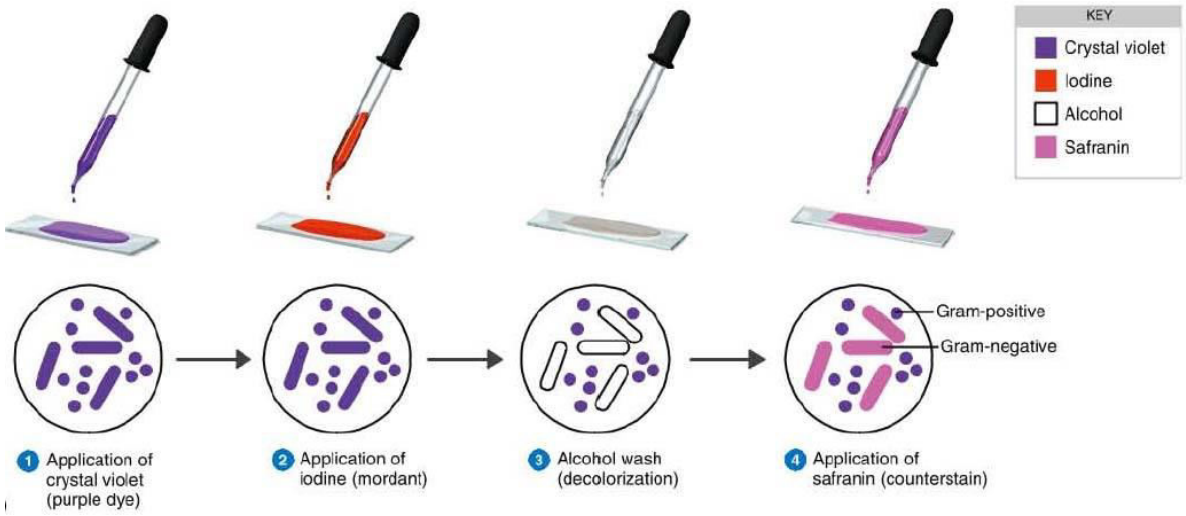

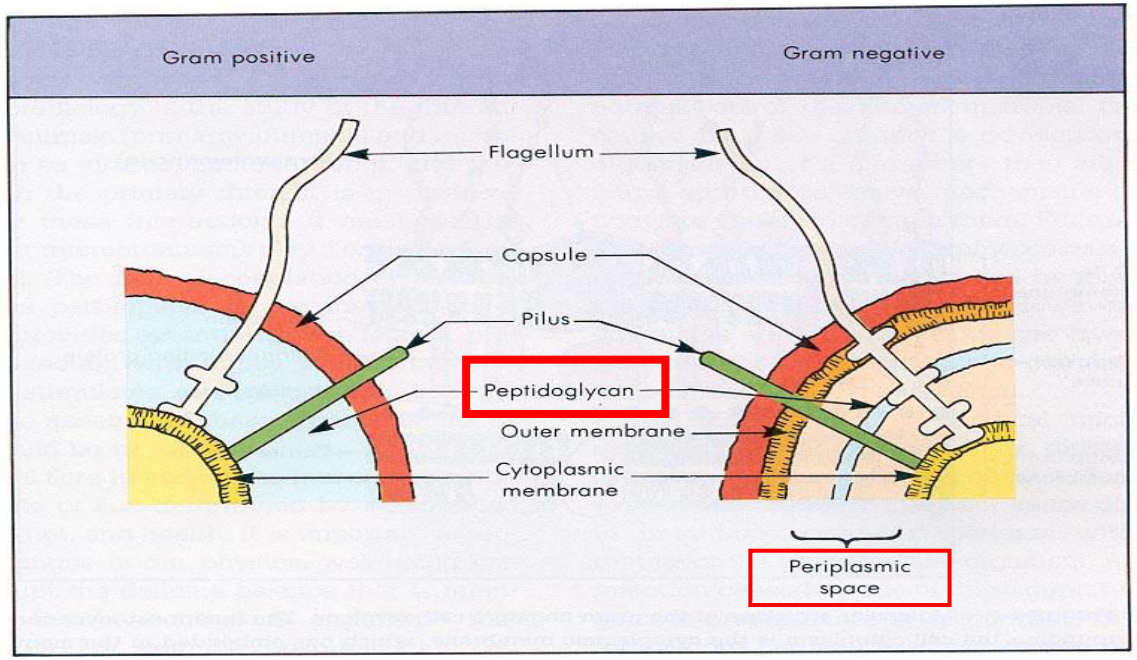

	Microscopic Appearance of Cell		Chemical Reaction in Cell Wall (very magnified view)	
Step	Gram (+)	Gram (–)	Gram (+)	Gram (–)
1. Crystal violet			Both cell walls affix the dye	
2. Gram's iodine			Dye crystals trapped in wall	No effect of iodine
3. Alcohol	تخفف اللون البنفسجي من الكحول و صار بدون لون		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

Acetone a type of alcohol

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Gram positive

Cocci

Anaerobe:

Peptostreptococci

capable of but not restricted to a particular function or mode of life

Aerobic /facultative Anaerobe:

1- Staphylococci (cluster catalase +)

2- Streptococci (chains catalase -)

3- Enterococci

Bacilli

Anaerobic:

1- Clostridium (SF)

2- Eubacterium (NSF)

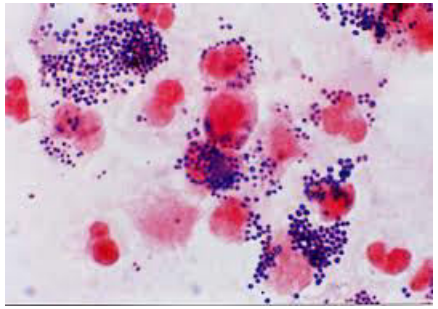
Aerobic/facultative anaerobe:

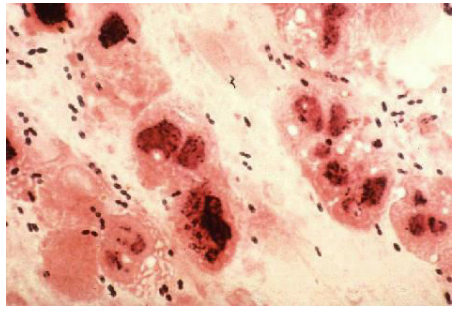
1- Cornybacterium (NSF)

2- Listeria Nocardia (NSF)

3- Latobacillus (NSF)

4- Bacillus Antheresis (SF)





Gram positive Cocci

Catalase: Biochemical test, to differentiate between streptococci and staphylococci.

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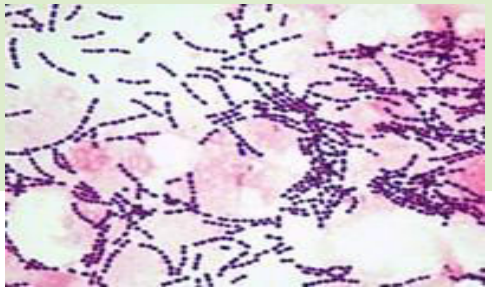
1- Staphylococci	2- Streptococci	3- Enterococci faecalis
Catalase-positive Gram-positive cocci in clusters ***A) Staphylococcus aureus:*** *coagulase positive most important* pathogen **B) Staphylococcus epidermidis:** Normal flora on the skin. other coagulase negative staphylococci ex, saprophyticus	**Catalase-negative** Gram-positive cocci in chains or pairs ***Examples:*** ***Strep. Pyogenes-*** Beta hemolytic Most clinically important of this group ***Strep. Pneumoniae-*** Alpha hemolytic and causes pneumonia and meningitis ***Viridans*** *streptococci* -Gamma hemolytic 	

Streptococcus

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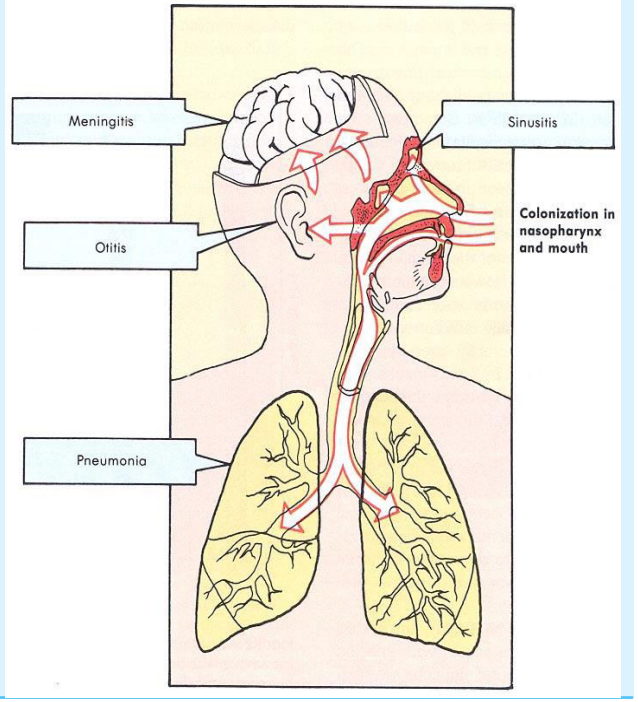
1- Streptococci viridans	2- Streptococci pyogenes divided by type of haemolysis	3- Streptococci pneumoniae Can cause meningitis
oral flora -infective endocarditis (infection of the heart)	**Group A, beta hemolytic strep** • Pharyngitis(most common) , cellulitis • rheumatic fever: (Autoimmune Disease) a) fever b) migrating polyarthritis c) carditis d) immunologic cross reactivity • acute glomerulonephritis (in the kidney) a) edema, hypertension, hematuria b) antigen-antibody complex deposition	

Gram Positive Bacilli:

1- Non spore forming
2- Spore forming which has the following:

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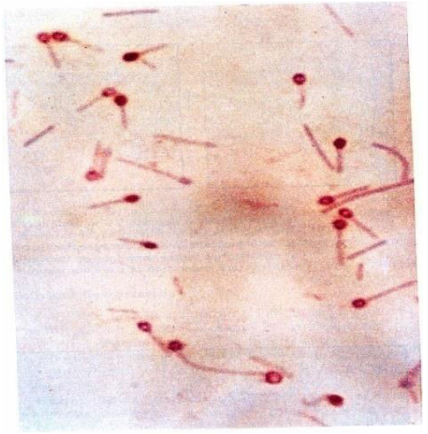
Anaerobic forming Example:

1- Clostridium tetani (C. Tetani) causes:

Tetanus



2- Clostridium perfringe (C. perfringens) causes:

Gas gangrene

3- Clostridium botulinum (C. Botulinum) causes:

a) botulism
b) Descending weakness-->paralysis
c) diplopia, dysphagia-->respiratory failure

4- C. diphtheriae causes:

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

Aerobic spore forming Example:

Bacillus anthracis, causes anthracis

Neisseria gonorrhoeae and Neisseria meningitis looks identical –kidney shaped-

Gram-Negative *Cocci*

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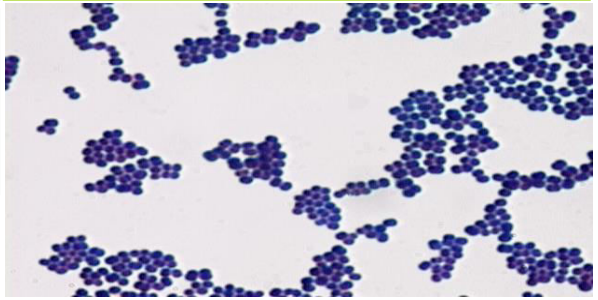
Neisseria gonorrhoeae	Neisseria meningitis (it is considered a potential pathogen in the orophyarynx)	Moraxella catarrhalis
The Gonococcus	1- The Meningococcus. 2- Both Gram-negative intracellular diplococci.	

Gram-Negative *Rods*

Enteric Bacteria they ferment sugars most important are:	Fastidious GNRs	Anaerobic GNRs
1- E. coli (normal flora but can cause diseases) 2- Salmonella (not normal flora "exogenous") 3- Shigella 4- Yersinia and Klebsiellosis pneumonia 5- Proteus	1- Bordetella pertussis 2- Haemophilus influenzae 3- Campylobacter jejuni 4- Helicobacter pylori 5- Legionella pneumophila	1- Bacteroides fragilis 2- Fusobacterium

Gram-Negative *Rods*

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437

Oxidase negative , non – fermentative i.e. they do not ferment sugars e.g:

- Acinobacter species

Oxidase positive comma shaped and also fermentative.

- The most important is Vibrio cholerae that causes cholera.

which is a disease characterized by:

1) Severe diarrhea.
2) Dehydration.

- Pseudomonas that causes infection in Immunocompromised patients (resistance to antibiotics)

Non-Gram-stainable bacteria

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Spirochaetes	Chlamydia	Unusual gram-positives (Mycoplasmas)
• Cause syphilis • Sexual transmitted	Obligate intra-cellular bacteria	- Smallest free-living organisms - No cell wall - M. pneumonia, M. genitalium

Quiz and references

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437

Which one of the following has periplasmic space?
A)Gram positive cell wall B)Gram negative cell wall C)both D)none

Which one of the following is not gram positive bacteria?
A)Staphylococci B)Streptococci C)Bacilli D)Mycobacterium TB

Oxidize positive bacteria have:
A)comma shaped B)rods shaped C)oval shaped D)spiral shaped

Non-Gram-stainable bacteria are:
A)Unusual gram-negative B)Usual gram-negative C)Unusual gram-positive D)Usual gram-positives

Mycoplasmas have:
A)no cell wall B)thin cell wall C)thick cell wall

B
D
A
C
A

Sherries Medical Microbiology, an Introduction to Infectious Diseases.

Latest edition, Kenneth Ryan and George Ray. Publisher : McGraw Hill .

لايقوى الإنسان في الحياة على هذه الأرض من دون أن يعاونه النّاس ويقفوا معه.

Team members:

فهد الفايز
سعد الهداب
خالد الدوسري
خالد المطيري
أنس السيف
عبدالجبار اليماني
عبدالله السرجاني
عبدالعزيز الدخيل
عادل العريني
محمد الدويغري

داود إسماعيل
عمر الفوزان
عبدالله الزهراني
معن شكر
عبدالمجيد الوردي
محمد إبراهيم
عمر السحيباني
سيف المشاري
سعد العقيلي
فهد الشغيرثي
حسين علامي

الهام العلامي
رناد المقرن
هديل عورتاني
اسراء النزاوي
لمياء القويز
شوق القحطاني
نورة القاضي
افنان المصطفى
رهف الشمري
الهنوف الجلعود

Team leaders:

غادة الحيدري ، علي الشحادة

For any corrections, suggestions or any useful information please contact us at: Micro.437@hotmail.com