# Mid Summary


اذكروا والداي بدعوة 🫶

By Hanadi MJ 🦋 & By Omar Debas 🤓

ملاحظة : بتقدروا تدرسوا منه و تعتمدوه لو درستوا من التفاريغ قبل لأنه شامل بإذن الله 🤍

مادة دكتور محمد كاملة ب ١٣ صفحة 👀

By Hanadi MJ

ايام ما كانت الحياة بسيطة

# Bacteria

Single celled prokaryotes

## Shape


## Gram stain

- Gram positive, G+
- Gram negative , G-

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## Lec 2

الترقيم بناء على رقم المحاضرة مش رقم الملف

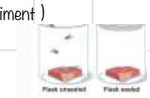
قبل ما نكمل عن البكتيريا ، خلينا ناخد تاريخ كيف بلشوا يكتشفوا هالمواضيع 👀

## History

**Aristole**
- things generate from non-living matters "Spontaneous generation".

**Robert Hooke**
- Cell theory – all living things are made up of cells , but still didn't see a microbe

**Francesco Redi**
- they claimed that fresh air was needed for spontaneous generation ( challenged the theory by an experiment )



**Anton van Leeuwenhoek**
- 1st person to see living microorganisms
- He's the father of microbiology

**Louis Pasteur**
- Disproving the Theory of Spontaneous Generation

**Robert kock**
- Koch's postulates used to prove that a specific microbe causes a specific disease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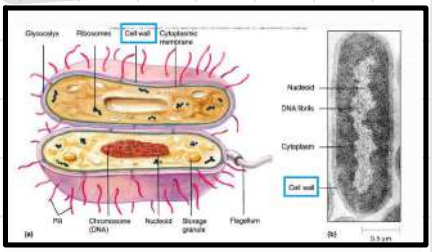
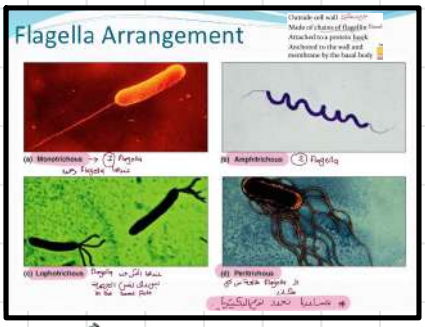
## Lec 3

### Prokaryotic cell




Spiral bacteria has endoflagella
it's rotation causes movement

### Bacteria's flagella for motility




الفرق بين نوعين الخلايا

| Prokaryote | Eukaryote |
|---|---|
| • One circular chromosome, not in a membrane | • Paired chromosomes, in nuclear membrane |
| • No histones | • Histones |
| • No organelles | • Organelles |
| • Peptidoglycan cell walls | • Polysaccharide cell walls |
| • Binary fission | • Mitotic spindle |

Filamentous appendages that are shorter, straighter and more numerous than flagella

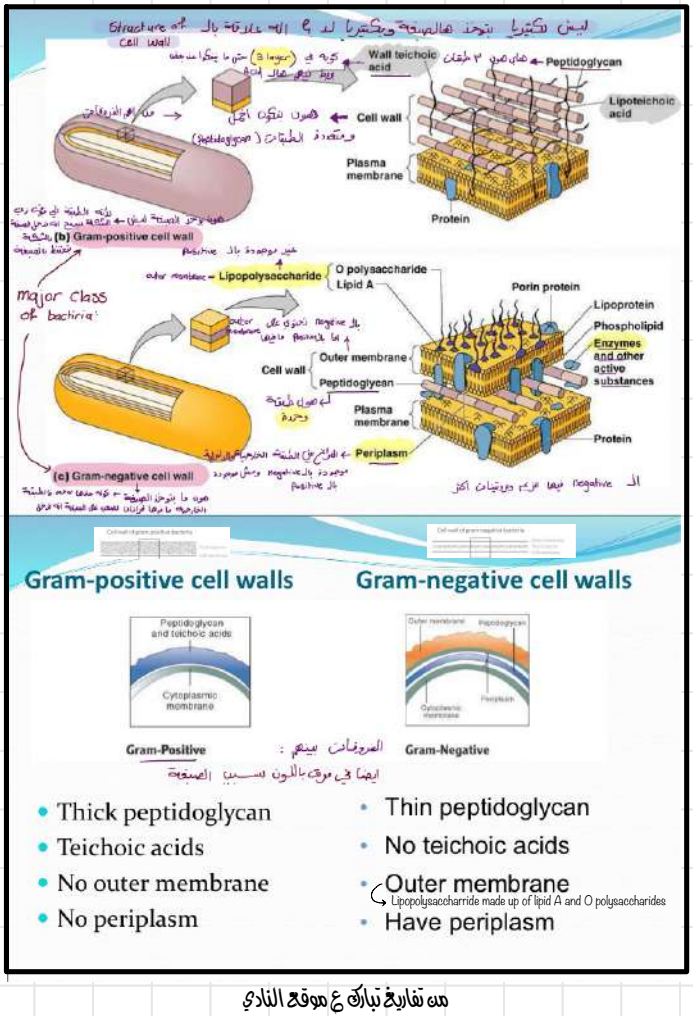
Found mostly in Gram (-) Bacteria

Fimbriae allow attachment while Pili are used to transfer DNA from one cell to another

: Pili & fimbriae

### Different cell walls

Cell wall Determines cell shape , Prevents osmotic lysis , Part of cell envelope & in some cases recognized by host immune system , the cell wall structure is made up of polymer of disaccharide of N-acetylglucosamine (NAG) & N-acetylmuramic acid (NAM) Linked by polypeptides




## Summary

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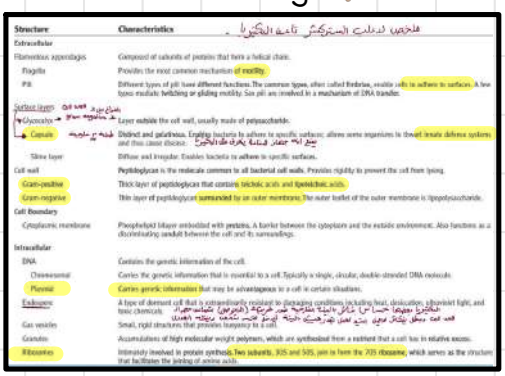
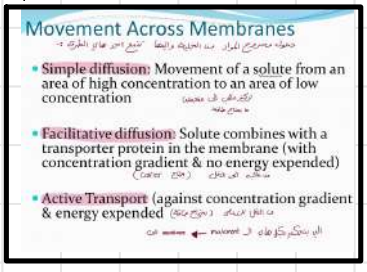



نفس اللي أختناه أيام البيو و الفسيو تقريبا



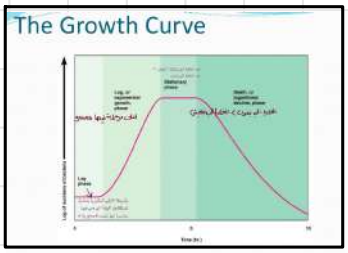

من تفاريغ تبارك ع موقع النادي

Lec 4

# Microbial growth or Binary Fission

**Generation time** is the time it takes for a single cell to grow and divide

الاسرع بالعلاج ⟵ الاسرع بالنمو

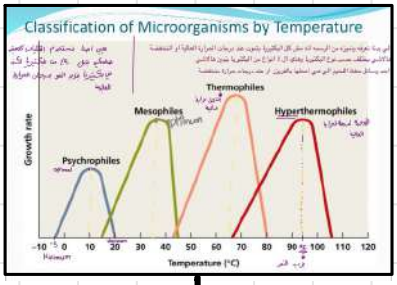


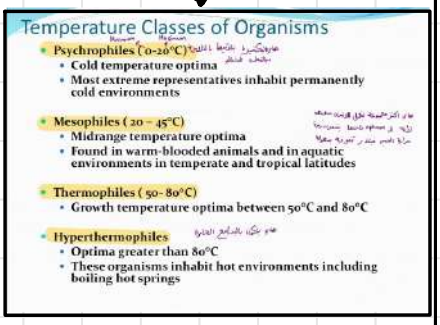

- During **lag phase**, cells are recovering from a period of no growth and are making macromolecules in preparation for growth
- During **log phase cultures** are growing maximally
- **Stationary phase** occurs when nutrients are depleted and wastes accumulate (Growth rate = death rate)
- During **death phase** death rate is greater than growth rate


## Factors Affecting Bacterial Growth

### 1) Temperature

- **Minimum Temperature:** Temperature below which growth ceases, or lowest temperature at which microbes will grow
- **Optimum Temperature:** Temperature at which its growth rate is the fastest
- **Maximum Temperature:** Temperature above which growth ceases, or highest temperature at which microbes will grow




### 2) PH

**Acidophiles:**
Grow optimally between pH ( 0 and 5.5 )

**Neutrophiles:**
Grow optimally between pH ( 5.5 and 8 )

**Alkalophiles:**
Grow optimally between pH ( 8 – 11.5 )

Examples :

Most bacteria grow between pH 6.5 and 7.5

Molds and yeasts grow between pH 5 and 6

Human blood and tissues has pH 7.2+0.2

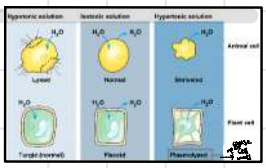
### 3) osmotic pressure

**Osmotic pressure** depends on the surrounding solute concentration and water availability

**Osmophiles:** organisms which thrive in high solute

**Osmotolerant:** organisms which tolerate high solute

Remember 👇



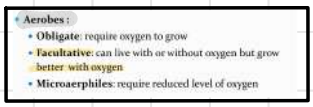

### 4) oxygen

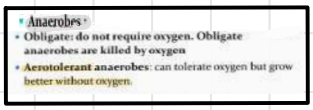
Using oxygen (O2 ) in metabolism creates toxic waste
Microbes that are able to use aerobic respiration produce enzymes to detoxify oxygen:

**Catalase:**
h2o2 > h2o & o2

**Superoxide dismutase** (SOD):
oxygen radical > h2o & o2

Classification of Organisms Based on O2 Utilization :




### 5) nutrition

Required nutrients:

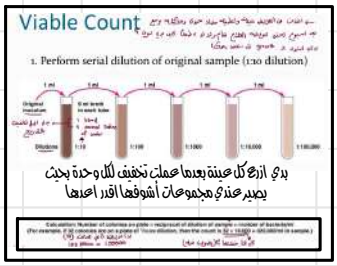
**Macronutrients :**
Elements required in fairly large amounts: Carbon , Nitrogen , Sulfur , Phosphorus

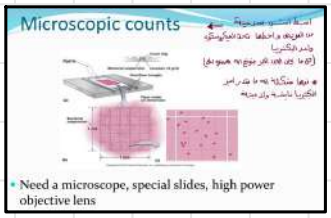
**Micronutrients :**
Metals and organic compounds needed in very small amounts, usually as enzyme and cofactors: Calcium, Copper, Iron, Magnesium, Manganese, and Iron

**Special requirements**
Amino acids , Nucleotide bases , Enzymatic cofactors or "vitamins"

## Methods Used to Measure Microbial Growth :

Count colonies on plate or filter (counts live cells) , Microscopic counts , Mass determination→ استخدام الوزن للتعبير كمية البكتيريا (الطريقة هي دقيقة) Turbitity , Measurement of enzymatic activity or other cell components



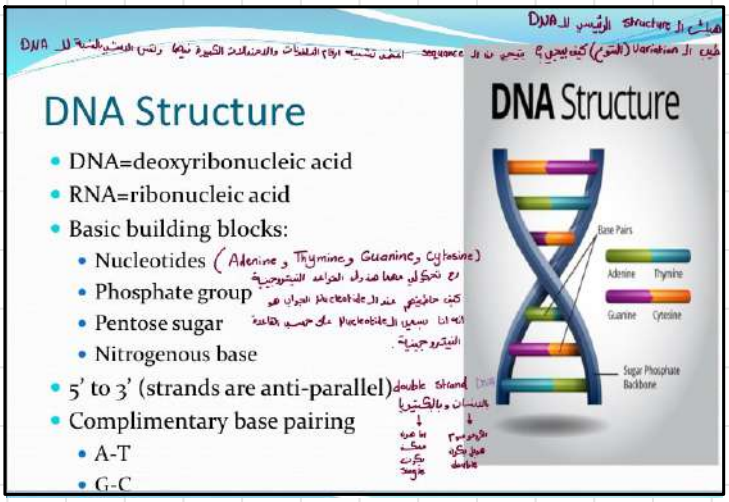


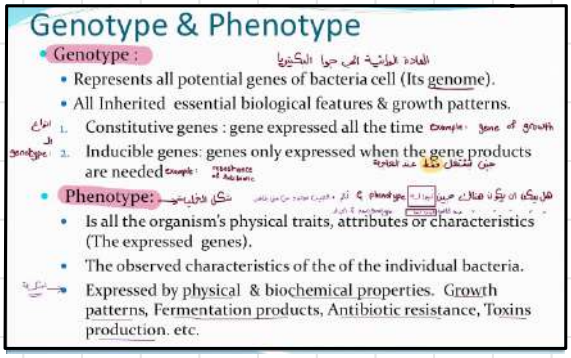
اللهم صلّ على سيدنا محمد

يلا ندخل شوية جينتكس على المايكرو

**Introduction** : Bacterial genome includes Chromosome & DNA Plasmid

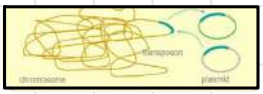
Genetic information is stored in DNA sequences found in : 1. Chromosomes 2. Plasmids

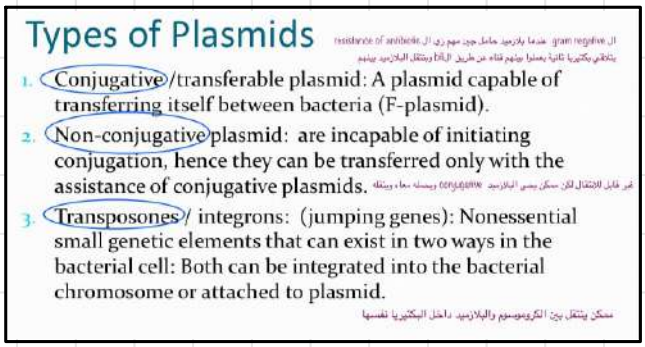



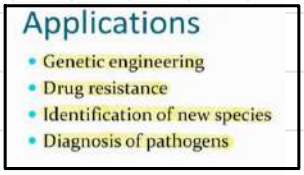

من تفاريغ تبارك ع موقع النادي

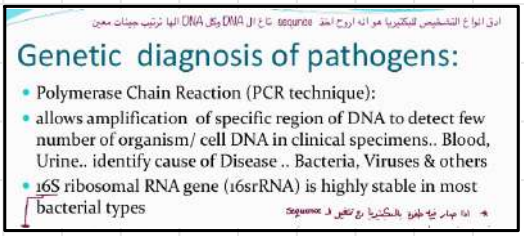
**Genetic information is encoded in DNA, transcribed into mRNA, translated on ribosomes through tRNA into various protein, polypeptides / structures and enzymes with diverse functions**



**Plasmid** : Extra-chromosomal piece of circular double-stranded autonomous DNA , Replicate by itself, It often carries nonessential genes such as resistance to antibiotics, virulence factors (enterotoxin, adhesion factor). Each contains 5-100 genes




**Genetic change: A major mechanism for the appearance of new pathogens/toxigenic strains, Development of antimicrobial resistance and can occur and become widespread over a short period of time**

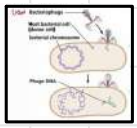
### Genetic recombination

Three processes bring bacterial DNA from different individuals together:

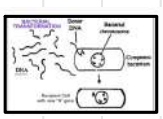
**Transduction**: Bacteriophages (bacterial viruses) transfer bacterial genes from one host cell to another

**Transformation**: Alteration of a bacterial cell's genotype and phenotype by the uptake of naked, foreign DNA from surrounding environment

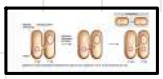
**Conjugation**: Direct transfer of DNA between live bacterial cells that are temporarily joined



Transduction



Transformation



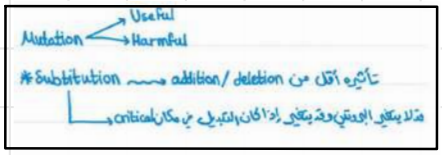
Conjugation

### By Mutations and genetic variation

Random, heritable, undirected variation due to a change in the nucleotide sequence of DNA .

Addition, deletion or substitution of a base pair

1. Spontaneous mutation
2. Induced mutation -mutagens



# Sterilization lec 6

**Note : British physician Joseph Lister (Father of antideptic)**



## Definitions

- **Sterilization:** A treatment that kills or removes all living cells, including viruses and spores, from a substance or object
- **Disinfection:** A treatment that reduces the total number of microbes on an object or surface, but does not necessarily remove or kill all of the microbes
- **Antiseptic:** A mild disinfectant agent suitable for use on skin surfaces
- **Sanitization** The process whereby pathogenic organisms are reduced to safe levels on inanimate objects
- **Biocide** A chemical or physical agent, usually broad spectrum, that inactivates microorganisms

## Resistant Microorganism

- **Bacterial endospores:** most resistant, only extreme heat or chemical treatment destroys them
- **Protozoan cysts and oocysts:** resistant to disinfectants; excreted in feces; causes diarrheal disease if ingested
- **Mycobacterium species:** waxy cell walls makes resistant to many chemical treatments
- **Pseudomonas species:** resistant to and can actually grow in some disinfectants
- **Non-enveloped viruses:** lack lipid envelope; more resistant to disinfectants

## Conditions Influencing Antimicrobial Activity

- Several critical factors play key roles in determining the effectiveness of an antimicrobial agent, including:
  - Population size
  - Types of organisms
  - Concentration of the antimicrobial agent
  - Duration of exposure
  - Temperature
  - pH
  - Organic matter
  - Biofilm formation

## Physical Methods

### Moist heat

Protein Denaturation and membrane disruption

**Boiling at 100°C:**
Effective against most vegetative cells; ineffective against spores; unsuitable for heat sensitive chemicals & many foods

**Autoclaving/pressure canning:**
Temperatures above 100°C achieved by steam pressure Most procedures use 121.1°C, achieved at approx. 15 psi pressure, with 15 - 30 min autoclave time

**Pasteurization:**
Used to reduce microbial numbers in milk and other beverages while retaining flavor and food quality
Traditional treatment of milk, 63°C for 30 min
Flash pasteurization (high-temperature short term pasteurization); quick heating to about 72°C for 15 sec, then rapid cooling

### Dry heat

**Incineration**
Burner flames
Electric loop incinerators
Air incinerators
**Oven sterilization** 1
Used for dry glassware & heat-resistant metal equipment Typically 2 hr at 160°C is required to kill bacterial spores by dry heat

### Low temperature.

**Refrigerator:**
around 4°C
inhibits growth of mesophiles or thermophiles;
psychrophiles will grow
**Freezer:**
"ordinary" freezer around -10 to -20°C
"ultracold" laboratory freezer typically -80°C
Generally inhibits all growth; many bacteria and other microbes may survive freezing temperatures

### Filtration.

Used for physically removing microbes and dust particles from solutions and gasses; often used to sterilize heat-sensitive solutions or to provide a sterilized air flow

**Depth filters:** Thick porous filtration material (e.g., cellulose), Larger pores
**Membrane filters:** Small pore size (0.2 µm) to remove bacteria, Thin, eg. Nitrocellulose
**HEPA filters:** High efficiency particulate air filters used in laminar flow biological safety cabinets

### Radiation

**Ultraviolet Radiation :**
DNA absorbs ultraviolet radiation at 260 nm wavelength & This causes damage to DNA in the form of thymine dimer mutations & it's Useful for continuous disinfection of work surfaces

**Ionizing Radiation :** 2
Gamma radiation produced by Cobalt-60 source
Powerful sterilizing agent; penetrates and damages both DNA and protein; effective against both vegetative cells and spores
Often used for sterilizing disposable plastic labware

## Chemical agents

قراءة

### Phenolics

Aromatic organic compounds with attached -OH
Denature protein & disrupt membranes
Commonly used as disinfectants e.g. "Lysol"

### Alcohols

Ethanol; isopropanol; used at concentrations between 70 – 95% that Denature proteins; disrupt membranes & Kills vegetative cells of bacteria & fungi but not spores

### Halogens

Act as oxidizing agents; oxidize proteins & other cellular components as Chlorine compounds ( disinfectant ) and iodine compounds ( antiseptics )

### Heavy metal

Mercury, silver, zinc, arsenic, copper ions it Form precipitates with cell proteins

Toxic

### Quaternary Ammonium compounds

Quaternary ammonium compounds are cationic detergents they Denature proteins and disrupt membranes

### Aldehydes

Formaldehyde and gluteraldehyde React chemically with nucleic acid and protein, inactivating them

Remember 👉

**GENERAL MECHANISMS OF BIOCIDE ACTION**
Disruption of the Cell Membrane or Wall
Protein Denaturation
Disruption of Free Sulfhydryl Groups
Damage to DNA
Chemical Antagonism

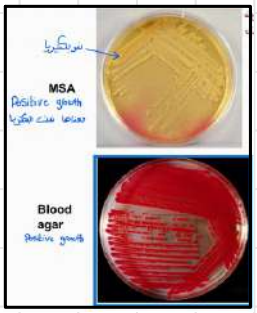
# Lec 7 : Diagnosis of microbial growth

ملاحظة : اختطرت اسلك هاي المحاضرة بهاي الطريقة لأنه بالعموم اللي جد محتاج تلخيص هو من محاضرة ٨ و بعدها

Summary

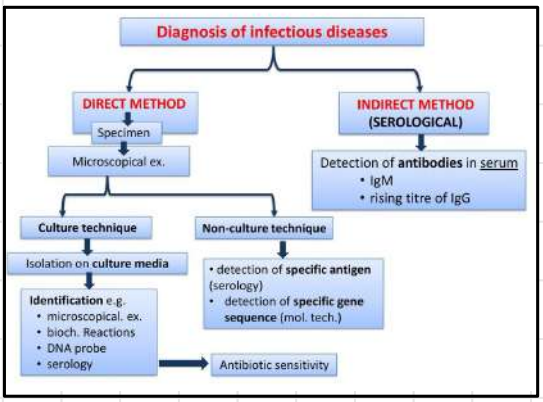
## 2. Culture

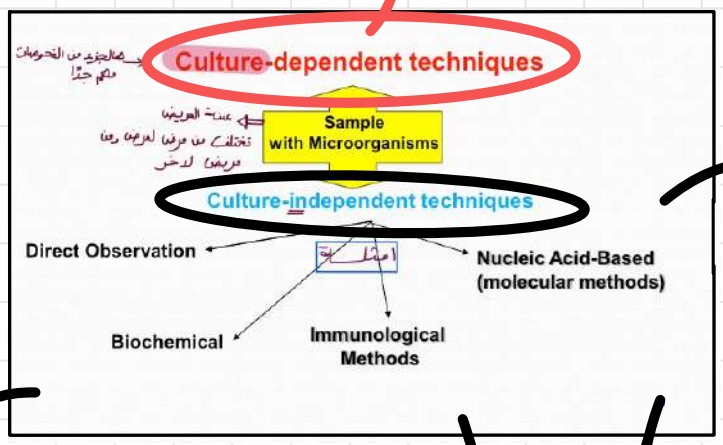
- Culture: Microbes growing in/on culture medium
- Culture Medium: Nutrients prepared for microbial growth
- Agar: Complex polysaccharide used as solidifying agent for culture media in Petri plates, slants, and deeps
- Agar is not metabolized by microbes, liquefies at 100°C and solidifies ~40°C




- Media can be classified on three primary levels

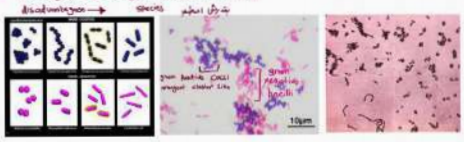
1. **Physical State**
   - Liquid Media
   - Semisolid
   - Solid (Can be converted into a liquid)
   - Solid (Cannot be converted into a liquid)
2. **Chemical Composition**
   - Synthetic – exact formula
   - Non synthetic or complex - No exact formula
3. **Functional Type**
   - General
   - Selective




## 1. Direct Observation

- Using light microscope to visualize bacterial shape and arrangement
- Using special stains to differentiates bacteria like gram stain and acid fast stain
- Quick and informative yet not definitive



## Molecular Methods

- Polymerase chain reaction (PCR)

Molecular methods make the species specific DNA visible . In PCR , Sequence specific primers are used in the amplification of DNA or RNA of specific pathogens.

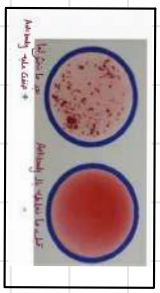
## 3. Immunologic Methods

- Any assay that relies on the characterization of antigen and/or antibody reaction
- Antibodies can reveal the history of a patient's contact with microorganisms or other antigens
- Serology: the branch of immunology that traditionally deals with *in vitro* diagnostic testing of the serum

Antigen: any "thing", foreign to the immune system. e.g. bacteria, viruses, (or their parts)
Antibody: proteins produced by the immune system which help defend against antigens

**Antigen/antibody interaction**

To visualize antigen-antibody interaction mutiple strategies can be applied: as Agglutination



## 5. Biochemical Tests

- The microbe is cultured in a media with a special substrate and tested for an end product
- Prominent biochemical tests include carbohydrate fermentation, acid or gas production and the hydrolysis of gelatin or starch
- Many of these test used in rapid system for quik detection of certain infection called Rapid test

## Catalase Test

- This test is used to identify organisms that produce the enzyme, catalase
- This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas
- Place a drop of $H_2O_2$ on the culture. A positive reaction show gas bubbles

## Agglutination Test

- Agglutination: antigens are whole cells such as red blood cells or bacteria with determinant groups on the surface
- Antibodies cross-link the antigens to form visible clumps
- Performed routinely to determine ABO and Rh blood types
- Widal test: tube agglutination test for diagnosing salmonella and undulant fever
- Latex agglutination tests: tiny latex beads with antigens affixed

اللهم صلّ على سيدنا محمد ، اللهم و اجبر بقلوب أهل غزّة و انصرهم

من هاي المحاضرة رح نبلش بالمايكرو عنجد

# Staphylococcus aureus

Staphylococci are gram-positive cocci which are Spherical cells arranged in irregular clusters (grape like clusters) . They're Common inhabitant of the skin and mucous membranes , Lack spores and flagella , May have capsules , Catalase-positive , About 40 species one common is S.aureus
And other 2 species in the pic ( zoom in ) 👉


هاي الصفحة شاملة اول نوع و هو aureus كامل ، النوعين التانيين هم staphylococcus هيهم بالصورة مش محتاجين تلخيص

The Genus is Staphylococcus and it has 40 species, how are they classified ?

**Based on coagulase production:**
1. Coagulase-positive: Staphylococcus aureus
2. Coagulase-negative: Staphylococcus epidermidis
Staphylococcus saprophyticus

**Based on pathogenicity:**
1. Common pathogen: S. aureus
2. Opportunistic pathogens: S. epidermidis & S. saprophyticus
3. Non pathogen: S. homonis

Lec 8 ( file 7 ) Gram +ve cocci

## General characteristics 👇

Optimum temperature of 37 Celsius
Coagulase-positive
Facultative anaerobe
Withstands high salt, extremes in pH and high temperatures
Produces many virulence factors

### Epidemiology : 👇

Source of infection:
A) Exogenous: patients or carriers
B) Endogenous: from colonized site
Mode of transmission:
A) Contact: direct or indirect
B) Inhalation of air borne droplets

## Virulence factors 👇

**Cell associated factors:**
**A) Cell associated polymers**
1. Cell wall polysaccharide 2. Teichoic acid 3. Capsular polysaccharide
**B) Cell surface proteins**
1. Protein A 2. Clumping factor (bound coagulase)

**Enzymes :**
Coagulase – coagulates plasma and blood
Hyaluronidase – digests connective tissue
Staphylokinase – digests blood clots
DNase – digests DNA DNA
Lipases – digest oils; enhances colonization on skin
Penicillinase – inactivates penicillin

**Toxins:**
Hemolysins (α, β, γ, δ) – lyse red blood cells
Leukocidin – lyses neutrophils and macrophages
Enterotoxin – induce gastrointestinal distress
Exfoliative toxin – separates the epidermis from the dermis
Toxic shock syndrome toxin (TSST) – induces fever, vomiting, shock, systemic organ damage

## Clinical presentations 👇

**Infections:** 1 ) **Skin and soft tissue**: Folliculitis, furuncle (boil), carbuncle, styes, abscess, wound infections, impetigo
2) **Musculoskeletal**: Osteomyelitis, arthritis, bursitis
3) **Respiratory**: Tonsillitis, pharyngitis, sinusitis, otitis, infection bronchopneumonia, lung abscess, empyema
4) **Central nervous system**: Abscess, meningitis
5) **Endovascular**: Bacteremia, septicemia, endocarditis
6) **Urinary**: Urinary tract infection
**Intoxications**: 1) Food poisoning 2) Toxic shock syndrome
3) Staphylococcal scalded skin syndrome


1: folliculitis
2: furuncle
3: carbuncle

Staphylococcal Toxic Shock Syndrome (STSS):  It is fatal multisystem disease
Menstrual associated STSS occurs in the vagina of menstruating woman
Staphylococcal Scalded Skin Syndrome (SSSS):  Exfoliative toxin produced by S.aureus is responsible for this

## Laboratory diagnosis 👇

1. **Specimens** collected: Pus, sputum, blood, stool
for the detection of carriers- Nasal swab
2. **Gram Stain**: Gram-positive cocci in grape like clusters
3. **Culture**: Culture media:
**Non selective**: Nutrient agar, Blood agar, MacConkey's agar
**Selective media**: Mannitol Salt Agar
Culture conditions: Ambient conditions, 37 °C, 18-24 h

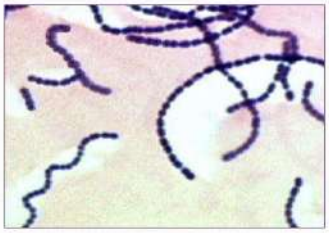
**Colonial morphology:**
Nutrient agar- golden yellow pigme 
MacConkey's agar- small & pink in colour
Blood agar- most strains produce β- haemolytic color 

**Biochemical tests:**
Catalse -positive , Coagulase-positive , Ferments mannitol
Drug **resistance** is common ( we may use penicillin, cloxamicillin,methicillin,vancomycin , but there still may be resistance to them as MRSA

Lec 9 , cont of Gram +ve cocci : **streptococcus**

## General Characters

- Gram-positive cocci
- Chains or pairs
- Usually capsulated
- Non motile
- Non spore forming
- Facultative anaerobes
- Fastidious
- Catalase-negative (Staphylococci are catalase-positive)



| Staph | Strepto |
|---|---|
| gram positive | gram positive |
| cluster (Arrangmet) * | chain * |
| May capsulated * | usually capsulated * |
| Non motile (Flagella) | Non motile |
| Non spore forming | Non spore forming |
| Facultative anaerobes | Facultative anaerobes |
| tough * | fastidious * |
| catalase positive * | catalase negative * |

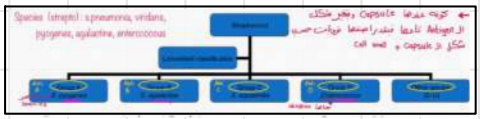
من تفريغ تبارك ع موقع النادي

# Streptococcus

## Hemolysis on blood agar

| α | β | γ |
|---|---|---|
| Partial hemolysis with green discoloration around the colonies ✓ | Complete hemolysis with clear zone of hemolysis around the colonies ✓ | No lysis ✗ |
| non-groupable streptococci : S. pneumoniae & S. viridans | Group A & B : S. pyogenes & S. agalactia | Group D Enterococcus spp |

## Serology



Classification based on C- carbohydrate antigen of cell wall

**Groupable streptococci**

A, B and D (more frequent)

C, G and F (Less frequent)

**Non-groupable streptococci**

S. pneumoniae (pneumonia) & viridans streptococci : causing S. mutans & Causing dental carries

## Oxygen requirements

*1.Anaerobic (Peptostreptococcus)*

*2. Aerobic or facultative anaerobic (Streptococcus)*

## Group B include S. agalactiae

Infections :

Nenistal disease on early and late onset

Others : UTI's , systemic, cutaneous

## Group A include only S.pyogenes

Virulence factors :

**Structural components (cross reactivity)**

M protein-interferes with lysis of the bacteria, Lipoteichoic acid & F protein-adhesion, Hyaluronic acid capsule

**Enzymes**-facilitate the spread of streptococci through tissues : Streptokinases, Deoxynucleases, C5a peptidase

**Pyrogenic toxins** mediates bacteremia and shock

**Streptolysins** (lyse red blood cells, white blood cells, and platelets) : Streptolysin O , Streptolysin S

Disease caused by S. pyogenes

- **Suppurative** :
- **Non-Invasive**

Pharyngitis "strep throat" & Skin infection: Impetigo

- **Invasive**

Scarlet fever-rash that begins on the chest and spreads across the body

Pyoderma- pus-producing lesion that usually occurs on the face, arms, or legs

Necrotizing fasciitis-toxin production destroys tissues and eventually muscle and fat tissue (flesh eating bacteria)

**Non Suppurative** : Rheumatic fever & Glomerulonephritis

## Group D is divided into :

that will grow in 6.5% saline (enterococci)

those that will not (non-enterococci)

**Associated infections:**

Bacteremia

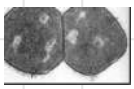
Urinary tract infections

Wound infections

Endocarditis

By S. pyogenes

## Streptococcus Pneumonia



**General characteristics :**

Inhabits the nasopharyngeal areas of healthy individuals

Typical opportunist

Gram-positive diplococci

**Virulence factors :**

Polysaccharide capsule

**Clinical infections :**

Pneumonia, **Meningitis** , Bacteremia , and Sinusitis/otitis media

# Neisseria gonorrhoeae ( gonococcus )

اذكروا والداي بدعوة

## General characteristics:

Gram-negative cocci often arranged in pairs (diplococci)
Oxidase positive
Most catalase positive
Non-motile
non spore forming
Sensitive, aerobic but grow better with low CO2

## Structure:

Pili (N-methylphenylalanine)
Outer membrane:
1. Phospholipids
2. Proteins: Outer membrane proteins (OMP I, OMP II, Opa)
3. Lipopolysaccharide (LPS) mainly as lipooligosaccharide (LOS)

Antigenic variation: Pili, OMPs, LOS

## Epidemiology & Pathogenesis :

Not part of normal human flora, only fond in mucous membranes of genitalia, anorectal area, oropharynex or conjunctiva during infection Transmission primarily by sexual contact or from infected mother during birth , Asymptomatic carriage is a major reservoir & reinfection can occur

Attachment and invasion: pili and outer membrane protein help the bacteria to attach
Survival and multiplication :
in the submucosa to establish infection
Spread and dissemination: The bacteria cause local cell injury and inflammatory response
Most common sites of inoculation:
- Cervix (cervicitis) or vagina in the female
- Urethra (urethritis) or penis in the male

## Virulence factors :

N. gonorrhoea secrets IgA protease that inhibit IgA antibodies function
Outer membrane proteins:
1. Prevents phagolysosome and promotes intracellular survival
2. Mediates firm attachment to epithelial cells and subsequent invasion into cells
3. Protects other surface antigens from bactericidal antibodies

Lipooligosaccharide (LOS) (Lipid A) has endotoxin activity Acquisition of antibiotic resistance:
1. Plasmid-encoded beta-lactamase production
2. Chromosomally-mediated changes in cellular permeability inhibit entry of antibiotics

## Laboratory diagnosis :

- Gram Stain: Grame –ve diploccoci inside polymorphonuclear leukocytes
- Culture : Media:

Thayer Marten Media (TM):
Enriched chocolate agar with antimicrobial colistin (to inhibit G- bacili) nystatin (to inhibit yeast) and vancomycin (to inhibt G+ bacteria)
Modified Thayer Marten Media (MTM):
as above plus trimethoprim (to inhibit proteus)
Martin Lewis medium (ML):
same as above except that anisomysin is substituted for nystatin and vancomycin concentration is increased
Colonial appearance: Small, grayish white, convex, translucent, shiny, with smooth or irregular margins

## Other tests :

Resistance to pencillin is quite common due to production of beta-lactemase but Resistance to ceftriaxone is not described

Oxidase positive
Glucose fermentation positive (while maltose and lactose fermentation is negative)
Nitrite reduction negative

Lec 11 اذكروا والداي بدعوة

# Neisseria meningitides( meningococcus)

## General characteristics:

Encapsulated small, gram-negative diplococci
Oxidase positive , Catalase positive
Can be a member of the normal flora of the upper respiratory tract
Causes life-threatening disease when the bacteria invade the blood or cerebrospinal fluid CO2 enhances growth but is not absolutely required
Less sensitive than Niesseria gonorrhoeae
Have a well developed highly antigenic capsule

## Structure:

Pili: attachment and enhance virulence
Outer membrane:
1. Porins
2. Outer membrane proteins (OMP)
3. Lipooligosaccharide (LOS)

Capsule contains polysaccharide with more than 13 known antigenic types
Types A, B, C, Y & W135 are more commonly associated with human disease

## Epidemiology & Pathogenesis :

Neisseria meningitidis found as nasopharyngeal flora in 10% of healthy individuals
Transmission occurs by inhalation of respiratory droplets
The most common cause of meningitis in under 20 and the second most common cause after pneumococci in all ages

Replicate intracellularly and migrate to subepithelial space , Organisms are internalized into phagocytic vacuoles, avoid intracellular killing and primarily infect the CNS to cause acute meningitis withh bacterremia

Immunity : Absence of antibody correlates with susceptibility

## Virulence factors :

Pili-mediated, receptor-specific colonization of nonciliated cells of nasopharynx
Antiphagocytic polysaccharide capsule allows systemic spread in absence of specific immunity
Toxic effects mediated by hyperproduction of lipooligosaccharide (Endotoxin)

ما عدا كثير انزيمات وعوامل تكسير وسموم بس خطورتها تكمن بالمكان الي بتعمل فيه الالتهاب (الدماغ)

## Clinical presentation

Meningitis:
Fever, fatigue, weakness
CNS: convulsion, motor disability, loss of consciousness
Thrombocytopenia results in bleeding and skin petchiae
Disseminated intravascular coagulation (DIC)
Fatal if not treated early

## Laboratory diagnosis :

Specimens: pharyngeal swab, cerebrospinal fluids, skin lesions and blood

Gram stain : encapsulated, small, gram negative diplococci and polymorphonuclear leukocytes (PMN's) can be seen microscopically in cerebrospinal fluid (CSF)

Culture same as previous

Biochemical Tests :
Oxidase positive
Glucose and maltose fermentation positive (while lactose fermentation is negative)
Nitrite reduction negative

penicillin can be used, but we need something stronger

Immunological test not recommended ,and for antibiotics Chloramphenicol or cephalosporins can be used

Lec 12

# Gram negative bacilli

## Haemophilus, bordetella , Pseudomonas

## Haemophilus

### General characteristics & Epidemiology

Aerobic gram-negative bacilli/coccobacilli liking blood & Are Major pathogens for which humans are natural hosts :
1. Haemophilus influenzae
2. Haemophilus ducreyi-induce sexually transmitted diseases (chancroid)
are Non motile & H. influenza has a polysaccharide capsule with six different serotypes (a-f) … 95% of invasive disease are caused by type b (Hib) which contain polyribose-ribitol phosphate (PRP) capsule

**Epidemiology**

Transmitted via respiratory droplets, or direct contact with contaminated secretions & is Normal flora of the human respiratory tract and oral cavity

### Virulent factors & vaccinations

Antiphagocytic polysaccharide capsule is the major pathogenesis factor
lipid A component from the cell wall (major role in non capsule strains)
All virulent strains produce neuraminidase and an IgA protease
Pathogenesis: Organism colonizes nasopharynx followed by :
1. Local invasion: otitis media and sinusitis
2. Systemic invasion: …bacteremia…meningitis

**Vaccinations**

Pre-vaccination: Meningitis
Post-vaccination: Most cases in unvaccinated
Non-encapsulated, non-typeable strains and serotype f are the most common
Children - Pneumonia and meningitis are less common
Hib are among the commonest causes of bacterial otitis media and sinusitis

### Laboratory diagnosis & treatment

Gram stain: Gram-negative bacilli/coccobacilli that Requires 2 erythrocyte factors for growth: X (hemin) and V (NAD)… X & V factors are released following lysis of RBCs
Culture: IsoVitaleX-enriched chocolate agar & Blood agar with S. aureus
Biochemical tests:
Catalase, oxidase, nitrate reduction, and glucose fermentation are all positive
Iridescence: different colors on transparent media due to the optical properties of the capsule
Serological tests for serotyping (anti-a, .. )

**Treatment**

Treatment with an effective 3rd generation cephalosporin , Ampicillin-resistant strains now common , Prevention by vaccination

## Bodetella

### General characteristics & transmissions

Gram–negative coccobacilli (rod-shaped) single or paired & Obligate aerobe, Optimum growth 35-37 ℃ , grow better on media with a slightly acidic reaction
Colonizes the respiratory tract . Incidence dropped significantly after vaccination & is Specific to human hosts & The main species :
1. B. pertussis: whooping Cough (Pertussis)
2. B. parapertussis & 3. B. bronchiseptica

**Transmission**

Spread through direct contact of respiratory secretions
Most contagious during first few days of infection
Resides in upper airway pathways, mostly the trachea and bronchi & is very contagious

### Clinical presentation : Whooping cough

Incubation period 4-21 days
3 Stages:
1. Catarrhal Stage 1-2 weeks: runny nose, sneezing, low fever, and a mild cough (common mistaken for cold)
2. Paroxysmal Stage 1-6 weeks: whooping cough, which consists of bursts of numerous, rapid coughs, that end with a long inspiratory effort with high pitched whoop, severity of the infection is at its greatest
3. Convalescent Stage: weeks-months, gradual recovery starts

### Laboratory diagnosis & treatment

1 Specimen: posterior nasopharynex
2. Gram stain: Grame-negative coccobacilli
3. Culture:
Media: Bordet-Gengou, or charcoal agar
4. Polymerase Chain Reaction: rapid, specific
5. Bordettela antibodies detection by ELISA
6. Slide agglutination

**Treatment**

Antibiotic Therapy :
Erythromycin, Azithromycin , Clarithromycin
Pertussis vaccine :
1st Pertussis vaccine , Acellular vaccine , Combination vaccines
CDC recoomends children be given the Diphtheria, Tetanus, and Pertussis (DTaP) vaccine

## Pseudomonads

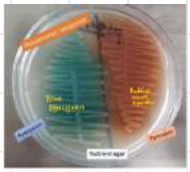
### General characteristics & Epidemiology

P. aeruginosa is the most common pseudomonad
Gram-ve rods arranged in pairs & are Motile by single or multiple polar flagella
Non-fermentative , Oxidase-positive obligate aerobes .. Some strains are mucoid contain (polysaccharide capsule)
Pseudomonads produce diffusible pigments, as:
1. Blue pyocyanin 2. Yellow fluorescein
3. Reddish-brown pyorubin 4. Black pyomelanin

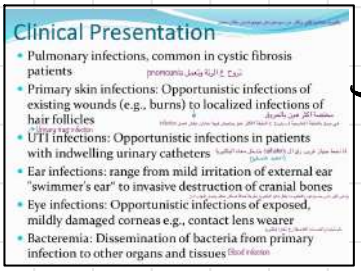
**Epidemiology**

Habitats in soil, water and plants
Rarely a part of normal flora in healthy individuals
Transmission by:
Ingestion or exposure to contaminated stuff
rarely transmitted by p2p





### Clinical presentations & Laboratory diagnosis




Specimens: according to infection
Gram stain: Gram-negative bacilli
Culture: nutrient or blood can grow at 42 ℃
Green color and fruity smell & for Biochemical tests: Oxidase, Catalase, and Nitrate reduction-positive & Pyocyanin green pigment production & non fermenters
Serological test: not usually used
Antibiotics sensitivity tests:
to prevent using resistant antibiotics commonly associated with P. aeruginosa

### Virulence factors

:Polysaccharide capsule
Mediated bacteria adhesion to epithelial cells, prevent phagocytosis and inhibits antibiotics
Endotoxin (lipid A) & Exotoxin A disrupts protein synthesis by blocking peptide chain elongation leading to necrosis >The toxin is also immunosuppressive
Pyocyanin : Can mediate tissue damage
Exoenzymes S and T: Cause epithelial cell damage
Elastases: Cause damage to elastin-containing tissues and lung parenchymal
Alkaline Protease: Causes tissue destruction and interferes with host immune response
Phospholipase C: Heat-labile hemolysin
Rhamnolipid: Heat-stable hemolysin
:Resistance to antibiotics
P. aeruginosa is one of the most resistant bacteria to many groups of antibiotics
:Mechanism of resistance
Mainly due to outer membrane porin proteins mutation & Production of many β-lactamases and carbapenemase

# Lec 13 : Anti microbial resistance

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it
Penicillin G: when first introduced only 3% of bacteria resistant, but now over 90% are resistant

## Natural & Acquired Resistance

1. Natural resistance — antibiotics البكتيريا من اول ما خلقت عندها natural resistance ليعض ال هاد اكتشفوه بالامافير يبا كنا مكتشفين ال antibiotics لسا
   - Intrinsic resistance: some species naturally insensitive
   - Chromosomic genetic support
   - Affect almost all species strains → resistance كلهم عادة بكونوا
   - Existed before antibiotic use (*Enterobacter sp.* - amoxicillin) هو اللي بهمنا وهو الأخطر
2. Acquired resistance (mutation)
   - Spontaneous mutation: happen as cells replicate
   - Gene transfer: usually spread through conjugative transfer of R plasmid → بنتقل من بكتيريا لبكتيريا ففي strain طريك ال resistance بتروح تعطيه ل strain تاني وهكذا
   - Affects a fraction of strains → جزء مهم من كلهم
   - Increased with antibiotic use (extended spectrum beta-lactamase producing *E. coli*)

## Contributing Factors to Resistance

- Misuse and overuse of antibiotics
- Modern live: travelers carry resistant bacteria
- There are more large cities in the world today
- Food is also a source of infection and resistance
- Increase in the number immunocompromised people
- Emerging and re-emerging diseases are another source of resistance.
- Hospitals are ideal reservoirs for the acquisition of resistance.
- Destruction of normal flora allows pathogenic pathogens to dominate

## Impact of Antibiotics Resistance

- Infections caused by resistance organisms result in prolonged illness, disability, or death
- Antimicrobial resistance reduces the effectiveness of treatment; thus patients remain infectious for a longer time, increasing the risk of spreading resistant microorganisms to others
- AMR increases the costs of healthcare
- AMR has the potential to threaten health security, and damage trade and economies
- resistant

## Slowing the emergence and spread of antimicrobial resistance

1. Responsibilities of Physicians: must work to identify microbe and prescribe suitable antimicrobials, must educate patients
2. Responsibilities of Patients: need to carefully follow instructions
3. Educate Public: must understand appropriateness and limitations of antibiotics ; antibiotics not effective against viruses
4. Global Impacts: organism that is resistant can quickly travel to another country, in some countries antibiotics available on non-prescription basis

## Approaches to Antibiotic Therapy To Prevent Resistance

- Use antimicrobials only when necessary
- Maintain high concentration of drug in patient for sufficient time
- Use antimicrobial agents in combination
- Develop new variations of existing drugs
  - Second-generation drugs
  - Third-generation drugs
- Search for new antibiotics, semi-synthetics, and synthetics
- Design drugs complementary to the shape of microbial

هاي المحاضرة ما بدها تلخيص فرح أحط سلايداتها عشان التلخيص يشمل كل المحايضرات

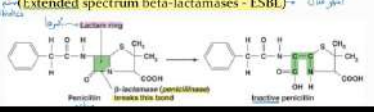
Summary

## Mechanisms of Resistance

1. Production of enzyme that destroys or deactivates drug
2. Pump antimicrobial drug out of the cell before it can act
3. Slow or prevent entry of drug into the cell
4. Alter target of drug so it binds less effectively
5. Alter their metabolic chemistry

### 1. Enzymatic Inactivation

- Inactivation involves enzymatic breakdown of antibiotic molecules.
- A good example is β-lactamase:
  - Secreted into the bacterial periplasmic space
  - Attacks the antibiotic as it approaches its target
  - There are more than 190 forms of β-lactamase
  - E.g of lactamase activity in *E.coli* and *S. aureus*
  - (Extended spectrum beta-lactamases - ESBL) → امثلة مثال




### 2. Efflux Pumping

- Efflux pumping is an active transport mechanism. It requires ATP
- Efflux pumps are found in:
  - The bacterial plasma membrane
  - The outer layer of gram-negative organisms → مثلا
- Pumping keeps the concentration of antibiotic below levels that would destroy the cell
- Genes that code for efflux pumps are located on plasmids and transposons

### 3. Decrease Permeability

- Some bacteria reduce the permeability of their membranes as a way of keeping antibiotics out
- They turn off production of porin and other membrane channel proteins
- Seen in resistance to streptomycin, tetracycline, and sulfa drugs

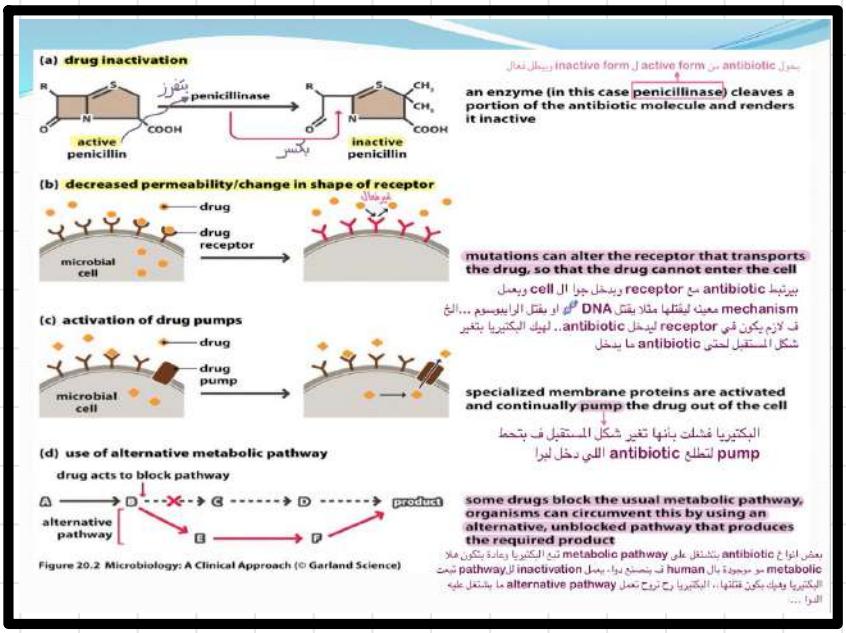
### 4. Modification of Antibiotics Targets

- Bacteria can modify the antibiotic's target to escape its activity
- Bacteria must change structure of the target but the modified target must still be able to function. This can be achieved in two ways:
  - Mutation of the gene coding for the target protein
  - Importing a gene that codes for a modified target
- Bacteria have penicillin- binding- protein (PBPs) in their plasma membranes. These proteins are targets for penicillin

- MRSA (methicillin- resistant - *S. aureus*) has acquired a gene (*mecA*) that codes for a different PBP
  - It has a different three-dimensional structure
  - MRSA less sensitive to penicillins
- MRSA is resistant to all β-lactam antibiotics, cephalosporins, and carbapenems
- *Streptococcus pneumoniae* also modifies PBP
  - It can make as many as five different types of PBP
  - It does this by rearranging, or shuffling, the genes
- Bacterial ribosomes are a primary target for antibiotics. Resistance can be the result of modification of ribosomal RNA so it is no longer sensitive

### 5. Alteration of Pathway

- Some drugs competitively inhibit metabolic pathways.
- Bacteria can overcome this method by using an alternative pathway
- Some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to using preformed folic acid

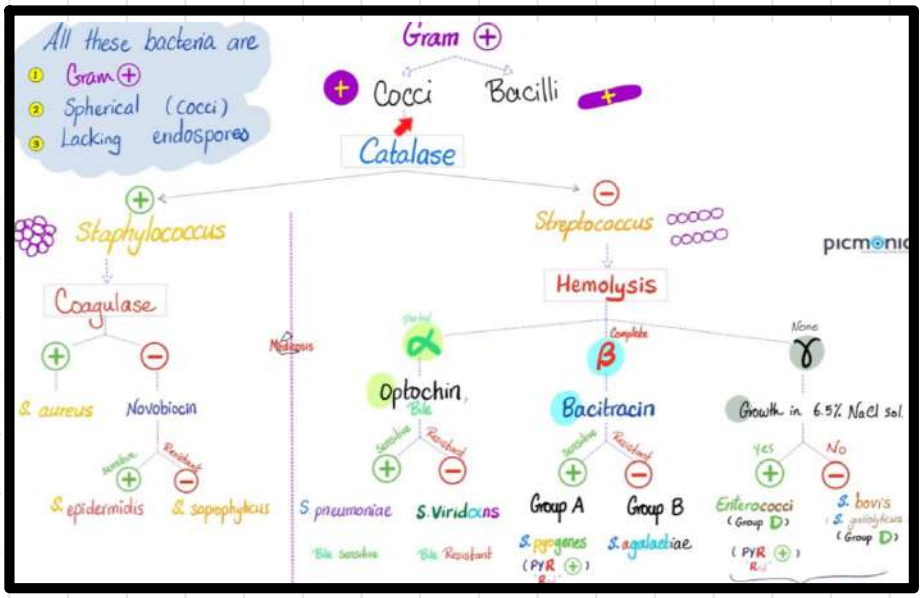


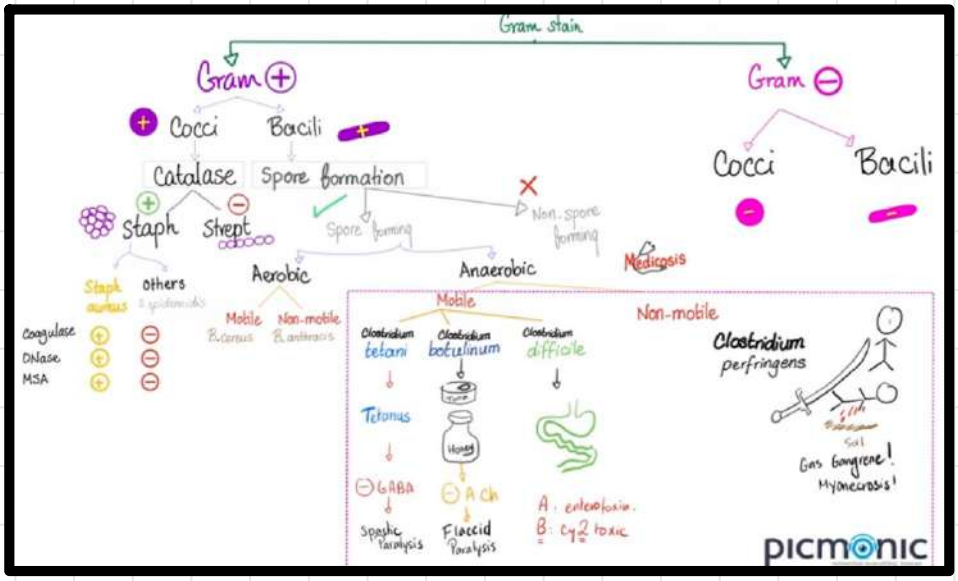

لتواريخ مو حفظ بس اعرفو انو زمان ال resistance كان ياخد وقت وهلأ هو سريييع

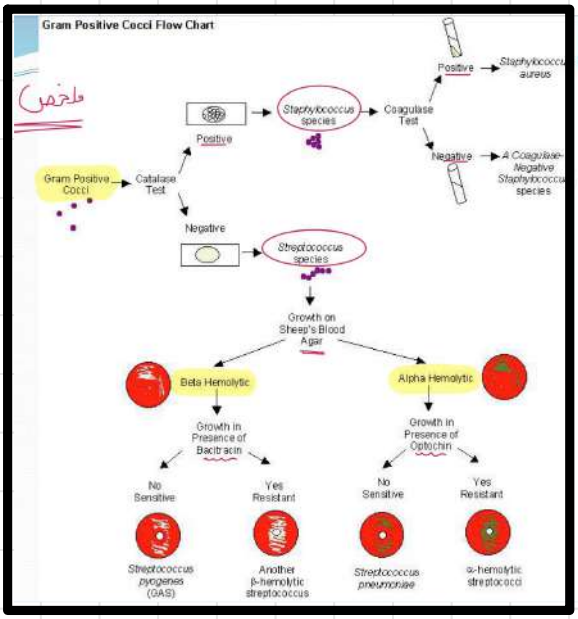
هيك بيكون خلص دوري بتلخيص كل مادة دكتور محمد و حطيت تلاخيص خارجية بالصفحتين هدول ، لا تنسوا ترجعولهم بس تخلصوا

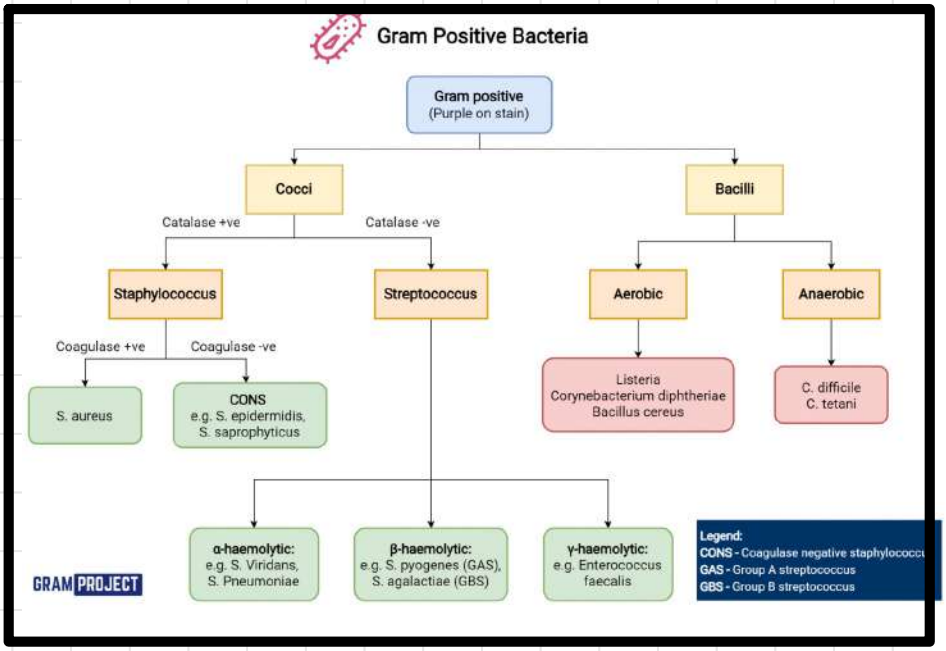
1 positive staphylococcus
2 positive streptococcus
3 negative cocci neisseria
4 negative bacilli
5 negative enterobacteriaceae
6 positive bacilli
7 mycology

ترتيب المحاضرات

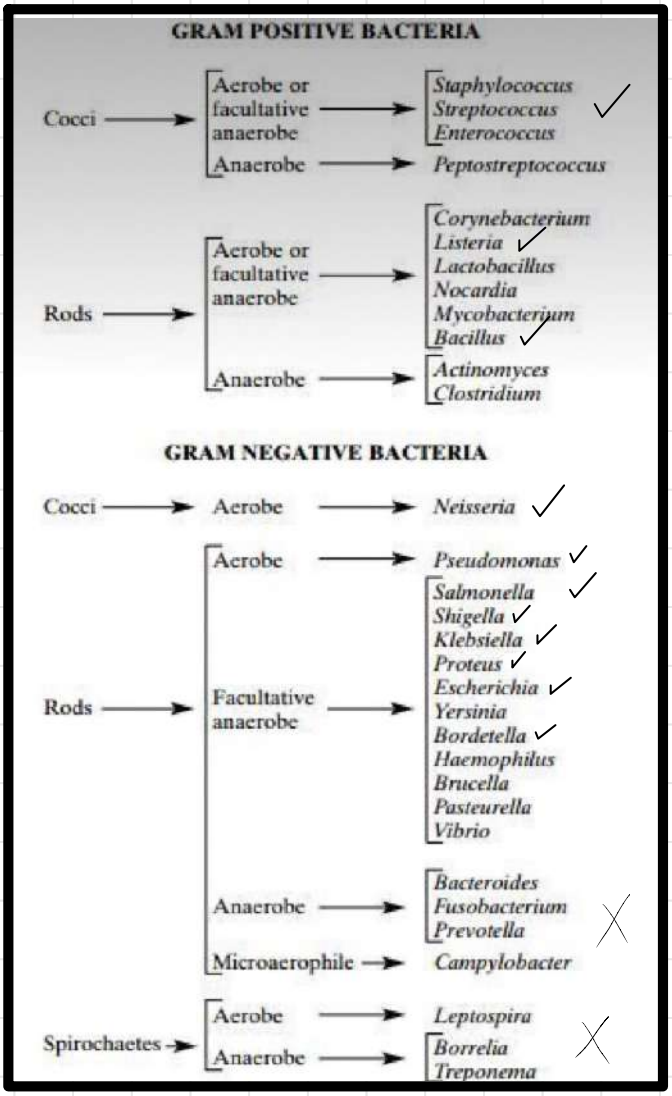


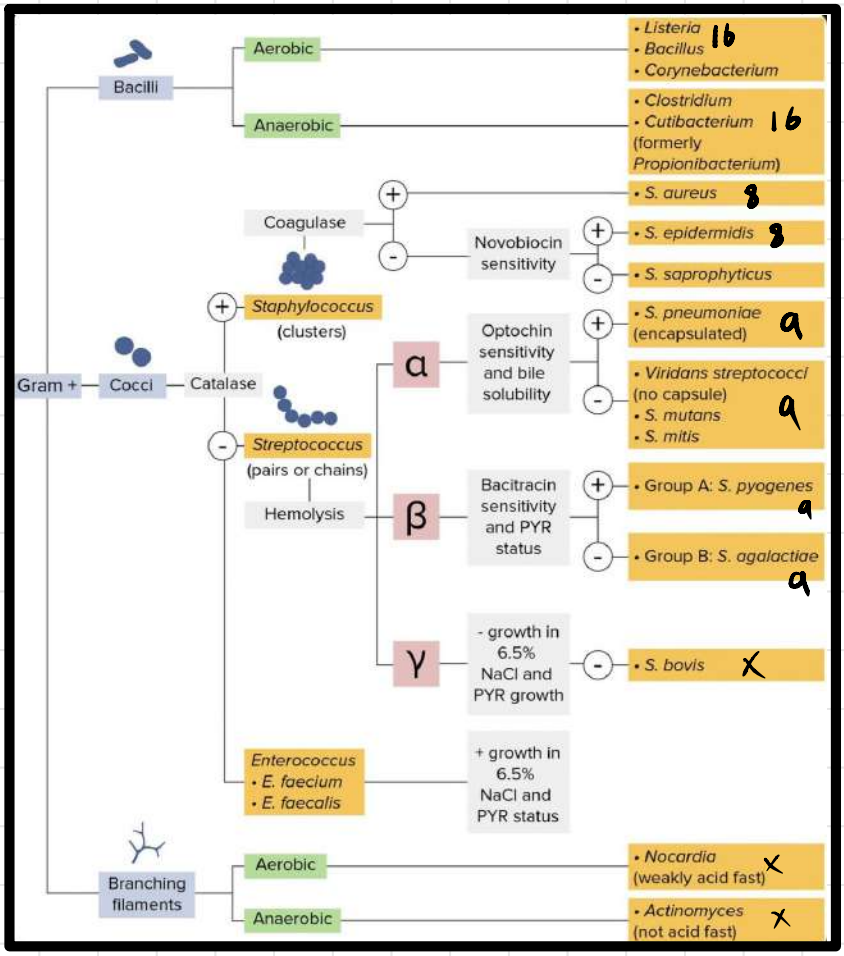



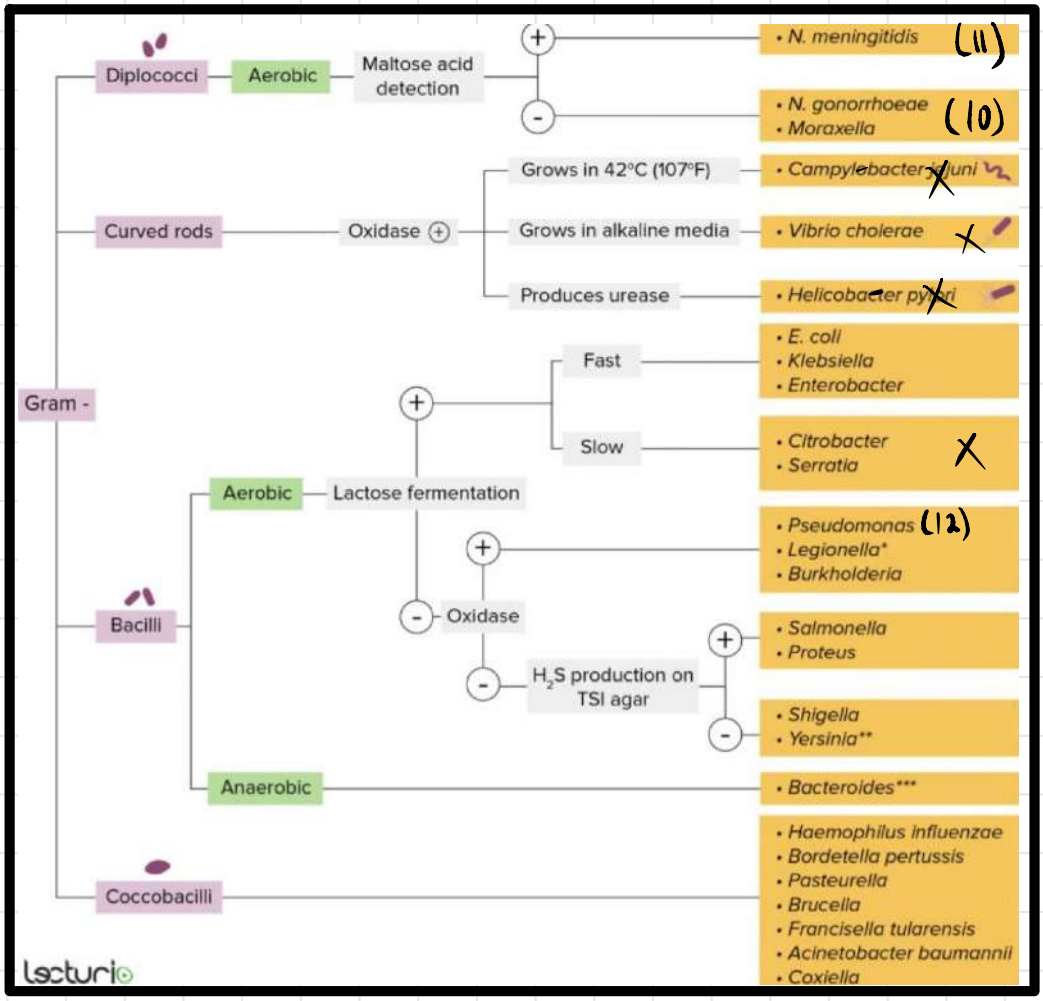



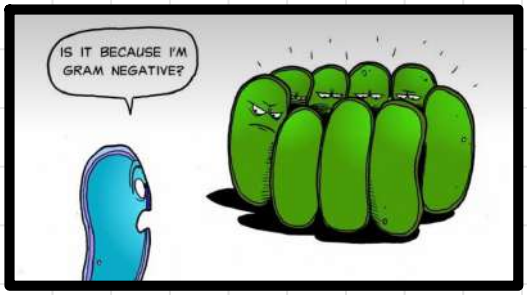
دعواتكم ، تاركين المتراكم و بنسوي التلاخيص ❤️🥲




هاي التلاخيص شاملة محاضراتنا حتى لو في أشياء لسة ما وصلتلها بالتلخيص




رح يكمل معكم زميلي عمر دعباس من ١٤ ل ١٨

| | Salmonella | Shigella | E. coli | Klebsiella | Proteus |
|---|---|---|---|---|---|
| Gram | G- | G- | G- | G- | G- |
| Normal flora | **not** part of normal flora | **not** part of normal flora | normal flora | normal flora | normal flora |
| Oxidase | Oxidase - | Oxidase - | Oxidase - | Oxidase - | Oxidase - |
| Motility | motile | **Non motile** | Motile | **Non-motile** | Very motile |
| Capsule | capsule | Capsule | Capsules | Capsulated | **Non-capsulated** |
| Anaerobes | Facultative anaerobes | Facultative anaerobes | Facultative anaerobes | Facultative anaerobes | Facultative anaerobes |
| spore | Non-spore forming | Non-spore forming | Non-spore forming | Non-spore forming | Non-spore forming |
| nitrite | Reduce nitrates to nitrites | Reduce nitrates to nitrites | Reduce nitrates to nitrites | Reduce nitrates to nitrites | Reduce nitrates to nitrites |
| Lactose | **Non-lactose fermenting** | **Non-lactose fermenting** | Lactose fermenting | Lactose fermenting | **Non-lactose fermenting** |
| Glucose | Glucose fermentation | Glucose fermentation | Glucose fermentation | Glucose fermentation | Glucose fermentation |
| Gas production | + | - | + | + | + |
| $H_2S$ | $H_2S$ positive | **negative** | **negative** | **negative** | $H_2S$-positive |
| urease | Urease negative | Urease negative | Urease negative | **Ureaese-positive** | **Ureaese-positive** |

H2S +ve bacteria produce black colonies.

Lactose fermentation +ve appear pink on MacConkey's agar, and lactose fermentation-ve appear yellow.

# E. Coli

O antigen-LPS
H antigen-flagella
K antigen-capsule

α-hemolysin, protein synthesis inhibitor, toxins that alter messenger pathways

CNF: α-β toxin that disrupts G protein signaling, causing apoptosis & cytoskeleton arrangements.

LT: A-B toxin that increases cAMP in the cell, which opens chloride channels

ST: small peptide that increases cGMP in the cell, pumps electrolytes out

Stx: binds to 28S rRNA and inhibits protein synthesis .
Its B unit binds to Gb3 receptor

By Omar Debas

# Uropathogenic E. Coli (UPEC): Most common cause of UTI

## ETEC

| |
|---|
| LT, ST and colonizing factor (CF) pili |
| Watery diarrhea, not invasive |
| Traveler diarrhea, diarrhea in infant (developing countries) |
| Food and water contamination, animals not involved |
| High infecting dose (p2p is unusual) |

| EPEC |
|---|
| effacement or loss of microvilli |
| Acute or chronic diarrhea in infants (20%) |
| Feco-oral route |
| Low infecting dose in infant, high infecting dose in adult |
| Bundle forming (Bfp) pili, microcolnies |
| degeneration brush border, loss of the microvilli, and changes in the cell morphology (pedestals) |
| attachment and effacing (A/E) lesion (intimin, and an injection (type III) secretion system) |
| modifications in enterocyte cytoskeleton proteins (actin-rich A/E lesion ) |
| mitochondrial injury and induction of apoptosis, change electrolyte transport across the luminal membrane |

| Enterohemorrhagic E. coli (EHEC) |
|---|
| Shiga toxin |
| O157:H7 |
| Hemorrhagic colitis<br>Crampy abdominal pain, little or no fever, bloody diarrhea, **HUS** |
| Animal (cattle), p2p, low infection dose (100) |
| More in developed countries |
| Hamburger (rare in the middle) |
| A/E lesion (intimin) and and Stx (extraintestinal features ) |
| long polar fimbriae [Lpf] (colon not intestine) |
| Stx<br>• production causes capillary thrombosis and inflammation of the colonic mucosa, leading to a hemorrhagic colitis<br>• glomerular swelling and the deposition of fibrin and platelets in the microvasculature |

## EIEC

| |
|---|
| Mild version of shigelosis related to Shigella |
| Contaminated food and water, high infection dose (low p2p) |
| Dysentery usually with blood |
| Invade intestinal epithelial cells, lyse the phagosomal vacuole, spread through the cytoplasm and infect adjacent cell similar to shigella |

| Enterotoxigenic | Enteropathogenic | Enteroaggregative | Enteroinvasive | Enterohemorrhagic |
|---|---|---|---|---|
| mild **watery** diarrhea (2-4d) last few days<br><br>High infecting dose (p2p is unusual) | mild **watery** diarrhea (2-4d)<br><br>last few days may chronic<br><br>Low infecting dose in infant, high infecting dose in adult | mild **watery** diarrhea (2-4d)<br><br>**last for weeks**<br><br>High infecting dose (p2p is unusual) | mild watery diarrhea (2-4d) last few days.<br><br>**Dysenteric**<br><br>**high infection dose (low p2p)** | mild watery diarrhea (2-4d) last few days **dysenteric**<br><br>vomiting, pain, bloody diarrhea<br><br>Colonoscopy :edema, hemorrhage, and pseudomembrane formation (3-10)day resolve)<br><br>low infection dose (100) |

## EAEC

| |
|---|
| autoagglutinate |
| No enterotoxins |
| prolonged watery diarrhea >14d +_blood mucus |
| aggregative adherence fimbriae [AAF]), no A/E lesions |
| thick mucus–bacteria biofilm |
| Stx toxin |

# Salmonella

## Typhi

Contains Vi antigen
Causes typhoid fever (enteric fever):

Fever and headache, slow pulse, chest and abdominal rash. Initial constipation followed by diarrhea.
Chronic infection can lead to myocarditis, encephalopathy, intravascular coagulation.
After progressing for over weeks, a hemorrhage may occur in the terminal ileum or proximal colon.

The vi antigen resists phagocytosis and allows intracellular replication.
S. Typhi causes chronic infection of biliary tree and uronary tract.

Strictly human

## Mutual

Salmonella alters the cell architecture of M cells by creating “ruffles” which endocytose the organism and the salmonella enters the lamina propria where they are phagocytosed.
Typhi salmonella continue on by surviving inside the macrophage and replicating.

## Non-typhi (S. enterica)

No vi antigen stops at the mucosa and submucosa causes gastroenteritis

Animals and humans

## Shigella

Shigella dysenteriae (Type A1) is the most potent Stx producer

Dysentery: inflammatory diarrhea: small volume stool, WBC’s, RBC’s, bacteria.
Stx-producing shigella cause HUS with high mortality rate.
Patient experiences: cramps, painful straining to pass stool (tenesmus) and small volume, bloody, mucoid fecal discharge

Strictly human disease with very low infection dose (10 micro-organisms)

# Corynebacterium diphtheria

Corynebacterium: pleomorphic gram +ve bacilli
Diphtheroids: Aerobic, Normal Flora; Respiratory, Urinary tract and Skin. Mostly Nonpathogenic.
Corynebacterium diphtheriae:
Diphtheria is an acute, serious, highly infectious disease. Strains are facultative aerobes, Non-motile, Non-sporing bacilli that appear like Chinese characters.

Pharyngitis, tonsillitis, Gray-white psudomembrane, cervical adenitis (bull neck).
complications: mechanical obstruction, myocarditis (2-3w), cardiac enlargement, arrhythmias, CHF

Gram stain, direct smear and throat culture on blood tellurite medium

Diphtheria toxin (DT): inhibit cytoplasmic protein synthesis irreversibly by targeting elongation factor 2 (EF2).
Results in inflammation and Necrosis in the throat, pharynx and larynx (pseudomembranes), with myocarditis having a high fatality.

## Listeria monocytogenes

Small Bacilli, flagellated, motile at 37°C ,
but active tumbling motility at 25°C. Catalase positive, Grow slowly in cold even below zero. Foodborn transmission. Transmitted transplacentally to the fetus and causes stillbirth.
It causes Invasive Infection (listeriosis), Septicemia, Meningitis and Abortion.


## Spore-forming bacilli

### Aerobic (bacillus)

Bacillus Ceres:
Food poisoning, vomiting and diarrhea

Bacillus Subtilis: Small, short, thin bacillus with rounded ends.
Motile and non-capsulated.
Opportunistic Pathogen, can cause wound infection and Sepsis in Infants and in Immunocompromised Patients.

### Anaerobic (clostridium)

Bacillus anthracis:
Large, non-motile, rectangular bacilli, arranged in chains.
Spores are oval and central.
Aerobe and facultative anaerobe. Vegetative cells are readily destroyed by heat, Spores are highly resistant to heat.

infected from animals and its products: Cutaneous Anthrax: Wound Infect,
Hemorrhagic Pneumonia & Septicemia by Inhalation.
Polypeptide capsule, Potent exotoxin complex capable of protein inactivation.

High Fatality. Biological War Agent.

Cutaneous anthrax: 2-5 day erythematous papule, 7-10 day ulcer(malignant pustule)

Pulmonary anthrax: distress, cyanosis, 1-5 day, fever, cough, edema, hemorrhagic anthrax, meningitis

# Clostridium

## C. botulinum

Food-borne botulism. BT: resistance to GI enzymes. No change in food (doesn't spoil)
Home canned, green bean, fish, mushroom
The most potent toxin in nature (a Heat-Stable Exotoxins-Protein (20min /100C))
Metaloprotease act on NM junction
Block release of NTM Acetyhcoline at presynaptic membrane causing flaccid paraysis of motor system.
Botulism: Clinical symptoms begin 18-36 hours after toxin ingestion with:
weakness, dizziness, dryness mouth, Nausea, Neurologic features.. blurred vision, inability to swallow, difficulty in speech, weakness of skeletal muscles and Respiratory Paralysis
Diagnosis: Clinical Features.. Rare Toxin Detection
Feeding honey to infants can result inbotulism

## C. tetani

Tetanus is a highly fatal disease with a High Mortality rate,
Localized infection/Surface or Deep in Tissues,
Wound: puncture wound with a splinter, unskilled abortion, female circumcision.
Release potent neurotoxin (Tetanus toxin / tetanospasmin) produced when spores germinate and vegetative cells grow in necrotic tissues.
The organism multiplies locally and symptoms appear remote from the infection site.
Toxin:
NTM: glycine and gamma aminobutyric acid and affects inhibitory neurons
Unopposed firing of active motor neuron resulting in spasm, spasmatic paralysis (botulism: flaccid paralysis)
masseter muscle affected first, unable to open mouth (lock jaw) or trismus.

## C. difficile

Antibiotic associated diarrhea (10 Days after anti- biotic) causing overgrowth of C. difficile.
Pseudomembraneuse Colitis..Bloody Diarrhea ( lethal inflammation of colon, toxic megacolon).
Treatment: Stop Antibiotics, Give metronidazol Vancomycin.

## C. perfringes

Gas gangrene:
Traumatic wound and muscle damage, compound wound and bullet wound.
Food poisoning, intense watery diarrhea, but No Fever.

# Mycology

The natural habitat of most fungi is the environment. An important exception is Candida albicans, which is part of the normal human flora.

Fungi are Eukaryotic organisms
1. Have a true nucleus with nuclear membrane.
2. Have membranous organelles
3. Their cell membrane containing ergosterol.
4. Their cell wall consists mainly of polysaccharides:
a) Chitin b) β-glucan
β-glucan is the target of the antifungal drug, Echinocandin (e.g.Caspofungin)
5. Cryptococcus have an antiphagocytic polysaccharide capsule

**Comparison between Fungi and Bacteria**

| Feature | Fungi | Bacteria |
|---|---|---|
| Diameter | Approximately 4 µm (*Candida*) | Approximately 1µm (*Staphylococcus*) |
| Nucleus | Eukaryotic | Prokaryotic |
| Cytoplasm | Mitochondria and endoplasmic reticulum present | Mitochondria and endoplasmic reticulum absent |
| Cell membrane | Sterols present | Sterols absent (except *Mycoplasma*) |
| Cell wall content | Chitin | Peptidoglycan |
| Spores | Sexual and asexual spores for reproduction | Endospores for survival, not for reproduction |
| Thermal dimorphism | Yes (some) | No |

| Prokaryotes (Bacteria) | Eukaryotes (Fungi) |
|---|---|
| 0.1-10 um | 10-100 um |
| No nuclear membrane | Nuclear membrane |
| Single chromosome | multiple |
| No histones | Histones |
| Binary fission | Mitotic division |
| No organelles | Organelles |
| Peptidoglycan | Chitin |
| No ergosterol | Ergosterol |
| 70 S ribosomes | 80 S ribosomes |

# Fungal reproduction:

(1) Sexual reproduction (perfect fungi):
When two parents' spores combine to produce a zygospore.
(2) Asexual reproduction (imperfect fungi):
Most of the common pathogenic species are imperfect fungi and propagate by forming conidia (Asexual spores).

# Morphology of fungus

Mold (filamentous fungi):
They are multicellular fungi which produce hyphae (i.e) microscopic long branching filaments.
There are 2 types:
a-Molds with septate hyphae (i.e) with cross walls in hyphae.
b-Molds with aseptate hyphae (i.e) without cross walls in hyphae.
Mycelium: a mass of hyphae.
Example: Dermatophytes, Madurella & Aspergillus.

Yeasts (Budding fungi):
Oval or rounded single cells, reproduce by budding (blastospore).
Have NO hyphae, but some yeasts may have elongated budding cells linked in branches called pseudo-hyphae.
Example: Candida and Cryptococcus.

Dimorphic fungi: (Dimorphism)
Some fungi can occur in 2 different forms: ➢In nature or in culture at room temperature, they occur in a filamentous form (molds).
In infected tissues or when incubated at 37°C they occur in a yeast form.
Example: Histoplasma capsulatum & Sporothrix schenckii

# Superficial mycoses

Affect the skin and / or mucous membrane, hair or nails.

## Pityriasis versicolor (Tinea Versicolor):

Caused by Malassezia furfur, Affect the skin and the infected areas are
recognized by their de-pigmentation especially on tanned skin in the summer, usually asymptomatic, only cosmetic importance.

## Candidiasis (Moniliasis):

Caused by Candida, a budding yeast. Candida albicans (the commonest cause of candidiasis) is a member of the normal flora of the mucous membranes of respiratory, gastrointestinal and female genital tracts.
Candida albicans are opportunistic fungi which may dominate and become associated with pathological conditions.


Candidiasis of the skin

Oral thrush Mucocutaneous Candidiasis

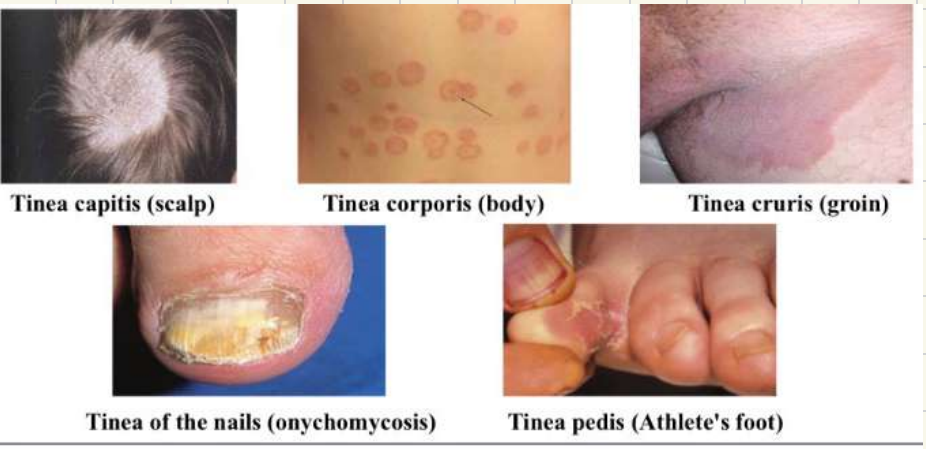
Superficial candidiasis includes:
1- Candidiasis of the skin especially in:
- Axilla, Groin, intergluteal folds, diaper rash (warm, moist areas). The lesion is itchy, flat, red with smaller "satellite lesions" nearby.
2- Candidiasis of mucous membranes:
- Oral thrush, esophagitis, vaginal thrush.
3- Mucocutaneous Candidiasis. - Angle of the mouth.

# Superficial mycoses pt. 2

## Dermatophytes (Ringworm):

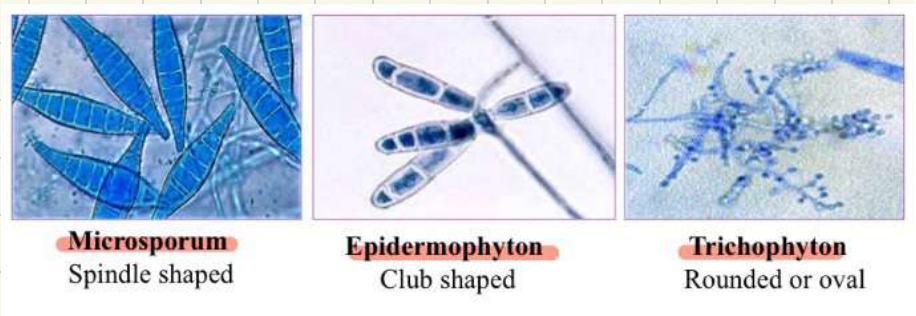
They are filamentous fungi and are classified into 3 genera:
1) Microsporum 2) Trichophyton 3) Epidermophyton

They infect only the superficial keratinized layers of the Skin, Hair and Nails. They never spread to deeper tissues. Infections are acquired from soil or active ringworm lesions (contagious disease).
The spores settle on the skin, germinate, and form a mass of branching hyphae which-grows out radially to produce circular or ring-like lesions.




Specimen is placed on a slide with a drop of 20% KOH to digest the keratin surrounding the fungus.
All species of dermatophytes appear as septate hyphae and arthrospores.

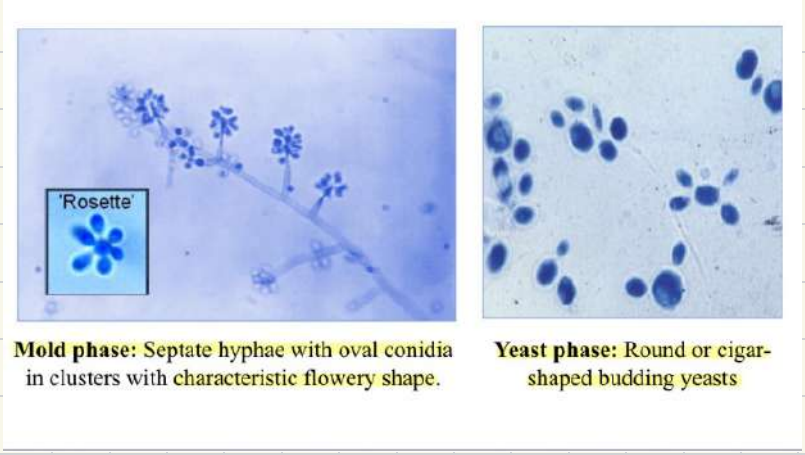
Culture
On sabouraud's agar: Incubate at room temp (22- 25°C) for up to 3 weeks



# Subcutaneous Mycoses

These are caused by fungi that grow in soil and on vegetation and acquired only when the fungus is implanted into subcutaneous tissues by trauma.

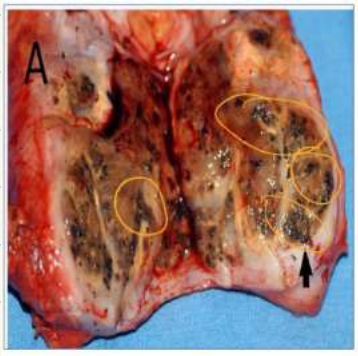
Sporotrichosis “Rose gardener's disease”:
Caused by Sporothrix schenckii, a dimorphic fungus. Spores of the mold found on rose thorns, hay, sphagnum moss, twigs, and soil, and introduced into the skin, typically by a thorn, cuts or abrasions, so, occurs most often in gardeners or farmers because they may be stuck by a rose thorn.
It occurs in the form of a local nodule (which may ulcerate) with nodules along the draining lymphatics.



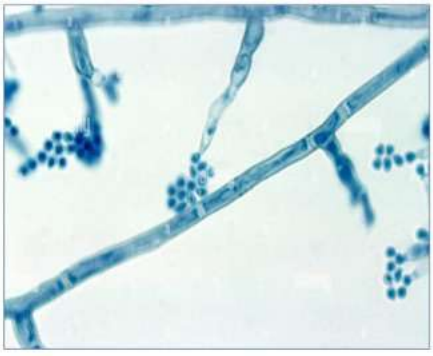

**Mold phase:** Septate hyphae with oval conidia in clusters with characteristic flowery shape.

**Yeast phase:** Round or cigar-shaped budding yeasts

Mycetoma
Caused by mold Madurella mycetomatis, and the foot is the common site affected and is usually called "Madura foot".
A localized chronic granuloma with progressive destruction of deeper tissues.
Mycetoma swelling is nodular and contains sinuses which usually open on the surface of the skin, discharging mucopus containing small black granules which are compact colonies of the causative organism.




**Black granules in infected tissue**

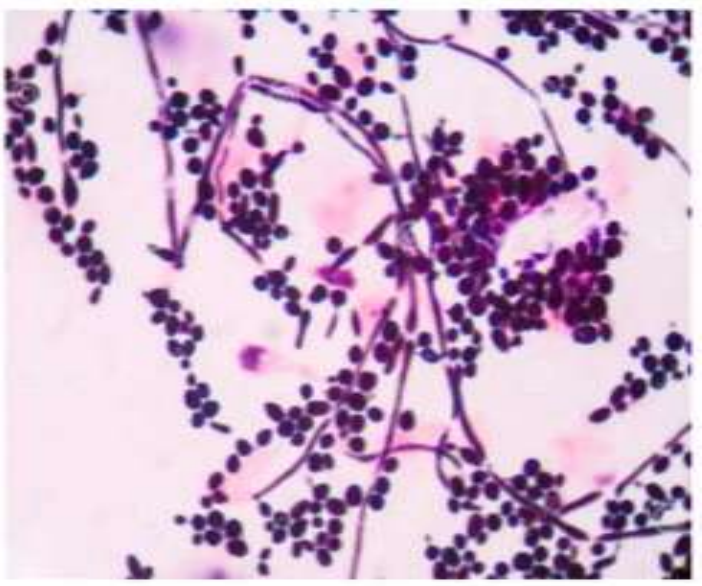


**Septate hyphae and chlamydospores**

# Deep Mycoses

## CANDIDIASIS

Systemic (invasive) candidiasis is a serious infection of the blood or other normally sterile sites. Affects mostly immunosuppressed and hospitalized individuals.
Candida albicans (budding yeast) is the commonest cause of candidiasis, but C. tropicalis and C. parapsilosis are important pathogens also.
Infection is mostly blood borne e.g. Central venous catheters, intravenous drug use, Major surgery,..)
Systemic Candidiasis include:
1- Candida bloodstream infection (candidemia).
2- Broncho-pulmonary infection.
3- Endocarditis.
4- Meningitis.
5- Endophthalmitis (infection within the eye).



Laboratory Diagnosis
Specimen: According to the lesion:
- In superficial candidiasis: Skin scraping, vaginal discharge, oral swab,..
- In deep candidiasis: Blood, Urine, Sputum,...

Direct microscopy:
In Gram stained film, candida appear as Gram + ve, oval, budding cells with pseudohyphae

Culture:
On sabouraud's agar at 37°C for 1-2 days, colonies are cream colored, pasty, with distinctive yeast smell.
Biochemical reactions:
C. albicans ferment Glucose & Maltose (acid&gas) and Sucrose (acid only).
Serology: A rise in antibody titer is of diagnostic value in diagnosis of deep candida infections.


Differential tests:
To differentiate between C. albicans and non-C.albicans species
1- Germ tube test:
When Candida is grown in human serum at 37°C for 3 hours, they forms a germ tubes (tubal outgrowth extending from yeast cells). C. albicans gives + ve test.
2- Chlamydospore formation:
When candida is cultured on corn meal extract agar medium (deficient medium), it produce pseudohyphae and chlamydospores. C. albicans gives + ve test.
3 Chrom agar (Indicator media):
Different candidas give different colored colonies.

# Aspergillus

## A. fumigatus:

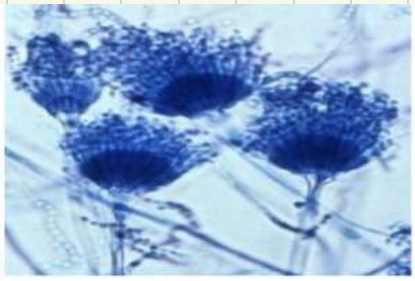
Causes pulmonary Aspergillosis, (in patients with a pre-existing lung disease). 1- Aspergilloma or " Fungus ball": Fungus grow in a pre- existing cavity e.g. T.B. cavity.
X- ray shows fungus ball.
2- Invasive Aspergillosis: Mainly occurs in immunocompromised persons, and usually fatal. Fungus invades lung tissues giving rise to pneumonia and hemoptysis. Dissemination to other organs occur leading to disseminated Aspergillosis
. 3- Allergic bronchopulmonary aspergillosis.

## A. flavus:

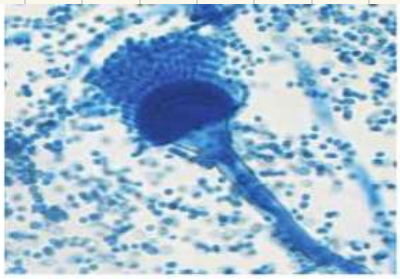
Produce aflatoxins which cause chronic damage & neoplasm in liver (Cancer liver)

## A. niger:

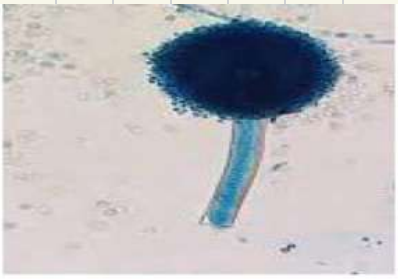
Causes otomycosis, chronic infection of the external auditory meatus.
Manifested by pain, itching and ear discharge.



***A. Fumigatus***
**Flask shaped head**

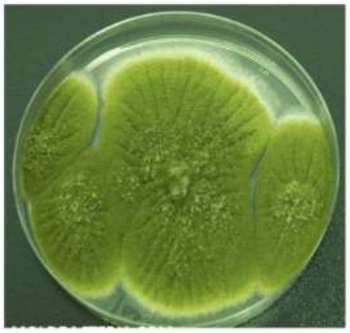


***A. Flavus***
**Hemi-spherical head**



***A. Niger***
**Rounded head**

***A. Fumigatus***
Smoky green



***A. Flavus***
Yellow-green

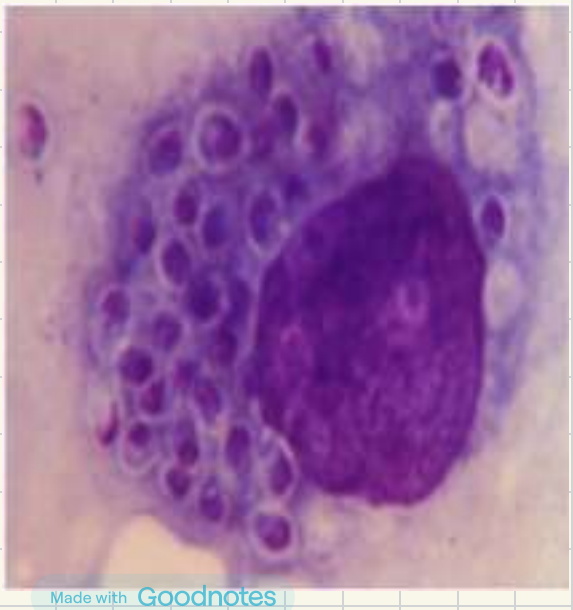
***A. Niger***
Black

# Histoplasmosis

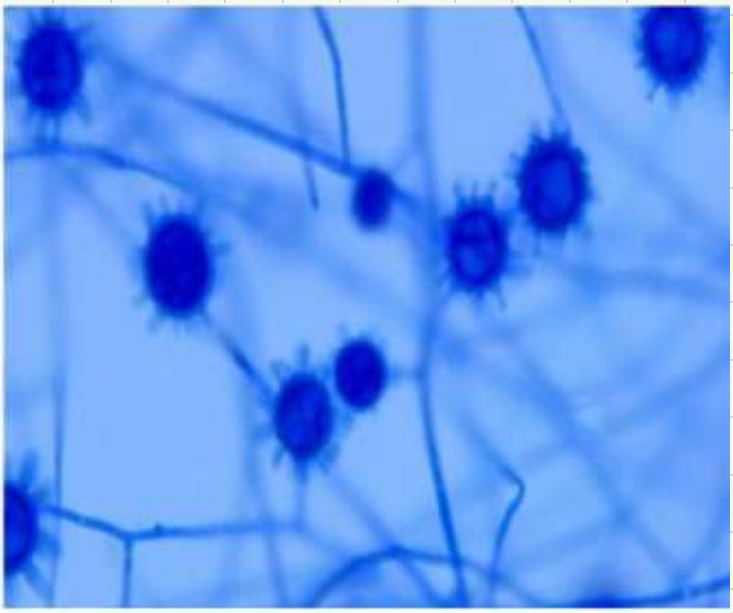
It is an intracellular mycoses of the Reticuloendothelial system caused by dimorphic Fungi called Histoplasma capsulatum.
Infection is acquired by inhalation of the spores. Inhaled spores are engulfed by alveolar macrophages, resist intracellular killing and develop into budding cells. Spores may spread from the lung to any part of the body.
Infection may be either:
Asymptomatic,
Acute: Due to heavy exposure. Similar to pneumonia,
Disseminated: into reticuloendothelial system with lymphadenopathy, enlarged liver & spleen, high fever and anemia.

Specimen is taken from sputum, Bone marrow aspirate or blood.

In Giemsa stained preparations, yeast form can be seen intracellular as round or oval budding cells.



On sabouraud's agar at room temperature for up to 3 weeks produce filamentous growth.
*A lactophenol cotton blue stained film show
septate hyphae and round spores with finger like projections.



# Cryptococcus

Opportunistic mycoses usually affecting the lungs, brain and meninges,
Caused by a budding yeast called Cryptococcus neoformans.
Found in large amounts in dry pigeon faeces and this is the main source of human infection. Human infection occur by inhalation of yeast cells or its spores.
In normal persons the infection may be:-Asymptomatic or mild pulmonary symptoms.
In immunocompromised Persons: A primary pulmonary infection occurs and then disseminates mainly to Meninges.
Clinically the commonest manifestation is chronic meningitis with a fluctuating course usually called:- "Cryptococcal meningitis ".
It is fatal unless treated.

Specimen: C.S.F. or Sputum.
Direct microscopy:
Yeast cells of cryptococcus neoformans can be detected best in an India ink preparation from C.S.F.
-Cells appear rounded surrounded by a wide capsule (white unstained halo) against a black background.


On sabouraud's agar at 37°C for 2-3 days, colonies are cream colored, mucoid & Shiny.
Serology:
Detection of capsular Ag by latex agglutination
in C.S.F of patients is of diagnostic value.