Tishk International University-Nursing
Medical Microbiology

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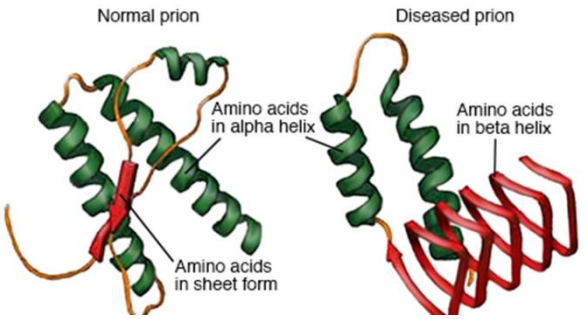
Lec.8-9

- **Medical microbiology:** Is a branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases.-Microorganisms that cause infectious disease: bacteria, fungi, parasites and viruses, and one type of infectious protein called prion.



-Medical microbiology also involves the study of beneficial microbes.

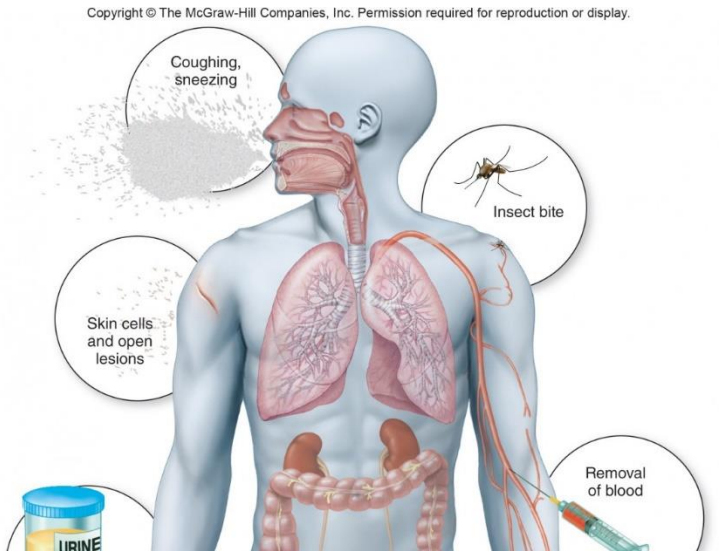
-Microbes have been shown to be helpful (microorganisms a source of antibiotics)

-Some may also act as probiotics to provide health benefits to the host, such as providing better gastrointestinal health or inhibiting pathogens.

- **Infection:**
- Infections may be caused by bacteria, viruses, fungi, and parasites. The pathogen that causes the disease may be
- **exogenous** (acquired from an external source; environmental, animal or other people, e.g. Influenza) or
- **endogenous** (from normal flora e.g. candidiasis).

-The site at which a microbe enters the body is referred to as **portal of entry**. These include the respiratory tract, gastrointestinal tract, genitourinary tract, skin..

- The portal of entry for a specific microbe is normally dependent on how it travels from its natural habitat to the host



- **Methods for disease transformation**

- Direct contact - Touching an infected host, including sexual contact
- Indirect contact - Touching a contaminated surface
- Droplet contact - Coughing or sneezing
- Fecal–oral route - Ingesting contaminated food or water sources
- Airborne transmission - Pathogen carrying spores
- Vector transmission - An organism that does not cause disease itself but transmits infection by conveying pathogens from one host to another
- Fomite transmission - An inanimate object or substance capable of carrying infectious germs or parasites
- Environmental - Hospital-acquired infection (Nosocomial infections)

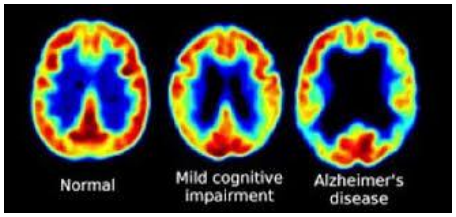
- **Diagnosis of infectious disease**

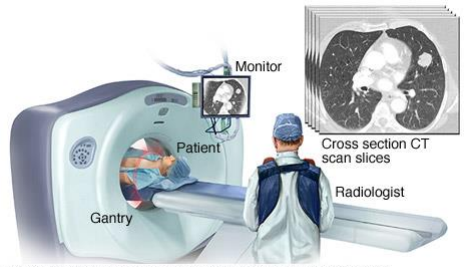
1- Is nearly always initiated by consulting the patient's medical history conducting a physical examination.

2- More detailed identification techniques involve

Microbial culture, microscopy, biochemical tests and genotyping.

3- Other less common techniques (such as X-rays, CT scans- computerize tomography, PET scan- positron emission tomography or NMR-Nuclear Magnetic Resonance) are used to produce images of internal abnormalities resulting from the growth of an infectious agent.





More detailed identification techniques involve

a- Microbial culture

- Microbiological culture is the primary method used for isolating infectious disease for study in the laboratory. Tissue or fluid samples are tested for the presence of a specific pathogen, which is determined by growth in a selective or differential medium.

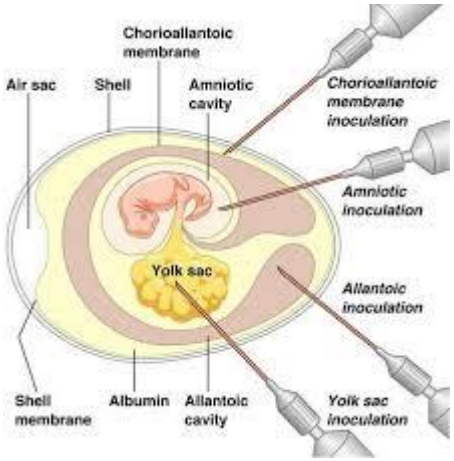
The main types of media used for testing are

- Solid culture(Bacteria and fungi)
- Liquid culture: (parasites and detecting mycobacteria)

On the base of function:

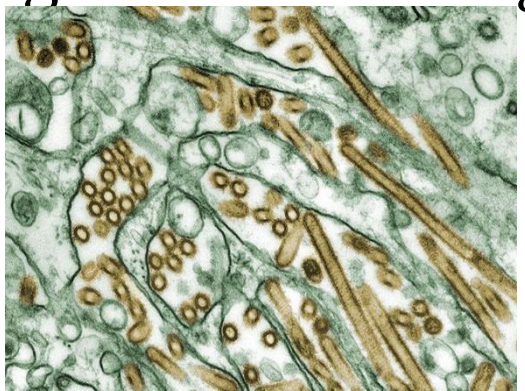
Selective, differential, enriched, enrichment ……..

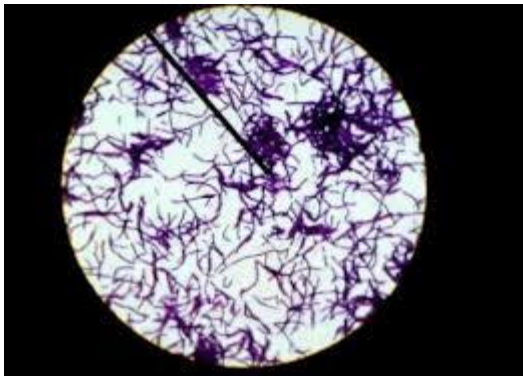
- Cell culture: animal cell cultures are infected with the microbe of interest. These cultures are then observed to determine the effect the microbe has on the cells. This technique is used for identifying viruses

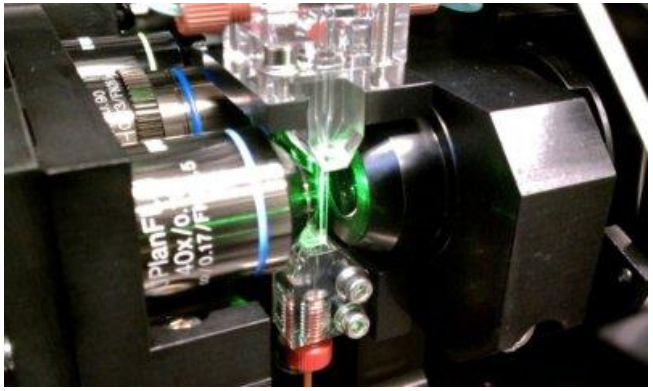


B-Microscopy

- Culture techniques will often use a microscopic examination to help in the identification of the microbe. Instruments such as compound light microscopes can be used to assess critical aspects of the organism. This can be performed immediately after the sample is taken from the patient and is used in conjunction with biochemical staining techniques, allowing for resolution of cellular features.
- Electron microscopes and fluorescence microscopes are also used for observing microbes in greater detail for research.







C- Biochemical tests

- Fast and relatively simple biochemical tests can be used to identify infectious agents.
- For bacterial identification, the use of **metabolic or enzymatic characteristics are common due to their** ability to ferment carbohydrates in patterns characteristic of their genus and species.
- Acids, alcohols and gases are usually detected in these tests when bacteria are grown in selective liquid or solid media, as mentioned above.
- In order to perform these tests an automated machines are used. Like (Api test)These machines perform multiple biochemical tests simultaneously, using cards with several wells containing different dehydrated chemicals. The microbe of interest will react with each chemical in a specific way, aiding in its identification.

Infor.

Gram (+) cocci

Catalase

(+)

Staphylococci

Coagulase MSA

(+)

S. aureus

(-)

CONS

(-)

Streptococci

Hemolysis (BAP)

Alpha

Esculin (+) NaCl (+)

Enterococci

Esculin (+) NaCl (-)

Group D Non-Enterococci

Esculin (-) NaCl (-) Taxo P sensitive

S. pneumoniae

Beta

Taxo A

Sensitive

Group A *S. pyogenes*

Resistant

CAMP Test

(+)

Group B *S. agalactiae*

(-)

Esculin (+) NaCl (+)

Enterococci

Esculin (+) NaCl (-)

Group D Non-Enterococci

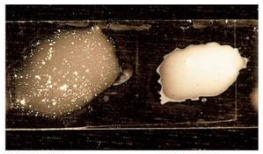
Gamma

Esculin (+) NaCl (+)

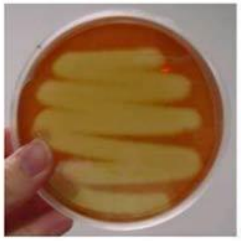
Enterococci

Esculin (+) NaCl (-)

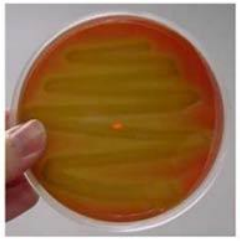
Group D Non-Enterococci



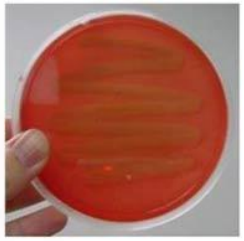
(a) Positive test (b) Negative test



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

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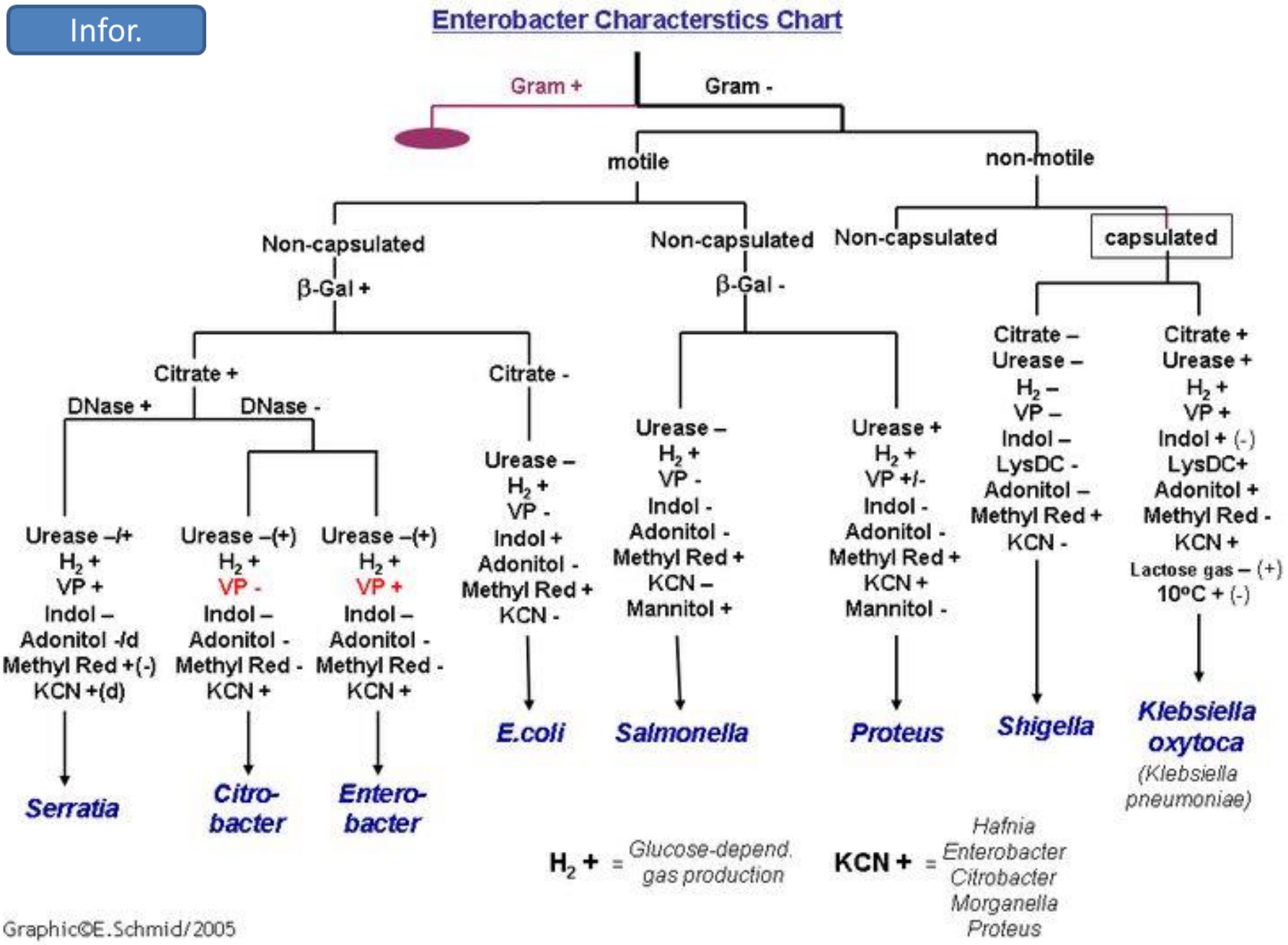
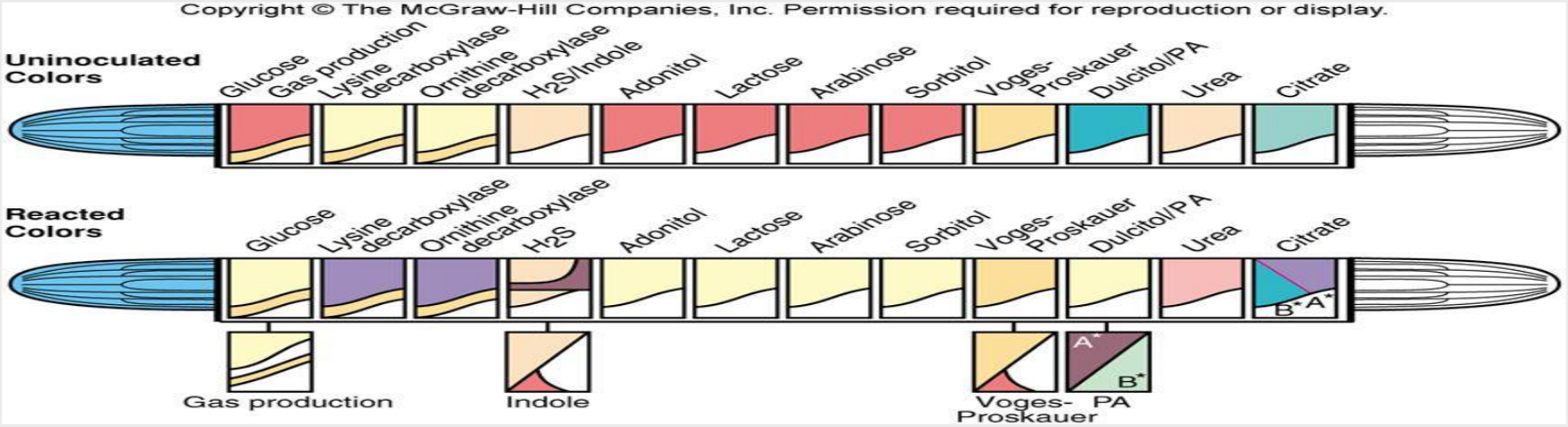

Enterobacter Characterstics Chart
Gram +
Gram -
motile
non-motile
Non-capsulated
β-Gal +
Non-capsulated
β-Gal -
Non-capsulated
capsulated
Citrate +
DNase +
DNase -
Citrate -
Urease –
H2 +
VP -
Indol +
Adonitol -
Methyl Red +
KCN -
E.coli
Urease –
H2 +
VP -
Indol -
Adonitol -
Methyl Red +
KCN –
Mannitol +
Salmonella
Urease +
H2 +
VP +/-
Indol -
Adonitol -
Methyl Red +
KCN +
Mannitol -
Proteus
Citrate –
Urease –
H2 –
VP –
Indol –
LysDC -
Adonitol –
Methyl Red +
KCN -
Shigella
Citrate +
Urease +
H2 +
VP +
Indol + (-)
LysDC+
Adonitol +
Methyl Red -
KCN +
Lactose gas – (+)
10°C + (-)
Klebsiella oxytoca
(Klebsiella pneumoniae)
Urease –/+
H2 +
VP +
Indol –
Adonitol -/d
Methyl Red +(-)
KCN +(d)
Serratia
Urease –(+)
H2 +
VP -
Indol –
Adonitol -
Methyl Red -
KCN +
Citro-bacter
Urease –(+)
H2 +
VP +
Indol –
Adonitol -
Methyl Red -
KCN +
Entero-bacter
H2 + = Glucose-depend. gas production
KCN + = Hafnia Enterobacter Citrobacter Morganella Proteus
Graphic©E.Schmid/2005

Infor.

Figure 20.12 BBL Enterotube II, rapid biochemical testing of enterics



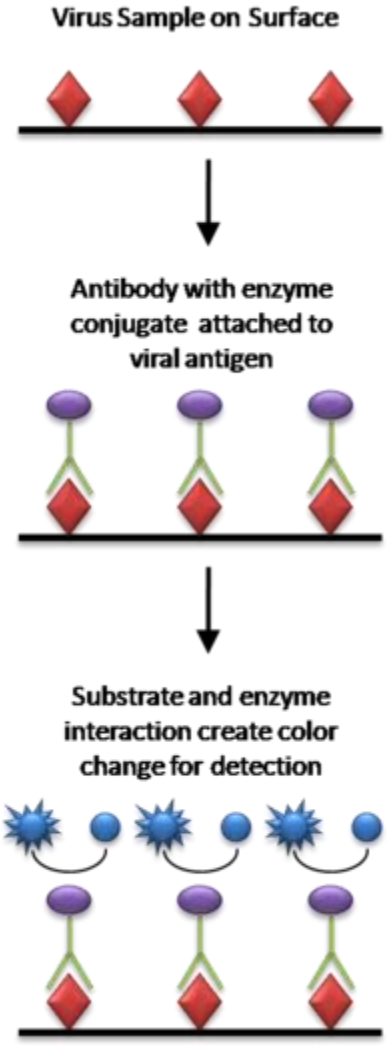
API(Analytical profile idex)

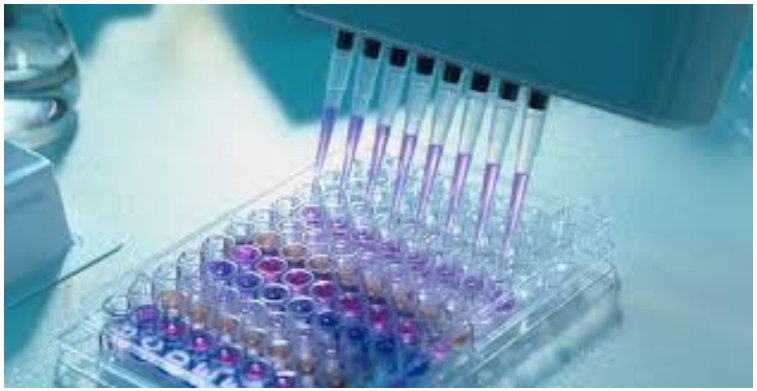
Info.

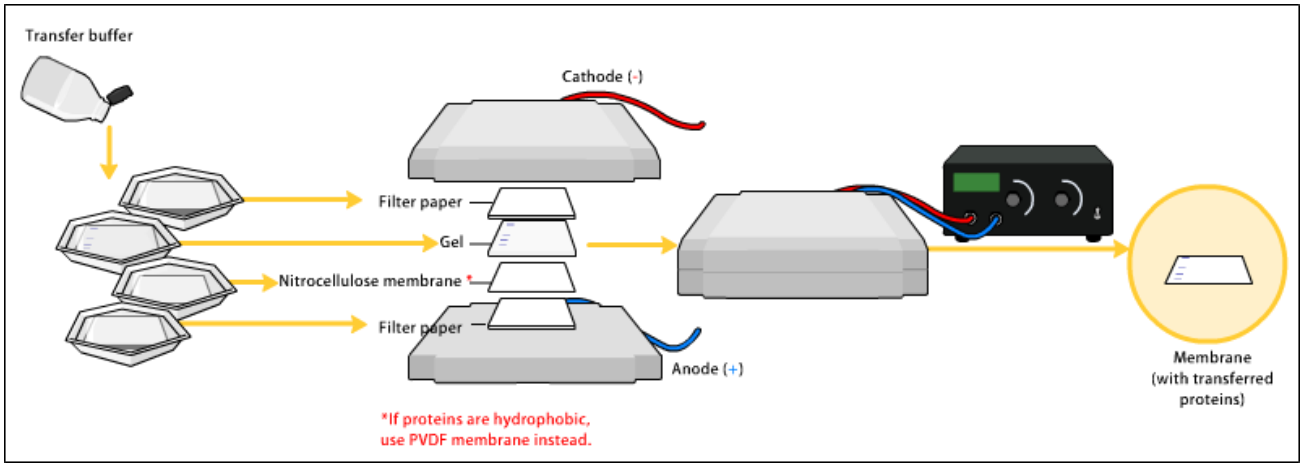
ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside
ADH: decarboxylation of the amino acid arginine by arginine dihydrolase
LDC: decarboxylations of the amino acid lysine by lysine decarboxylase
ODC: decarboxylations of the amino acid ornithine by ornithine decarboxylase
CIT: utilization of citrate as only carbon source
H2S: production of hydrogen sulfide
URE: test for the enzyme urease
TDA (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent to put- Ferric Chloride.
IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase . Reagent- Indole is detected by addition of Kovac's reagent.
VP: the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway
GEL: test for the production of the enzyme gelatinase which liquefies gelatin
GLU: fermentation of glucose (hexose sugar)
MAN: fermentation of mannose (hexose sugar)
INO: fermentation of inositol (cyclic polyalcohol)
SOR: fermentation of sorbitol (alcohol sugar)
RHA: fermentation of rhamnose (methyl pentose sugar)
SAC: fermentation of sucrose (disaccharide)
MEL: fermentation of melibiose (disaccharide)
AMY: fermentation of amygdalin (glycoside)
ARA: fermentation of arabinose (pentose sugar)

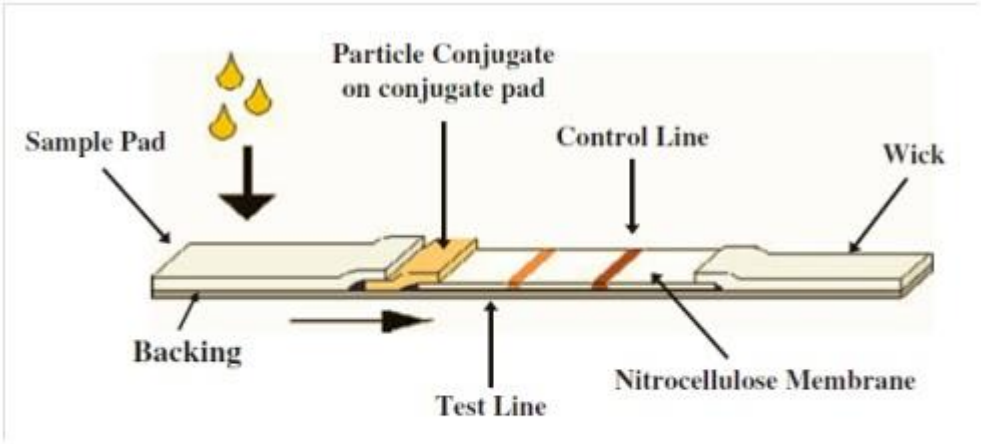
- **Serological methods** are highly sensitive, specific and often extremely rapid laboratory tests used to identify different types of microorganisms.
- Serological tests, involving the reactions of microorganisms with specific antibodies, are useful in determining the identity of strains and species, as well as relationships among organisms.
- Slide agglutination, ELISA, and Western blotting are examples of serological tests.
- flow cytometer also employs serological methods, but doesn't have to.

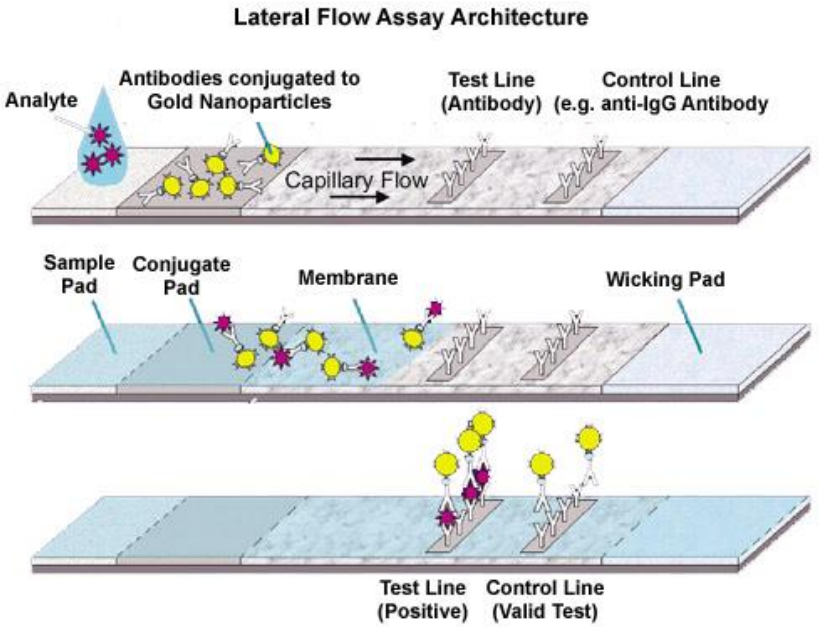
- More complex serological techniques are known as immunoassays., Immunoassays can detect or measure antigens from either infectious agents or the proteins generated by an infected host in response to the infection


Virus Sample on Surface
Antibody with enzyme conjugate attached to viral antigen
Substrate and enzyme interaction create color change for detection




Transfer buffer
Cathode (-)
Filter paper
Gel
Nitrocellulose membrane *
Filter paper
Anode (+)
Membrane (with transferred proteins)
*If proteins are hydrophobic, use PVDF membrane instead.





Molecular test (genotyping): The DNA and RNA based methods (including % G+C comparisons, PCR, rRNA sequencing,

- **Treatment:**
- Medical microbiologists often make treatment recommendations to the patient's physician based on the strain of microbe and its antibiotic resistances, the site of infection, the potential toxicity of antimicrobial drugs and any drug allergies the patient has.

Biofilm and biofilm formation

A biofilm is any group of microorganisms in which cells stick to each other and often these cells adhere to a surface.
These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS).

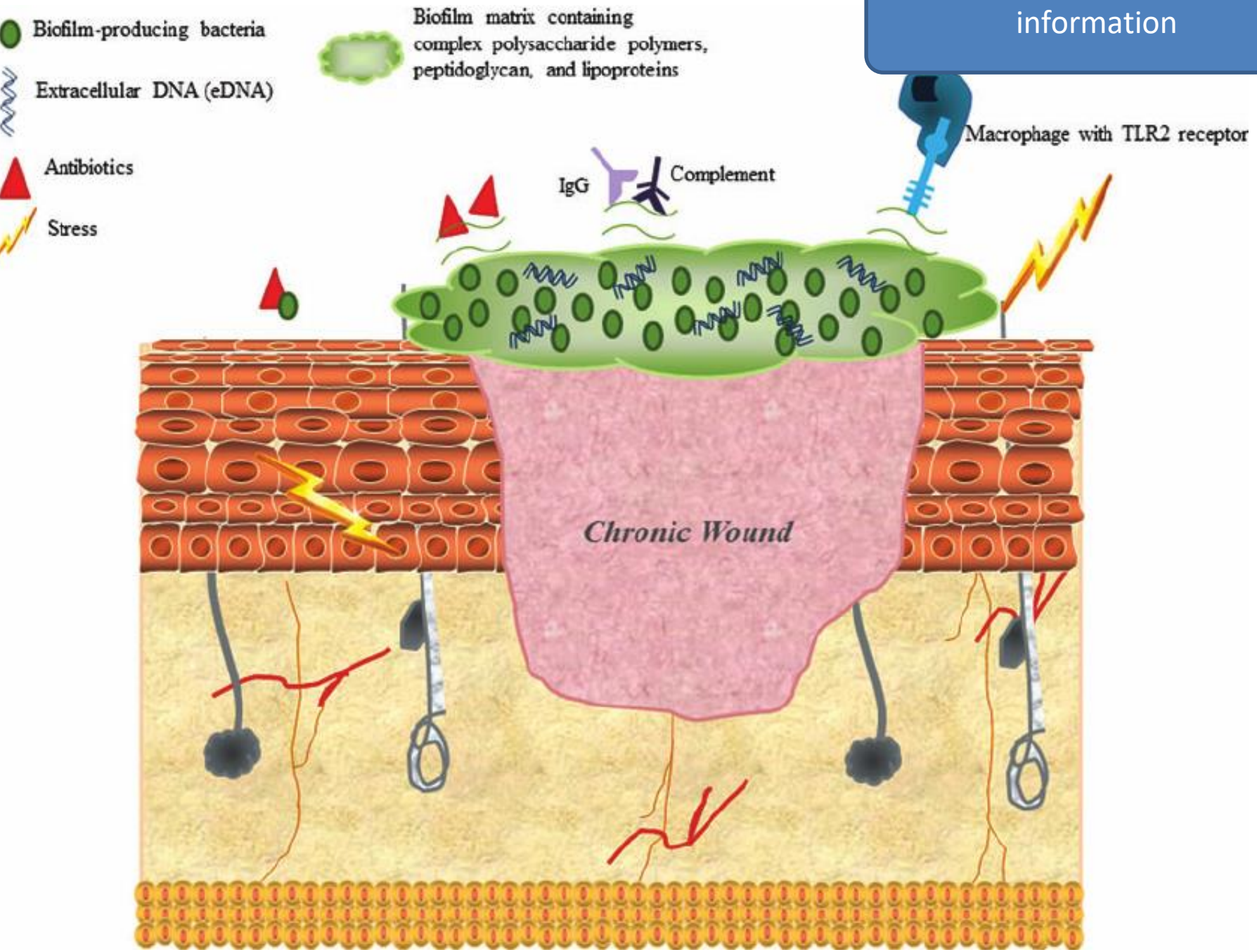
Biofilm extracellular polymeric substance is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides.

Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings
The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single-cells that may float or swim in a liquid medium.

Why do bacteria join biofilms?

Nutrient acquisition, Protection

information

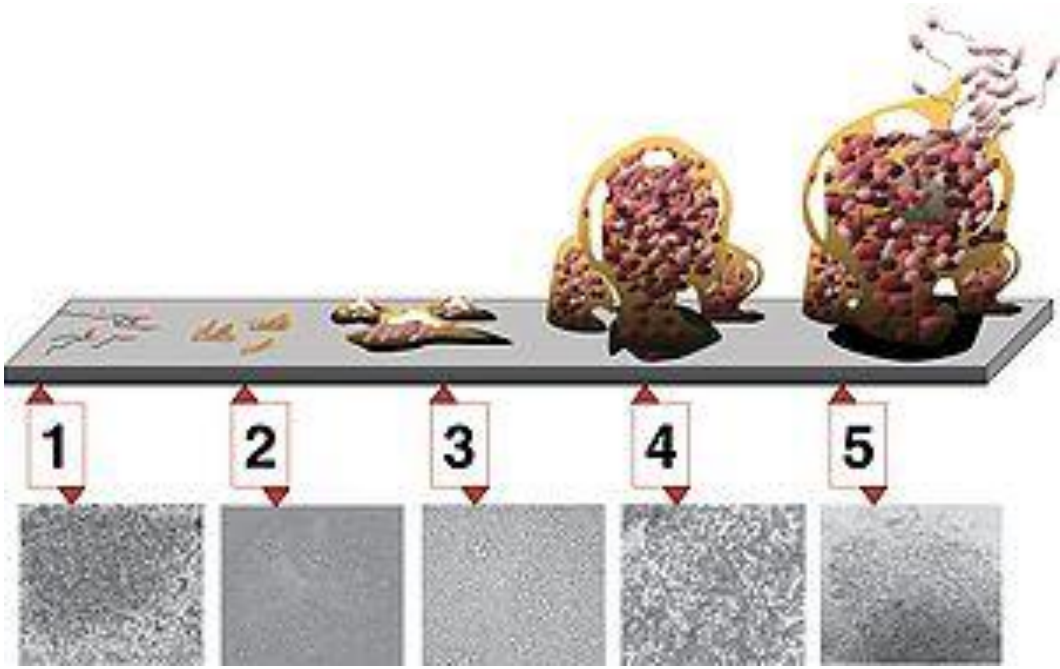


- Form in places with access to water
- Attach to a solid surface using several means:

  Flagella, Hydrophobic Cell Walls, Sticky Polymers.

There are five stages of biofilm development:

1.Initial attachment.
2.Irreversible attachment.
3.Maturation I.
4.Maturation II.
5.Dispersion.



Formation of Biofilms

- Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface.

- the first colonists of a biofilm adhere to the surface initially through weak, reversible adhesion via van der Waals forces and hydrophobic effects. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

- Some species are not able to attach to a surface on their own but are instead able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing (QS) using products such as N-acyl homoserine lactone (AHL).

- Some bacteria are unable to form biofilms as successfully due to their limited motility.
- Non-motile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria.

Uses of Biofilms

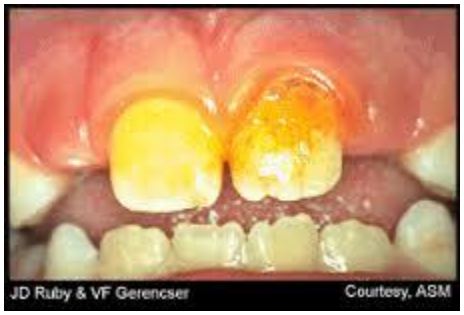
- Often used to purify water in water treatment plants
- Used to break down toxic chemicals
- Used to produce useful biological compounds, including medicines

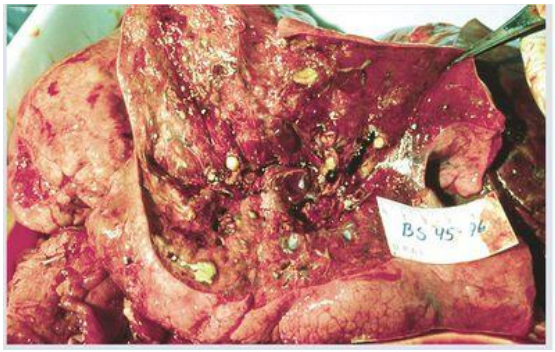
Problems Caused by Biofilms

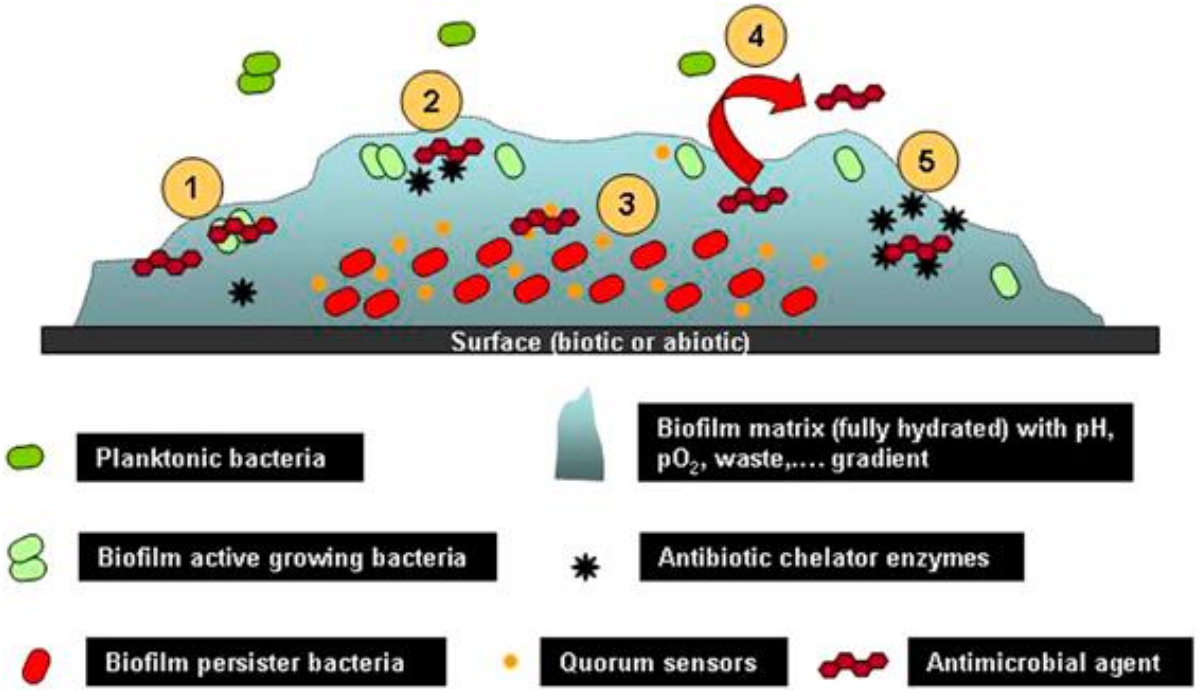
- Tend to clog pipes and water filters
- Can cause numerous diseases, including many diseases prevalent in hospitals
- Extra-resistant to antibiotics
- Can form almost anywhere that water is present, including catheters, kitchen counters, etc.

Chronic infection with biofilm association

- Plaque
- Infectious Bacterial Endocarditis
- Urinary tract infections
- Cystic Fibrosis
- Tuberculosis
- MRSA





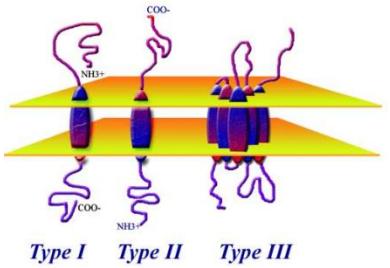


- **Virulence factor:** Virulence factors are molecules produced by pathogenic bacteria, viruses, fungi, and protozoa that add to their effectiveness and enable attachment to cells, evasion of the host's immune response, inhibition of the host's immune response .obtain nutrition from the host
- Virulence:
  - defined as quantitative measure of pathogenicity, and is measured by minimum number of bacteria to cause the disease.
- LD 50:
  - Number of organisms required to kill 50% hosts.
- ID 50:
  - Number of organisms required to cause infection in 50% of host population.

- Organisms with lower LD 50 are more virulent than higher ID 50, as fewer bacteria are required to cause death or disease.
- Infectious dose varies among organisms.
- *Shigella* (ID 50 less than 100) and *Salmonella* (100,000) cause diarrhea by producing infection in GIT.

Bacterial VF can be divided into several groups on the basis of the mechanism of virulence and function:

Membrane Proteins



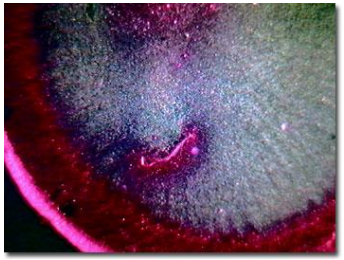
Adhesion, colonization and invasion

Promote adherence to the host cell surface

Responsible for resistance to antibiotics

Promote intercellular communication

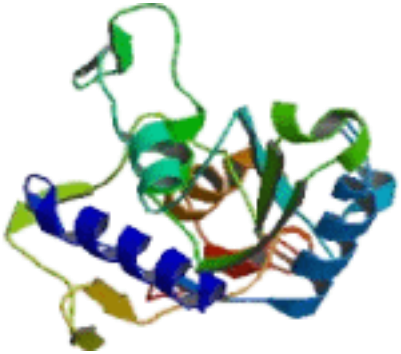
Polysaccharide Capsules



surround the bacterial cell and

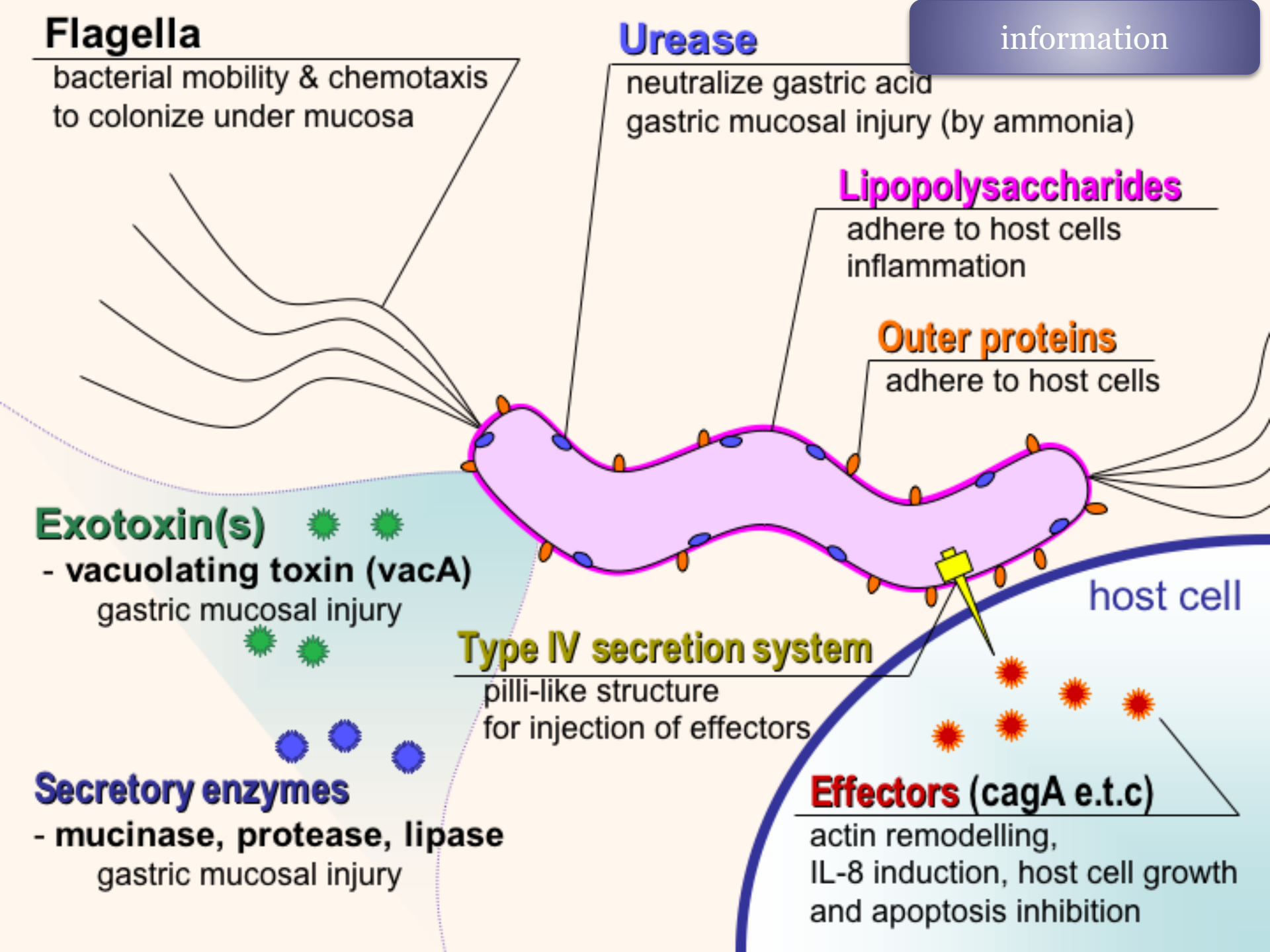
have anti-phagocity properties

Secretory Proteins



can be toxins

can modify the host cell environment and are responsible for some host cell-bacteria interactions


Flagella
bacterial mobility & chemotaxis
to colonize under mucosa
Urease
neutralize gastric acid
gastric mucosal injury (by ammonia)
information
Lipopolysaccharides
adhere to host cells
inflammation
Outer proteins
adhere to host cells
Exotoxin(s)
- vacuolating toxin (vacA)
gastric mucosal injury
host cell
Type IV secretion system
pilli-like structure
for injection of effectors
Secretory enzymes
- mucinase, protease, lipase
gastric mucosal injury
Effectors (cagA e.t.c)
actin remodelling,
IL-8 induction, host cell growth
and apoptosis inhibition

- **Bacteria causes disease by one of the 3 mechanisms:**
  1. Immunopathogenesis. Immunopathogenesis is the process of disease development involving an immune response or components thereof.
  2. Invasion of the tissue, followed by inflammation.
  3. Toxin production

Enzymes secreted by invasive bacteria play role in producing the disease These are :

-Collagenase & Hyaluronidase: these enzymes degrade collagen and hyaluronic acid allowing the bacteria to spread through subcutaneous tissue e.g *Streptococcus pyogenese.*

-Coagulase: it accelerates the formation of fibrin clot e.g *Staphylococcus aureus.*

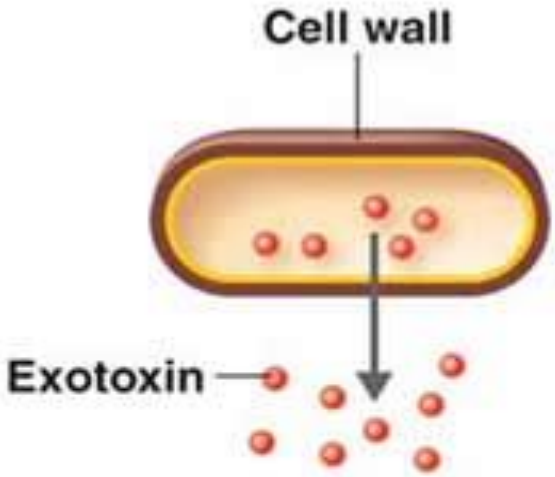
-Immunoglobulin A (IgA) protease, it degrades IgA allowing the organism to adhere to the mucousmembrane e.g N. *gonorrhae, Haemophilus influenzae, strept. Pneumonae.*

-Leucocidine, it destroys neutrophilic leukocytes and macrophaeges.

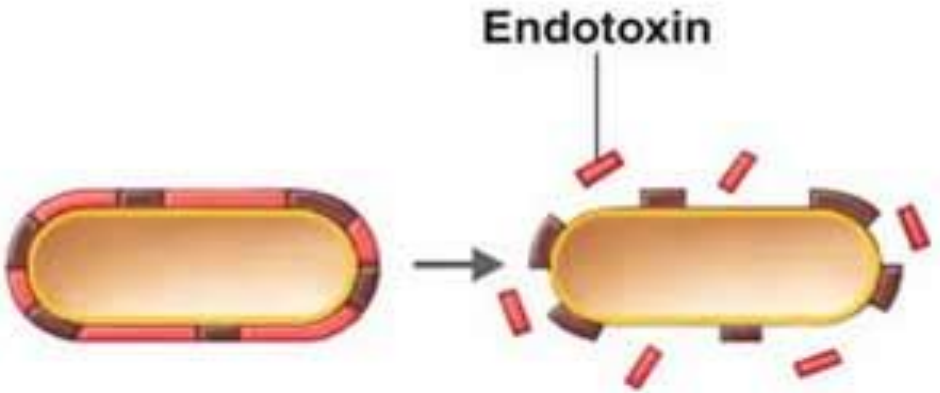
Toxin Production

It is the second major mechanism for disease production.

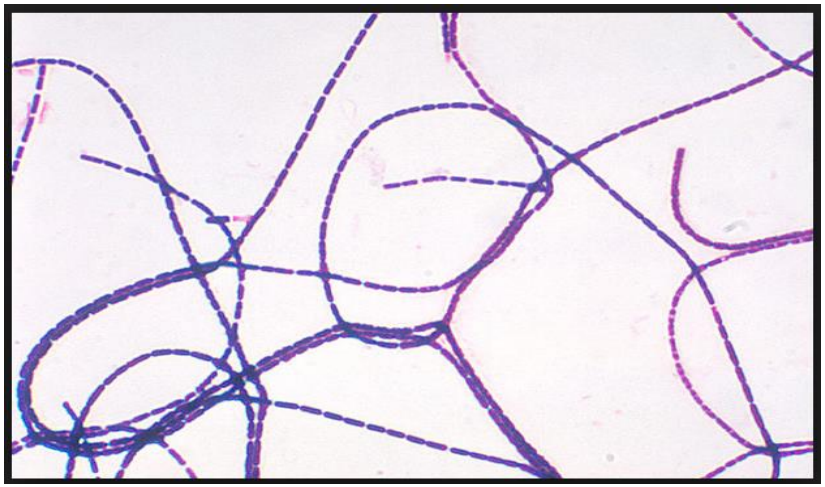
Differences Between Exotoxins and Endotoxins



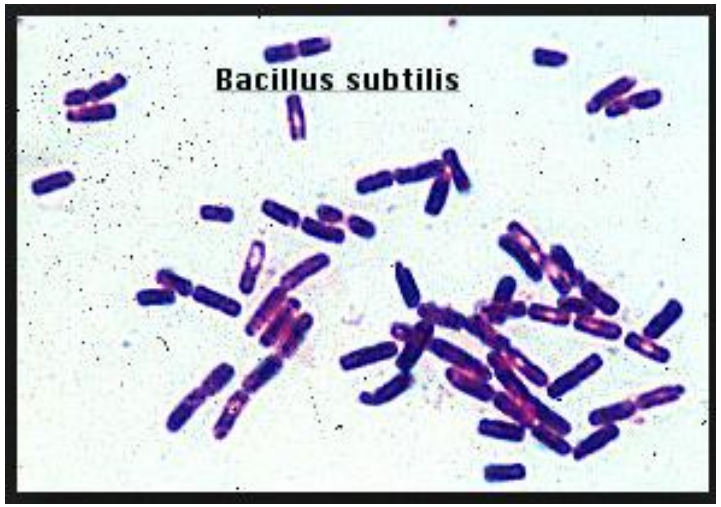
(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.

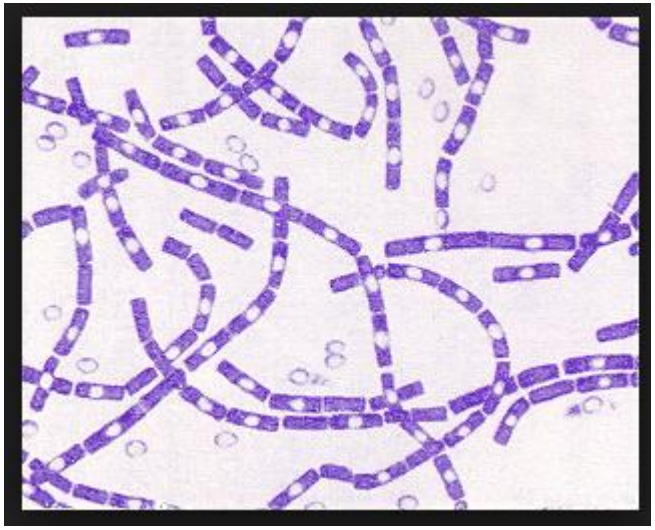


(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of ative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.



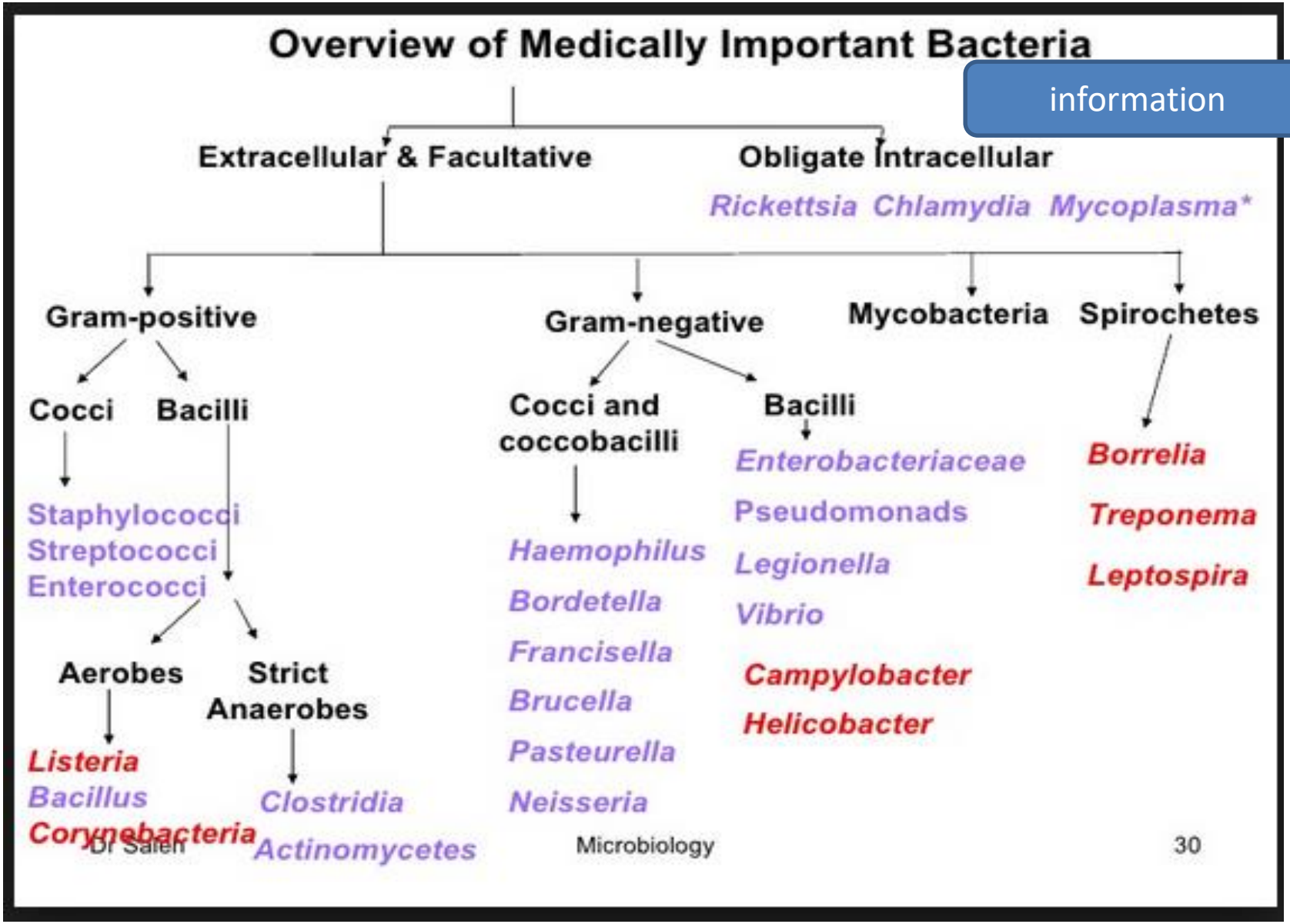
Gram positive rods





Overview of Medically Important Bacteria

information



- **Member of Disease causing microorganism**

1- Rod shaped spore forming bacteria :

-Bacillacea: this family consists of 2 general groups (*Bacillus* sp., *Clostridium* sp.)

-These bacteria have endospor which is resistance to heat so these resistances make bacterium a problem in the canning food and other food preservation.

-Also they produce different enzyme which hydrolyze carbohydrate, lipid and protein so they can cause spoilage of food.

For information

- *Clostridium tetani*- tetanus
- *Clostridium botulinum*- botulism
- *Clostridium sporogenes* – spoilage the protein food
- *Clostridium perfringens*- produce interotoxin
- *Bacillus anthracis* – anthrax
- *Bacillus congulunas* responsible for spoilage of pasteurized milk.

Bacillus species

Two Bacillus species are considered medically significant:

1- ***B. anthracis***, which causes anthrax
2-***B. cereus***, which causes food poisoning similar to that caused by *Staphylococcus*.

An easy way to isolate *Bacillus* species is by placing non sterile soil in a test tube with water, shaking, placing on mannitol salt agar, and incubating at room temperature for at least a day. Colonies are usually large, spreading, and irregularly shaped. Under the microscope, the *Bacillus* cells appear as rods, and a substantial portion of the cells usually contain oval endospores at one end, making it bulge

Character of Bacillus spp.

1- Gram-positive

2-Rod-shaped (bacillus) bacteria

3- *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic)

4- positive for the enzyme catalase when there has been oxygen used or present

5- Ubiquitous in nature

6- Bacillus includes both free-living (nonparasitic) and (parasitic) pathogenic species.

7-Under stressful environmental conditions, the bacteria can produce oval **endospores** that are not true 'spores', but to which the bacteria can reduce themselves and remain in a dormant state for very long periods.

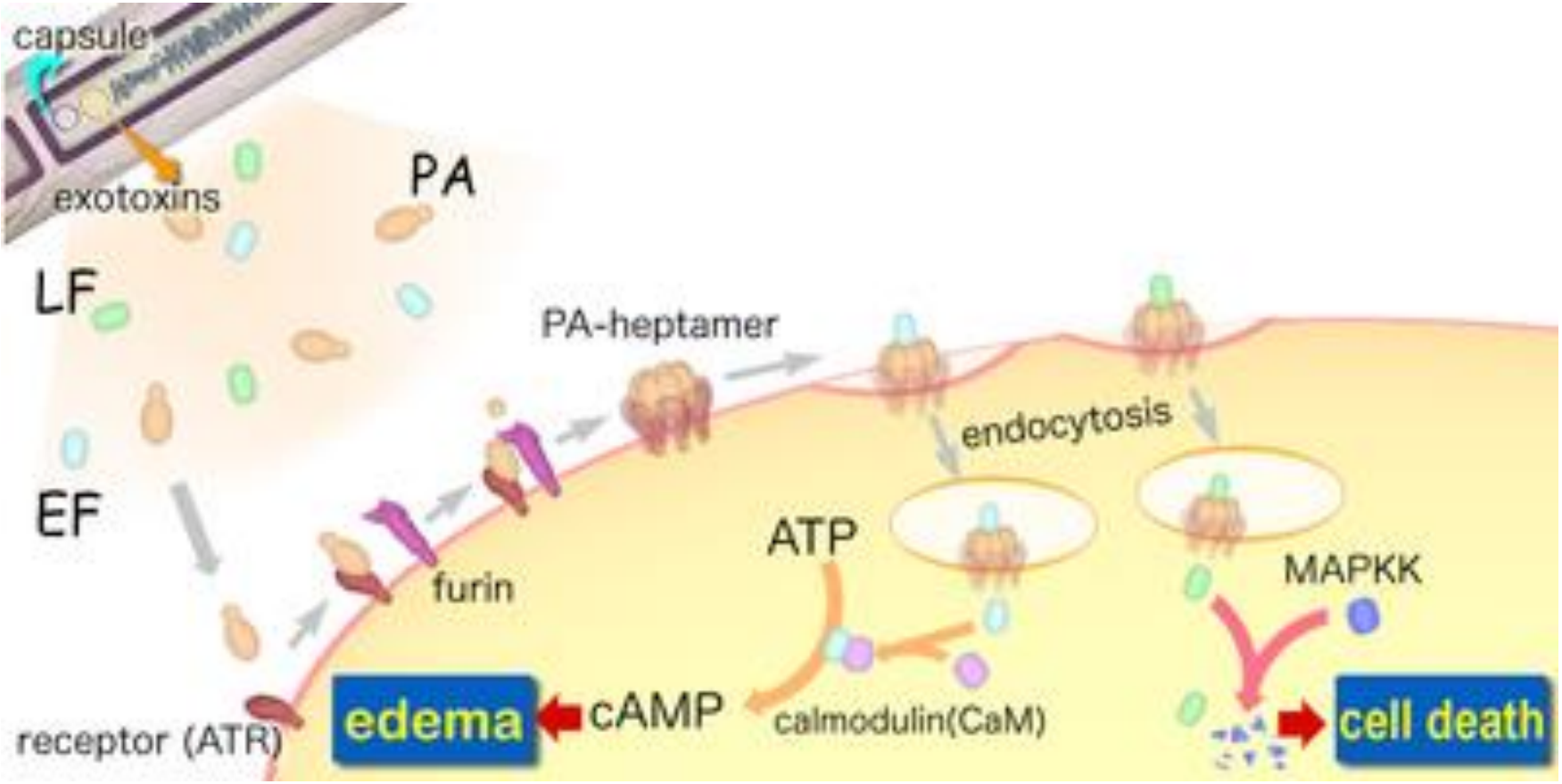
Bacillus antharcis (Anthrax disease)

Virulence:

The capsule is present in virulent strain . Virulent strains also produce three exotoxins that combine to form edema toxin (combination of protective antigen and endema factor) and lethal toxin (protective antigen with lethal factor).

Epidemiology:

- *B.anthracis* primarily infects herbivores with humans as accidental hosts.
- Rarely isolated in developed countries with humans as in impoverished areas where vaccination of animals is not practiced.
- Individuals at risk include people in endemic area in contract with infected animals or contaminated soil, people who work with animal materials imported from endemic area.
- There is significant concern that the spores will be used in Bioterrorism.


capsule
exotoxins
PA
LF
EF
PA-heptamer
endocytosis
furin
ATP
MAPKK
receptor (ATR)
edema
cAMP
calmodulin(CaM)
cell death

Disease:

Cutaneous anthrax is the most common form

Inhalation anthrax is the most deadly form

Gastrointestinal anthrax is a rare but commonly fatal disease

Diagnosis:

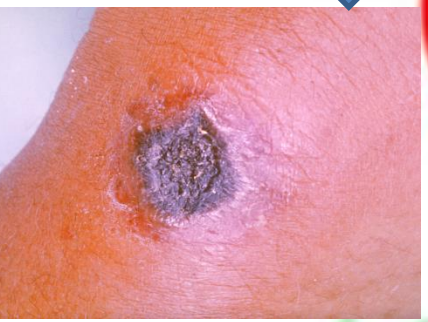
Isolation of the organism from clinical specimens (eg; papul or ulcer, blood)

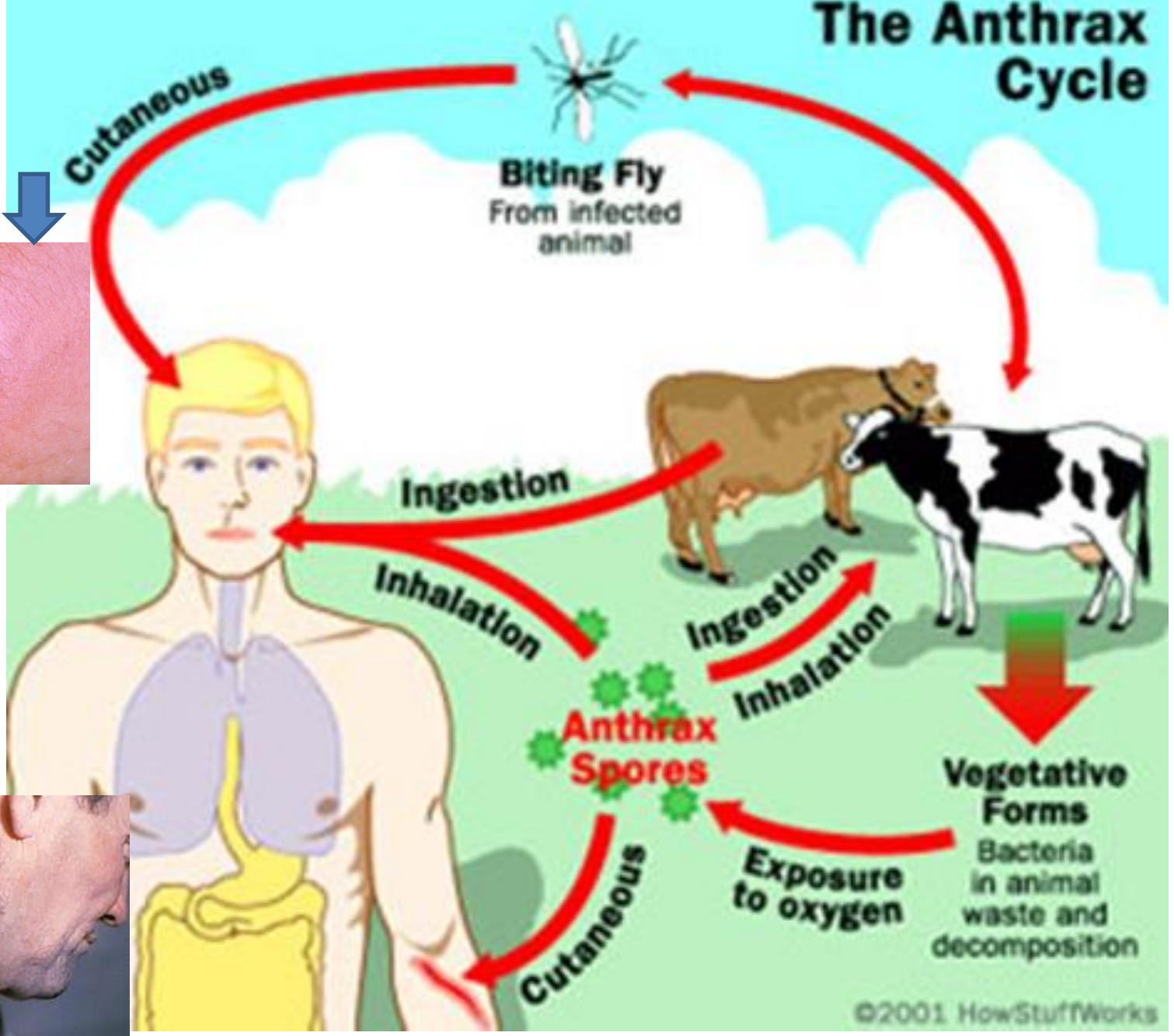
Treatment and prevention and control:

Ciprofloxacin is the drug of choice; penicillin, doxycycline, erythromycin or chloramphenicol can be used (if susceptible), but the bacteria are resistant to sulfonamides and intended-spectrum cephalosporin's.

Vaccination of animals herbs and people in endemic areas can control disease, but spores are difficult to eliminate from contaminated soil.

Animal vaccination is effective, but human vaccines have limited usefulness.




The Anthrax Cycle
Cutaneous
Biting Fly
From infected animal
Ingestion
Inhalation
Ingestion
Inhalation
Anthrax Spores
Vegetative Forms
Bacteria in animal waste and decomposition
Exposure to oxygen
Cutaneous
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