SHEET NO. 9

MICROBIOLOGY (Bacteriology)

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

DOCTOR : Dr.Anas

- In the last lecture we talked about the **Microbiota**, and we identified it as the collection of all microorganisms (including bacteria, fungi, and viruses) that reside within a certain part or organ in our bodies. In today's lecture we're going to talk about the pathogenicity of bacteria and how it infects us.

- Most bacteria are **harmless** or often **beneficial** (e.g. the microbiota in the gut), only a minority are **pathogenic** (disease causing).

- **Several thousand** species exist in the human digestive system without causing disease. In contrast, the number of species that are seen to cause infectious diseases in humans are estimated as **fewer than a hundred**.

> Pathogenicity is, in a sense, a highly skilled trade, and only a tiny minority of all the numberless tons of microbes on the earth has ever involved itself in it; most bacteria are busy with their own business, browsing and recycling the rest of life. Indeed, pathogenicity often seems to me a sort of biological accident in which signals are misdirected by the microbe or misinterpreted by the host.
>
> —Lewis Thomas, *The Medusa and the Snail*

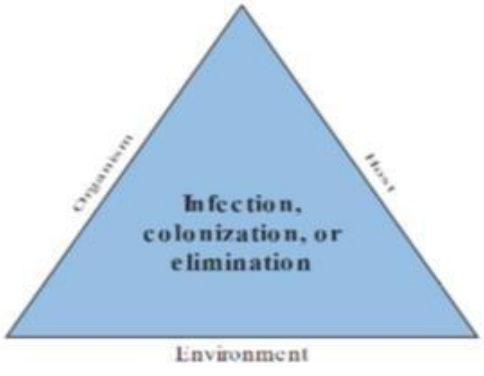
- So the main idea here is that there isn't a conscious mechanism for **Pathogenicity**, it's not directed by the microbe or even the host itself, the bacteria basically sends signals to get to their nutrients, and whenever the host senses the signals it will miss interpret these signals as danger and will recruit the immune system causing the manifestations of the disease.

- In order for a bacterium to cause a disease it needs 3 factors:

- **Organism**
- **Host**
- **Environment** (in which the microbe and the host cell meet, e.g. if they meet in the skin there wouldn't be a problem, if they meet in GI tract it would cause a disease).

- These 3 factors would give us 3 possibilities:

- **Infection**
- **Colonization** (may be part of the microbiota or a dormant colonized pathogen)
- **Elimination**



- This was mentioned in Ibn Sina's book 1000 thousand years ago:

(Pathogen)

وليس كل سبب يصل إلى البدن يفعل فيه بل قد يحتاج مع ذلك إلى أمور ثلاثة: إلى قوة من قوته الفاعلة، وقوة من قوة البدن الإستعدادية، وتمكن من ملاقاة أحدهما الآخر زماناً في مثله يصدر ذلك الفعل عنه.

(Environment) (Immune system)

-Before we move on, we have to clarify some terms:

- a **Pathogen** is a microorganism in which when it meets the host cell it will cause a disease, **Microbiota** are microorganisms in which when they meet the host cell they'd be harmless or even beneficial.

- **Opportunistic Pathogen** is a microorganism in which usually when it meets the host cell it wouldn't cause a disease unless the host was immunocompromised, e.g. a pathogen called Pseudomonas Aeruginosa, in healthy individuals it's very rare to cause infection, but in people who are suffering from burns the integrity of the epithelial skin surface is compromised which will may lead to an infection by pseudomonas aeruginosa

- Pathogenesis of bacterial infection

- For a bacterium **to cause disease (to be pathogenic)**, it needs to have some attributes to help it reach the host, persist within the host and replicate, while causing harm (disease) to the host.

- Characteristics of bacteria that are pathogens are sometimes referred to as **virulence factors**, and they include:

- **Transmissibility**
- **Adherence to host cells**
- **Motility**
- **Persistence**
- **Invasion of host cells and tissues**
- **Toxigenicity**
- **Iron uptake mechanisms**
- **The ability to evade or survive the host's immune system.**
- **Resistance to antimicrobials and disinfectants.**

- Some of the Virulence factors can be shared with non-pathogenic bacteria (microbiota), such as: Transmission, adhering, motility.

- Virulence factors can be referred to as steps of infection (although sometimes the order of these steps can be disturbed).

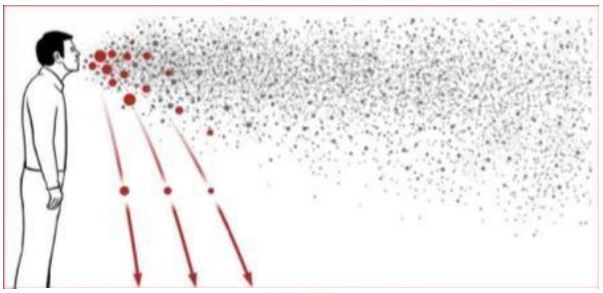
- Transmission

- Bacteria can come from variety of sources, such as: soil and animals. We refer to the bacteria that are found everywhere as **ubiquitous**, e.g. Clostridium bacteria

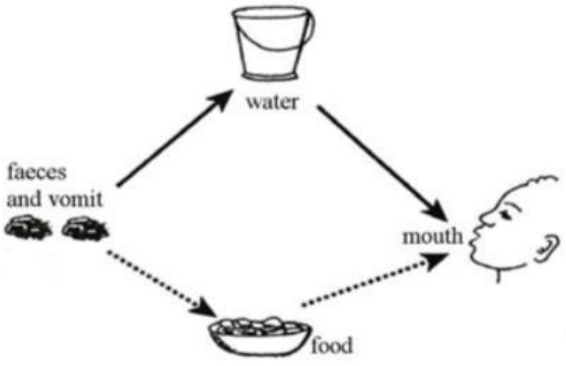
- Bacteria can adapt to a variety of environments that include external sources such as **soil, water and organic matter** or internal milieu as found **within insect vectors, animals and humans.** (The bacteria could be pathogenic or reside as normal flora in their original source)

- **The clinical manifestations of diseases** (e.g. diarrhea, cough, genital discharge) produced by microorganisms often **promote transmission of the agents**.

- The diseases that cause **less symptoms are easier** to be transmitted because it would not kill the host, so there's more time for the pathogen to thrive, hence be transmitted. Besides, the infected individual wouldn't notice the infection he has which will make him interact with others normally; thus promoting the infection.



- The first picture on the right illustrates a man sneezing, the respiratory droplets coming out could reach up to 3 meters, these drops could carry the pathogen, and depending on the type of pathogen the **inoculum dose** differs.

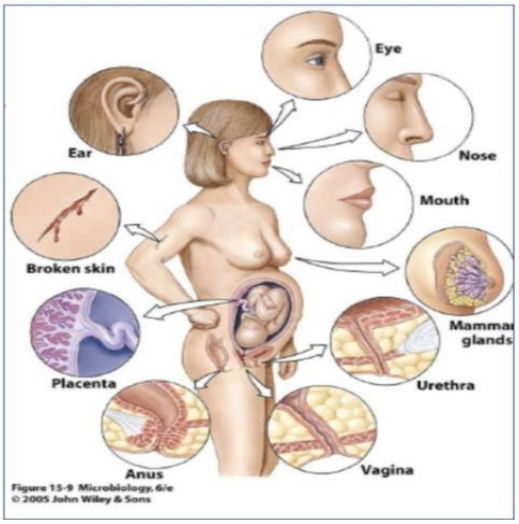


(Certain pathogens need a higher inoculum dose to infect the host, others with lower inoculum dose need only few droplets to infect)

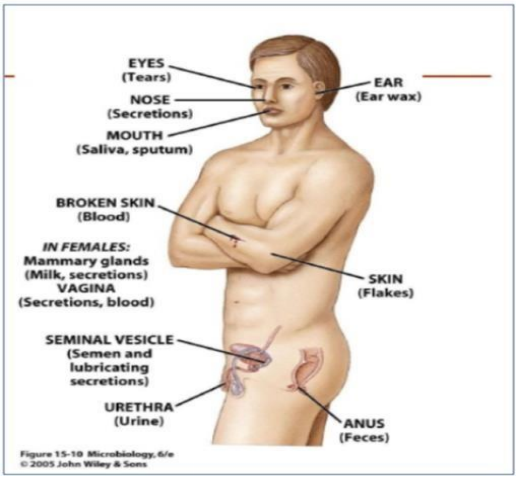
- The sites where the bacteria enter the host body are called **Sites of entry.** The respiratory (upper and lower airways), gastrointestinal (primarily mouth), genital, and urinary tracts. Abnormal areas of mucous membranes and skin (e.g. cuts, burns, and other injuries) are frequent **sites of entry**.

- In contrast to the portals of entry, there are **portals of exit,** where the bacteria exit the body. Usually, bacteria are carried out with a certain secretion, e.g. Tears, Saliva, Feces

Portal of entry



Portal of exit



- Adhesion

- Viruses need a primary site of infection, they have specific binding receptors which facilitates their entrance to the host cell. In contrast, bacteria have general appendages for attachment (it also lead by receptors to a smaller extent).

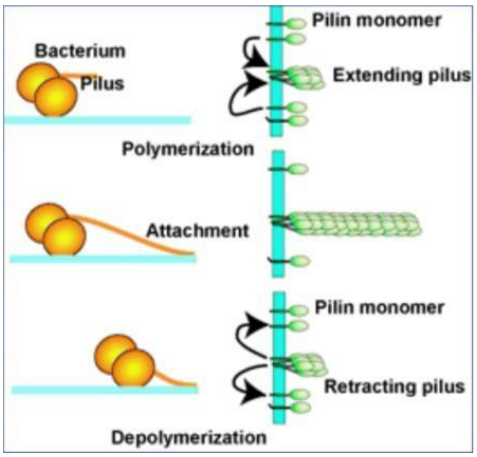
- Bacteria are more versatile when it comes to adhesion, they have the **Fimbria (Pili)** that help them to attach to surfaces, because adherence is an essential step for infection.

- The bacteria wouldn't be swimming around in an open space in the body because there's a continuous movement in the body, e.g. in the GI tract there's peristalsis, and in the respiratory tract we have cilia continuously moving mucous. If any fluid in the body becomes stagnant it would be susceptible for infection.

-In Cystic Fibrosis when the respiratory mucous becomes thick and sticky it would be a potential infection site. Another example is in the urinary bladder, the urine is continuously flushing out any potentially infection causing pathogen, if there was a problem in urine secretion the bladder would a potential site of infection. It can even happen in the blood in individuals that have congenital anomalies in certain valves, the blood will become stagnant and will clot making it a place to establish infection.

- **Pili** is Composed of structural protein subunits (monomers) termed **pilins.** At the tips of the pili minor proteins termed **Adherins** are responsible for the attachment properties**.**

- The formation of pili is a **polymerization** process, in which pilins are added from the inside out (the pilins are added intracellularly and the pili will extend extracellularly).



- The process of adhesion is mediated by the **attachment** of proteins on the tips (Adhesins) of pili with the surface, this will induce **depolymerization** of the monomers from inner end shortening the pili. The result is that the bacterium moves in the direction of the adhering tip. This kind of surface motility is called **twitching** and is widespread among piliated bacteria.

- Pili are also used in inhibiting the phagocytotic activity, probably by interfering sterically or physically with phagocytotic cells of the host.

- Motility

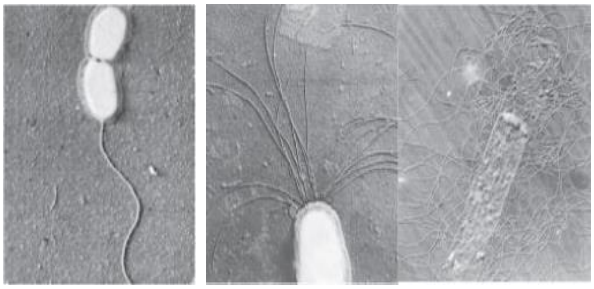
A huge advantage for bacteria to reach the host, maneuver in the host and evade the immune system is for a bacterium to be **motile** – to have the ability to direct its own movement.

- keep in mind that not all bacteria are motile but all of them can adhere

- The bacterial **flagellum** (plural is Flagella) is a complex molecular machine with a diversity of roles in pathogenesis including reaching the optimal host site, **colonization** or **invasion**, **maintenance** at the infection site, and post-infection **dispersal.**

- There's a microorganism called H pylori which establish colonization in the stomach, it uses its flagella to dig into the mucous of the stomach to hide from the acidic environment.

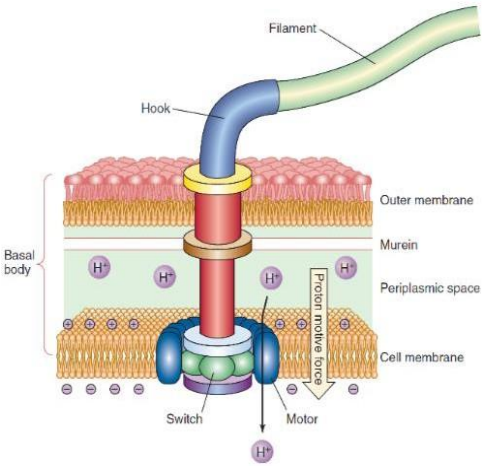
- As we took before, we can classify bacteria according to their number of flagellum and their distribution.



- Bacterial flagella are thread-like appendages composed of a protein subunit (monomer) called **flagellin.**

- The bacteria contain certain antigens that are recognized by the human immune system, e.g. the O antigen (cell wall), the highly antigenic **H antigen** (flagella) immune responses to infection can be directed against these proteins.

- The mechanism of how flagella work depends on a **proton gradient** in the periplasmic space, protons start to go down their electrochemical gradient, into the cell and by doing that they activate a motor that will cause the flagellum to rotate so as to move the bacteria in certain direction.



- The direction in which the bacterium moves depends on the availability of nutrients. Meaning that the bacterium can sense where the nutrients are, and moves towards them in a process called **Chemotaxis.**

- **Chemotaxis**: the net movement of the cell toward the source (a sugar or an amino acid). Cell behavior brought about in response to a change in the environment is called **sensory transduction.**

- Invasion

- Some bacteria invade the host cell after they adhere to it, but it's not obligatory to do so, bacteria follow various infection pathways unlike viruses which is obligated to certain life cycle.

- Some bacteria adhere to the host cell and establish infection in the extracellular matrix (superficial infection), others invade the host cell and reside intracellularly, some other types could cross through cells to reach a deeper tissue, some types can do all of the following. Depending on the virulence factor the bacterium has, e.g. Staph. Aureus, could change its behavior (Staph. aureus is usually an extracellular pathogen, but with certain virulence factors it becomes better intracellularly, or it can become invasive).

- The invasion process is referred to as an **Active process between cells and pathogen,** usually requires **actin polymerization** (induces changes in the exoskeleton to facilitate the entrance of the pathogen).

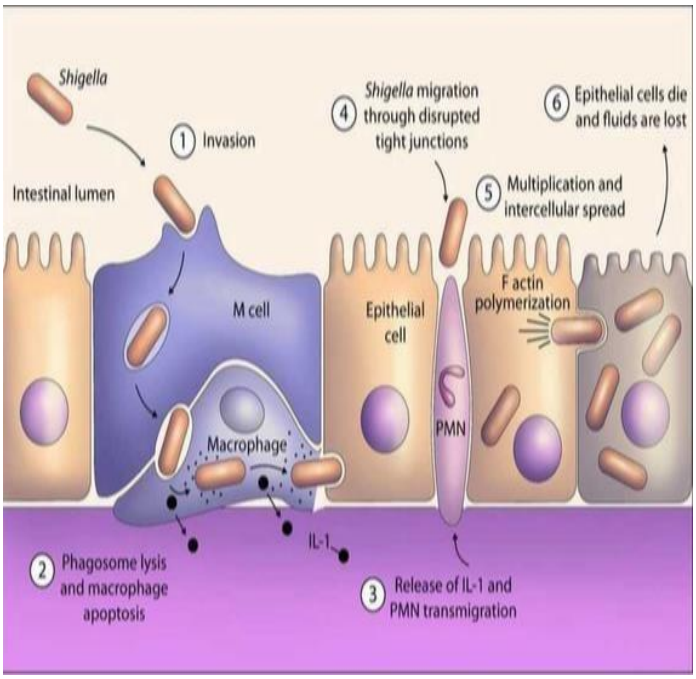
- Invasion can happen **through tight junctions of epithelial surfaces**, or **through internalization** into epithelial cells, it can happen in various tissues even immune cells.

- Once inside the cells, the bacteria can be transported by **vesicles to the lysosome, or can remain or escape the vesicles to multiply in the cytoplasm, or be released to the extracellular space to invade other cells**. Bacteria can also induce **apoptosis** in cells they invade.

- In the Shigella example we have here, the mechanism of invasion is illustrated. Firstly, the shigella slightly **adheres** to the cell surface causing **membrane ruffling**, then it gets **internalized** into **M cells**, and through it, Shigella **reaches the macrophage** where it **lyses the phagosome and induces macrophage apoptosis** (it gets out of the phagosome before it merges with the lysosome) and goes into the cytoplasm, **activating** certain pathways that **kill** the **macrophage**.

-As the **macrophage** is dying it will release **IL-1,** together with other cells. IL-1 would draw in **polymorphic mononuclear cells (PMN)** e.g. Neutrophils, these cells that are recruited by the immune system (to kill the pathogen) will disturb the tight junction, allowing the shigella to even migrate through these disturbed junctions, and invade deeper into the tissue.

-Bacteria can also move from one cell to another, having intercellular spread by repolymerizing the actin. They might replicate there or even induce **apoptosis**.



-Toxigenicity/ Exotoxins

-Another virulence factor that bacteria share is the production of toxins. Toxins are divided into **exotoxins** which are actively secreted by contact or by cell death, and

endotoxins which are parts of the bacterial cell wall eg: LPS that is found in Gram negative bacterial cell wall.

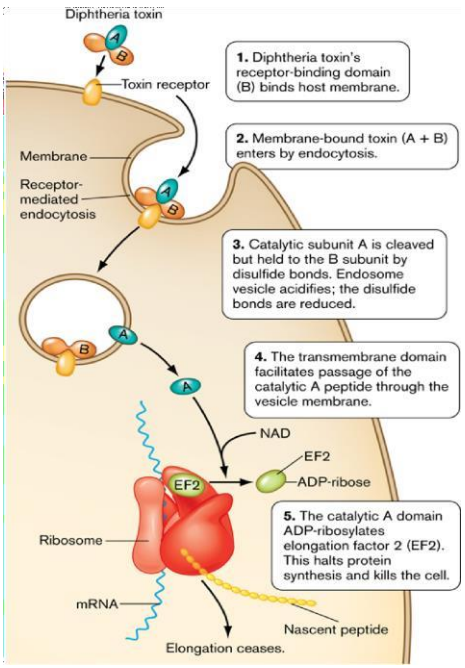
-Each exotoxin works differently (as seen in the table below); however, most of them share the same structure of having two subunits: **A subunit** for toxic activity and **B subunit** for binding to target cell receptor.

-By referring to diphtheria toxin, we notice the two subunits, A and B, along with a target cell receptor which is usually different among different exotoxins. The mechanism starts with Diphtheria toxin's receptor-binding domain **(B subunit) attaching to the receptor** on host membrane, then the **toxin (A+B subunits) gets internalized** into the cell by the process of endocytosis, forming a vesicle. Afterwards, the **A subunit gets cleaved** due to changes occurring in the phagosome such as acidification, and passes through the vesicle membrane into the cytosol to **cause its toxic effects**, which in the case of Diphtheria are the inhibition of protein synthesis (translation) by affecting an **elongation factor**, leading to the death of the cell.

-Exotoxins that affect the GIT, and thus are associated with diarrheal diseases are known as **enterotoxins**. (The prefix entero- means it affects the GIT)

-Some exotoxins can be weakened through heating or denaturation to form **toxoids**, which are bases for some vaccines (eg: Tetanus vaccine). This is due to the fact that exotoxins are basically foreign antigens that are capable of inducing the immune system.

Toxin	Organism	Gene Location	Subunit Structure	Target Cell Receptor	Biological Effects
Anthrax toxins	*Bacillus anthracis*	Plasmid	Three separate proteins (EF, LF, PA)	Tumor endothelial marker-8 (TEM-8); capillary morphogenesis protein 2 (CMG2)	EF + PA: increase in target cell cAMP level, localized edema; LF + PA: death of target cells and experimental animals
Bordetella	*Bordetella* spp.	Chromosomal	A-B	Unknown, probably glycolipid	Adenylate cyclase toxin. Increase in target cell cAMP level, modified cell function, or cell death
Botulinum toxin	*Clostridium botulinum*	Phage	A-B	Polysialogangliosides plus synaptotagmin (co-receptors)	Decrease in peripheral presynaptic acetylcholine release, flaccid paralysis
Cholera toxin	*Vibrio cholerae*	Chromosomal	$A\text{-}B_5$	Ganglioside (GM_1)	Activation of adenylate cyclase, increase in cAMP level, secretory diarrhea
Diphtheria toxin	*Corynebacterium diphtheriae*	Phage	A-B	Growth factor receptor precursor	Inhibition of protein synthesis, cell death
Heat-labile enterotoxins	*Escherichia coli*	Plasmid	Similar or identical to cholera toxin		
Pertussis toxin	*Bordetella pertussis*	Chromosomal	$A\text{-}B_5$	Surface glycoproteins with terminal sialic acid residues	Block of signal transduction mediated by target G proteins
Pseudomonas exotoxin A	*Pseudomonas aeruginosa*	Chromosomal	A-B	α_2-Macroglobulin receptor (α_2-MR)	Similar or identical to diphtheria toxin
Shiga toxin	*Shigella dysenteriae*	Chromosomal	$A\text{-}B_5$	Globotriaosylceramide (Gb3)	Inhibition of protein synthesis, cell death
Shiga-like toxins	*Shigella* spp., *E. coli*	Phage	Similar or identical to Shiga toxin		
Tetanus toxin	*Clostridium tetani*	Plasmid	A-B	Polysialogangliosides plus 15-kDa glycoprotein (co-receptors)	Decrease in neurotransmitter release from inhibitory neurons, spastic paralysis



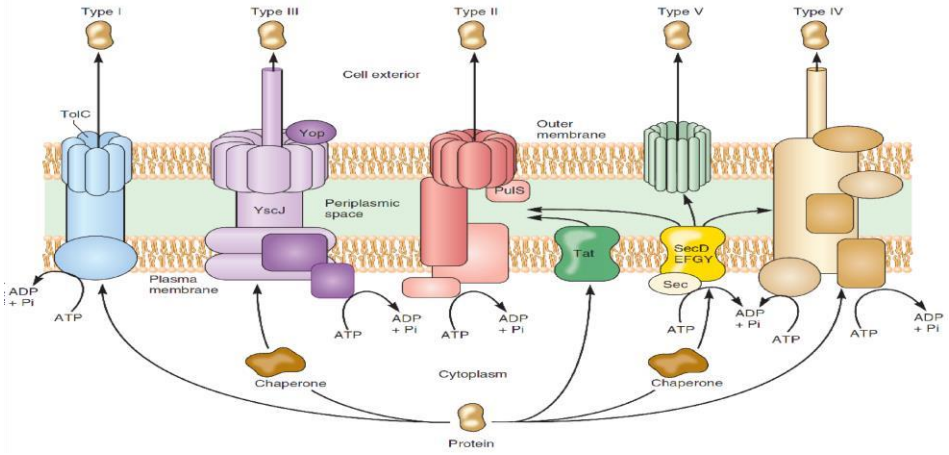
PS: the table isn't required from us now; however, the doctor referred to diphtheria toxin for the example above.

-Secretion System

-Substances, including toxins, are actively secreted outside the bacterial cell through **secretion systems** which are protein complexes present on the cell membranes of bacteria (spanning the membrane). These systems allow bacteria to efficiently introduce their toxins into the environment. Basically, they are considered from the virulence factors of bacteria, suggesting that if a bacterium has more than one secretion system, it is more virulent.

-A lot of the secretion systems depend on the presence of **ATP** for their activation although there are some that depend on **chaperones** which have the function of carrying the toxin protein to the system to be secreted to the extracellular compartment.

Types of secretion systems:-



Stressing on type III pathway since it is associated with many pathogenic bacteria:-

Type III secretion pathway: known as contact-dependent system due to the fact that it gets activated by the contact of the bacterium with a host cell. It is a needle-like structure that injects toxin proteins through its piston either directly into the host cell or in the vicinity of the cell.

Different secretion systems are found in different bacteria. For instance, **type I and IV** secretion systems have been described in **both gram-negative and gram-positive bacteria**. While type **II, III, V, and VI** secretion systems, they have been found **only** in **gram-negative bacteria**. (Notice that gram-negative bacteria have more secretion systems than gram-positive)

-Enterotoxins that are found in the GI tract depend on type III system found in bacteria such as Shigella species, Salmonella species and E. coli.

As seen, there are several bacteria with different secretion systems.

Secretion System	Genus Species	Substrate and Role in Pathogenesis
Type 1 (Sec-independent)	*Escherichia coli*	α Hemolysin makes holes in cell membranes
	Proteus vulgaris	Hemolysin
	Morganella morganii	Hemolysin
	Bordetella pertussis	Adenylate cyclase which catalyzes synthesis of cAMP
	Pseudomonas aeruginosa	Alkaline protease
	Serratia marcescens	Zn protease yields host cell damage
Type 2 (Sec dependent)	*Pseudomonas aeruginosa*	Elastase, exotoxin A, phospholipase C, others
	Legionella pneumophila	Acid phosphatase, lipase, phospholipase, protease, RNAse
	Vibrio cholera	Cholera toxin
	Serratia marcescens	Hemolysin
Type 3 (Sec-independent; contact-dependent)	*Yersinia* species	Ysc-Yop system; toxins that block phagocytosis and induce apoptosis
	Pseudomonas aeruginosa	Cytotoxin
	Shigella species	Controls host cell signaling, invasion, and death
	Salmonella enterica subspecies *enterica* serotypes Choleraesuis, Dublin, Paratyphi, Typhi, Typhimurium, and so on	Effectors from *Salmonella* pathogenicity islands I and II (SPI1 and SPI2), which promote attachment to and invasion of host cells
	Escherichia coli	Enterohemorrhagic (EHEC) and enteropathogenic (EPEC); disruption of epithelial barriers and tight junctions
	Vibrio parahaemolyticus	Direct cytotoxicity

-Toxins/ Endotoxins

-Endotoxins such as lipopolysaccharides of gram-negative bacteria are bacterial cell wall components that are liberated when the bacteria lyse.

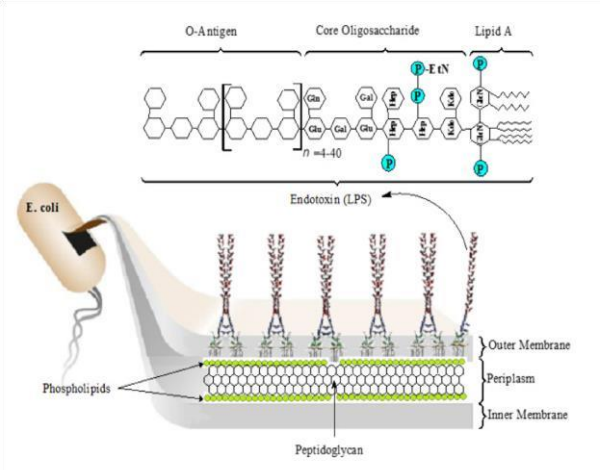
-In comparison to exotoxins, endotoxins are rather **more heat-stable**.

-In addition, LPS is **highly immunogenic,** and causes severe immune responses. Basically, LPS is **detected** by sensors like **TLR** which get the **immune system activated**, and thus lead to the **production of proinflammatory cytokines** such as IL-1 and TNF-α which, as a result, causes the **activation of complement and coagulation cascades**. (Some of the complement component such as MBL (mannose-binding lectin) can recognize sugar moiety and activate the complement system).
The following downstream effects can be observed:

-Fever, leukopenia, and hypoglycemia; hypotension and shock resulting in impaired perfusion of essential organs (eg, brain, heart, kidney); intravascular coagulation; and death from massive organ dysfunction.

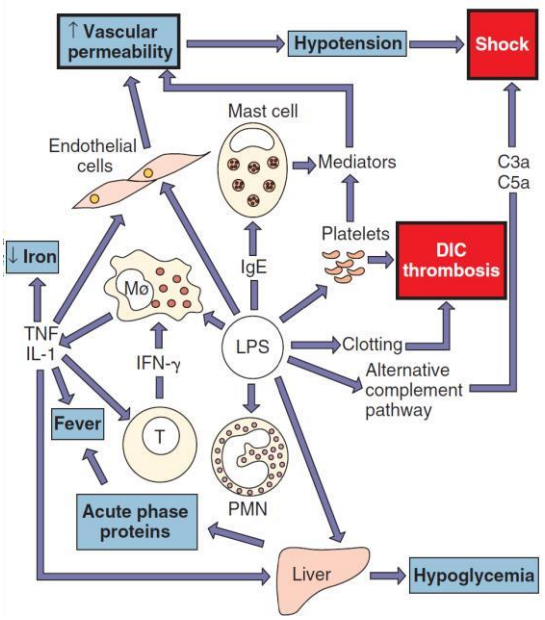
-The endotoxin **peptidoglycan** released from gram-positive bacteria can cause **similar immune responses**, but much **less potent** than endotoxin (LPS).

-The image demonstrates the structure of the endotoxin (LPS) found on the outer membrane of gram negative bacteria, E.coli, which consists of:
lipid A, oligosaccharide and O-Antigen.



-Effects of LPS:

-Immune and non-immune cells that have receptors such as TLR get activated; for example, neutrophils, macrophages, endothelial cells and mast cells. Consequently, they activate cascades of inflammatory events with the release of the cytokines, **TNF and IL-1**, which result in fever as they act upon the hypothalamus, as well as the release of acute phase proteins from the liver (**systemic protective effects**).



-LPS also activates platelets and clotting leading to DIC thrombosis. Additionally, it activates the alternative complement pathway, inducing the release of complement **C3a and C5a**.

- It is quite problematic if gram negative bacteria were found in the blood, because then there will be continuous activation of the complement system, releasing C3a and C5a which lead to the increase in vascular permeability and the decrease of vascular pressure (hypotension) causing the body to enter a condition known as shock.

The following table illustrates the differences between exotoxins and endotoxins

Through secretion systems

Exotoxins	Endotoxins (LPS)
Excreted by living cell; high concentrations in liquid medium	Integral part of the cell wall of gram-negative bacteria; released on bacterial death and in part during growth; may not need to be released to have biologic activity
Produced by both gram-positive and gram-negative bacteria	Found only in gram-negative bacteria
Polypeptides with a molecular weight of 10,000–900,000	Lipopolysaccharide complexes; lipid A portion probably responsible for toxicity
Relatively unstable; toxicity often destroyed rapidly by heating at temperatures above 60°C	Relatively stable; withstand heating at temperatures above 60°C for hours without loss of toxicity
Highly antigenic; stimulate formation of high-titer antitoxin; antitoxin neutralizes toxin	Weakly immunogenic; antibodies are antitoxic and protective; relationship between antibody titers and protection from disease is less clear than with exotoxins
Converted to antigenic, nontoxic toxoids by formalin, acid, heat, and so on; toxoids are used to immunize (eg, tetanus toxoid)	Not converted to toxoids
Highly toxic; fatal to animals in microgram quantities or less	Moderately toxic; fatal for animals in tens to hundreds of micrograms
Usually bind to specific receptors on cells	Specific receptors not found on cells
Usually do not produce fever in the host	Usually produce fever in the host by release of interleukin-1 and other mediators
Frequently controlled by extrachromosomal genes (eg, plasmids)	Synthesis directed by chromosomal genes

The ones labile/sensitive to heat are sometimes used as toxoids for vaccines. Although there are some that are stable with heat.

Since exotoxins are not essential for the survival of the bacteria, they can be controlled by plasmids, and thus be acquired by other bacteria

Since endotoxins are an integral part of the cell wall, they can't be controlled by plasmids or else the bacteria might lose it. Therefore, it is directed by chromosomal genes.

PS: the doctor only talked about the differences within the red border.

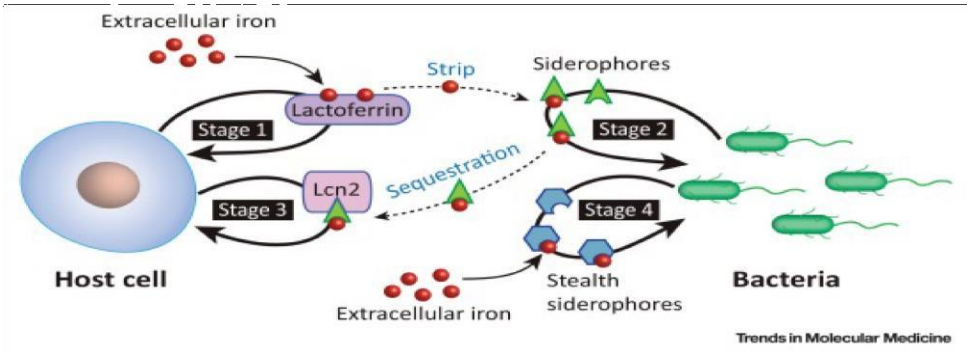
-To further emphasize the concept of exotoxins being transferred from one bacterium to another using plasmids, an example can be given regarding the bacterium Vibrio cholerae which causes cholera. Some of this bacterium swim freely while causing no harm; however, they acquire few genes through transduction (with the help of bacteriophage) which transfers genes of toxic bacteria responsible for the production of toxin into nontoxic bacteria, converting the nontoxic bacterium into toxic vibrio cholerae that causes disease. **In other words, such plasmids are capable of changing nonpathogenic bacteria into pathogenic bacteria by giving them virulence factors.**

-Iron Uptake mechanisms

-Moving on to another characteristic of pathogenic bacteria, iron uptake is necessary for the growth and survival for bacterial, which is why the availability of iron in a mammalian body is **reduced** in both extracellular and intracellular compartments **in response to infection.** (to fight the infection)

-In good normal conditions, **iron** is attached to **transporter protein** such as transferrin and lactoferrin for the purpose of sequestering the iron content into a compartment **not** available for bacteria. For that reason, bacteria have evolved to compete for iron, and steal it through the production of siderophores.

-Siderophores are small, high-affinity iron-chelating compounds; meaning that they strip the iron out of the transporter. But at the same time, host cells produce proteins that can take away siderophores altogether. To overcome this, some bacteria can produce **stealth siderophores** that **cannot** be detected by the immune cells; hence, take the extracellular iron.



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Evasion of the host immune system:

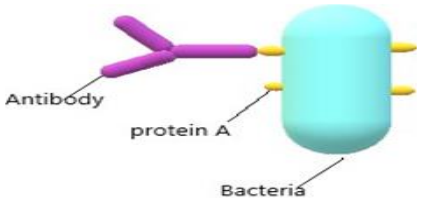
Pathogenic bacteria can evade phagocytosis in many ways. Examples include **capsule production**, **Protein A in Staph aureus binds antibodies in an inactive manner.** Some bacteria produce proteins that **inhibit complement activation,** thereby decreasing immune signaling and opsonization* of bacteria**.** Intracellularly, some bacteria **inhibit phagolysosome fusion.** all these ways are called virulence factors; factors that increase the effectiveness of the bacteria to cause disease.

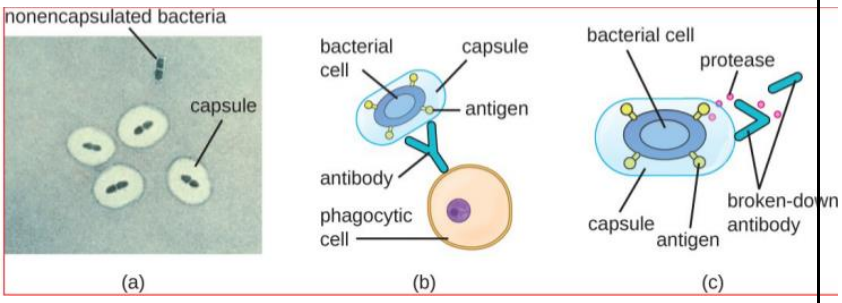
1-*The formation of the capsule*:

Capsules are most commonly made of polysaccharides, but some can be made of polypeptides. We have unencapsulated & capsulated bacteria. Capsulated bacteria are bacteria with a surrounding space full of carbohydrates / polypeptides that can help the bacteria in many processes:

- **Physically:** by creating a barrier around itself which makes it harder for antibodies and other opsonins to reach the antigen and deposits on the surface of it. So, it inhibits the pathogen opsonization.
- It inhibits the pathogen phagocytosis; thus, inhibiting an important part of the immunity.

Be aware that a capsule is so important to the point that **some bacteria can be nonpathogenic without a capsule.** Ex: Streptococcus pneumonia.





An important example is protein A in Staph aureus; which can bind the antibody in an inactive manner by holding it inversely so that the FC (effector portion) is reversed (blinding it). Therefore, the immune system won't benefit from this bound antibody.
In researches, protein A is used in order to purify antibodies. They put a column and a protein A is put on top of it to catch antibodies. This is a very efficient mechanism, because the binding of antibodies to antigens is very strong, and the only way to restore antibodies from those antigens is by denaturing the antibody (thus losing it). Meanwhile using protein A will preserve the antibodies by binding them in reverse. This could help bacteria in preventing opsonization.

Opsonization is the process in which bacteria is covered by substances to enhance phagocytosis. For example, antibodies bound to bacterial surface, as well as activated complement components depositing on bacterial surfaces are considered" opsonins" since they let bacteria get phagocytosed more easily.

In our circulation, we have complement inhibitors that bacteria can hijack to prevent their deposition and therefore, inhibiting opsonization. Ex: (**CD46, CD55, CD59**)
The point is that the immune system produces complement inhibitors in order to monitor the complement system so it's not active 24/7. So, when those CI are bound to the bacteria, an opsonin convertase is formed on its surface & immediately those CI's will start cleaving complement proteins on the surface of the bacteria, so it's not opsonized anymore & its phagocytosis is prevented.

Even if bacteria got inside the phagosome & phagocytosis happened; it also has ways to escape.

It can secrete enzymes that can lyse the phagosome & let itself out to the cytoplasm; or it can inhibit the fusion of the phagosome with the lysosome so it'll keep sitting in the phagosome.

Sometimes a bacterium reaches the cytoplasm and induces apoptosis in the cell.

Gram negative strains bacteria keep shifting their antigens making it harder on the immune system to recognize them; it will need qualified adaptive immunity to recognize them & they'll keep shifting (antigenic variation).

What we are saying is that the pathogen has ways to overcome the different levels of the immune system depending on different virulence factors that help it to cause disease; causing a problem to the immune system. This is why diseases are observed even in people with a healthy functional immune system.

The inhibition of phagocytosis & opsonization can happen in many other ways. Ex:

2-*bacterial enzymes*: some bacterial cells can produce enzymes that breaks down antibodies at the hinge region.

Bacteria can produce enzymes and some of them can be classified as exotoxins (because they're of the same concept). Eg. **Hyaluronidase and collagenase** are enzymes that hydrolyze hyaluronic acid and collagen respectively, constituents of the ground substance of connective tissue & extracellular matrix. These enzymes are necessary for tissue invasion; helping the bacteria to break down junctions between epithelial cells & break down the basement membrane; helping the bacteria to invade deeper in the interstitial tissue.

Some of those enzymes can lyse immune cells (cytolysis). Ex: **hemolysin** which breaks down RBC & epithelial cells and **leucocidins** (a killer of white blood cells).

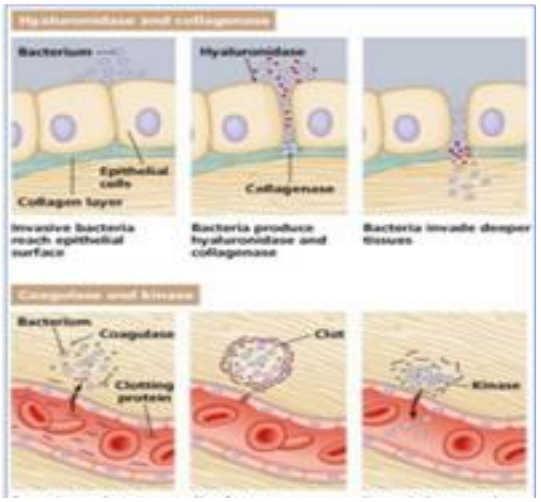
How do they usually work?

They form pores within the membrane of host cell making the cell compromised & then cell lyses.

Other examples are coagulases & kinases. We have 2 types of bacteria regarding coagulase:

- -coagulase +ve: Staph. aureus.
- -coagulase -ve: part of the normal microbiota (Staph. epidermidis).

When a bacterium gets into the tissue, it starts inducing coagulases, so that a clot forms around the bacterium (it hides itself). Immune cells will try to enter & phagocyte this bacterium, but they will be stopped by the presence of the clot. After a certain period of time, the bacterium breaks the clot by kinases & gets back to the circulation.



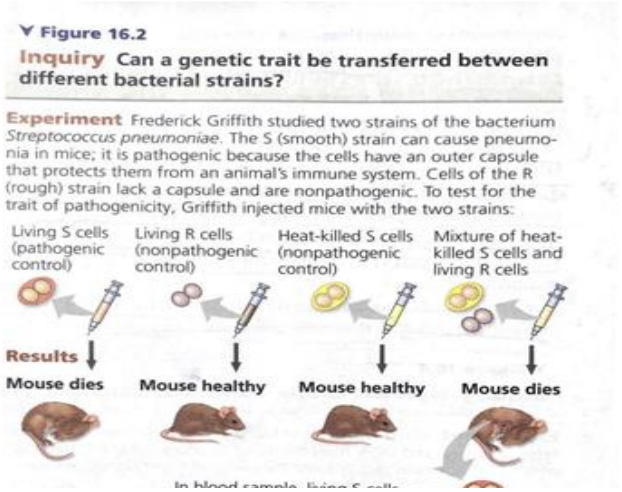
Clinical Application: This kinase is extracted and used to synthesize heart & thrombosis drugs. Ex: streptokinase which is a kinase produced by some strains of streptococcus bacteria during their pathogenesis to disturb the surrounding clot, we extract it and use it to disseminate clots in arteries.

We can exploit bacteria in many regions in medicine; ex. We can use the bacterial enzymes (which break down antibodies) in curing diseases that involve antibodies components like in transplantation. We give the patient enzymes against alloantibodies (the antibodies against allograft) before he undergoes transplantation.

Pathogenicity islands

Definition: Chromosomal or extra chromosomal discrete genetic units that encode genes that aid in the virulence of a bacteria by coding for adhesins, secretion systems (like type III secretion system), toxins, invasins, capsule synthesis, iron uptake system.

The **G-C content** of pathogenicity islands is usually different from the rest of the genome. Most of the time, these stretches are found extra chromosomally on plasmid; meaning that it is commonly found on mobile genetic elements **(passed through plasmids, transformation, transduction, transposons**). If a pathogenic and nonpathogenic bacterium conjugate together successfully, the plasmid that have pathogenic island gene will be transmitted from the pathogenic to the nonpathogenic bacteria making it pathogenic.



Remember

*Being together enhances the ability to control them; pathogenicity islands are usually activated by environmental causes; like temperature change, pH change, ..etc.

For example, a bacterium living in soil won't benefit from secreting enzymes that break down antibodies; so their genes will be shut down. But when it enters the body & senses the change in temperature or in pH, it activates the pathogenicity island, adhesins, secretion systems (**like type III secretion system**) and produces toxins & coagulases. All these things were not needed when the bacterium was in the soil, but it needs them to survive in the body (the sensitivity isn't the same).

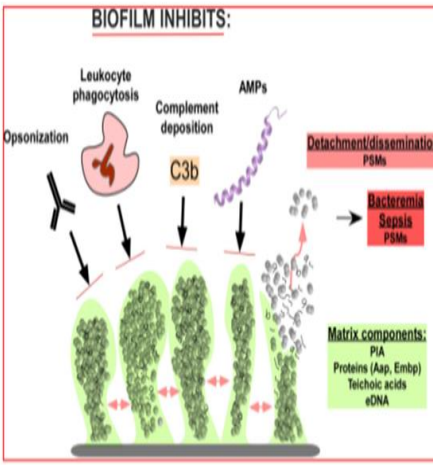
-Be aware that pathogenicity island genes don't exist in nonpathogenic bacteria.

Bacterial Communities / Biofilms:

Bacteria communicate together. Those bacterial cells group together exhibiting the behavior of a multicellular organism resulting in the formation of something we call a Biofilm. Those bacteria can secrete extracellular polymeric substances (polysaccharides, proteins, DNA segments & lipids) forming a thin layer film made of biological material (polymeric conglomeration)

-Note// Scientists had long held the view that bacterial cells behaved as self-sufficient individuals, unable to organize themselves into groups or communicate. But it was later discovered that **bacteria are found in communities that aid the survival of the whole**, through providing new characteristics.

Now, how does the biofilm help bacteria?



- It enhances communication between bacterial cells.
- It creates a microenvironment that protects the bacteria from the surrounding circumstances like a home for it, which is most commonly made of a mixture of proteins, lipids, polysaccharides, DNA, or it can consist of a single type of them.
- It inhibits opsonization, phagocytosis, complement deposition (C. inhibition) & attacks of antimicrobial peptides.
- It inhibits antibiotics; because it doesn't allow them to penetrate well enough throw this big layer of biological material affecting the susceptibility tests.

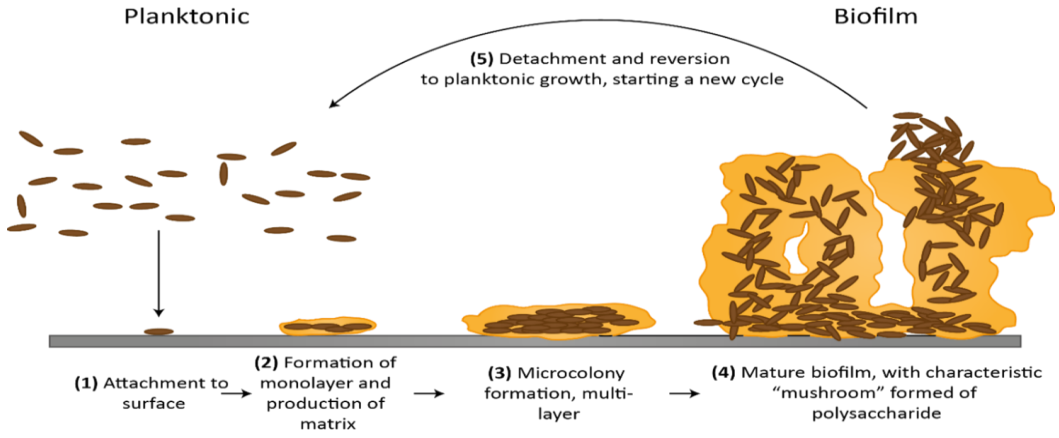
In susceptibility testing, we extract bacteria and try antibiotics on the specimen to see which antibiotics are effective and which aren't. Sometimes, results differ between the vitro(lab) and the human body, meaning that some antibiotics can kill bacteria in the lab but can't kill them in the body; one of the reasons is biofilm formation.

It's because of that, the biofilm can mess up with the susceptibility of the antibiotic. Agar can't induce the formation of the biofilm, that's why antibiotics could be active in the lab but not inside the human body.

Some biofilms can hold different bacterial species, others can be formed depending totally on one type of bacteria, which happens usually.

When the bacteria mature, the biofilm can sometimes break apart & release some bacteria; those single bacteria are called planktonic bacteria (free living/not in a community) that attaches somewhere else, colonizes and starts forming new biofilm.

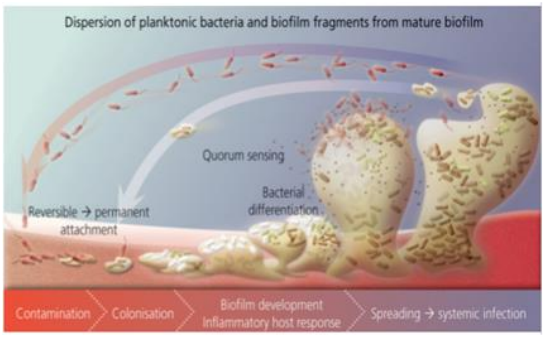
To sum up, those communities aid in survival; in a way similar to a multicellular organism in biofilms and they can distribute nutrients more beneficially.



The bacterial cells recognize its presence in a community by something we call **Quorum Sensing**; which means that bacteria can sense its environment and send signals to other bacteria communicating with them as we said before.
One of the simplest forms of communication is the concentration of AHL's molecules that catch certain transcription factors activating certain genes. When the no. of the bacteria increases while multiplying, the AHL's concentration will increase, thus the amount of AHL's getting inside cells will increase & more genes will become activated. Finally, this results in the formation of biofilm, because of the increase in the no. of bacterial cells (BCs).

Keep in mind that AHL activates different types of genes, not only the AHL gene.

10 BCs→ low AHL, 100 BCs → high AHL & biofilm formation, 1000 BCs → very high AHL &planktonic cells formation (detachment of some BCs out of the biofilm).

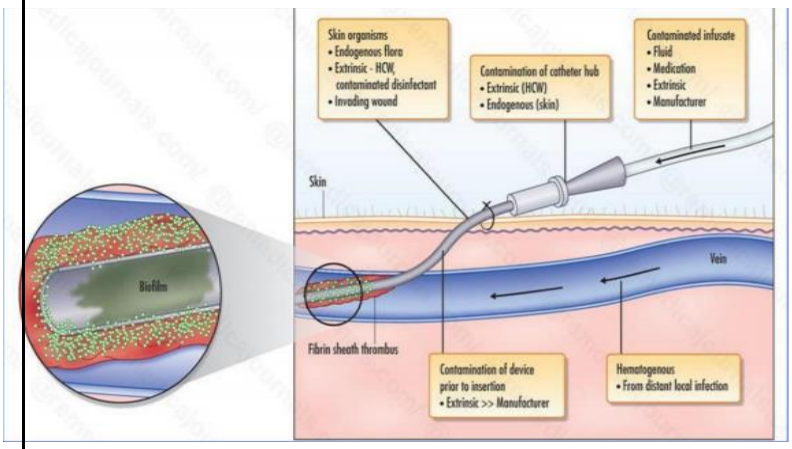


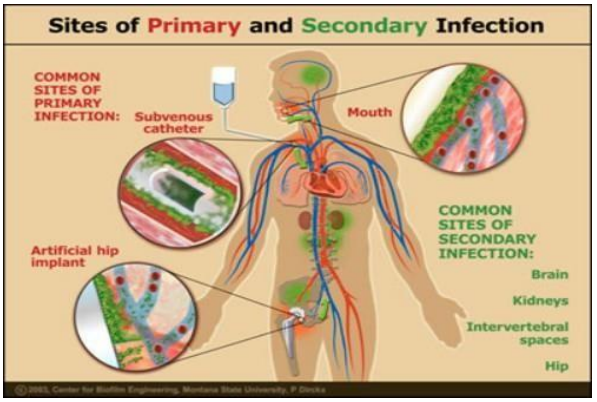
Some scientists relate this to pathogenesis saying that if we have a small number of bacterial cells, they won't cause diseases.

In medicine, we're interested in studying the biofilm because it's formed a lot on the plastic surfaces. EX: Catheters that are inserted in the urinary bladder is an excellent surface for the bacteria to form biofilm; as it's a nonbiological surface which won't face a strong response from the immune system.

Eradication of these bacteria in this form is quite difficult & antibiotics can do nothing if the patient has sepsis.

Whenever we sense weakness in a patient under clinical examination that has a catheter & make blood test and it turns out that the patient has microbes in blood(sepsis), we immediately remove the catheter ; because even if a biofilm doesn't exist as soon as microbes in the blood find that surface they will bind to It (formed hematogenously). This can be a sign of urinary tract infection (if the catheter is in the urinary tract)





Note:- catheters are used widely with patients that need continuous vein supplements like in dialysis

When the catheter is obtained from the company, it is very sterile and dealing with it must be upon aseptic techniques; by cleaning your hands, wearing gloves, and cleaning the site of insertion.

Rarely, the infection could occur prior to the insertion where equipment is contaminated from the manufacturer, or it can come from the skin of the patient, thanks for the normal flora (microbiota) present there. Ex: Staph epidermidis, Staph aureus. In the operation room simple mistakes could cause it.

Even in your toothbrush after 2-3 times of using, biofilms will start forming. Biofilms can even form on teeth as plaques!!

People with prosthetic limbs have a prominent sub-venous catheter and are highly exposed to risk infections.

What we should do is clean the area of insertion before sterilizing it (disinfecting it) to decrease the amount of the biological material interfering with alcohol; because starting with sterilization (spreading alcohol) won't have enough concentration to kill all the

bacteria, so we start with cleaning & follow it with disinfection.

***Factors that affect the efficacy of both disinfection and sterilization include:**

-Prior cleaning of the object.

-Organic and inorganic load present.

-Type and level of microbial contamination.

-Concentration of and exposure time to the germicide.
-Physical nature of the object (e.g., crevices, hinges, and lumens).

-Presence of biofilms.

-Temperature and pH of the disinfection process.

TABLE 9-1 Guidelines for Establishing the Causes of Infectious Diseases

Koch's Postulates	Molecular Koch's Postulates	Molecular Guidelines for Establishing Microbial Disease Causation
1. The microorganism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.	1. The phenotype or property under investigation should be significantly associated with pathogenic strains of a species and not with nonpathogenic strains.	1. The nucleic acid sequence of a putative pathogen should be present in most cases of an infectious disease and preferentially in anatomic sites where pathology is evident.
2. The microorganism should be grown in pure culture in vitro (or outside the body of the host) for several generations.	2. Specific inactivation of the gene or genes associated with the suspected virulence trait should lead to a measurable decrease in pathogenicity or virulence.	2. The nucleic acid sequence of a putative pathogen should be absent from most healthy control participants. If the sequence is detected in healthy control participants, it should be present with a lower prevalence as compared with patients with disease and in lower copy numbers.
3. When such a pure culture is inoculated into susceptible animal species, the typical disease must result.	3. Reversion or replacement of the mutated gene with the wild-type gene should lead to restoration of pathogenicity or virulence.	3. The copy number of a pathogen-associated nucleic acid sequence should decrease or become undetectable with resolution of the disease (eg, with effective treatment) and should increase with relapse or recurrence of disease.
4. The microorganism must again be isolated from the lesions of such experimentally produced disease.		4. The presence of a pathogen-associated nucleic acid sequence in healthy subjects should help predict the subsequent development of disease.
		5. The nature of the pathogen inferred from analysis of its nucleic acid sequence should be consistent with the known biologic characteristics of closely related organisms and the nature of the disease. The significance of a detected microbial sequence is increased when microbial genotype predicts microbial morphology, pathology, clinical features of disease, and host response

A tweaked version of Koch's postulates❼ molecular guideline for establishing microbial disease causation.

Optical Microscopy

Historically, the microscope first revealed the presence of bacteria and later the secrets of cell structure. Today it remains a powerful prominent tool in cell biology as the simplest type of microscopes.

The basic components of light microscopes consist of a light source used to illuminate the specimen positioned on a stage, a condenser used to focus the light on the specimen, and two lens systems (objective lens and ocular lens)

Resolving power is the distance that must separate two-point sources of light if they are to be seen as two distinct images. The best brightfield microscopes have a resolving power of approximately 0.2 µm, which allows most bacteria, but not viruses, to be visualized.

The resolving power is greatest when oil is placed between the objective lens (typically the 100× lens) and the specimen, because oil reduces the dispersion of light.