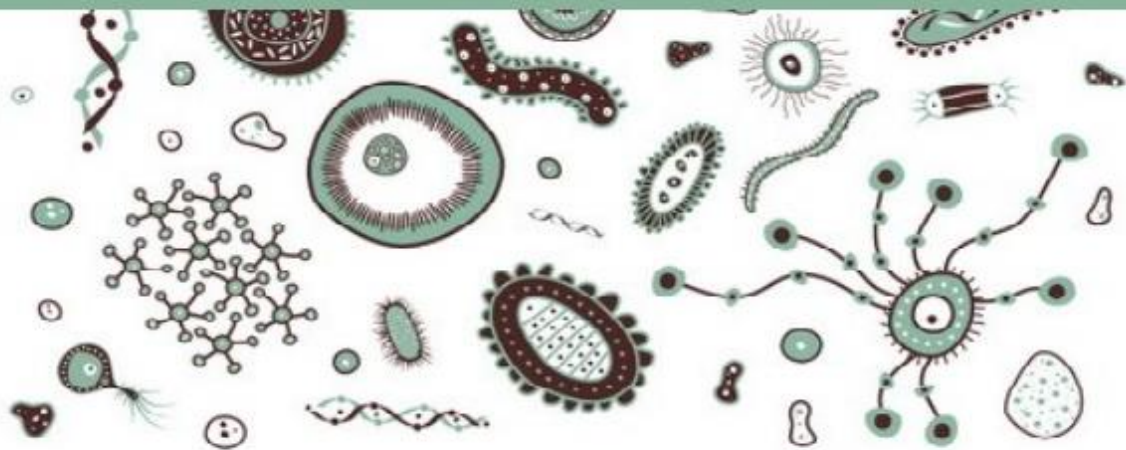




Microbiology



☒ Sheet

☐ Slides

Number : 5

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Subject: Disinfection & sterilization

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Normal Body Flora

What are normal body flora?

They are microorganisms that normally reside at a given site and under normal circumstances do not cause diseases. But they can cause infection if they are introduced to unusual sites, or even if the immunity drops.

Now these microorganisms can be bacteria, viruses, spores of fungi, and we might have yeast.. etc.

But mainly bacteria are more important in relation to developing what we call localized infection, and in association with contamination of devices used in the patient.

e.g., we have bacteria on our skin that under normal conditions usually cause us no harm, but if you got a wound for example then some of these bacteria will reach subcutaneous tissue or the blood and produce infection.

So you should to put in mind that you can be a source of infection for your patients, and you have to be careful not to introduce what you have of organisms to the blood of your patient during surgical manipulations or injection of a drug or whatever.

Fun fact: There are more bacteria living in or on our bodies, than we have cells of our own.

NOTES:

- 1- Normal body flora colonize the human body.
- 2- We don't live in a sterile environment but we're actually in contact with a cloud (huge variety) of microorganisms.
- 3- The normal flora/bacteria in our body are mostly anaerobes.
⇒ 95% anaerobes & 5% facultative anaerobes.



4- Disinfection is important to avoid infections -that can be caused by normal flora sometimes- in general.

How do we benefit from normal flora?

1- They compete with pathogens and prevent their adherence:

Think of it this way, if our normal flora reside and colonize on a tissue then simply the pathogen will find no place to adhere to that tissue. So they protect our tissues.

e.g., the E.coli (a normal flora) are found in our intestines. And if we took Salmonellae, Which are dangerous bacteria, then they won't find any room to adhere. So simply they will leave our body. But if there's a defect in E.coli found in the intestines, then Salmonellae will definitely cause us a disease.

2- They help in ingestion of some of the nutrients, and in the absorption of minerals.

3- They produce some vitamins and provitamins (e.g., E.coli produces vitamin k).

4- They prime the immune system:

What do we mean by priming the immune system?

The immune system in newborns is still immature, so normal body flora help the system to recognize the antigens better. So they contribute in the developing of the immune system.

5- They can eliminate toxins. And sometimes they produce toxins but not against us, they're against other pathogenic bacteria competing with their territory.

6- They are adapted to the mucosa and they protect our bodies through producing certain materials specially acids (e.g., acetic acid).

7- Normal flora (endogenous bacteria) can reduce the number of pathogens (exogenous bacteria / come from outside the body).



NOTES:

- Sometimes the exogenous bacteria can adapt the human body environment and become beneficial -through evolution-.
- There is always a contribution relationship between the flora and the body.
- If the bacterial extent in our body has changed, by any kind of injury, then the equilibrium of our body system will be changed. Which may lead to infections or even further complication.

Examples of Normal Flora:

As you'll see we have large numbers of organisms, and some of these are very rarely to cause infections under normal healthy conditions. But other organisms "might" under certain conditions become opportunistic and cause us some problems.

Skin Flora:

- *Staphylococcus spp.* can contaminate wounds and cause skin localized infection and in case of immuno-deficiency it can cause fatal diseases if it reached the body fluids specially the blood.
- *Propionibacterium*: which play a role in forming acnes during puberty.

Oral Cavity and Nasopharyngeal Flora:

- *Streptococcus*: causes tooth decay.
- *Neisseria*: in the nasopharynx.
- *Corynebacterium*
- *Haemophilus*: if introduced to the blood or the CNS it causes Meningitis (مرض السحايا).

Intestinal Flora:

It's the most common place for bacteria. Specially anaerobes:

- *Bacteroides*



- *Bifidobacterium*, and *Lactobacilli*: their presence is very good for the health of the intestines, and their secretion might help digesting the food.
- *Streptococci*
- *Clostridia*: about 5% of the people have this bacteria.

And we have also facultative anaerobes:

- *Enterobacteriaceae family* (e.g., *E.coli*, *Enterobacter*, and *Klebsiella*).
- *Yeast*: like *Candida*. (note: yeast can be obligate aerobic also).

Note about intestinal flora:

If we studied when each bacteria will be found in the intestine, we'll find that its presence depends on what kind of a diet the child is on as he grows up. So when a newborn starts drinking milk we'll notice the intestine has *Bifidobacterium* and *Lactobacilli*, and then as gets older we'll even find the others like *Bacteroides*, *Streptococci*, *E.coli*.. etc.

Urogenital Flora:

The urogenital tract is normally sterile. Like it's a disaster if we found bacteria in the bladder or in the kidney since they cause infections.

But yet we do have bacteria in two places:

- *In males*: the lower part of the urethra contains certain organisms found on the skin but they can't cause localized infections in normal conditions due to the flow of urine.
- *In females*: the normal flora is important to keep the vagina healthy. And sexual contact can transfer certain organisms that may cause infection.

e.g., *Lactobacilli* are found in the vagina, which produce lactic acid and preserve the acidity of 4.5 pH, but these *Lactobacilli* might die or decrease in number when we take certain antibiotics, or maybe because we got a cold that caused our immunity to drop. So accordingly the pH there is changed. Which may allow *Candida* to overgrow and cause infections.

Notes:

- 1- Susceptible bacteria extent can change in case of using antibiotics and drugs.
- 2- There's no antibiotic and no possible other means to eradicate all intestinal flora. We can't survive without them.

Some terminology before we go into “Disinfection & Sterilization”:

- Sepsis: this means there's a foreign microorganism that entered our subcutaneous tissue (that must be sterile), or the blood stream. (e.g., wound sepsis, blood sepsis).

There is a close term to Sepsis which is Bacteremia.

Bacteremia is the presence of bacteria in the blood too, but its destination is not the blood, but somewhere else.

Sepsis → its destination is the blood.

Bacteremia → its destination is not blood. (so it's just passing through).

- Asepsis: is the absence of these foreign microorganism, and these microorganisms are antigens that usually produce a cascade of reactions in our body.
- Antiseptic techniques: used to destroy microorganisms on living tissues, mucosa, skin, wounds...
- Disinfection (sanitization): using disinfectant agents to reduce the extent of microorganisms to a safe level (99% will be killed). And it's for non-living objects.
- Sterilization: to kill 100% of the microorganisms (contaminating microbes of any type; like spore-forming bacteria, viruses...etc.).

Sterilization is widely used in the medical field.

e.g., if the patient must be given glucose then the solution must be sterile (“not” disinfected). And if it wasn't then blood sepsis might occur.

All surgical devices must be sterile.



Now the end results of all available sterilization methods are:

- ⇒ Damaged cell wall.
- ⇒ Damaged cell membrane.
- ⇒ Damaged nucleic acids of microbes.

- Bactericidal effect: killing effect.
- Bacteriostatic effect: inhibitory effect.

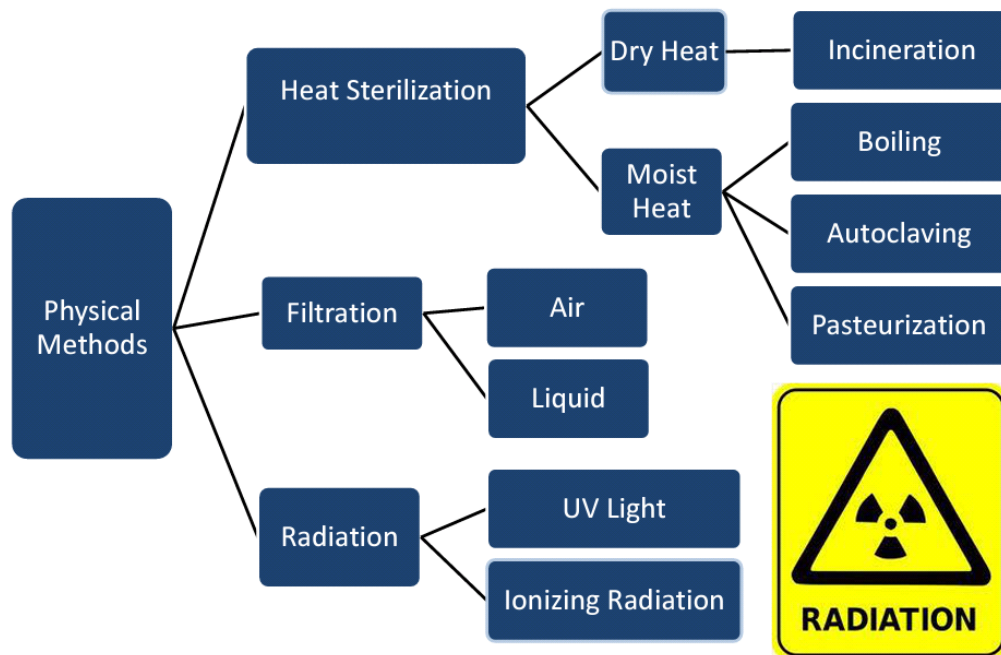
These two terms can be applied to different techniques (e.g. disinfection).

Note: everything ends with “-cidal” means killing.

- Lipolization: removing water to inhibit bacteria by lowering its metabolic rate. And if we give it the water back then it restores its activity. (sometimes we use it to preserve the bacteria).
- Refrigeration and Deep Freezing slow the growth of bacteria.

Methods to Control Microbial Growth:

Methods to Control Microbial Growth



First we're going to discuss physical methods, which are widely used.

Heat Sterilization: we have two important factors:

- Time.
- Temperature.

Sometimes we increase the temperature and reduce the time. And sometimes we reduce the temperature and increase the time.

- **Dry Heat**: is the heat without moisture.

Incineration: is burning, or if you put it in the oven.

Incineration (or direct flaming) can be applied to things that we want to get rid of (e.g., things contaminated with blood). And these methods are widely used in labs (to sterilize flasks, tubes...). And they're less used in the medical field.

- **Moist Heat**: is the heat with vapor. (more effective).

Boiling:

Boiling water (100 °C/for 2-3 min) kills nearly all human pathogens. Only the spore-forming bacteria might survive → disinfection method.

But if you want to give a patient water (in the form of injectable water) then you can't rely on boiling. You have to use a sterilization process, like autoclaving.

Autoclaving: (steam under pressure)

It is widely used in laboratories & hospitals to prepare many types of culture plates, devices for usage...etc. and sterilize them. The autoclave provides 121 °C/15 PSI for sterilization for a short period of time ~15min will be enough. This method often doesn't cause damage to the material that you want to sterilize. And it can't be used to sterilize surgical devices that are made of plastic or other materials that can't stand such conditions.



Pasteurization:

a very effective method of disinfection for some types of materials (e.g., milk, juices and food). It reduces the number of pathogenic organisms –heat sensitive ones- but NOT FOR A LONG TIME (because it doesn't kill the spores).

So it has bacteriostatic effect thus increasing the shelf life for products, like milk.

How to pasteurize milk?

- Boiling it at home (form of pasteurization).
- In industry: by heating it to $\sim 62^{\circ}\text{C}$ for 30 min in a closed container, or for 72°C for 15 sec using UHT (ultra-heat treatment technique) in special Aluminum plates / this allows evaporation to occur to collect the milk powder.

Pasteurization is an excellent way to kill certain pathogens and inhibit the others for a short time.

Filtration:

For liquids. Using nitrocellulose membrane with pore sizes in the rang of 0.01-0.2 μm (but doesn't filtrate viruses).

The technique is related to researches and widely used in labs.

Radiation:

Ionizing Radiation: (cold sterilization)

- high-energy electromagnetic beams in form of gamma rays usually produced by special equipment and under certain conditions.
- Radioactive cobalt 60 is used.
- Takes few minutes.
- Important method in the industrial field, and used in the pharmaceutical industry in preparations of intravenous fluids with disposable materials.

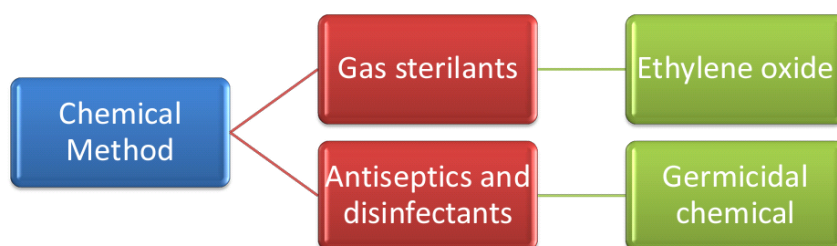


- The object you want to sterilize using this method must be thin-walled (can be penetrated).
- this method is used to kill the microorganisms of the frozen food.
- All radiation methods damage cellular DNA.

UV Light:

- Is not enough to kill all types of microbes, so it is a disinfection method /it is a slow process/ used in clinics for certain some orally-using objects.
- UV light is harmful for the skin and eyes so it is not advisable to expose to it for long time.
- Why is it slow? Due to the nature of the UV light (its wavelength =240-280 nm), so the penetration distance is short (at level of surfaces / can't penetrate liquids). => so it can't be applied to devices and such objects.
- Used to reduce the microorganisms found in the air of surgery theaters, and also used in the labs to reduce microbes in preparation of tissues.
- It's not 100% enough to be used for sterilization.

Chemical Microbial Control



Alkylating Gases:

Ethylene Oxide:

- It's very toxic for humans, so we don't like to use it unless it's very needed. (harms the respiratory tract, eyes, and sometimes it causes death in case of long exposure).
- For example it's used to disinfect rooms that are highly contaminated with bacteria or maybe spores.
- Because it's very toxic as we mentioned, so it's preferable to be mixed with 10% of CO₂ (we do that because this way it's easier to get rid of this gas after it did its function).
- We use it on fiber endoscopes, Heart-lung machine, Textiles, and disposable plastic articles.

Formaldehyde Gases:

- Formaldehyde evaporates at low temperatures (around 70 °C).
- We usually call it Formalin.
- Also highly toxic for humans –long time exposure may result in lung toxicity, cytotoxicity and eye or skin harm.
- Excellent chemical agent to kill all microbes of any type.
- (3-5%) formalin is used to preserve tissue free of microbes and to avoid morphological changes.
- Widely used in labs/ can be used in clinical medicine but it's very dangerous.

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