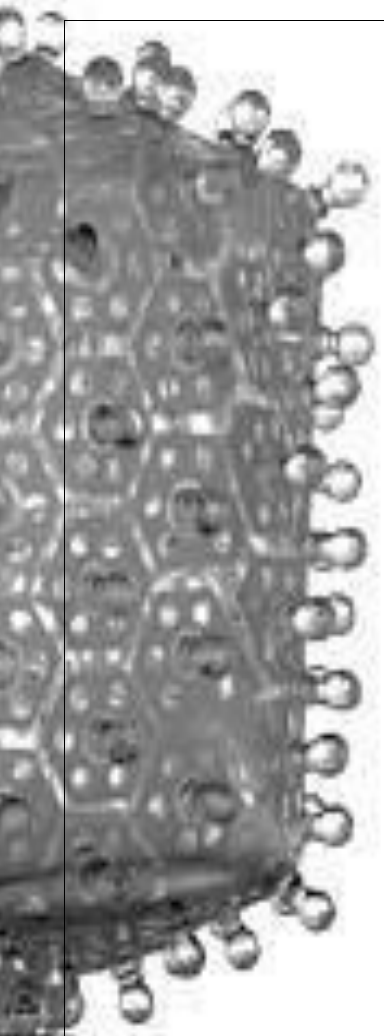

Microbiology

Lecture No: 8

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Sheet ■ Slide □

Normal Body Flora and

Control of Microbial Growth

We don't live in sterile environment; we are surrounded by the presence of various types of microorganisms which may be present anywhere even in small dust particles (viruses, bacteria, spores of fungi, etc).

This is important to take into consideration during the process of surgery (to the skin or to the internal organs) or injections, we can't proceed with operations on patients or inject them with any material if the targeted area -the skin for example- isn't properly prepared (killing the contaminants/ microorgansims), and we must make sure we're using sterile surgical instruments (needles, blades or any devices).

A large number of microorganisms constitute the normal body flora, these organisms can be found as commensal in many parts of our bodies like the oral cavity, the skin, the intestinal tract, the vagina and the lower urethra. The majority of these microorganisms are bacteria but there are few viruses and ectoparasites (ectoparasite: a parasite that lives on or in the skin but not within the body).

In the normal body flora, the number of microorganisms in the intestinal tract is much larger than that in any other part of the body.

The total bacteria in the normal body flora of any part of the body (whether in the respiratory tract, the intestinal tract, the skin, or any other part of the body) is divided as follows:

- Up to 95% are anaerobic bacteria
- 5% are facultative bacteria and obligate aerobic bacteria

Obligate aerobic bacteria constitute a percentage of 0.1% or less

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Bacteria colonize in any part of our body especially those parts exposed to the atmosphere or subject to food intake, like the oral mucosa and the respiratory mucosa.

Recently, they have discovered the presence of few numbers of bacterial cells in our lungs (in the past they thought that the lungs' tissue is free of any organism, YES it's free to some extent but not always, for example during aging some folding and damage occur because of the presence of viruses and fibrosis which might attach certain types of bacteria without causing diseases (but at certain conditions these might contribute to a type of inflammation in the lungs).

-The importance of the normal body flora:

- The production of useful end products which help our body function well like provitamins and certain minerals during the break down of food.
- They occupy a place on the surface of body parts like the mucosa of the respiratory tract or the intestinal tract which prevents the attachment of pathogens.
- Under normal conditions in healthy people, commensal bacteria can cope with the presence of few numbers of pathogens.

Refer to the slides to see the normal and abnormal conditions

*****black: pathogens *****white: commensal bacteria

Example:

If we ingest food contaminated with bacteria called Salmonella (which produce gastrointestinal symptoms), if the intestinal tract carries sufficient amounts of commensal bacteria which produce bacteriocins (anti-biotic-like substance), they might kill the salmonella and prevent the development of gastrointestinal symptoms like diarrhea.

If two people eat from the same food, one may have diarrhea the other might not, this response is related to *healthy commensal bacteria* in the intestinal tract, which has managed to suppress in presence of few numbers of pathogens. Contact with these types of bacteria (especially gram -ve commensal bacteria) results in developing immunoresponse against pathogens.

Factors that affect our **response** to microorganisms:

- Physiological factors.
- pH.
- Presence of healthy bacteria which produce end products related to other types of bacterial pathogens.

Together, these might –to some extent- control infection.

If the normal body flora is disturbed (lost its content of bacteria) due to toxic drugs or materials, our body parts will be more vulnerable to pathogens and we might develop diseases especially immunaodeficiency in the intestinal tract.

- The Normal Body Flora:

-1) Skin Flora: (bacteria that reside as commensal)

- **Staphylococcus:** (coagulase positive staph, coagulase negative staph)
- **Propionobacterium/ Propioni bacteria**: these produce propionic acid as an end product to inhibit the attachment of pathogens.

If damage occurs to the epidermis, dermis, or subcutaneous tissue due to injury, these bacteria might enter and produce infection and inflammation.

- 2) The Oral Cavity:

There is a large number of bacteria in our oral cavity, they contribute to the production of acids, these acids prevent the presence of yeast which might produce fermentation products (fermentation products produce bad smell of oral cavity).

- 3) The Intestinal Flora:

There is a huge number and types of bacteria in our intestinal tract (90% of the total bacterial cells of the body are found here, and around 50-60 types of anaerobes alone are present).

- **Anaerobic Bacteria:**

About 50-60 types present in the intestine, like 1)Bacteroides which is composed of many species like bacteroide fragilis 2) Fusiform bacteria. Anaerobes are less important as causative agents of infections, (around 10% stand for malignancy or surgery).

- **Aerobic Bacteria:**

Some of these bacteria might contribute to certain infections specially related to surgery in the intestinal tract.

- **Facultative Bacteria:**

A group called enterobacteriaceae, (gram negative facultative anaerobes), these manage to survive in the intestinal tract especially in the upper part of the colon like Escherichia coli (E.Coli), Klebsiella, Enterobacter and many others.

These 3 types together compete against types of non-commensal bacteria (which might reach our intestines via water, food, etc) especially against yeast; accumulation of yeast in the intestinal tract results in fermentative products, this might produce disturbances or gastrointestinal symptoms (not necessarily diarrhea) but might produce other intestine-related symptoms.

- 4) Urogenital Tract:

Lactobacilli in young women are important as they lower the pH of the vagina (keep it between "4.5-5") and prevent infection with other organisms or accumulation of organisms which may cause 1) irritation in the mucosa of the vagina and mucosal damage 2) production of vaginal discharge (fluid expelled from the vagina) 3) pain during urination. Such organisms require neutral or slightly alkaline pH, (yeast requires alkaline pH), they are present in few numbers but any change in pH results in the previous problems.

Sexual contact might transfer certain microorganisms from males to females, so it might sometimes cause superinfection, localized infection, ?? at16:30

The number of lactobacilli decreases with aging due to change in hormones, at around 50 years of age the pH of the vagina becomes more neutral and not acidic enough.

-5) The Urethra: (especially the lower part)

The urogenital tract has two parts:

- Upper: urinary bladder, ureter, kidneys
- Lower: urethra, vagina.

In men and women, there is always a number of organisms, these organisms are considered part of the normal flora of the urethra and are related to the skin flora, sometimes, (especially in ladies because they have shorter urethra), infection might be acquired from the rectum, some organisms manage to reach the opening of the urethra and with time produce ascending infection from the lower part to the higher part of the urogenital tract, which results in urinary bladder infection and the infection might reach the ureters and the kidneys as well. Most urinary tract infections are acquired from outside through the urethra. So the presence of microorganisms/ the bacterial flora there can to some extent control the presence of pathogens.

- Physical Control of Microbial Growth:

In order to understand the physical and the chemical methods of disinfection and sterilization, we have to know the difference between the 2 terms:

- Disinfection (التطهير) :

- ✓ Reduces the number of microorganisms to a safe level.
- ✓ 99% of microorganisms are eliminated or eradicated.
- ✓ 1% includes spore forming bacteria and certain types of parasites which cannot be easily killed by disinfection agents or methods.
- ✓ We can disinfect parts exposed to bacteria in our body, but not sterilize them.

- Sterilization (التعقيم):

- ✓ Kills/ eliminates all available living cells (bacteria, viruses, fungi, or parasites).
- ✓ 100% free of microorganisms
- ✓ we don't sterilize body parts (usually used for instruments)

Why can't the methods of sterilization be applied to our bodies (skin, oral cavity)?

Because using sterilization methods means we have to use eliminating agents such as chemical substances (acidic or basic) or heat to kill ALL microorganisms which produces irritation and damage to our mucosa, skin, etc. We can only use methods of disinfection to prevent damage.

We disinfect the targeted area in surgery or injections to prevent sepsis تسمم الدم

- Sepsis and Asepsis:

- Sepsis:

- ✓ the presence of any organism in a sterile part of the body like (subcutaneous tissue, body fluids like blood or CSF –Cerebrospinal Fluid-, upper part of urinary bladder).
- ✓ Sepsis also applies for the presence of organisms which causes inflammation (to the skin for example)

- Asepsis:

In contrast to sepsis, asepsis is the absence of organisms, aseptic techniques are the methods used to achieve asepsis or disinfection.

In relation to the surface or our skin or the mucosa, we can't use sterilization methods so we use disinfection methods. Aseptic technique is an important process in hospitals and we can't perform any operation before applying aseptic technique, as to prepare the skin for surgery or to prepare it for drug injections, or to prepare the oral cavity for operation.

*Note: Sanitization is like disinfection but it's related to the environment (to kill contaminants off materials), the term isn't used in clinical medicine.

The killing effect of disinfection and sterilization is directed against vegetative bacterial cells, meaning the living cells that can replicate and produce more cells, and more growth of bacteria.

However, getting rid of spore forming bacteria is difficult, for example if we want to inject a patient with a saline solution, we have to sterilize that to kill spore forming bacteria, these cannot be killed easily by boiling at 100 C for 1-2 minutes, they require boiling for at least 60 minutes at 100C to be eliminated. Along with some parasites and some viruses like: hepatits B or hepatitis C which are heat resistant, they can't be easily killed especially if they're associated with organic compounds like the liver.

Other terms used in relation to labs, research or treatment with antibiotics:

- Bacteriostatic:

Inhibition of the metabolic activity of the microorganism and not necessarily killing it.

- Bactericidal/ Microbiocidal:

The end result is killing the organism. This is often used in relation to sterilization (killing all types of organisms including spore forming bacteria) and in relation to preparation of pharmaceutical products and intravenous fluids.

- Refrigeration or Deep Freezing:

★ Refrigeration or deep freezing aren't equal to disinfection or sterilization.

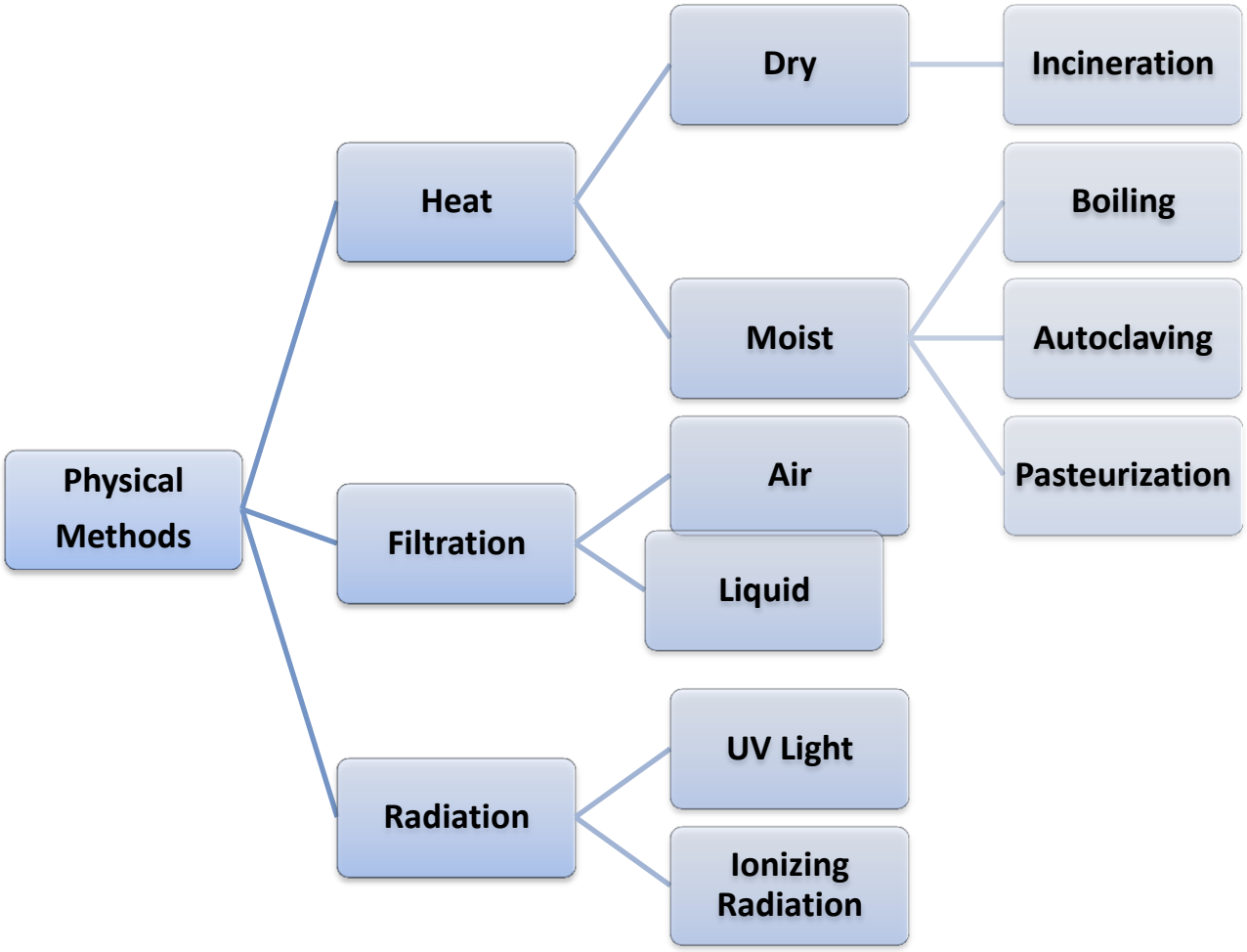
★ Refrigeration and freezing only inhibit the activity of certain types of organisms, and preserve it (for example we preserve our culture at -70 -80 degrees).

★ **(**there are few numbers of pathogenic organisms which can't tolerate low temperature like haemophilus and neisseria**)**

★ But in general, refrigerating and freezing don't usually kill microorganisms, they only inhibit the activity of the organism and control its growth, because the majority of pathogens can tolerate low temperatures.

**Note: Desiccation التجفيفlon the other hand is to some extent similar to disinfection, because it reduces the level/ number of micro organisms.

- Physical Methods:



- Dry Heat:

- to directly use the atmospheric oxygen or nitrogen only**,** without the presence of any water
- Excellent methods for sterilization.
- an application that uses dry heat is the hot air oven, it can be with UV or with electrical heating, you place the instruments or materials in, keep them for 1-2 hours at temperature between 160-180C, in order to kill all bacterial cells
- used more in industries and research than in hospitals

in hospitals, the majority of pharmaceutical products, surgical instruments can be sterilized by using the Autoclave (which we will come to in a moment)

- Moist Heat:

Utilizes hot air that is heavily laden with water vapor and where this moisture plays the most important role in the process of sterilization.
An application on moist heat is the AutoClave

– The Autoclave:

★ Using moist heat under certain conditions controls the presence of organisms.
★ It's a sterilization method
★ For materials that are expensive and should be reused in hospitals
★ Temperature not 100 but we increase that to 121degrees
★ Pressure increases from 1.5 atmospheric pressure to 2
★ Time needed 15 minutes
★ This saves energy because if we use the lower values for temperature and pressure we might need 1 hour of time in order to achieve the aim of sterilization, so increasing those will decrease the time needed for sterilization.

Note:

Boiling water for 1-3 minutes is enough to kill the majority of pathogens, and it makes the water safe to drink, (free of viruses, most bacteria etc) but spore forming bacteria of the genus bacillus and clostridia might not be killed, so moist heating→ boiling is an excellent method of disinfection and not sterilization.

- Incineration:

- burning contaminated materials resulting from operations or hospital wastes
- For disposable materials, approximately 50% of the materials used during operations are disposable and are sent to incineration and not to the autoclave, but other instruments are very expensive and can't be disposed so these should be sent to the autoclave.

- Ionizing Radiation:

- (xrays aren't physically active enough to kill lots of micro organisms)
- High-Energy Electromagnetic Beams
- ex : Gamma Rays , Radioactive Cobalt 60
- can damage any type of bacteria , viruses , etc
- this method aims to damage genetic material "DNA", and thus results in a killing effect
- Radioactive cobalt 60 is used in industry for preparation of all disposable materials used in surgeries and hospitals (like blood and urine containers), it's also used to prepare intravenous fluids especially glucose and saline.
- In any hospital, hundreds to thousands of liters of these 2 IV fluids are daily used to control conditions of patients.

- Filtration:

- By using special filters with pore sizes between 0.01-O.2um.
- Used in labs to prepare certain amino acids or polypeptides for research which can be damaged by sterilization.
- Hospitals don't filter fluids or drugs anymore, autoclaves or radiation is used there.

- Pasteurization (البسترة):

- Pasteurization was used to produce non-acidic wine, (to inhibit the activity of acid bacteria in wine)
- Later it was applied to milk production, to produce safe milk that can be preserved for 1-2 weeks.
- Pasteurization means: killing the most dangerous organisms which may be found in milk especially pathogens and the other types of bacteria which might have contaminated the milk during collection or transport.
- There are certain types of bacteria found on the skin of animals or even within their bodies, and might contaminate the milk during milking and collection like Brucella, the disease Brucella is a very serious disease which occurs as a result of drinking non pasteurized milk.

- Pasteurization is using low temp of 62-63C for 30 minutes.
- Pasteurization will not eliminate spore forming bacteria, but usually this doesn't contaminate milk and if it does it will not change the taste of the milk or produce fermentation.

  Two aims of pasteurization:

  1) Kill pathogens.

  2) Preserve milk for a short period of time (at least 1 week).
- Long term Pasteurization involves the use of gamma rays, it produces long lasting milk that can be kept for 1 year without being damaged or causing diseases.

- UV (Ultraviolet) Light:

- UV light can achieve the level of disinfection only, (excellent level of disinfection)
- UV can reduce the number of contaminants from the material especially off the surface of materials. (however it doesn't kill all organisms for example spore forming bacteria)
- cannot reach level of sterilization and so, can't be used for intravenous fluids
- Used on the dust particles in the atmosphere of operation rooms (because they might have microorganisms)
- The penetration of UV light is limited to the surface; it can't reach the deeper tissue of the body or bacteria that reside deep in the body.
- Exposing our skin to UV light for a long time might damage our skin, eyes, DNA of our tissue and of course kill some bacteria along the way.
- UV in general causes break down of the structure of the nucleotide sequence of the DNA and so inhibit the replication of the vegetative form of the bacteria.

- Chemical Methods:

- **Sterilization Methods..**
  - Alkylating Gases (Ethylene Oxide)
  - Formaldehyde Gas
- **Disinfection Methods..**
  - alcohol, iodine, detergants used in our homes

To be continued next lecture (the dr. says read the slides)

"ان الذين في قمة الجبل لم يسقطوا فجأة من السماء :)"
Done By: Hiba Al-Atrash