

***Title of Lecture: Introduction into Microbial growth***

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***Refer to slide no. : 4***

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**Introduction into Microbial growth**

* To control the growth of bacteria and microorganisms, we want to suppress the growth of them, it can actually be done on inanimate objects or on the skin, this is known as Sterilization and Disinfection. We use disinfectants, and sterilizing agents (Antibiotics are used to control bacteria inside the body.) to control the growth of bacteria outside the body, for various reasons.
* For example: 1. Surgical instruments are used inside the body, we want the clear and sterile. 2. We want bacterial growth under control in food because bacteria will spoil the food.
* Early civilizations practiced salting, smoking, pickling, drying, and exposure of food and clothing to sunlight to control microbial growth and preserve food.
* Use of spices in cooking was to mask taste of spoiled food. Some spices prevented spoilage.
* In mid 1800s Semmelweiss and **Lister** helped developed **aseptic techniques** to prevent contamination of surgical wounds especially in hospitals.
* We have 2 types of infections:
 1. Nosocomial infection : which is an infection acquired in hospitals, especially in surgeries.
2. Community acquired infections, picked up outside hospitals.
* Before then:
	+ Nosocomial infections caused death in 10% of primitive surgeries (ex: lead amputation)
	+ Up to 25% mothers delivering in hospitals died due to infection (sepsis) this is very serious.

**Definitions**

* **Sterilization:** Killing or removing ***all forms of microbial life*** (Bacteria, viruses, protozoa, including **endospores**) in a material or an object.

\*Endospores are very resistant, and hard to kill, sterilization should remove all endospores, because if not removed, they will evagenate giving rise to new bacteria.
\*Endospores: spores that will give rise to bacteria.
\*Spores: could give rise to other types of microorganisms. Ex:fungi.
* **Disinfection:** Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases. Usually involves the removal of **vegetative** or **non -endospore forming** pathogens, fungi as well.
disinfection could get rid of spores of fungi, but not endospores.
so, disinfection will give us a clean and safe instruments but not sterile.
* **Sepsis: it is an infection.** Comes from Greek for decay or putrid. Indicates bacterial contamination.
* **Asepsis**: Absence of significant contamination(absence of infection).
* **Aseptic techniques:** techniques in which you can handle a patient without the risk of contamination. used to prevent contamination of surgical instruments, medical personnel, and the patient during surgery.(scissors, forceps, masks, gloves)
* Aseptic techniques are also used to prevent bacterial contamination in food industry. And in healthy restaurants.
* **Disinfectant**: chemical substance used to eradicate/kill microorganisms applied to inanimate objects.
\* Doesn’t have to be harmless to human body.
* **Antiseptic**: chemical substance used to eradicate/kill microorganisms applied to living tissue (***antisepsis***).
\*Should be harmless. Because it’s used on living tissue.
* **Degerming**: Mechanical removal of most microbes in a limited area without killing them. Ex: washing the hands with soap.
* **Sanitization**: Use of chemical agent on food-handling equipment to meet public health standards and minimize chances of disease transmission.

**General Considerations in Sterilization & Disinfection:**

1. **Population of the bacteria is unknown**, we need a substance that can kill all kinds of bacteria without having to test the surface each and every time and culturing the bacteria to identify it and then finding the right sterilizing agent for it.
2. **Determination of the substance’s level of kill.**
3. **Heterogeneity within population**. (we have thousands and millions of different types of bacteria) we need a substance that would kill as much as possible.
4. **Troublesome forms.** Some types of bacteria are harder to kill than others (like TB ‘tuberculosis’ bacteria) others are easier (like gonococcus bacteria)
5. **Environmental factors**. Ex: ph. It affects the efficiency of the disinfectant that’s used.
6. **No Universal Method for sterilization.** That’s why we have various methods for sterilization.
7. **Careless handling.** If we handle the samples, or the tools used, carelessly, it might get infected. For example: a nurse might open the cap of a syringe, place the syringe on a table for 5 minutes then use it on a patient, now the syringe was contaminated because the table isn’t sterilized.
8. **Effect on Bacteria as the principal criteria.** Whether the disinfectant is going to kill or not to kill the bacteria.
9. **Critical** (MUST be sterilized. Tools used in surgery like scissors an d clamps) **, semi-critical** (To have clean an disinfected instruments but not necessarily sterilized, like Laryngoscope and thermometers. They have minimum contamination) **, and non critical items** (things that are going to be used by the patient, like electrodes. Should be clean but not sterile)**.**

**Methods of sterilization: 1. Physical Methods of S/D:**

1. Heat
2. Moist heat

**Note**: these are the first 6 physical methods mentioned in today’s lecture.

1. Steam at atmospheric pressure
2. Steam under pressure
3. Pasteurization
4. Dry heat
* **Heat**
* Kills microorganisms **by denaturing their enzymes and other proteins.**
* **2 types of heat sterilization:** 1. Moist heat. 2. Dry heat.
* Heat resistance varies widely among microbes.

\*\*the following terms are not for memorizing.
	+ **Thermal Death Point (TDP)**: the temperature at which all of the test microorganisms in a 24 hour broth culture are killed in ten minutes.
	+ **Thermal Death Time (TDT):** the time required to kill all microorganisms of a known population at a given temperature under specific conditions.
	+ **Decimal Reduction Time (DRT):** Time in minutes at which 90% of bacteria at a given temperature will be killed.
* **Moist Heat**
* Kills microorganisms by **coagulating** their proteins.
* In general, moist heat is much more effective than dry heat.
* **Boiling:** -Some sort of moist heat. Heating to 100oC at sea level. Kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within 10 minutes or less.
- not considered as a sterilizing method completely, because it doesn’t kill endospores, and some types of viruses.

 **- Hepatitis B virus**: Can survive up to 30 minutes of boiling.

 **- Endospores**: Can survive up to 20 hours or more of boiling.

* Reliable sterilization with moist heat requires temperatures above that of boiling water.
* **Steam at atmospheric pressure**
* Steam at 100°C has 540 calories of latent heat as compared to the 80 calories for boiling water at that temperature.
* This is why steam is more effective that boiling, it has higher temperature and more calories.
* It can kill endospores but not completely.
* Used to disinfect pipes, vats, in diaries and breweries.
* **Steam under pressure( Autoclaving)**
* At a temperature of 121°C of moist heat for 15 minutes spores are destroyed. (Reaches high temperature in less time than at atmospheric pressure)
* The best method of moist heat. It can kill all endospores.
* In order to obtain this T° saturated steam is placed under a pressure of 15 Psi (15 libra/inch2 or 1.05 kg/cm2).

Pressure (Psi) ***T°C*** Time (minutes)

 15 121° 15

Note that with increasing pressure time decreases, and temperature increases, this saves time on factories.

 20 126° 10

 30 134° 3

-Autoclave: is a machine that accomplishes steam under high pressure. Steam enters at high temperature and pressure, and achieves sterilization.

**Autoclave: is a closed chamber** **with High Temperature and Pressure** in the middle. Surrounded by a jacket with inlets and outlets.

We can use it to sterilize everything: from beddings, to towels, gauzes, and tools for surgery.

* Usually :
1. Instruments (or whatever we want to sterilize) must be cleaned.
2. Packaged with green cloth.
3. Sealed very well.
4. Now we can put them in the chamber.
* when we put them in the chamber, we must leave enough space for the steam to circulate the chamber freely. Don’t over-crowd the chamber with instruments, because no sterilization will occur.
* once done, we apply suction pressure to get rid of all the air.( they will keep the steam from reaching the temperature and pressure we need)
* once we have the air out, we let the steam and pressure inside, through the steam supply, it’ll go around the jacket first then in the chamber itself.
* we keep it at 121C, pressure= 15, and for 15 minutes.
* the safety valves: to release the extra pressure when it exceeds what is needed.
* after 15 minutes: we release all the steam inside first (to prevent burning of steam when we open the chamber) then we open the chamber and get the sterilized things out.
* Now we must make sure the sterilized instruments got actually sterilized through Quality Control.

-2 methods:

1. ***Colour indicators***: if the colour changes to the required degree we know that the temperature has been reached.
2. ***Using endospores of stearothermophilus***: we put them with the packages, after sterilization, we take them and culture them, if they grow then the autoclaving was unsuccessful if they didn’t grow then it was successful.
\*\*the agar plates are also sterilized because we only want one type of bacteria to grow (the one we want to study)
* **Pasteurization: Developed by Louis Pasteur** to prevent the spoilage of beverages. Used to reduce microbes responsible for spoilage of milk, juices, beer, wine, etc.
* Not considered as sterilization, because some bacteria and endospores are not killed.
* Usually used for liquids .
	+ **Classic Method of Pasteurization**: exposure to a T° of 65oC for 30 minutes.
* Not preferable by factories. Because it takes long time.
* Bacteria that causes diseases found in milk:
	+ - 1. **Brucellosis by a bacteria called Brucella, from milk from goats.**
			2. **Tuberculosis from cows.**
	+ **High Temperature Short Time Pasteurization (HTST):** Used today. Exposure to a T° of 72oC for 15 seconds.
	-this method is now used in some factories, where they put the milk on a steel bridge-like thing. But the milk will not last long before expiry.
	+ **Ultra High Temperature Pasteurization (UHT):** Exposure to a T° of 140oC for 3 seconds and then cooling very quickly in a vacuum chamber. Most canned milk nowadays is made this way.
	\*\***Advantage**: can be stored at room temperature for several months.
	+ **Fractional sterilization (Tyndalization** ) old fashioned method used for making jam, we heat/boil the substance, and then you put it in a warm place, now we’ve killed the bacteria, but the endospores are still there. The 2nd day we repeat the process because the endospores have bud off into new bacteria, and repeat it for a 3rd day to make sure no endospores are still there at all
* **Dry Heat:** Kills by **oxidation** effects.
* **Direct Flaming:** Used to sterilize inoculating loops and needles. Heat metal until it has a red glow.
* **Incineration**: kills everything.
\*\* Effective way to sterilize disposable items (paper cups, dressings) and biological waste.
* **Hot Air Sterilization:** Place objects in an oven. Require 2 hours at 170oC for sterilization. Dry heat transfers heat less effectively to a cool body, than moist heat.