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Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

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Bacterial Death

Death of any living organism can be defined as its **irreversible inactivation**, so it does not **grow** nor **reproduce** anymore.

To confirm the death of bacteria:

We grow it on a **suitable media** and see if it **forms colonies** on agar or increases turbidity in broth. If no growth is observed, we can confirm bacterial death.

Note: *The setup of the experiment to confirm death, like the type of media used or time of experiment after exposure to the killing agent must be optimized. Otherwise, confounding results can happen. (ex. growing viable bacteria in the wrong media can result in no colony formation, and assuming bacterial death here is not correct)*

Exponential decrease through bacterial death

As seen in the following graph, an exponential decrease in microbial survivors takes place and varies in degree according to **the killing agent.**

This happens because the probability of a given cell's dying is constant per unit time. For example, the **rate** of bacterial death is **90%** per **minute in the graph to the right**. This means that at any given minute, each bacteria has a 0.9 chance of dying. The dashed line corresponds to the right Y axis (arithmetic number of survivors), while the continuous line corresponds to the left Y-axis (logarithm of number of survivors)

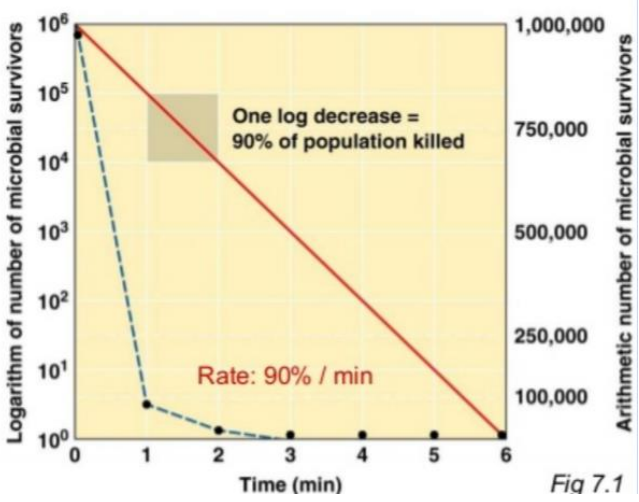


Fig 7.1

Note: *Bacteria can become resistant to the killing agent after some time (ex. Emergence of antibiotic resistant mutants), leading to a change in the constant rate of death, and a change in the slope of the line.*

Formula of cell death

$$S = S_o * e^{-kt}$$

S → Number of cells **survived after** time (t) of using a killing agent

S^o → Number of cells **originally**

E^{-K} → Depends on the **susceptibility of the bacteria to the killing agent**

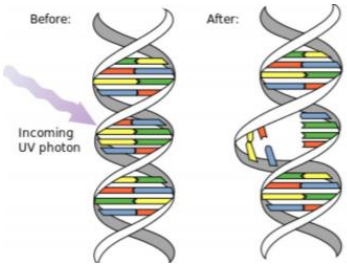
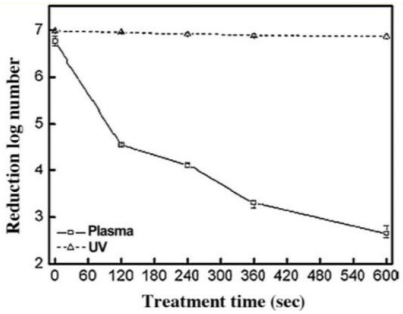
Antimicrobial Agents Definitions

1	**Sterilization**	A process to make an object **free from any living organism** on a surface.
2	**Disinfection**	Removal of **some microorganism** to get a surface that is appropriate to **use level**. It is **less effective** than sterilization. ***Note:*** *Some microorganisms like bacterial **spores** and **prions may persist** since they are **highly resistan**t microbes.*
3	**Cleaning**	Removal of **organic and inorganic materials** *(biofilm)* from objects mechanically with **water** and detergent for example.

Bacteriostatic: It is the **reversible inactivating** of an organism process.
*Once we remove the agent the bacteria will **resume** its growth and replication.*

Bactericidal: It is the **irreversible inactivating** of an organism process.
*Even if we **remove** the agent the bacteria will still be **dead**.*

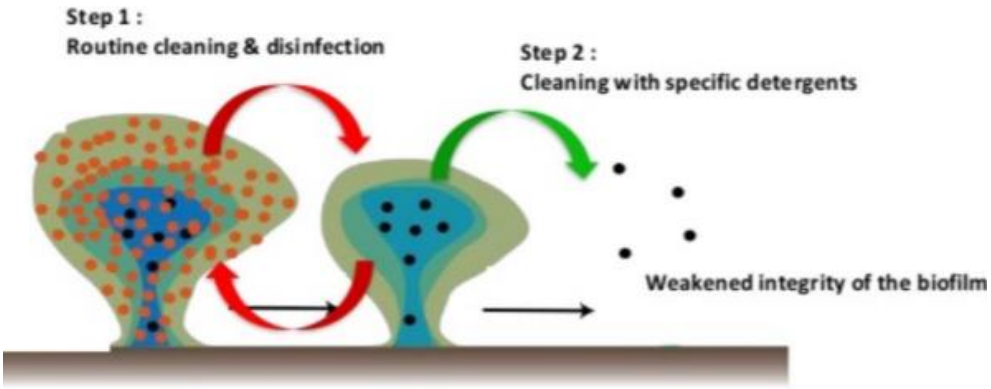
Sterilization methods

1	**Autoclaves**	They are **strong containers** that use **pressurized** and **saturated steams** at almost 121 C^o for around 15-20 min. It's the most **efficient** way to kill microorganism using **heat and pressure.** 
2	**Filters**	With **pores** size of **0.2 µm** filters mostly, microorganisms will be **retained**. Smaller pore
3	**Chemicals**	**Hydrogen peroxide** is used in high concentrations (10%-30%). **Glutaraldehyde** is used with long contact times (3-12hr). ***Note:*** *Both when used at **lower concentration/time** may be considered **disinfectants** since they will not be as **effective** to be **considered** sterilizing agents.*
4	**Radiations**	**UV radiation** damages DNA by **crosslinking adjacent** pyrimidines. **Ionizing radiation** like **Gamma radiation** for example causes **strand breaks**  ***Note:*** *Gamma radiation has **better penetration into materials** than UV radiations.*
5	**Gas plasma /Vapor phase**	This process shows **high efficiency** in killing **spores**. **Example:** Hydrogen peroxide and Peracetic acid in **vapor form can be used to sterilize endoscopes.** *The graph shows that **Plasma** kills bacterial spores **better** than **UV*** 

Factors to consider in disinfection and sterilization:

1) **Prior cleaning**

The presence of **biofilm** makes it harder for sterilizing agents and disinfectants to **penetrate** into deeper bacterial layers and even sometimes **inactivates** the agents, so we have to **clean** the object before sterilizing/disinfecting to get rid of biofilms, that's why cleaning is **important**.



2) **Organic and inorganic load present**

This **decreases** the **efficacy of action** for both disinfection and sterilization.

3) **Type and level of microbial contaminations.**

Example: Prions, whom are **highly resistant.**

4) **Concentration of and exposure time to the germicide** *(killing agent)* as discussed before.

5) **Physical nature of objects**

If they have **crevices, hinges or lumens**, its hard to **reach** them properly.

> ***Note:*** *Sterilization methods are used* ***depending on the objects' nature****, for example some objects are* ***heat sensitive*** *therefore we cannot use* ***autoclaves****, so filters or gas plasma are considered* ***more suitable****.*

6) **Presence of biofilm**

7) **Temperature and pH of the disinfection process**

Antimicrobial Agents Definitions part 2

1	**Septic**	Is the presence of **pathogenic microbes** in **living tissues** or **associated fluids**. ***Note:*** *A* ***septic shock*** *is a serious medical condition that occurs when* ***living tissues*** *are* ***damaged*** *in response to* ***infection*** *by* ***microbes****.*
2	**Antiseptic**	**Destroys or inhibits** the **growth** of microorganisms in or on **living tissues** or fluids. They are similar to disinfectants, but they work on **living tissues** unlike disinfectants. ***Example:*** *Alcohol, Iodine and Chlorhexidine.* 
3	**Aseptic**	Is an adjective describing a **technique free of microorganisms** ***Example:*** *Surgery*
4	**Preservation**	Is the **prevention** of multiplication of microorganisms in formulated products using **preservatives**.

Mechanism of action of antimicrobial agents

1) **Denaturation of proteins**
   Heat destroys the **tertiary structure** which gives a protein its **function**.

2) **Oxidation damage** interferes with **most biological processes** of the cell *(membrane/DNA synthesis)*
   Example: Hydrogen peroxide interferes with **some enzymes** disturbing essential metabolic activities in the cell.

3) **DNA damage**
   Example: UV and Ionizing radiation.

4) **Cell membrane and cell wall disruption**

Example: Alcohol dissolves membrane lipids.

This table is only for further explanation of how each Antimicrobial agent affect different microorganism and others

Agents	Bacteria	Mycobacteria	Bacterial Spores	Fungi	Viruses
Disinfectants					
Alcohol	+	+	–	+	+/–
Hydrogen peroxide	+	+	+/–	+	+
Phenolics	+	+	–	+	+/–
Chlorine	+	+	+/–	+	+
Iodophors	+	+/–	–	+	+
Glutaraldehyde	+	+	+	+	+
Quaternary ammonium compounds	+/–	–	–	+/–	+/–
Antiseptic Agents					
Alcohol	+	+	–	+	+
Iodophors	+	+	–	+	+
Chlorhexidine	+	+	–	+	+
Parachlorometaxylenol	+/–	+/–	–	+	+/–
Triclosan	+	+/–	–	+/–	+

+ → The microbe is **killed**

_ → The microbe is **not killed**

+/- → Means that the effect **depends** on the **concentrations/time** used.

Good Luck ☺