9

Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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NOTE: the underlined notes are not mentioned in the lecture I've added them just for further clarification so they are not required .m

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The doctor clarified this point from the previous lecture:

Typing is the identification to the strain level (subspecies) , <u>we use typing to identify different strains of bacteria within a single species</u>

Do we need to do typing for every sample we have ?

No, just in certain cases like some infections, researches , outbreak of infection

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Sterilization and disinfection

-methods of sterilization :

1.heat : dry and moist

2.radiation : UV rays , ionizing radiation and infra-red rays

3.filtration

4.chemical agents

We've talked about the first one and now we well continue :

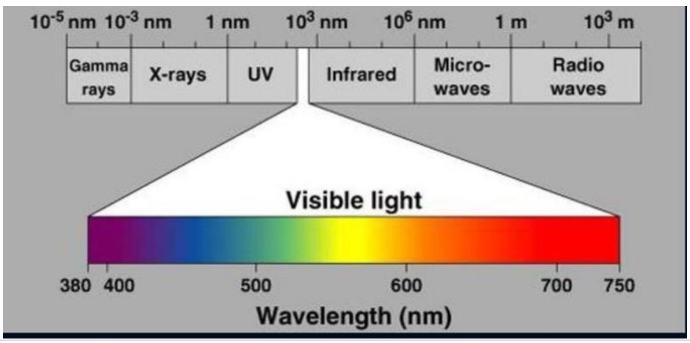
2.Radiation

a.UV light:

bactericidal (it affects and kills bacteria)

not efficient for complete sterilization

reduce number of bacteria in certain areas such as operating theaters



b. ionizing radiation:

rays of short wave length and high penetration power e.g gamma rays

in moderate doses lethal to microorganisms

sterilize pre-packed disposable plastic items that can't stand heat such as plastic catheters and gloves

note: doctor said to ignore the Syringes (a needle used to inject liquid in the body) because it's an old way

C. infra-red rays :

Act through heating temperature that reaches 180c

Sterilize mainly glass ware and syringes

3.Filtration

Mechanism

Mechanically removes microorganisms by passage of a liquid or gas through a screen like material with small pores may be done under either positive or negative pressure

But what does + and – pressure mean?

According to the purpose that we want to sterilize a certain room for , we either use + or – pressure

****positive pressure environment :** Air pressure in the room under positive pressure is higher than outside, so contaminants (particles, viruses, bacteria) are kept out. The positive pressure environment is used to protect patients in operating theatres, so that infection does not enter open body cavities, or other conditions linked to a compromised immune system, being nursed in isolation rooms. Positive pressure rooms also often known as a **protective** room. (so we use + pressure when we want a room to be sterilized and to protect it from any contaminants from outside)

****negative pressure environment :** The air pressure in the room under negative pressure is lower than outside so that contamination from the room does not flow out into surrounding areas. The negative pressure environment is used for airborne infection control to protect people from patients with very contagious disease

(we try to prevent anything inside a certain room to flow out)

Application

Filtration is the preferred method of sterilizing certain solutions , that likely be damaged by heat (if we sterilize them with heat all of their properties will be changed) e.g : IV fluids , antibiotic solutions , vaccines , enzymes and some culture media .

Most common types :

a.membrane filters : uniform pore size , used in industry and research

different sizes :

1) 0.22 and 0.45um pores used to filter most bacteria. don't retain spirochetes ,mycoplasmas and viruses (they can pass through the pores)

2) 0.01 um pores: retain all viruses and some large protein (we use this type of membrane filters if we don't want the viruses to enter)

''we're done with the physical methods of sterilization, now we will talk about the chemical methods''

1.Ethylene oxide:

Denature proteins and DNA by cross-linking functional groups

Ethylene dioxide used in sterilizing heat sensitive materials such as surgical instruments and plastics , it is used for sterilizing endoscopes and anesthetic apparatus.

Q: Do we need endoscopes to be sterile (100% free from living organisms)?

NO, because it enters areas that are not sterile , but we use a disinfectant to reduce the number of microorganisms to a level that is NOT harmful to the patient .

**disadvantages: highly inflammable , potentially explosive gas.

2.Aldehydes:

-Denature proteins and inactivate nucleic acids

-formaldehyde as gas used to sterilize operations theaters and other spaces

-glutaraldehyde a chemical relative to formaldehyde less irritating and more efficient than formaldehyde

-glutaraldehyde used to disinfect hospital instrument , including endoscopes and respiratory therapy equipment.

3. Halogens :

-The halogens particularly iodine and chlorine, are effective antimicrobial agents

-They damage enzymes via oxidation or by denaturing them

Iodophores (Betadine®), chlorine treatment of drinking water, bleach, swimming pools.

4.O2xidizing agents :

-Peroxides and ozone, kill by oxidation of microbial enzymes

-Hydrogen peroxide can disinfect and sterilize surfaces of objects

-Ozone treatment of drinking water (but we prefer to use chlorine because it's less expensive)

5.Alcohol :

-powerful disinfectant and antiseptic

-effectively kills bacteria and fungi but does not inactivate spores

Mode of action : denatures protein , dissolves lipids and can lead to cell membrane disintegration

-swabbing of skin with 70% ethanol prior to injection :

There is normal flora on our skin like **staphylococcus aureus**, **staphylococcus epidermidis** and **hay bacillus** this normal flora if it enters a place that's not its original one it will become pathogenic that's why before any injection we use alcohol to disinfect the area.

most commonly used alcohols are ethanol and isopropanol.

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The choice of method of sterilization or disinfection depends on:

• The nature of the item to be treated

• The likely microbial contamination

• The risk of transmitting infection to patients or staff in contact with the item.

Measurement of microbial death:

<u>(Thermal death is a concept used to determine how long it takes to kill a specific bacteria at a certain temperature and it is determined by D-value)</u>

survivor curve, D value: dose required to inactivate 90% of the initial population. See figure

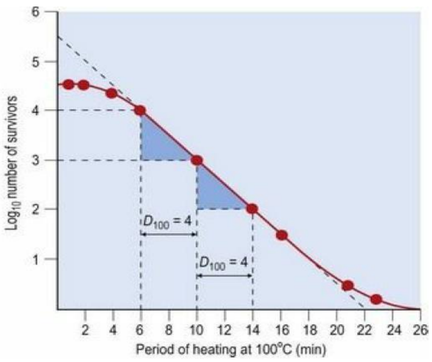

Fig. 4.3 Rate of inactivation of an inoculum of bacterial spores showing the decimal reduction time (*D* value) at 100°C and the nonlinear 'shoulder and tail' effects.

Resistance to Sterilization and disinfection

• Vegetative bacteria and viruses are more susceptible

• Bacterial spores are the most resistant, to sterilizing and disinfecting agents.

• Within different species and strains of species there may be wide variation in intrinsic resistance (structure difference, growth conditions)

• Prions: Highly resistance, use disposable instruments

Glutaraldehyde is least effective and considered as a disinfectant, usually we use it in cases of Fibrooptic endoscopes

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Bacterial genetics

We will talk about:

• bacterial genome and replication

• Mutations and effects on bacteria

• Genetic exchange and role in virulence

• Transposons definition and significance

The bacterial genome: (these are the source of genes)

• Chromosome (a must)

• Plasmids(an extra chromosome)

• Bacteriophage

• Transposons

They give the bacteria extra genes

Do all bacteria have plasmids /bacteriophage/transposons ?

No, but all bacteria should have chromosome

Significance of bacterial genetics?

1*Structure, Pathogenicity and virulence. 2*Antibiotic resistance.
3*Toxins. 4* Enzymes. 5*Molecular cloning

• Bacterial Genome: Chromosome, single circular double-stranded DNA.

Gene expression :

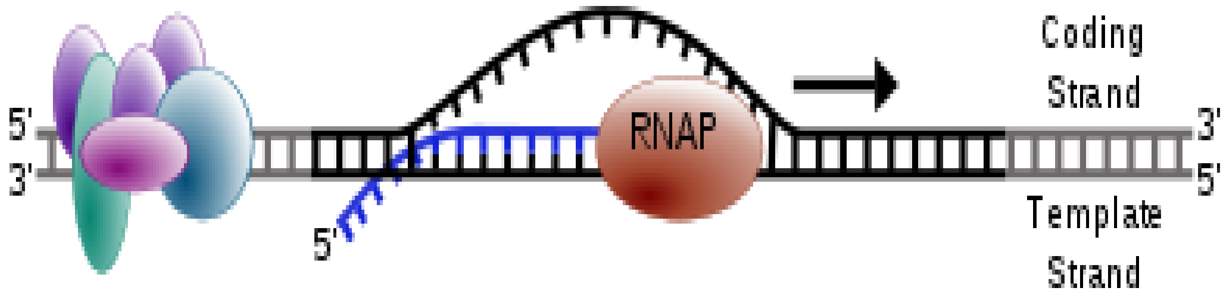
• is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA , Several steps in the gene expression process may be modulated, including the transcription, RNA splicing, translation, and post-translational modification of a protein.

Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism.

In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype, i.e. observable trait.

Transcription:

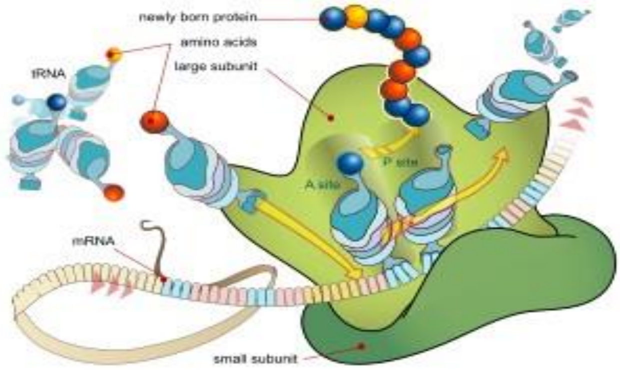
The process of transcription is carried out by RNA polymerase (RNAP), which uses DNA (black) as a template and produces RNA (blue).



RNA processing :

• While transcription of prokaryotic protein coding genes creates messenger RNA (mRNA) that is ready for translation into protein, transcription of eukaryotic genes leaves a primary transcript of RNA (premRNA), which first has to undergo a series of modifications to become a mature mRNA.

Is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis. The genetic code describes the relationship between the sequence of base pairs in a gene and the corresponding amino acid sequence that it encodes.



During the translation, tRNA charged with amino acid enters the ribosome and aligns with the correct mRNA triplet. Ribosome then adds amino acid to growing protein chain. In prokaryotes translation generally occurs at the point of transcription (co-transcriptionally), often using a messenger RNA that is still in the process of being created.

Gene regulation in bacteria

• Bacterial genes are often found in operons (specific regulatory molecules that control whether a **particular gene** will be transcribed into mRNA.

Often, these molecules act by binding to DNA near the gene and helping or blocking the transcription enzyme, RNA polymerase Genes in an operon are transcribed as a group and have a single promoter(is a region of DNA that leads to initiation of transcription of a particular gene) .

Each operon contains regulatory DNA sequences, which act as binding sites for regulatory proteins that promote or inhibit transcription. Regulatory proteins often bind to small molecules, which can make the protein active or inactive by changing its ability to bind DNA. Some operons are inducible, meaning that they can be turned on by the presence of a particular small molecule. Others are repressible, meaning that **they are on** by default but can be turned off by a small molecule.

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• **Genotype / Wild Type:** Represents all potential genes of bacteria cell (Its genome) All Inherited essential biological features & growth patterns.

• **Phenotype:** The expressed gene9s.The observed characteristics of the of the individual bacteria species/strain. Expressed by physical & biochemical properties. Growth patterns, Fermentation products, Antibiotic resistance, Toxins production. .etc.