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Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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Doctor

Ala'a

Quick recap:

In the last lecture we talked about Identification of Bacteria, and we categorized the methods of identification into three methods:

1- **Phenotypic** : morphology (macro and microscopic)

2- **Immunological** (serological) tests

3- **Genotypic** (molecular techniques)

-**Microscopic morphology** : includes a combination of cell shape, size, Gram stain, Acid fast stain, special structures e.g. endospores, granules and capsules can be used to give an initial putative identification

-**Macroscopic morphology** : bacterial cultivation (isolation of bacteria from the specimens) which includes : 1- nutritional requirements (fastidious and non-fastidious bacteria)

2- streaking for isolation

3- streaking for quantitation

4- colony characteristics (texture, shape, pigment, growth pattern)

5- biochemical tests

-**Types of culture media** : 1- Basal media (used for bacteria that do not need enrichment of the media)

2- enriched media (by adding blood, serum or egg)

3- selective media (contains agents that inhibit the growth of all agents except that being sought

4- differential media (an indicator is included and a particular organism causes change in the indicator

5- transport media

6- storage media

Now let's talk about today's lecture:

Biochemical tests :

-The microbe is cultured in a media with a special substrate and tested for an end product, so we add the special substrate and we wait for a specific reaction to happen which will indicate the presence of a certain type of bacteria.

-Prominent biochemical tests include enzymes (catalase, oxidase, decarboxylase), fermentation of sugars, acid or base production and the hydrolysis of gelatin.

- different biochemical tests are used for different characteristics of the obtained bacteria.

-Other biochemical tests of interest include: • Indole test

• Methyl Red / Vogues-Proskauer

• Citrate utilization

• Coagulase test

• H2S production (TSA)

• Urease test

• Phenylalanine deaminase test

-The doctor didn't talk about the tests listed above, but focused on the Catalase and Coagulase tests.

1- Catalase test

- Catalase is an enzyme that catalyzes the decomposition of Hydrogen peroxide into Oxygen and water. According to this equation: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

- This test is used to differentiate between Staphylococci and Streptococci, due to the presence of **Catalase enzyme** in Staphylococci, and the absence of it in the Streptococci

- What we do in the test is that we'll have two plates (still unknown bacteria type) and take a colony from each one , then I'll add Hydrogen peroxide, If bubbling occurs this

indicates presence of catalase enzyme (reaction indicator) this means its Staphylococci , if it doesn't bubble then its Streptococci.

2- Coagulase test

- This test is used to differentiate between Staphylococcus Aureus (Coagulase positive) and other Coagulase negative Staphylococci

- Coagulase is an enzyme (which is obtainable in Staphylococcus Aureus) that catalyzes the conversion of Fibrinogen to Fibrin, which results in blood clotting .

- What we do in this test is: the test is done on a slide or in a test tube , then I will make a colony for each sample , add Plasma to the sample, If clotting took place then its Coagulase positive Staphylococci Aureus , If not then its Coagulase negative Staphylococci.

Now let's move to another topic :

Classification of Bacteria

- Taxonomy : is the science of classification of organisms

- Bacterial taxonomy consists of three separate, but interrelated areas :

1- Classification : is the arrangement of organisms into groups on the basis of similarities or relationships

2- Nomenclature : is the assignments of names to the taxonomic groups according to international rules

3- Identification : is the practical use of a classification scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species

Taxonomic Rank :

- Kingdom or Domain
- Division or Phylum
- Class
- Order

Formal rank	Example
Domain	Bacteria
Phylum	Gracilicutes
Class	Scotobacteria
Order	Eubacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*Coli*

•Family

•Genus

•Species

-**Species** : The basic and most important taxonomic group in bacterial systematics

- The boundaries of species are rather difficult to define precisely, however; the boundaries of some genera are sharply defined. For example, the genus Bacillus and the genus Escherichia. (differentiation between species requires additional test)

- **Typing :** is the identification of organisms to strain level (sub-species)

- The main purpose is to control nosocomial (hospital acquired infections) infection and also can be used in different epidemiological purposes (cholera, meningitis)

- What information can be typing supply?

1- Similarity between isolates

a- wide range of characters that could be examined

b- significance of difference needs to be understood

c- significance of difference needs to clearly expressed

2- Relationship to other strains

- Comparator strains must be recognizable by the typing methods, having known characteristics

Methods of Typing:

- **Phage typing**
- **Bactericin typing**
- **Resistotyping**
- **Biotyping**
- **Serotyping**
- **Plasmid typing**

-**Bactericin typing:** is antibiotic-like substance produced by certain organism that can inhibit the growth of some close related bacteria. Eg: Colicin typing used for typing of Shigella sonni

-Biotyping: it depends on biochemical reactions (tests), as we took biochemical reactions is an identification method but it can be also used for typing classification
-It's part of the Analytical profile index (API) which is a classification method for bacteria depending on experiments. Eg. API 20 E

-We put the bacteria through several chemical reactions and observe if it's a positive reaction or not, until we identify the bacteria type

-**Advantages:** easy to perform – standardized

-Disadvantages: cannot be used for all organisms. Like in this example, I only used it when the family is Enterobacteriaceae

-Serotyping: it depends on serological reactions
- its used mainly for identification and can be used for typing
-The main idea here is that we classify the bacteria according to the cell surface antigens (which will produce an immunological response)
- **Advantages:** standardized – specific – easy to perform
- **Disadvantage:** Cannot be used for all organisms

-Resistotyping: also called Antibiogram
-It's a sensitivity test for typing
-What we do here is that we add antimicrobial agents (antibiotics) and we observe how the bacteria deals with it and classify them accordingly
- Uses a different type of antimicrobial agents that differ from the ones used for treatment (toxic dyes)

- **Advantages:** easy to perform – simple – cheap
- **Disadvantages:** limited sensitivity (some organisms are highly sensitive or resistant)

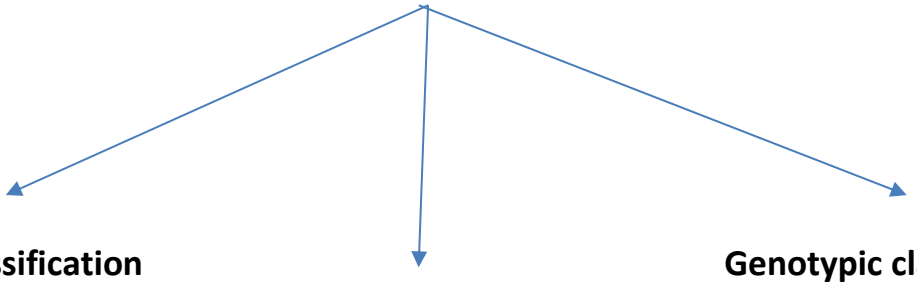
-Plasmid typing: also called molecular typing, Eg. PCR

- the bacteria is classified according to their Plasmid content, after it has been sequenced

-Advantages: very sensitive – very specific (it provides me with the sequence of genes)

-Disadvantages: need special machines – very expensive method

Classification of Bacteria



Phenotypic classification **Genotypic classification**

Environmental reservoirs

(mode of transmission)

-Adansonian classification:

-In most systems of bacterial classification, the major groups are distinguished by fundamental characters such as cell shape, Gram-stain reaction and spore formation (which are initial step for identification)

- Genera and species are usually distinguished by properties such as fermentation reactions (Lactose fermenting bacteria), nutritional requirements and pathogenicity

-Similarity coefficient when shared positive characters are considered or a **matching coefficient** when both negative and positive shared characters (matches) are taken into account.

-Phenotypic classification:

- Morphology and Gram staining characteristics

- Growth requirements and metabolic behavior

- Morphology: Cocci, Bacilli, Curved or Spiral, Filamentous

-It was found that there is some correlation between morphology and disease:

- **Spiral bacteria** (Treponemes, Borrelias, Leptospiras) **tend** to cause systemic diseases
- **Pathogenic Filamentous bacteria** (Actinomyces, Nocardia, Mycobacteria) **tend** to cause chronic diseases
- **Gram positive bacteria** (Staphylococcus, Streptococci) **more likely** to cause skin infection

- Growth requirements and metabolic behavior :

-Nutritional requirements: - Organism synthesizes organic compounds = Autotrophic
-Organism requires organic compounds = Heterotrophic

-Gaseous requirements: -Aerobic bacteria
- Anaerobes
- Facultative anaerobes

-Thermal conditions: -Psychrophiles
- Mesophiles
- Thermophiles

- Genotypic classification:

- DNA hybridization, used to designate species and similarities between species : (when there is two microorganisms one its gene sequencing is known and labeled , while the other is not , I hybridize the DNA they notice that similar genes are bounded together , so I can identify the sequence)
- Genomic 'G+C' content (Guanine + Cytosine ratio)
- Ribosomal RNA (rRNA) sequence analysis

Naming Microorganisms

-**Binomial** (scientific) **nomenclature**

- Gives each microbe 2 names:

- **Genus :** noun, always capitalized
- **Species:** adjective, lowercase

-Both italicized or underlined

Staphylococcus aureus (S. aureus)

Bacillus subtilis (B. subtilis)

Escherichia coli (E. coli)

Bacteria	
Actinobacteria	(High G+C Gram positives) *Actinomyces, Streptomyces, Corynebacterium, Nocardia, Mycobacterium, Micrococcus*
Firmicutes	(Low G+C Gram positives)
Gram-positive bacilli	*Listeria, Bacillus, Clostridium*, Lactobacillus*, Eubacterium**
Gram-positive cocci	*Staphylococcus, Streptococcus, Enterococcus*
Gram-negative cocci**	*Veillonella*, Mycoplasma*
Proteobacteria	(a very large group with 5 sub-divisions)
~~Gram-negative cocci~~	~~*Neisseria, Moraxella*~~
Gram-negative bacilli	Enterobacteria – *Escherichia, Klebsiella, Proteus, Salmonella, Shigella, Yersinia*
	Pseudomonads – *Pseudomonas, Burkholderia, Stenotrophomonas*
	Haemophilus, Bordetella, Brucella, Pasteurella *Rickettsia, Coxiella*
Gram-negative curved and spiral bacilli	*Vibrio, Spirillum, Campylobacter, Helicobacter*
Bacteroidetetes	*Bacteroides*, Prevotella**
Spirochaetes	*Borrelia, Treponema, Brachyspira, Leptospira*
Chlamydia	*Chlamydia*

* Anaerobic organism

* The table shows similarities between certain types of bacteria

* The doctor stated that the table has to be memorized, but it can be postponed until we take the bacteria in details.

Sterilization and disinfection

- Sterilization: The inactivation of all self-propagating biological entities (e.g. bacteria, viruses, prions) associated with the materials or areas under consideration 100% killing.

- **Disinfection:** The reduction of pathogenic organisms to a level at which they no longer constitute a risk.

-Antisepsis: Term used to describe disinfection applied to living tissue such as a wound. E.g Dettol

-Methods of Sterilization:

1- Heat (Dry / Moist)

2- Radiation (U.V rays / Ionizing radiation)

3- Filtration

4- Chemical agents

* chemical agents can be used in Sterilization and disinfection depending on the concentration used

Heat

-The only method that is both reliable and widely applicable

- temperatures above 100°C to ensure that bacterial spores are killed.

-This temperature is insufficient to destroy prions.

- Shorter applications of lower temperatures, such as in pasteurization can effectively remove specific infection hazards (Milk, Juice)

- Dry Heat :

- Most common method

- It kills microorganisms by destroying their oxidative processes

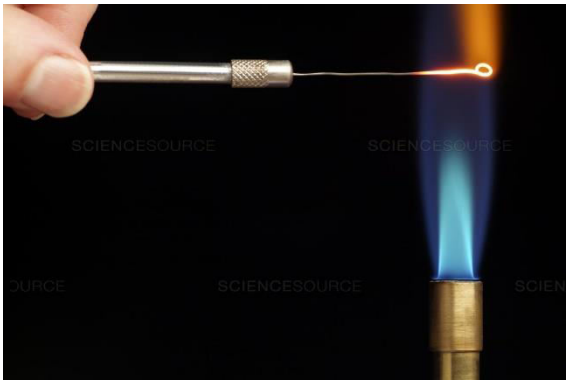
* killing microorganisms is done by 2 main methods: Oxidative or Coagulative. This can visualized by the cooking of an egg.

-If you take an egg, crack it open and drop it into a pot of boiling water, you will cook the egg by poaching it. The white part of the egg starts to stick together and becomes hard, a process called **coagulation**, which occurs at 52°C. When the egg coagulates, it becomes denaturalized, thus killing all proteins inside the cells of the egg.(the same thing happens in bacteria , I denaturate all proteins inside the cell so I kill the bacteria)

-Another way to cook an egg is to fry it in a frying pan. At first the egg will coagulate, but if you leave the egg in the pan and let it continue frying, it will all eventually turn black. This burning is called **oxidation**, and occurs at much higher temperatures than coagulation. Oxidation is a chemical process in which electrons are removed from the atom, and the end result is death of that organism.

-Dry Heat is divided into :

1- Red Heat: The item to be sterilized is directly held in the flame and heated till it becomes red hot.



- applications: Bunsen burner used for sterilizing bacteriological loops, knives and blades

2- Flaming: killing of organisms present on the surface of slides, mouth of culture tubes,…etc

-Incineration: is a huge metal compartment used for burning all the objects that cannot be cleaned. Used for disposal hospital waste. I burn it converting it into ash .

-Hot air oven: it expose items to 160-170 C for 1-2 hours. It has electric element in chamber as source of heat plus a fan to circulate air for even distribution of heat in the chamber. Used to sterilize items that are lacking water such as metals and glassware

- Moist Heat :

- Steam is non-toxic and non-corrosive, but for effective sterilization it must be:

1 -Saturated: which means that it holds all the water it can in the form of a transparent vapor.

2 -Dry, which means that it does not contain water droplets.

-When dry saturated steam meets a cooler surface it condenses into a small volume of water and liberates the latent heat of vaporization. The energy available from this latent heat is considerable: For example, 6 L of steam at a temperature of 134°C (and a corresponding pressure of 3 bar absolute) will condense into 10 mL of water and liberate 2162 J of heat energy.

- **Moist heat kills microorganisms by Denaturation of proteins.**

***Moist heat at temperature below 100 C :** Pasteurization, inspissation

***moist heat at temperature at 100 C :** Boiling, Tyndallization

*** Moist heat at temperature above 100 C :** Autoclaving

-Pasteurization:

- Used heat at temperature sufficient to inactivate harmful organisms in milk

- Temperature may be 72 C for 20 secs. Followed by rapid cooling (flash method)

Or 63 C for 30 mins (Holder method)

-Inspissation:

- Exposure of the media to humid heat at 75 C for

2 hours on three successive days (sterilization of media containing proteins e.g. Serum)

-Boiling: simple boiling at 100 C for 5-10 min. Is used to sterilize some glassware, forceps, scalpels.

-Tyndallization : Exposure to steam (100 C) for 20-30 min. for three consecutive days. It is used for materials which can not withstand prolonged boiling (media containing sugar and gelatin)

-Autoclaving: is the standard (most efficient and reliable method) sterilization method in hospitals

- It works under the same principle as the pressure cooker where water boils at increased atmosphere pressure, because of the increased pressure the boiling point of water is >100 C

- The autoclave is a tough double walled chamber in which air is replaced by pure saturated steam under pressure

- Equipment subjected to high pressure saturated steam at 121 C around 15-20 minutes

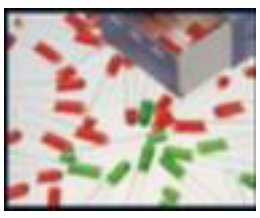
- Autoclave is used to sterilize most of the instruments and culture media

-monitoring of Autoclaves:

- We monitor the efficiency of the autoclave (was it a successful sterilization or not) either Chemically or Biologically

-**Chemically**: it consists of heat sensitive chemical that changes color at the right temperature (121 C) and exposure time. Done by : Autoclave tape, Browne's tube

-Biologically: where a spore-bearing organism is add the sterilization process and then cultured later to ensure that it has been killed, these biological indicators



contain Bacillus Stearothermophilus spores

‘Man cannot discover new oceans unless he has the courage to lose sight of the shore’.

Good luck.