Medical Microbiology. Dental 4th semester.

Mid-term assignment paper.

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Instruction; Write briefly and up to the point. All questions carry equal marks.

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1) Explain Structure of bacteria in detail ? also Explain some cell organelle of bacterial cell and its function.

Bacteria

Bacteria are small single celled organism that are not visible to human naked eye and can only be observed with the aid of microscope. It was observered by Vqn Leeuwenhoek in 1618.Although bqcteria are microscopic yet they have tgeir own characteristic shapes and structure. The structure of a typical bacterium and their important components can be explained as follows:

=) Structure of Bacteria:

The main components of bacyeria are

1) Capsule; most species of bacteria posses a tight protective layer covering around the cell called capsule.It is a gelatinous and sticky layer composed of polysaccharide, but some species use other materials, such as poly-D-glutamic acid in Bacillus anthracis.The capsule prevents bacterial cell from dehydration.

2) cell wall:

The cell wall is the outer most component common to all bateria (except Mycoplasma species whic lack cell wall).The cell wall is located externally to the cytoplasmic membrane and is composed of peptidoglycan. Peptidoglycan provides structural support and maintain the characteristic shape of bacterial cell.it allows bacteria to with lstand media of low osmotic pressure,such as water.This layer is compose of N-acetylmuramic acid & N-acetylglucoseamine molecules. Attached to each muramic acid is a tetrapeptide consisting of D-and

L-amino acids .The D-alanine is an amini acid involved in the cross linking between tetrapeptide and in tge action of penicillin.On the basis of cell wall Hans Christian Gram divided bacteria into two major grouos i.e Gram positive and gram negative

Gram +ive bacteria which can be stained blue-purple, possess a thick cell wall composing of many peptidoglycan layers containing techoic acid, surface proteins and Lipoyechoic acid. In the Gram-positive Bacteria, the cell wall is thick (15-80 nanometers) .On the other hand Gram -ve bacteria have a thinner layer of 10 nanometers. These bacteria have and inner peptido glyacan layer followed by a Lipoprotein layer which is further sorrounded by and outer Lipoploysacchride membrane..

3) Cytoplasmic membrane

Just below the peptidoglycan layer of cell wall lies the cell membrane, which is compose of phospholipid bilayer. They are chemically similar to eukaryotic cell membrane except that eukaryotic membranes contain sterols .This membrane has 4 basic functions i) active transport of molecules ii) energy generation by oxidative phospgorylation iii) synthesis of precursors of cell wall iv) secretion of enzymes and toxins.Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphiphilic molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments.

4) Flagella:

Most bacteria posses a thin hair like appendages which helps them in motality. Flagella are anchored in the cell wall and spin like a propeller,pulling the cell through medium. The structure of flagella is that it consit of a basal body which is anchored in the cytoplasmic membrane and cell wall. It consist of rings sorrounded by a pair of protein called Mot-protein. It helps in tge rotation of flagellar motor which is composed of S,M and P rings. The Fli proteins reverse the rotation of flagellar motor in response to stimulus.

Hook: it is made up Flg-E proteins which connects the basal body ro the Filament of flagella

Filament: It is made uo of flagellin protein.

5) Cytoplasm:

The cytoplasm of bacteria has two distinct areas :

-An amorphous matrix that contains ribosomes, metabolites , nutrients and plasmids.

-An inner nucleoid region containing tge genetic material of that cell

CELLULAR ORGANELLES OF BACTERIA & THEIR FUNCTIONS:::

1) RIBOSOMES :

These are the site of protein synthesis . They are different in size and chemical composition from eukaryotic ribosomes. These are 70s in size in bacteria with 50s and 30s subunits . During protein synthesis a ribosome moves along an mRNA molecule, reading the codon and adding the correct amino acid (from the corresponding aminoacyl tRNA) to the growing protein. When a stop codon is reached,translation ceases, and the mRNA and protein are released.

2) Nucleoid:

This is the area of cytoplasm in which DNA is located .The DNA of prokaryotes is a single ,circular molecule that contains about 2K genes. Bacterial DNA has no introns. It contains the whole genetic infromation of the cell.

3)Plasmids

Plasmids are extrachromosomal ,double stranded,circular DNA molecules that are capable of replicating independently of the bactrial chromosome.They can also be integrated into bactrial chromosome. They occur both in gram positive and negative bacteria. A typical plasmid consit of an origin of replication, Antibiotic resistant genes, Promoter region, Selectable marker and Multiple cloning sites.

Plasmids are of several types and they are used as vector molecules to carry piece of DNA or genes from cell to cell in Biological research projects.

Some types of plasmids are as follows

1) transmissible plasmid: They can be transfered from cell to cell by conjugation. They a have MW of 40-100 million. They are present in 2 to 3 in number per cell

2)Non transmissible : They have MW 3-20 million. Tgey do not contain transfer genes. They contain genes for antibiotic resistance , Resistance to heavy metal and UV rays, genes for pilli and exotoxins and Bacteriocins.

Q2)What is Bacterial culture media ? write down some types of bacterial culture media in detail?

Bacterial culture media / microbial growth:

" A suitable platform for the growth of a pathogen/bacteria."

OR

Bacterial culture media is "a liquid or gel or semi-solid medium designed to support the growth of microorganisms .growth media have those nutrients and specific environment which is required for the growth of pathogen and microorganisms .

Types: there are two types of composition of growth media :

i) solid media

ii) liquid media

Solid Media :- that type of media which contains composition of a liquid media and solidifying agent like agar . Solid media is used to grow colonies on its surface for the identification of characteristics of various bacteria .

Solid media is useful to determining the characteristics of the isolated bacterium from the colony .

Examples :

Nutrient broth , nutrient agar and peptone water.

Uses: staphylococccus and Enterobacteriaceae grow in these media .

Liquid media :

It is also a type of microbial growth which contains specific amount of nutrients but don't have any tracesof gelling agent like gelatin or agar .

These media remain liquids at room temperature .Liquid media are used for different purposes i.e,

Propagation of large number of organisms , fermentation studies , and various other tests .

Examples :

Nutrient broths (liquid nutrien broth) and LB medium (lysogeny broth) .

Uses :

Use for the Escherichia coli cultures .

Classification of media :

There are further six types of media .

I) Basal media ii) Enriched media iii) Selective media iv) Indicator media v) Transport media vi) Storage media

i) Basal Media :

They are pre-made or simple media generally used for the primary isolation of microorganisms.Basal media supports most non-fasitidious bacteria (those bacteria which grow faster in agar and do not required specific nutrion or environment) .

No further substances /addictives are required to be added to this media .

It have both media (liquid media) , semi-solid.

Nutrients:

Carbohydrates in the form of glucose

various salts . A source of amino acids and nitrogen (e.g beef , yeast extract), and agar .

Agar is used as solidifying agent

ii) Enriched Media :

Enriched media is used to favor the growth of a specific organism over others .By introducing nutrients and environmental condition from the outside to this media .

It only allows the growth of an organism of interest .

Nutrients are:

* Blood (for energy)

*Egg (for carbohydrates and protein)

*Serum (for Antibiotic portion)

It is also known as blood agar media .

Straptococi grows on blood and do haemolysis (breakdown of blood cells) .

So, it will grow in Enriched media because it needs nutrients and blood .

iii) Selective media :

This type of media contains specific ingredients to inhibit the growth of certain species of microorganisms in a mixed culture while allowing others to grow.

This type of media is used to find which type of bacteria is present .

It courages the particular bacteria and dis-courages the non-particular bacteria .

It is also known as L.J media (levanstion janson) .

It detect TB in sputum .

iv) Indicator Media :

Indicator media is a type of culture media used for identification of particular bacteria .It contains an indicator , and a particular organism cause change on that inficator .

Streptococi grwo and breakdown and do a chemical reaction which indicates the presence of bacteria .

This media is also known as MacConkey Agar Media.

v) Transport Media :

This type of media is used to transport bacteria from one place to another . It is composed of solutions of buffers with carbohydrates , peptone and other nutrients (excluding growth factors) .

This media is used minimize the growth of collected specimen during transporting the sample to the laboratory to be processed . The sample is collected and putted in a peptone water media .

vi) Storage Media :

It is used to store bacteria for a long time .It is also known as egg saline media .

Examples :

Egg saline medium , chalk cooked meat broth .

Q3)What is the difference between Sterilization and disinfection ? write down some methods used for sterilization ?

Ans 3: **Sterilization and Disinfection**:::

Disinfection:

Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Disinfection is a process that eliminates only pathogenic microorganisms. It only destroys viable cells. It means that the method does not destroy spores. Hence, the survived spores can germinate to cause contamination later.

Types of Disinfection::

^Sporicides kill spores when used in high concentrations.

^Hospital-strength disinfectants kill viable microbes in the presence of 400 ppm hard water and 5% organic serum.

^Broad spectrum disinfectants apply for a variety of microorganisms.

^Narrow spectrum disinfectants are used for several specific types of microorganisms.

=>Sterilization:

The process by which all type of microorganism (whether pathogenic or no pathogenic) are destroyed including their spores.

Difference b/w Sterilization and disinfection:::

i) The main difference disinfection & sterilization is that the sterilization is the complete removal of microorganisms with their resistant structures such as spores whereas disinfection is the elimination of pathogenic microorganisms. Morover, sterilization is an extreme level of cleanliness while disinfection is an adequate level of cleanliness.

ii)Sterilization uses chemicals, heat,filtration, filtration and high pressure techniques while for Disinfintion we use Hydrogen peroxide, alcohol,chlorine,phenolics and some heavy metals.

iii) Sterilization is a methods that gives extreme cleanliness while disinfection give an adequate but not complete one.

iv) sterlization destroys both living structures and their resistant components while disinfection can kill only the living microbes.

Methods used for sterilization:

Sterilization is an effective process which can be achieved by using chemical,physical and Gaseous methods which are explained as follows:

1) Physical Methods::

iThe main physical method of sterilization is Heat sterilization.

Heat Sterilization::::

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required. This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used. The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

i) Dry Heat Sterilization:

Some Examples of Dry heat sterilization are:

1. Incineration

2. Red heat

3. Flaming

4. Hot air oven It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments. It is also used for sterilizing non-aqueous thermo-stable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically. Hot-air oven Dry heat sterilization is usually carried out in a hot air oven.

ii) Moist Heat Sterilization:

Moist heat may be used in three forms to achieve microbial inactivation.Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. Steam for sterilization can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets). It is carried out in an Autoclave machine.

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation. Autoclaves should be tested periodically with biological indicators like spores of Bacillus stearothermophilus to ensure proper function. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures.

2) **Gaseous Sterilization**::

The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide (CH2)2O possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas. The mechanis of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids. The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 7075°C respectively. Both of these gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa.

(a) **Ethylene oxide sterilizer**:

An ethylene oxide sterilizer consists of a chamber of 100-300-Litre capacity and surrounded by a water jacket. Air is removed from sterilizer by evacuation, humidification and conditioning of the load is done by passing sub-atmospheric pressure steam, then evacuation is done again and preheated vaporized ethylene oxide is passed. After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system. Ethylene oxide gas has been used widely to process heat-sensitive devices, but the aeration times needed at the end of the cycle to eliminate the gas made this method slow.

(b) **Low temperature steam formaldehyde (LTSF) sterilizer:**

An LTSF sterilizer operates with sub atmospheric pressure steam. At first, air is removed by evacuation and steam is admitted to the chamber.

C) **Hydrogen Peroxide Sterilization**:

This method disperses a hydrogen peroxide solution in a vacuum chamber, creating a plasma cloud. This agent sterilizes by oxidizing key cellular components, which inactivates the microorganisms. The plasma cloud exists only while the energy source is turned on. When the energy source is turned off, water vapor and oxygen are formed, resulting in no toxic residues and harmful emissions. The temperature of this sterilization method is maintained in the 40-50°C range, which makes it particularly well-suited for use with heat-sensitive and moisture-sensitive medical devices. The instruments are wrapped prior to sterilization, and can either be stored or used immediately.

3) **CHEMICAL METHODS OF STERILIZATION**:::

Those are the chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used at apropriate concentration for appropriate duration can be used for sterilization and are called sterilant liquids. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

=>Alcohols:

Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein. Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.

=>Aldehydes:::

Mode of action:

Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleicacids. It kills all microorganisms, including spores.

Examples: Formaldehyde, Gluteraldehyde Application: 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers and operation theaters. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc.

=> Phenols:::

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes. Examples:

5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol)

Heavy Metals

Mode of action

Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic. Examples: Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate).

Q4)Write a note on Structure of fungi in detail ?

Fungi belongs to their own kingdom (The Kingdom Fungi). As Compared to higher plants and animals, they obtain their nutrition through a range of ways including degradation of organic material and symbiosis (as forming lichen) among others.

They are categorized as heterotrophic because they are unable to synthesize their own food because they lack chlorophyll. They can reproduce sexually or

asexually with a majority of fungi being spore producers. Fungi are generally sporophytes and are the most important decomposers in terrestrial ecosystem

STRUCTURE OF FUNGI::

Fungi lack cholorphyll that abosorbs different wavelengths of visible spectrum . They are not truly multicellular. The cytoplasm of one fungal cell is continous through pore with the cytoplasm of next adjacent cell.

Fungi exist in two fundamental forms; The single celled budding forms (yeast) and the filamentous (hyphal) . But, for the sake of classification they are studied as moulds, yeasts, yeast-like and dimorphic fungi.

All fungi have typical eukaryotic morphology. They have rigid cell wall composed of chitin, which may be layered with mannans, glucans and many other polysaccharides acconpanied by polypeptides.Some lower fungi possess cellulose in their cell wall. Some fungi such as Cryptococcus and yeast form of Histoplasma capsulatum possess polysaccharide capsules that help them to evade phagocytosis.

Inner to the cell wall is the cytoplasmic membrane that is a typical bi-layered membrane in addition to the presence of sterols. Fungal membranes possess ergosterol in contrast to cholesterol found in mammalian cells. The cytoplasm consists of various eukaryotic organelles such as mitochondria, golgi apparatus, ribosomes, endoplasmic reticulum, lysosomes, microtubules and a true nucleus enclosed in membrane.

A specific property of nuclear membrane is that it persists throughout the metaphase of mitosis unlike in animal and plant cells where it dissolves and re-forms. The nucleus may possess paired chromosomes.

Main Body of Fungi::

The main body of most fungi is made up of fine, branching, usually colourless threads called hyphae. Each fungus will have vast numbers of these hyphae, all intertwining to make up a tangled web called the mycelium.

The mycelium is generally too fine to be seen by the naked eye, except where the hyphae are very closely packed together.

Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella. Those hyphae that don't possess any pigment in their cell wall are called hyaline.They are transparent and do not develop fruiting body. Hyphae may have some specialised structure or appearance that aid in identification. Some of these are:

a) Spiral hyphae: They are spirally coiled structures commonly seen in Trichophyton mentagrophytes.

b) Pectinate body: These are short, unilateral projections from the hyphae that resemble a broken comb. Can be seen in Clavate microconidia.

c) Favic chandelier: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (thats why also known as antler hyphae). They occur in Trichophyton violaceum.

d) Nodular organ: This is an enlarged structure that consists of closely twisted hyphae. Often seen in Trichophyton mentagrophytes.

e) Racquet hyphae: There is regular enlargement of one end of each segment with the opposing end remaining normally thin. Seen in Epidermophyton floccosum and Trichophyton mentagrophytes.

f) Rhizoides: These are the root like structures seen in portions of vegetative hyphae in some members of zygospore former known as zygomycetes.

g) There are structures in the hyphae, which arise out of modification of a single cell and transform into thick walled resting cells. Chlamydospore (or chlamydoconidia), which are produced by Trichophyton schoenleinii and Trichophyton verrucosum are thick walled cells that are larger than other cells and arranged singly or in groups. In some fungi such as Trichosporon beigeilli and Coccidioides immitis some alternating cells become thick walled and

subsequently the intervening cells disintegrate leaving behind arthrospores (or arthroconidia).

Structure of mycellium:

Fungal mycelium is mostly hidden from human view, not only because of its small size, but also as a result of its location. The tangled mycelial mass is usually hidden deep within its food sources, such as rotting matter in the soil, leaf litter, rotting wood, or dead animals. The mycelium remains undetected until it develops one or more fruiting bodies, containing the reproductive spores.

In most fungi such as Rhizopus stolonifer,the hyphae form a white or grey mycelium which develops into an upright sporangiophore during asexual phase which bears a sporangium at its tip. This sporangoum contains thousands of spores

Mycelium are of three kinds:

1. Vegetative mycelium are those that penetrates the surface of the medium and absorbs nutrients.

2. Aerial mycelium are those that grow above the agar surface

3. Fertile mycelium are aerial hyphae that bear reproductive structures such as conidia or sporangia.

Q5) What are few Hospital based infections that can be transfer to others due to un hygienic condition ? Explain with an example ?

Hospital Acquired Infection:::

An infection that is acquired by a patient in hospital or other otyer health care facility is kbown as Hospital Acquired infection(HAIs) . It is also known as Nosocomial infection or Health care associated infection(HCAI).

Causes of nosocomial infections:

Bacteria, fungus, and viruses can cause HAIs. Bacteria alone cause about 90 percent of these cases. Many people have compromised immune systems during their hospital stay, so they're more likely to contract an infection.

A nosocomial infection is contracted because of an infection or toxin that exists in a certain location, such as a hospital. People now use nosocomial infections interchangeably with the terms health-care associated infections (HAIs) and hospital-acquired infections. For a HAI, the infection must not be present before someone has been under medical care.

One of the most common wards where HAIs occur is the intensive care unit (ICU), where doctors treat serious diseases. About 1 in 10 of the people admitted to a hospital will contract a HAI. They're also associated with significant morbidity, mortality, and hospital costs.

As medical care becomes more complex and antibiotic resistance increases, the cases of HAIs will grow. The good news is that HAIs can be prevented in a lot of healthcare situations.

Symptoms of nosocomial infections:::::

For a HAI, the infection must occur:

up to 48 hours after hospital admission

up to 3 days after discharge

up to 30 days after an operation

in a healthcare facility when someone was admitted for reasons other than the infection

Symptoms of HAIs will vary by type.

The most common types of HAIs are:::::

¡)Staphylococus aureus which cause blood infections(septicemia)

¡¡) E.Coli main cause of urinary tract infections

¡¡¡)Enterococci may cause infections like UTIs , Septicemia and infections in wounds

¡V)Pseudomonas aeruginosa causes kidney infection, UTI and resporatory tract infections

urinary tract infections (UTIs)

surgical site infections

V) Gastroenteritis

Vi)Meningitis

ViiPneumonia

The symptoms for these infections may include:

.discharge from a wound

fever

.cough, shortness of breathing

burning with urination or difficulty urinating

.headache

.nausea, vomiting, diarrhea

People who develop new symptoms during their stay may also experience pain and irritation at the infection site. Many will experience visible symptoms.

Example of HAI that can be transfered due to unhygeinic condition:::

The best example of infection that can be spread by unhygienic condition in the hospital is COVID-19. This is infection which has spread pandamically throughout the world recently by Novel virus it was started in Wuhan China caused by a new species of virus called Novel Coronavirus . They belong to a member of the corona virus family , that was first identified in the 1960s .They are responsible for causing a range of disease in humans . Including the common cold and more severe and life threating forms like SARS and MERS .Well unfortunately , all this is a very new illness , no treatment for it is available so far , and because it is a virus antibiotics won'twork against it . it is always advisable to practice good hygiene and caution by avoiding close contact with individuals suffering from acute respiratory infections , or wild animals or livestock , dead or alive Also it would be best if you wash your hands with warm and soapy water regularly , especially , after being in direct contact with an infected person.