Practical

Microbial Toxin

4th Class

Microbial toxin

There are many factors that determine the pathogenic bacteria virulence or ability to cause infection : -

1. Toxin Production

2. Adherence factors

3. Invasion of host cells and tissues

4. Enzyme Production

Definition of pathogenicity as: Capability of Microorganisms to cause the disease or contributing to the events of natural or experimental methods in your host.

Toxicity: the ability of bacteria to cause harm or damage to the tissue.

Severity of the disease (virulence) : the number of Microorganisms or the number of micro grams of poison (toxin) sufficient to kill the host when he entered it in different ways and are usually described **Lethal dose 50 (LD 50)** the dose of toxin, or pathogen is required to kill half the members of a animal tests such as laboratory mice.

TOXIN (poison): A metabolic materials produced from metabolic processes, whether beneficial or harmful to cells and tissues and are produced in the process of **Idio phase** which is located between the Logarithmic phase and Stationary phase.

These toxins are either excreted by the Microorganisms to the cutler media or toxic substances secreted by bacteria and released outside the cell where such then called **Exotoxin** or keep these toxins associated with the surface of pathological cells and are part of the components of the cell wall and then called **Endotoxin**.

Since the process of metabolism in bacteria similar pathological and non-pathological So enjoy pathogenic bacteria additional specifications to enable them to cause about the disease.

1. The ability of bacteria to ingress in host body(vivo) .

2. Concentration of bacteria at the site of a permanent or temporary in host body (vivo).

3. Ability of bacteria to multiply.

4. The ability of bacteria to compete with the natural bacteria (normal flora) in the body.

5. Ability of bacteria to overcome the resistance of the host body (immune system).

The ability of bacteria to overcome these stages lead to the creation of the disease, and the most important of these factors is the ability of bacteria to produce substances known as toxins.

Microbial toxin : a secondary metabolic products produced by microorganisms and be harmful or fatal effect on cells and tissues.

Toxoid: is a bacterial toxin (usually an exotoxin) whose toxicity has been inactivated or suppressed either by chemical (formalin) or heat treatment, while other properties, typically immunogenicity, are maintained. Thus, when used during vaccination, an immune response is mounted and immunological memory is

Aghsh Samihasan

formed against the molecular markers of the toxoid without resulting in toxin-induced illness. In international medical literature the preparation also is known as anatoxin or anatoxine.

There are toxoids for prevention of diphtheria, tetanus and botulism. and can be accessed through the treatment of a number of substances, including toxins and formalin by specific temperature 37 and pH (6 - 9) for several weeks.

- **Toxigenicity**: the ability of microorganisms to produce toxins.
- **Toxemia:** the case of poisoning caused by the presence of the toxin in the blood.
- **Bacteremia:** is a disease caused by the presence bacteria in the blood circulation.
- **Septicemia:** is a presence of bacteria with their toxins in the bloodstream.

There are several types of toxins, which can be divided depending on several criteria: -

1. <u>According to chemical structure.</u>

a - Protein toxin .

b – Lipopolysacchaeide (LPS) .

<u>2. According to mechanism.</u>

A - Block protein synthesis ⟶ Ex: Diphtheria toxin

B - Block nerve function ⟶ Ex: Tetanus toxin

C - Toxin which help microorganism to separate in tissues ⟶ Ex: Hyaluorinidase

D - Toxin that lysis cells and killed them ⟶ Ex: Lecithinase

<u>3. According to side of action.</u>

A - Enterotoxin ⟶ Ex: Staph toxin

B – Neurotoxin ⟶ Ex: tetanus toxin

C - Cytotoxin ⟶ Ex: Shigella toxin

<u>4. According to role of enter.</u>

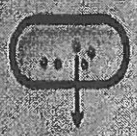
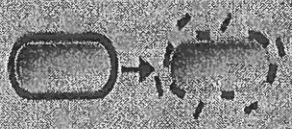
A - Toxin which pass through wound infection ⟶ Ex: tetanus

B - By blood invasivnase ⟶ Ex: Endotoxin

C - Initiated disease though intestinal tract ⟶ Ex: Salmonella

Asst. Sami Hassan

Finger 1 : comparison between Exotoxin and Endotoxin

Property	Exotoxin	Endotoxin
		
Bacterial Source	Mostly from gram-positive bacteria	Gram-negative bacteria
Relation to Microorganism	Metabolic product of growing cell	Present in LPS of outer membrane of cell wall and released with destruction of cell or during cell division
Chemistry	Proteins, usually with two parts (A-B)	Lipid portion (lipid A) of LPS of outer membrane (lipopolysaccharide)
Pharmacology (Effect on Body)	Specific for a particular cell structure or function in the host (mainly affects cell functions, nerves, and gastrointestinal tract)	General, such as fever, weaknesses, aches, and shock; all produce the same effects
Heat Stability	Unstable; can usually be destroyed at 60-80°C (except staphylococcal enterotoxin)	Stable; can withstand autoclaving (121°C for 1 hour)
Toxicity (Ability to Cause Disease)	High	Low
Fever-Producing	No	Yes
Immunology (Relation to Antibodies)	Can be converted to toxoids to immunize against toxin; neutralized by antitoxin	Not easily neutralized by antitoxin; therefore, effective toxoids cannot be made to immunize against toxin
Lethal Dose	Small	Considerably larger
Representative Diseases	Gas gangrene, tetanus, botulism, diphtheria, scarlet fever	Typhoid fever, urinary tract infections, and meningococcal meningitis

AS [c/1 Sami Nasser

Methods of exploration for toxins

The diseases caused by microorganisms are still problems that researchers must be find solutions to them so engaged researchers over the centuries to learn how the incidence of disease and the microorganism that causes her and the role of agents pathogenic owned by the creation of the disease, and perhaps that toxins produced by microorganisms is one of the factors of ferocity mission and is responsible for pathogenicity so it was necessary to find appropriate ways for the purpose of investigation: -

You Can be divided into methods of exploration for toxins to four axes

1. Culture method (bacteriological)

2. Biological method

A - Tissue culture.

B - Rabbit legated loop assay method (intestine tethered to the rabbit).

C - Suckling mice assay.

D - Mice lethality assay.

E - Vascular permeability assay.

3. Immunological methods .

A - Enzyme linked immuno sorbent assay (ELISA).

B - Latex agglutination test.

C - Cold haemagglutination.

D - Counter immuno electrophoresis.

4. Molecular methods .

which includes the way Polymerase chain reaction (PCR).

First: Culture method (bacteriological)

Using selective cutler media where those cutler media are used depending on the type of microorganism and the poisons (toxin) are investigating the microorganism.

Advantages

1. High sensitivity.

2. specialty.

3. cheap.

Disadvantages

1. Could not differentiate (discrimination) between the toxin-producing bacteria from non-productive.

2. Takes several days to isolate and diagnose the bacteria.

Secondly, Biological method

A - Tissue culture

This method is used for the detection of toxins to the cells and Tissue this is a way of

1. High sensitivity.

2. specialty.

3. Reveals about the disease and its causative organism.

But sometimes it may give false negative results due to dilution of samples significantly.

Either false positive results may occur when the patient is infected with disease-causing.

Disadvantages

1. Laboratory method is expensive.

2. Takes a long time for the conduct of (24-72) an hour depending on the type of poison and pathogen.

B - Vascular permeability assay .

This method involves the following steps: -

1. Injection toxin in the Rabbit through the skin on either side of the back of the rabbit (after shaving the back area).

2. After 18 hours of injection measured areas skin reaction zone.

3. Either vascular permeability estimated by Injection intravenous by colored dye (Evans blue).

4. Measured diameter blue areas after 3 hours of the start of the experiment.

C - Rabbit legated loop assay

This method involves the following steps: -

1. Knot (node) work in the small intestine for a length of 5-7 cm in rabbit.

2. Poison injected into the node.

3. After 10 hours are killing the rabbit is estimate the proportion of the size / thickness (ml / cm) for each node.

D - Suckling mice assay

This method involves the following steps: -

1. Toxins are given to suckling mice through the gastrointestinal tract.

2. Shut the anus using the a waxy substance (cyanacrylat ester) .

3. Conduct the experiment after 6 hours to kill rats to separate the neck area.

4. Ratio is estimated accumulated liquid bowel measuring the size / thickness (ml / cm).

E - Mice lethality assay

1. Injected into adult mice in the inside layer Intra peritoneal or inside of the vein(Intra venous).

2. 16-24 hours after the destruction of the mice were observed (Mice lethality).

Third: Immunological methods

A- Enzyme linked immuno sorbent assay (ELISA) .

This method relies on the use of Monoclonal antibodies to detect antigens (toxins) to the worker and the nurse in this way can investigate effectively poisons or ineffective either previous methods are investigating the biological toxins effective only.

So be (+) manner (ELISA) with the negative (-) in other ways.

B - Latex agglutination test .

The method is based on the investigation of pathogen antigens, and this is the way

1. Less sensitivity and specificity.

2. Did not discriminate between toxin-producing isolates.

C - Cold haemagglutination .

1. Taken (50 lμ) of toxins and undergo a series dilution decimal using Tris pH = 7.5 and a concentration of 0.1 M containing 0.05 M of Nacl and occlusion using the calibration micro titer plat.

2. Added each dilution (50 lμ) of 1% of the red blood cells stuck to the rabbit.

3. plate incubated at a temperature freezer 4 C° for three hours.

4. The results you read clumping is observed to be bloody.

Ashraf sami hassan

Fourth: molecular methods, which includes the way

Polymerase chain reaction (PCR)

This method is used to investigate the microbial toxin-producing, by inflating (repeat) any production preparation endless sequences of DNA (gene responsible for toxins) in a manner CHEMICAL for, and is used for this test device is small, this method is used in microbiology for the diagnosis of diseases such as hepatitis B and tuberculosis, as well as the diagnosis of parasites such as *Toxoplasmo gondii* as well as the detection of food pathogens

The advantage of this method is high in sensitivity.

Asst. Lecturer Hassan

Staphylococcus aureus toxin

Bacteria: *Staphylococcus aureus* (Also called pus-generating bacteria Pyogenic bacteria)

Characteristics

1. aerobic & facultatuive anaerobic.
2. Positive for the dye gram (G +ve) .
3. Spherical, grouped in Clusters.
4. non motile.
5. non spore forming.
6. non capsule.
7. Grow on Culture media easily and does not require complex nutritional requirements.
8. catalase (+) and oxidase (-).
9. Their ability to grow in Nacl up 0.01 and the temperature of 18-40 C°.
10. Are found in water, air and can be isolated from milk and animal waste.

Coexistence

There is such a naturally symbiotic bacteria on the skin, as well as found in the mucous membranes inside the nose, and a large percentage of the population carry this bacteria in the nasal cavity naturally (Nasal carriers).

Shape may be single or Diploid or be a gathering random arrangement and why it collects in this way is that the axis of cell division are in different directions A cell may split longitudinally(Vertically) becomes two cells then horizontally becomes four then divided laterally becomes more than four and after the split there remains a thread physicist draws cells together.

Advantage of these bacteria in its ability to bring the disease in addition to cases of poisoning and by their ability to Production of many toxins

Production of many Exotoxin .

1. Hemolysin

This poison lysis red blood cells(RBCs) and is considered as an important pathogenetic bacteria and the bacteria are classified into several types according to their ability to decomposition of RBCs which α, ß, γ, δ.

ß: Hemolytic of blood fully.

α, γ: partially blood Hemolytic

Be genetic determinants responsible for the production of hemolysin mounted on the chromosome in strains that affect humans, either that infect the animal shall be mounted on the plasmid.

Asst sami Hassan

A. Alpha toxin (α)

lysis a few types of cells with a lethal effect and cause skin necrosis, smashes Macrophage cells and Platelets for humans either cells Monocytes are resistant to them

B. Beta toxin (β) or (B-hemolysin)

Affect the specificity of the Sphingomylin a substance fall within the class of fatty membranes of red blood cells, and the effectiveness of this type has more against the red blood cells of sheep

C. Delta toxin (δ) or (δ - hemolysin)

lysis multiple types of cells where smashes Erythro cyte and Lympho cyte and Macrophages and Platelets and inhibits the absorption of water from the intestines

D. Gamma toxin (γ) or (γ - hemolysin)

lysis a few types of cells and inhibits the poison material Agar and cholesterol

2. Leucocidin

Works to kill the cells where (Cytolysis) kills or lysis white blood cells(WBCs) , leading to the formation of pus, and is considered his property antigenic protein can be converted into high-Toxoid.

3. Enterotoxin

Affects the intestines, causing food poisoning and there are nine types of this poison which (A, B, C1, C2, D, F, E, G, H), it is important in the case of food poisoning this bacteria if the food container on carbohydrate and proteins and the absence of competing bacteria (normal flora) so they are able to produce the poison in people who are taking anti-life as a result of contracting certain diseases, which leads to the absence of digestive bacteria competition.

4. Exfoliatin

Cause the separation of layers of the skin surface and observed in newborns Scalded skin syndrome (SSS) syndrome is (any number of symptoms at one time).

5. Toxic shock syndrome

Produced by the *Staphylococcus aureus* bacteria and the intervention by the wounds in surgical operations and lead to death.

6. Coagulase toxin (clumping factor)

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of Staphylococcus isolates. Importantly, S. aureus is generally coagulase-positive, meaning that coagulase negativity usually excludes S. aureus. However it is now known that not all S. aureus are coagulase-positive

Adil sami hussain

Detect the production of bacterial enzyme hemolysin

1. Plate method

A - attend the Culture media (Blood agar)and distributed in sterile occlusion and then incubated at 37 º C for 24 hours to ensure the purity of the center and the lack of contamination.

B - Staphylococcus aureus bacteria are grown on the plates and incubated at 37 º C for 18-24 hours.

C - read the results of the apparent decomposition of the areas (clear zone) that surround bacterial colonies, and by the size of the decomposition.

NOTE: -

• The Components of the Culture media affect the analytical capability of blood since the presence of serum cholesterol or cholesterol working on the inhibition of decomposition leading to the non-disclosure of Hemolysin.

• the proportion of bacteria rely analyst blood on several variables, most notably

a - the source of blood used b-place isolate bacteria c-test method used d-type Hemolysin.

2. Tube method

a-*Staphylococcus aureus* bacteria are grown in test tubes on container Nutrient broth at 37 º C for 18-24 hours.

b-convey the amount of 0.1 ml of the former farm into a test tube containing 10 ml of downtown Brain heart infusion to ensure the purity of grown for 24 hours and the temperature of 37 º C in an incubator rocker.

c-transmits 0.05ml (50 lμ) of the former farm into a test tube on the container (1 ml) of human RBCs stuck containing 0.02 of Normal slain.

d- incubate in a water bath for 15 minutes at 37 Cº.

e-works centrifuge tubes quickly in 3000 r / min for 5 minutes.

f-decomposition seen with the naked eye and compares with a control tube Control (absence of bacteria), and may be either a tube to control the absence of bacteria or the presence of bacteria in control.

Assam Hassan

Toxins secreted by the bacteria *Streptococcus pyogens*

Bacteria: *Streptococcus pyogens*

Is a genus of species of bacteria, pyogenic, a pellet positive for the dye gram , gather in chains may be short or long depending on the type of culture media in liquid media to be much longer than in the solid media, facultatuive anaerobic, gives a positive result for the examination oxidase, and negative for examination catalase, is non capsule , the bacteria are becoming increasingly demanding complex media (Fastidious bacteria) they need any dietary requirements, such as the complex media rich in blood.

Form colonies

The advantage of being small-sized colonies half transparent convexity spaced a few, have the ability to cause disease because of its production to Exotoxin (do not produce endotoxin), causing inflammation of the larynx Tonsillitis, and scarlet fever, rheumatic fever

These bacteria cause the decomposition of the blood type ß production as a result of bacterial toxins are dissolved in the blood shall be a large area transparent (clear zone) as a result of the decomposition of the blood. Is sensitive to the : Bacitracin more than the anther type of hemolytic *Streptococcus*.

The most important toxins produced

1. Streptolysin - O (SLO)

Features

1. Toxins is alysis red blood cells (RBCs), analyst of the cell (cytolytic).

2. Sensitive to oxygen which works in anaerobic conditions.

3. Poison a substance protein molecular weight 70,000 Dalton, where is the substance antigenic good stimulates the immune system to form antibodies called Anti streptolycin - O (ASL-O) of these antibodies are working on the equation of poison measured titer upturned highest dilution of antibodies, measuring the titer in serum-infected persons where the idea of the severity of the infection.

4. Titer that are up to (200 international units) is the highest natural limit and increased it indicates the severity of the infection .

5. Plays a role in the incidence of rheumatic fever as it leads to the demolition and destruction of tissue in the heart, so is one of the toxins attack cardiotoxin also be toxic to white blood cells (WBCs).

6. Detected the poison in two ways

a. Serological method:-using latex granules (Latex agglutination) for the detection of antibodies Anti - (SLO) appeared as if titer more than 200 high toxicity, it shows the incidence of meningococcal infection .

Asst. sami. hassan

b. culture method:- Through cutler toxin-producing of bacteria in test tube contain the Blood agar media base skim Glucose and using stabbing inside agae appeal to the depth of the Blood agar media is implanted bacteria after a lap 37 º C for 24 hours, to grow colonies surrounded by areas of decomposition.

2.Streptolysin - S (SLS)

1. The kind of toxins responsible for the decomposition of blood fully lysis in terms of blood type ß appears transparent regions at the center of the implant within the Blood agar.

2. Not affected by the presence of oxygen, which he was working under aerobic conditions and anaerobic .

3. heat-resistant.

4. The toxin is a protein with a Lowland molecular weight and (28000 Dalton), is non-antigenic material which does not stimulate the formation of Antibody in serum.

5. Poison killer white blood cells where he works to remove particles and thus cell death

This poison dissolves in serum.

Mechanical work poison

Be responsible for decomposition zones (clear zone)around the colonies located in blood agar media.

3.Erythrogenic toxin

1. protein Lowland molecular weight (29000 Dalton) generates antibodies increase but a few.

2. Constant heat does not damage easily rising temperatures, which is responsible for the so-called (Skin rash) any appearance of granules red color to the skin and this occurs when the infection pharangitis and tonsillitis and this case notes when infection scarlet fever scarlet fever as well as the so-called this examination on behalf of the poison which is used to know him and called Dick toxin and examination called Dick test.

Dick test

Is the examination depends on the Erythrogenic toxin Works Intradermal in human terms is injected poison by (0.2 ml) of the toxin per diluted (1000/1 ml) and is commonly used leaky grown bacterial containing poison where relieves poison by 1000/1 (water / toxin)

The result is the emergence of the positive area to be red and swollen diameter of about 10 mm and this region fries appear during 6-24 hours after injection.

Mechanical work

This poison affects on human mononuclear cells where the incentive to produce the (Tumor necrosis factor) (TNF), responsible for the rise in body temperature and shock leading to toxic shock.

Shigella toxin

Causative bacterium: *Shigella dysenteriae*.

Characteristics

1. Bacteria belong to the family Enterobacteriaceae and intestinal toxins that cause bloody diarrhea, and be the type of toxins Exotoxin.

2. Negative bacteria to dye gram G-ve.

3. Rod shaped bacteria coccobacilli, non casuals.

4. These bacteria possess genes help to the invasion of epithelial cells in the intestine but does not have the genes to help them spread into the bloodstream.

5. Ability to resist acidity, which can resist the acidity of the stomach.

Types of toxins produced by: -

1. Endotoxin

All types of bacteria have the ability to produce this type of toxins, which are responsible for scratching happening in the intestinal wall causing injury when the blood out, this type of toxins Lipopolysaccharide.

2. Exotoxin

This type of toxins consists of two parts:

Part (A): which has done enzymatically where inhibit the process of protein synthesis.

Part (B): It is responsible for the association of bacterial cells to receptors present on the special epithelial cells (target).

This type of toxins is a protein, as part (B) associated with glycolipid to a host cell in the gastrointestinal tract and then part (A) prevents making of the protein and thus cell death epithelial and sabotage cells of capillary blood vessels of the intestine cause mild bleeding that comes with out stool and loss carbohydrate and bicarbonates cause mild acidity of the blood and lead to the death of a person.

Extraction Shigella toxin

1. Develops the bacterium *Shigella dysenteriae* at the culture media Trypticase soy agar at 37 º C for 24 hours.

2. transplantation bacterial colony in Brain heart infusion broth and incubated at 37 º C for 24 hours.

3. Working centrifuges cells taken earlier where sludge (bacterial content) and Supernate (center pea) and then re-suspended neutral saline solution and re-washed 3 times.

4. Breaks the bacterial cells using a sonicatar for 3 minutes, where to put the poison out of the cell (in the sediment).

5. Centrifuge to precipitate bacteria Broken Neglects , where sludge is taken filtrate containing toxin where sterilizes bacteria filters precise diameter 0.45 mμ to get rid of the remnants of broken cells, if any.

Ashraf Sami Hassan

Shigella toxin

Structure

The toxin has two subunits—designated A (mol. wt. 32000 D) and B (mol. wt. 7700 D)—and is one of the AB5 toxins. The B subunit is a pentamer that binds to specific glycolipids on the host cell, specifically globotriaosylceramide (Gb3). Following this, the A subunit is internalised and cleaved into two parts. The A1 component then binds to the ribosome, disrupting protein synthesis. Stx-2 has been found to be about 400 times more toxic (as quantified by LD50 in mice) than Stx-1.

Gb3 is, for unknown reasons, present in greater amounts in renal epithelial tissues, to which the renal toxicity of Shiga toxin may be attributed. Gb3 is also found in central nervous system neurons and endothelium, which may lead to neurotoxicity. Stx-2 is also known to increase the expression of its receptor GB3 and cause neuronal dysfunctions.

The toxin requires highly specific receptors on the cells' surface to attach and enter the cell; species such as cattle, swine, and deer which do not carry these receptors may harbor toxigenic bacteria without any ill effect, shedding them in their feces, from where they may be spread to humans.

Mechanism

Shiga toxins act to inhibit protein synthesis within target cells by a mechanism similar to that of ricin. After entering a cell via a macropinosome, the protein cleaves a specific adenine nucleobase from the 28S RNA of the 60S subunit of the ribosome, thereby halting protein synthesis.

The toxin acts on the lining of the blood vessels, the vascular endothelium. The B subunits of the toxin bind to a component of the cell membrane known as Gb3 and the complex enters the cell. When the protein is inside the cell, the A subunit interacts with the ribosomes to inactivate them. The A subunit of Shiga toxin is an N-glycosidase that modifies the RNA component of the ribosome to inactivate it and so bring a halt to protein synthesis leading to the death of the cell. The vascular endothelium has to continually renew itself, so this killing of cells leads to a breakdown of the lining and to hemorrhage. The first response is commonly a bloody diarrhea. This is because Shiga toxin is usually taken in with contaminated food or water.

Interestingly, the bacterial Shiga toxin can be used for targeted therapy of gastric cancer, because this tumor entity expresses the receptor of the Shiga toxin. For this purpose an unspecific chemotherapeutical is conjungated to the B-subunit to make it specific. In this way only the tumor cells, but not healthy cells, are destroyed during therapy.

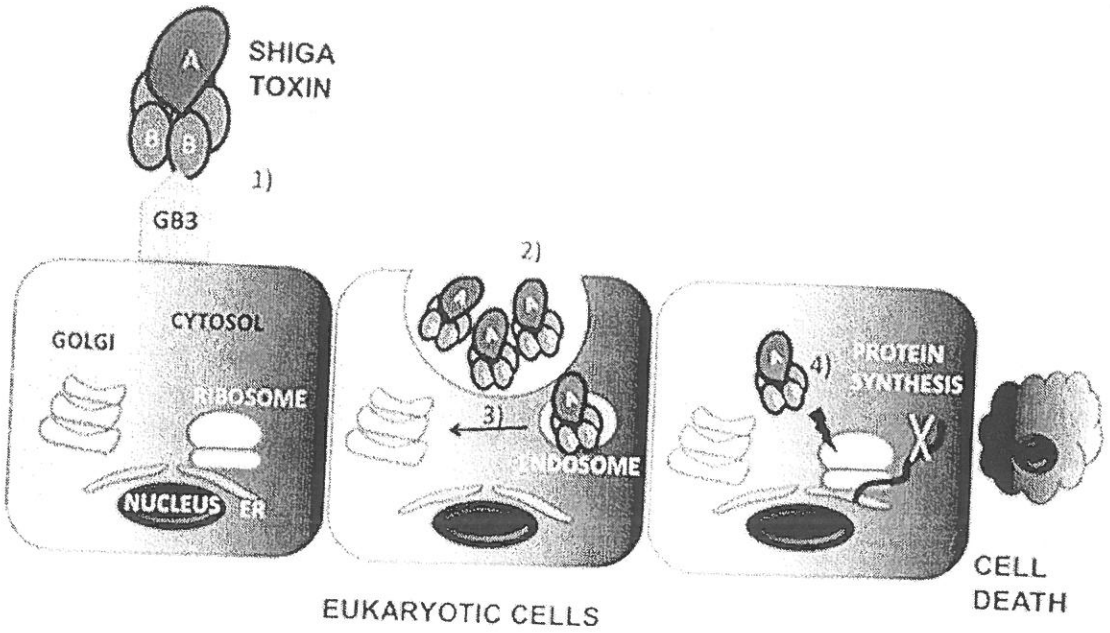
Q/ The toxin ~~[illegible]~~ produced by shigella

* resistanc to stomach acidity

Q/ bloody diarrhea

* shigella toxin.

Mechanism of action of Shiga Toxin



Diphtheria toxin

Bacteria causing the disease diphtheria *Corynebacterium diphtheriae*

Characteristics

Bacteria is Bacillus and cylindrical straight or slightly curved, the ends of the bacilli either pointed or bulbous , non motile, positive for the dye gram, positive to examine catalase , not be gases when fermentable sugars, non spore forming, is resistant to the acidity, causing Diphtheria disease.

The danger of bacteria in being productive is an external Diphtheria toxin and the toxin is the only factor that causes the disease.

Form colonies

Small, granular, shiny, gleaming at the edges that are irregular.

Bacteria do not have the ability to invasion, but remain in the region of the pharynx (localized infection) where secrete diphtheria toxin that spreads to distant places, causing the disease.

Features diphtheria toxin

1. A protein with a molecular weight of 63,000 Dalton

2. Poison consists of two parts, A, B

Part B is a function of the delivery of Part A effective to target either Part A is the part that enters toxic to the cell and cause harm

3. Monotypic toxin have any all strains of this bacteria secrete one type of poison and thus facilitates the formation of antibodies against him

4. Affected by this poison heat and formalin, a safe period of 4-6 weeks after losing its effectiveness

5. Require the production of this toxin in culture media to provide some important factors, including

Ph: who is up to (7.8 -8), and an abundance of O_2, provide peptone, either provide iron is considered factors very sensitive shall be better to focus him in the culture media (0.1 mg / ml) either if increased to (0.5 mg / ml) shall be this focus disincentive for this poison.

Mechanical work of toxin

Works on the inhibition of protein synthesis in the cell, either, affecting its impact on cardiac muscle tissue (Myocardial tissues) and Nerve ending.

Pathogenicity (disease caused by poison)

Be an incubation period of 3-4 days and is one of the casualties of topical mucous membranes in the throat and possible that infected throat and nose.

[illegible] membranes and secrete a small amount of the toxin to adjacent cells leads to the crash of these cells and

Abdelsamad Hussain

kill her within a few hours and this region crashed be suitable for the growth of an increasing number of bacteria and toxin production and thus spread of infection.

Features of infection

The presence of Suppuration Fibrous (pus) , the membrane thick gray consists of fibers and tissues of dead and WBCs white blood cells and a large number of bacilli bacteria down quickly turns this membrane to the membrane thick white opaque so-called pseudo membrane, which works on packaging the entire region has been working Packaging on the tonsils leads to stop the passage of oxygen to the lungs and O2 patient death from suffocation and this came label

Prevention

Taking DPT (DPT) Dephtheria , Pertusis , Tetanus

Differential test of diphtheria infection

Must distinguish between Diphtheria from the other diseases that give the same Clinical symptoms such as tonsillitis or mumps and inflation salivary glands and Candida infection in other diseases this membrane can elevation forceps easily either pseudomembrane of Diphtheria can not elevation, also through the work swab and b Methylene blue pigmentation appears coli cells inside granules (a screening laboratory) to differentiate between Candida and bacteria down.

Toxicity tests to make sure the isolates produced the poison or unproductive so conducting the following tests

1. In vivo tests (inside the body)

These tests are conducted on laboratory animals and is used for this purpose, rabbit or guinea pig two ways

a- Intra dermal

b-subcutaneous (Sub cutan)

This is done by injecting poison in small quantities in these areas, which leads to the appearance of the area Necrosis at the injection site due to the effect of the poison and the appearance of symptoms and death of the animal.

2. In vitro tests (outside body) Elek's test screening

Conducted outside the animal's body any laboratory within culture media , called method gel presiptation used for this purpose amid transplantation Elek's medium that contains serum horses placed where on the surface of the culture media strip of filter paper measured 15x60 mm be immersed in advance in anti diphtheria toxin concentration (1000 units per ml) and the surface must be dry before the culture media where the implant is implanted bacteria heavily angles and perpendicular to the tape after 48 hours notice sedimentation lines that appear on the white lines in the middle. In this method, anti-venom is spread from the area of the filter paper into the culture media

Either poison spread of the line shall be transplanted at the confluence of the bacterial and anti-poison with poison, and you get an equation where the deposition is born white line as a result of this interaction

Tetanus toxin (Tetanospasmin)

Toxin-producing bacteria: ***Clostridium tetani***

Characteristics: -

1. Bacteria positive for the dye gram G + ve , rod shape.

2. obligate anaerobic.

3. cell shape resembles a drum stick (terminal spore) because spore diameter greater than the diameter of the cell.

4. Bacteria are motile and analyzed for protein (protolytic) , toxin produces a very effective exotoxinl causes tetanus.

5. Present in soil and feces and transmitted through the spore to the wounds of pollution or contamination during surgical operations and then move to other areas of the body.

Toxins produced by the bacteria: -

Bacteria produces external cm of two types: -

A - Tetanolysin

Be sensitive for O_2 and heat, works on multiple types of RBCs for the animals where analyzes of rabbit RBCs and horses either cycle in pathogenicity is kill for the WBC (Leucotoxin) as well as a longer Necrotizing nostril and Sam to the heart muscles (cardio toxin).

B - Tetanospasmin

This is the poison responsible for pathogenicity, which is a protein sensitive to temperature constant oxygen (oxygen staple) and molecular weight 160,000 Dalton, an strong antigen this poison works centrally on the nerve cells in the brain and spinal cord and the impact less on peripheral nerves, is influenced by enzymes analyzed so he destroys ,infectious when taken for oral is a neurotoxin affects the nervous system.

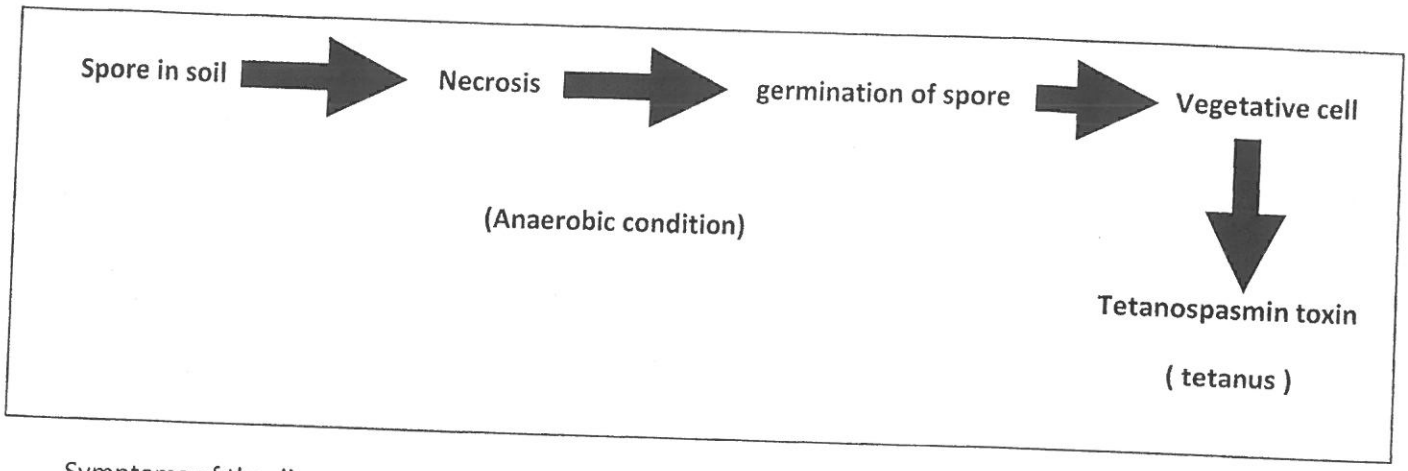
Mechanism (mode of action) : -

Consists of the venom of the two part (A) which is effective portion that runs on the target cell, and the part (B) aimed at connecting portion (A) to the target cell where the absorbed part (A) by the blood and goes to the central nervous system and in particular the spinal cord where the associated matrix nerve and prevents the transmission of meta nerve cell to another, and thus of the muscles to the brain and vice versa, and this is to prevent the brain from giving any injunction to keep the muscles in the case of muscle contraction.

Asly sami hassan

How the emergence of the disease: -

After a wound intervention spore in the soil to the body and when it is available anaerobic conditions in the affected area can spore germination to form vegetative cells producing poison after causing bacteria necrosis in that region.



Symptoms of the disease: -

Happens contraction in the muscles supplied by the cranial nerves, such as muscle, tongue, inability to swallow and speak with affected first the muscles of the head and neck and the consequent application of the jaws of the mouth is called the disease (Lock jaw) where we can not open the mouth to get stiffness in the neck muscles and then hardening the rest the body's muscles and the back muscles and the whole body becomes a piece of wood non-movement in any direction and this is the stage that causes death.

Note NOTE 1: -

The infection can get into the uterus in women causing what is known as an abortion or miscarriage (abortion) and can occur for children newborn through contamination of the umbilical cord is called the disease (Tetanus neonatium) any congenital tetanus.

Note NOTE 2: -

Infection remains localized in the area of the contaminated wound and poison that is produced by these bacteria, which is to be met so that the spread up to the nervous system and produce adverse effects.

Note NOTE 3: -

Needle container on Antigen vaccine to stimulate the immune system to form Antibody in case of injury.