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*Mi microbiology Sheet no:-21*

*Refer to slide :- 12 &13*

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**Vibrionaceae**

* Curved or Straight bacilli that are facultative anaerobes, gram negative, oxidase positive and non spore-forming.
* They are primarily found in water and are well known for their ability to produce gastrointestinal disease.
* Three Genera: Vibrio, Aeromonas and Plesiomonas. the most important one is vibrio-cholera which causes cholera disease.
* They are very motile .usually have one flagellum situated anteriorly
* They are among the most common bacteria in surface waters worldwide.
* They grow on alkaline media (pH 8.5-9.5).
* Many groups and serotypes

Cholera

* Usually cholera occurs in [outbreak];spreads within population not one or two persons because of contaminated food or water. Back in 1977 there was an outbreak of cholera in Jordan.
* Infectious dose is very high if water is the vehicle(since vibrio only grows in alkaline media they can't stand the acidity of the stomach ; killed easily by gastric acid )and low if food is the vehicle(they will hide inside the food so the acid won't affect them.) in individuals with normal gastric acidity.
* Achlorhydria: the absence of hydrochloric acid in gastric secretions . it have advantages and disadvantages. one of the disadvantage;you might be infected by cholera very quickly. Advantage; injected cyanide normally is fatal, but a person with achlorhydria won't die! to explain that : potassium cyanide reacts with hydrochloric  acid to form hydrocyanic acid and potassium chloride. hydrocyanic acid is poisonous and cause death. if there is no acid to react with it won't produce hydrocyanic acid
* V.colerae causes cholera by a heat labile enterotoxin (A-B toxin)
* B subunit:binds to the receptor promoting entry of subunit A into the cell which activates adenylate cyclase yielding high levels of intracellular cAMP that results in prolonged hypersecretion of water and electrolytes.
* A-toxin: which go inside the cell,then the cell (enterocyte) starts producing a lot of fluid and the fluid goes to the lumen ending up with watery diarrhea that can be so sever about 10-20 litters of fluid in stools resulting in acidosis(acidosis occurs cuz all bicarbonate is secreted with the diarrhea) and dehydration.
* people are killed by cholera(because of this extensive loss of fluid and acidosis) not by the organism itself, people die within~4 hours they become dehydrated, shocked and then die.
* So,the treatment is not antibiotic ,you have to give IV fluid and correct acidosis. Although it's an easy treatment but people used to die In outbreak ( a whole population ) >not enough IV fluids for all .
* 60-75% of infections are asymptomatic .
* The incubation period is 1-4 days for persons who develop symptoms followed by sudden onset of nausea, vomiting, and profuse diarrhea with abdominal cramps.
* Stools, which resemble “rice water” contain mucus, epithelial cells, and large number of vibrios,that are very very motile.
* **Diagnosis:-** Stool culture: peptone water and TCBS agar.
* **Epidemiology**: Major reservoir is infected humans(carrier;asymptomatic) if the carrier,especially who lives in estuaries((مصبات الأنهار passes the organism into water (or food), others become infected and that’s how its spread.

**Campylobacter :**

-in Europe countries

-Lives in gastrointestins of animal , They are commensals of cattle, sheep, dogs, cats, rodents, and fowl

- *Campylobacter* *species* are curved, comma shaped, oxidase – positive , catalase – positive, microaerophilic gram- negative bacilli that are motile by means of a polar flagellum.

- Contaminated poultry is responsible for >50% infections.

- Humans are infected due to consumption of contaminated food, milk or water.

- crampy abdominal pain, profuse diarrhea that may be grossly bloody, headache, malaise and fever.

-The illness is self limited within few days

- Systemic disease may be caused by *C. fetus* in immunocompromised individuals.

**Helicobacter pylori**

-It's spiral in shape ,gram –ve , motile (one polar flagellum )

-Pylori: because it has something to do with pyloric antrum in stomach

-Three pathogenicity factor help helicobacter to grow in stomach wall

1-mucinase it can dissolve mucine

2-adherence factor which adhere to the cell

3-Urease which break down the urea in the blood and produces ammonia which helps to neutralize acids so that bacteria can survive.

* So, mucinase with adherence factor and urease they are really pathogenicity factor that help the organism to live in wall of stomach, protected against the stomach acids.
* unknown Spread mechanism.
* It causes gastritis, peptic(duodenal) ulcers, and is associated with gastric cancer.
* most cases of peptic ulcer is usually caused by H.pylori … not due to smoking ,acid , spicy food…

**Mycobateria**

* This type of bacteria when they grow in a media they look *like fungus* that's why it is called mycobacteria [ acting like fungi but being truly bacteria ]..nocardia and actinomycin also grows like fungus.
* Mycobacteria are aerobic, slender, curved rods in stained clinical specimens.
* gram positive, but it's not stained by gram stain; because They are characterized by the presence of long-chain fatty acids, called mycolic acids in their cell wall, this acid interferes with staining and also interferes with the action of disinfectant and sterilizing. So; mycobacteria they are very difficult to stain because of that mycolic acid and also they are resistant to drying and disinfection.
* Mycobacterium tuberculosis can stay alive in dust for 6 months!..inhalation of this dust causes tuberculosis disease.
* Staining of mycobacteria :it’s a procedure in which the sample is spread on a glass slide and stained by carbolfuchsin ,carbolfuchsin can't penetrate by itself , so heat is used to drive the stain through the wall , this heating and staining is done multiple times, followed by decolorizing using acid , but here the acid can’t remove the stain . because Mycobacteria is resistance to decolorizing .it is called Acid Fast Bacillus (AFB) ;bacteria that once it become stained by carbolfuchsin it can't be decolorized using acid.
* Other important wall components are trehalose dimycolate (so called cord factor as it is though to induce growth in serpentine cords on artificial media; makes it grows like filaments ) and mycobacterial sulfolipids, which may play a role in virulence.   
  is this a pathogenicity factor ? some people say it is but indeed there is NO particular toxins or something poisons or enzyme has been identified in mycobacteria that causes damage ! so the damage that is caused by mycobacteria it's a type of HYPERSENSITIVTY reaction type 4 not an actual toxins produced by the mycobacteria itself.
* (type 4 ;delayed type hypersensitivity DTH : the body becomes hypersensitive and produces a lot of TH1 that produce a lot of inflammatory cytokines ,these cytokines activate microphages and a lot of destruction happens that results in tissue damage ,this damage along with macrophage and lymphocyte accumulate at the site of infection producing what is known as granuloam )
* Very slow growing : unlike other bacteria that have a doubling time of 20 minutes mycobacteria’s doubling time is about 18-20 hours .[ and this is an indication of the chronicity of the disease ,TB needs a long time to appears and also the symptoms gradually get worse and worse ,treatment prolongs for at least 6 months]
* In culture, it needs incubation for 4-6 weeks( because of the extremely slow growing) to observe the colonies and to confirm the Mycobacteria infection in a sputum sample.(the media :green and creamy media Löwenstein–Jensen medium,shown in the lab)
* Mycobacteria >Aerobic.
* They are intracellular ; stays in cytoplasm of the cell and multiply, so antibodies are useless ! indeed body needs to produce TH1cells that can activate microphages
* Another important consequence of the unique cell wall structure of mycobacteria is the adjuvant action of whole cells when mixed with a wetting agent (e.g. tween).
* The action of Mycobacteria in an oil-water emulsion (Freund's complete adjuvant), is mediated by muramyl dipeptide of peptidoglycan.

* because it's irritating it can be used as **adjuvant** (adjuvant :it's something that promotes the immune response ) we mix it with vaccine to produce irritation that attracts a lot of macrophages; release the vaccine slowly to the environment and this will give a better immune response .
* so we can mix the bacteria with some kinds of surfactant ,or mixing it with oil-water emulsion and this is known as fraud's adjuvant but these adjuvants are useful for animal experiment, not used in humane; because it can produce granuloma at the site of injection ,it's not very nice .
* so; for that in adjuvant for humane vaccine we use aluminum hydroxide  based on their ability to enhance antibody production ,these are alkaline ,they also release the vaccine slowly.

Summary   
for *animal vaccine* we use adjuvant which is either made by mixing mycobacteria with oil or tween( which is wetting agent )   
adjuvant for *human vaccine* we use aluminum hydroxide instead of mycobacteria.

* Tuberculosis vaccine
* known as BCG vaccine (Bacillus Calmette–Guérin).
* now is given for babies at one month old.
* the bacteria that is used is a related organism to mycobacteria tb ; Same antigenicity but different pathogenicity.
* it's alive vaccine .so it can really carry on dividing in the shoulder(the vaccination site); that's why it takes 6 weeks to heal after vaccination (until it stops dividing ).
* Once you are vaccinated you become allergic to the mycobacteria , skin test is done for this allergy known as PPD: purified protein derivatives
* PPD procedure >>Take the protein from the wall of mycobacteria , then inject PPD intradermally and ask the pt's to come again after 2 days to examine the site of injection ; if the site of injection becomes irritated and surrounded by red area this means that patients is allergic for PPD which is derived from mycobacteria.
* *positive PPD indication*:

1- The person is vaccinated or..2-The body have been infected and recovered (Antibodies were produced against PPD and so they react with the injected PPD) or..3-Patient has active disease (TB)

* The disease is mainly transmitted by droplet infection.

we have talked about mycobacterium tuberculosis …..**Mycobacteria bovis** another type of mycobacteria (causative agent of tuberculosis in cattle it can cause TB in human through unpasteurized milk) here the disease will star in *the* *intestine.*

while **Mycobacteria tuberculosis** >> from droplet infection , contaminated dust , some carrier cough in your face so it will get into *the lungs*. once it is inhaled, the mycobacteria goes to the lower lobe of the lung and there is taken up by macrophages ,but the macrophage can't kill them because they escape in the cytoplasm and prevent diffusion of lysosome with phagosome ;so you need helper T -cell (TH1- cell ) once they come they attract and activate more macrophages also they increase the oxidative pairs ; to kill as much as possible of these mycobacteria .

>>some of macrophage become fused togather and become multi nucleated giant cell known as Langerhans cell so now we have granuloma ; in 95% of cases the granuloma will become walled by fibrosis tissue and these bacteria remain inside. here the patient is okay with no problem at all,and may live until age of 90 ,this is known as **primary TB** .

**Primary TB**: it's an infection that occur locally in the lower lobe usually, and eventually will becomes walled granuloma and all the bacteria are isolated inside but they don't produce any disease ,this happens in 95% of cases .

Some unlucky people they have primary infection first but then the bacteria go to the blood and spread all over the body, it might get to meninges causing meningitis or to the kidney causing infection there, joint , skin ,bone.. this type of TB known as **miliary tuberculosis;** also known as **"disseminated tuberculosis**"; direct treatment is required ,people who remains untreated will die ,only a small portion of babies who gets infected in this way show miliary TB about 2-3% of cases.

**post primary TB**

* some people when they grow up and become old ,for some reason their primary TB becomes activated, either because their immune system becomes less effictive or for other reasons .
* usually involves the lung apex where bacteria will grow and express them self,, why there ?? some say because they are aerobic and oxygen level is higher at upper lobe or because of the slow lymphatic drainage and that encourages the growth .

but whatever the reason usually reactivation of TB gives an open cavity in the upper lobe and when the patient coughs these open cavities ( known as open phthusis,in this case the patient must be isolated) when the person cough they release sputum and mycobacteria (infecting other ppl) ,sometime there is blood stain may be due to TB or cancer of bronchi.

Ppl who are infected, the majority of them 95% by [primary TB] will live normally ,but 2-3% who develops[miliary TB] if left untreated they'll die cause it is a very sever, a small proportion as they grow older may develop [post primary TB].

treatment:

you treat them by verity of antibiotic a cocktail of antibiotics starting with 3 continue with 2 ,tretemnt should last for at least 6 months.

when you become allergic to TB ? after 2-3 week from primary infection