Programme: T. Y. B. Sc.

Subject: Microbiology

Paper Code: MIC 105

Paper Title: Medical Microbiology

Unit: 03 – Bacterial Diseases

Module Name: Gastrointestinal Diseases - Cholera (*Vibrio cholerae*)

Module No: 28

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Notes

Outline: Cholera: Gastrointestinal disease: *Vibrio cholera:* Mode of transmission; Pathogenesis; Symptoms; Laboratory diagnosis; Chemotherapy; Prophylaxis

Gastrointestinal Diseases: Cholera: *Vibrio cholerae*

Morphology: Gram -ve short curved rods, comma shaped, actively motile with polar flagellum, vibrios are seen arranged in parallel rows, described by Koch as having a fish-in-stream appearance.

Cultural characteristics: aerobic; temperature range of 16-40^{o} C, optimum of 37^{o}C. Growth is better in an alkaline medium, the range of pH being 6.4-9.6 (optimum 8.2), NaCl 0.5-1% is required for optimal growth, though high concentrations 6% and above are inhibitory. Therefore, they are halotolerant.

Biochemical reactions: Carbohydrate metabolism is fermentative, producing acid, but no gas. Cholera vibrios ferment glucose, mannitol, maltose, mannose and sucrose but not inositol, arabinose or lactose. Indole is formed and nitrates are reduced to nitrites. These two properties contribute to the cholera red reaction, which is tested by adding a few drops of concentrated sulphuric acid to a 24 hour peptone water culture. With cholera vibrios, a reddish pink colour develops due to the formation of nitroso-indole. Catalase and oxidase tests are positive. Methyl red and urease tests are negative. Citrate, H_2S negative. Vibrios elaborate several enzymes including collagenase, elastase, chitinase, nucleotidase, decarboxylase, lipase, mucinase and neuraminidase (receptor destroying enzyme).

Mode of transmission: Cholera is a major water-borne disease. Exclusively human disease where Infection originates from patients. Direct person-to-person spread by contact: Through faecally contaminated water, food and flies. Natural habitat: saline waters of coastal seas and brackish estuaries; shellfish beds contaminated by untreated sewage. Drinking contaminated water or vegetables washed with contaminated water; raw shellfish consumption, can lead to epidemics in humans. Vibrios survive in water for up to three weeks.

Pathogenesis: Cholera is not an invasive infection. *Vibrio cholerae* do not reach bloodstream, remains within the intestinal tract. Ingestion of 10^8 to 10^9 Cholera vibrios are required to cause disease. Vibrios are highly susceptible to acids; gastric acidity provides an effective barrier against small doses of cholera vibrios. 10^6 pathogenic vibrios administered to achlorhydria volunteers with food or sodium bicarbonate, predisposes to cholera. Even lower cell numbers can initiate infection if *V. cholerae* is ingested with food.

Mechanism of action: In the small intestine, vibrios can cross the protective layer of mucus and reach the epithelial cells by chemotaxis, motility, mucinase and other proteolytic enzymes. A haemagglutinin-protease cleaves mucus and fibronectin; also helps in releasing vibrios bound to bowel mucosa, facilitating their spread to other parts of the intestine and also their faecal shedding. Adhesion to the epithelial surface and colonization may be facilitated by special fimbriae. Throughout the course of infection, Vibrios remain attached to the epithelium but donot damage or invade the cells. The changes induced are biochemical rather than histological.

Enterotoxin: Vibrio multiplying on the intestinal epithelium produce an enterotoxin called Cholera toxin very similar to heat labile toxin of *E. coli* in structural, chemical, biological and antigenic properties, though cholera toxin is far more potent than heat labile toxin of *E. coli* in biological activity. The toxin molecule consists of one A (active) and five B (binding) subunits. B units attach to the GM_1 ganglioside receptors on the surface of jejunal epithelial cells; which promotes entry of subunit A into the cell. Subunit A on being transported into the enterocyte, dissociates into 2 fragments: A_1 and A_2 : A_2 fragment only links biologically active A_1 to the B subunit. The A_1 fragment causes prolonged activation of cellular adenylate cyclase and accumulation of intracellular cAMP, leading to outpouring into the small intestinal lumen, of large quantities of water and electrolytes, and the consequent watery diarrhea. The fluid secreted is isotonic with plasma but contains much more of potassium and bicarbonate. Toxin also inhibits intestinal absorption of sodium and chloride.

Pathogenesis: Disease, in treated cases, may last for 4-6 days, during which the patient may pass a total volume of liquid stool – as much as 20-30L per day. Rice water stools, in composition, is a bicarbonate rich isotonic electrolyte solution, with little protein. Its outpouring leads to diminution of extracellular fluid volume, hemoconcentration, hypokalemia,

base-deficit acidosis, shock and death. Incubation period varies from less than 24 hours to about five days. Mortality rate without treatment is between 25% and 50%.

Symptoms: Profuse, painless, watery diarrhoea – colourless watery fluid, with flecks of mucus, said to resemble water in which rice has been washed, hence called rice water stools, has characteristic inoffensive sweetish odour. Massive loss of fluid and electrolytes. Copious effortless vomiting, Fever, muscular cramps, renal failure, pulmonary edema, cardiac arrhythmias and paralytic ileus.

Laboratory diagnosis: 1. Specimen: Stool collected in the acute stage (10^6 - 10^9 vibrios/ml) of the disease, before the administration of antibiotics. Specimen collected by introducing a lubricated catheter into the rectum and letting the liquid stool flow directly into a screw-capped container. Stools from pans not recommended. Vomitus is not useful.

2. Transport: As cholera vibrios may die in a few hours at tropical temperatures, necessary to preserve the specimen at 4°C or in holding medium. Stool samples may be preserved in Venkatraman Ramakrishnan (VR) fluid or Cary-Blair (transport medium for *Salmonella, Shigella, Vibrio*) medium for long periods. If specimen can reach the laboratory in a few hours, it may be transported in enrichment media such as alkaline peptone water or Monsur's medium. If transport media are not available, strips of blotting paper may be soaked in the watery stool and sent to the laboratory packed in plastic envelopes.

3. Microscopy: Direct microscopic examination of cholera stool not recommended. Characteristic darting motility of the vibrio and its inhibition by antiserum can be demonstrated under dark field or phase contrast microscope using cholera stool.

4.Culture: On arrival in the lab, specimens sent in enrichment media should be incubated for 6-8 hours including transit time. Specimens sent in holding media/transport media, should be inoculated into enrichment media, to be incubated for 6-8 hours before being streaked on a selective and non-selective medium. Do direct plating before enrichment.

Plating Media: Alkaline Bile salt agar, pH 8.2; MacConkey's agar; thiosulphate citrate bile salt sucrose (TCBS) agar.

Antigenic structure: Have a single heat-labile flagellar H antigen; has O lipopolysaccharide *V. cholerae* serotypes are based on somatic (O) antigens - serotype O1. Serotypes- Ogawa, Inaba, Hikojima. Biotypes of cholera vibrios - Classical, El-Tor. New non-O1 strain (South India) was responsible for causing eighth pandemic strain of cholera.

El-Tor biotype was named after quarantine camp. Vibrios were isolated from pilgrims returning from Mecca; responsible for seventh pandemic. It is now spread throughout the world and has

largely displaced Classical biotype. It differs from Classical *V. cholerae* in several ways: causes only mild diarrhoea, has a higher ratio of carriers to cases, carriage is more prolonged.

5. Identification: Slide agglutination: Colonies suggestive of vibrios tested by slide agglutination with cholera O subgroup I serum. If slide agglutination is positive, the isolate is further tested by biochemicals for differentiation between El Tor and classical cholera vibrios.

Biochemical reactions: Oxidase test +ve; Utilization of amino acids - lysine +ve, arginine -ve, ornithine +ve; Fermentation of sugars - sucrose +ve, mannose +ve, arabinose +ve; Hemolysis; MR -ve, VP +ve; Polymyxin B sensitivity.

Cholera red reaction: To 24 hour peptone water culture, add few drops of conc H_2SO_4 – red (Indole +ve, nitrate +ve – contribute to cholera red reaction).

Susceptibility to cholera phage IV. Strain may be sent to International Reference Centre for vibrio phage typing at National Institute of Cholera and Enteric Disease (NICED) at Kolkata.

Chemotherapy: Prompt and adequate replacement of lost fluid and electrolytes. Oral administration of fluid containing glucose and electrolytes, either alone or supplemented by intravenous fluids is a highly successful method of treating cholera. Electrolyte replacement therapy consists of: 20g glucose, 4.2g NaCl, 4.0g $NaHCO_3$, 1.8g KCl, dissolved in 1L of water and Tetracycline/ Streptomycin used for reducing the period of vibrio excretion.

Prophylaxis: prevention of cholera essentially requires avoidance of untreated water/ice, raw foods, fish, shellfish; personal and environmental sanitation; protected water supply

Vaccination: 1. Parenteral (killed) - 8000 million *V. cholerae*/ml: composed of equal numbers of Ogawa and Inaba serotypes: subcutaneous/ intramuscular injection; 2. Oral vaccines – killed oral whole cell vaccines; live oral vaccines