

## Vibrio

General characters: They are thin, curved comma shaped Gram-negative, rigid and actively , motile (polar flagellum) bacilli. They are non-lactose fermenting, growing at alkaline pH, produce indole and are oxidase positive.

They ferment glucose with the production of acid only. Most important pathogenic members in man are:

1. *Vibrio cholerae*
2. *Vibrio El Tor*
3. *Non-agglutinable vibrio (NAV)*
4. *Vibrio parahemolyticus*

Classification of Vibrio

**Non halophilic vibrio** (Grow in media without sodium salt NaCl)

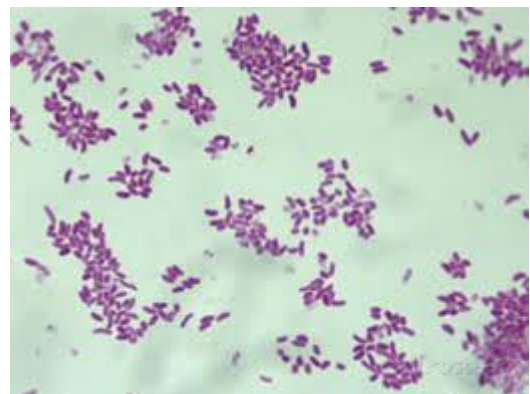
- a. *Vibrio cholera* (01 classical and E1 tor biotypes)
- b. Non 01 *Vibrio cholera* (Non cholera vibrio or no agglutinating vibrio )

**Halophilic vibrio** (Grow in media containing sodium salt NaCl )

- a. *Vibrio para-* c. *Vibrio vulnificus hemolyticus*
- b. *Vibrio alginolyticus* d. *Vibrio mimicus*

### **VIBRIO CHOLERA**

Morphology: It is short curved, comma shaped about  $1.5 \mu \times 0.2$  to  $0.4 \mu$  in size and Gram-negative bacilli. It may occur singly or as "S" shaped semicircular pairs . It is actively motile with a single flagellum. It shows darting type of motility.



**Culture characters:** It is strongly aerobic Growth occurs in alkaline pH (7.5 to 9.6) between 22 and 40°C (optimum 37°C). It grows well in ordinary media

**Alkaline peptone water:** Rapid growth in about 6 hours is with formation of thick surface pellicle. Turbidity and powdery deposit on prolonged incubation may be present .

**Nutrient agar:** Colonies are moist, translucent round 1 to 2 mm in size with bluish tinge in transmitted light. The growth has distinctive odor

**MacConkey's agar:** Colonies are colorless

**Blood agar:** Colonies show zone of green coloration around them which later become clear due to hemodigestion .

### Special Media

For cultivation of *Vibrio cholerae*. Venkat Raman (VR) medium: It contains 5gm peptone and 20 gm crude sea salt per liter distilled water adjusting pH 8.5 to 8.8. In this medium *Vibrio* remain viable for weeks but do not multiply

ii. Cary-Blair medium: It is a buffered solution of sodium chloride, sodium thioglycolate, disodium phosphate and calcium chloride at pH 8.4 .

#### b. Enrichment media:

i. Alkaline peptone water pH 8.6

ii. Monsur's taurocholate-tellurite peptone water (pH 9.2).

#### c. Plating media

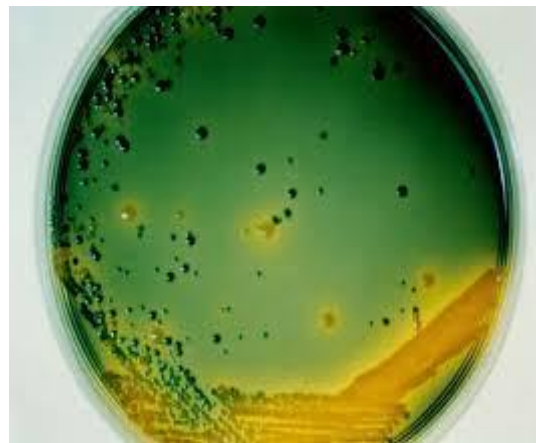
i. Alkaline bile salt agar pH 8.2 (BSA)

ii. Monsur's gelatin-taurocholate trypticase tellurite agar (GTTA) .

iii. TCBS medium: It contains thiosulphate citrate bromothymol blue and sucrose . It is widely used .



Nutrient agar



T.C.B.S agar



Blood agar

**Biochemical tests:** Acid without gas is produced from glucose, sucrose, maltose and mannitol. Oxidase is positive, indole may be produced, nitrate reduction positive, liquefies gelatin and urease negative. Cholera red reaction is tested by adding few drops of  $H_2SO_4$  to 24 hour growth in peptone water. With *Vibrio cholerae* red pink color is produced due to formation of nitrosoindole .

**Resistance:** It is killed by heat at 55°C in 15 minutes. It is destroyed by drying. The acidity of gastric juice at once kills them. It is susceptible to streptomycin, chloramphenicol and tetracycline.

### **Laboratory Diagnosis**

#### **Hematological Investigations**

It is not diagnostically significant in early stages. However, there may be increase of packed cell volume up to 65 to 85 percent hemoglobin contents are increased from 15 to 25 gm percent and there may be polycythemia (count may be more than 7 million per cu mm ).

#### **Bacteriological Investigations**

**a. Specimen:** Feces may be collected with a spoon in a sterile container free from antiseptic. If there are chances of delay transport media or holding media may be used (VR media). One to three gm stools are emulsified in 10 to 15 ml (VR media) .

In case of rectal swab, trypticase taurocholate tellurite broth (pH 9.2) or alkaline peptone water is used. If media is not available strips of blotting paper soaked in watery stool may be sent to Laboratory.

**b. Smear examination:** Hanging drop preparation shows darting type motility Gram-staining shows them to be Gram negative and comma shaped .

**c. Culture:** Specimen is inoculated in Monsur's medium. A tube of alkaline peptone water is inoculated simultaneously with fecal matter. After 6 to 8 hours Gram's stain shows curved Gram-negative bacilli with darting type motility. Sub-inoculation in Monsur's medium is done. The colonies in this medium are tested for biochemical reactions. It shows oxidase positive, nitrate reduction positive, fermentation of glucose , sucrose, mannose and arabinose, cholera red reaction positive, indole positive and slide agglutination with O group polyvalent or mono specific sera differentiates it into Ogawa, Inaba and Hikojima types .

**Serology:** It is of little use in the diagnosis of cholera. It may be helpful in assessing the incidence of cholera in an area. Indirect hemagglutination vibriocidal test and antitoxin assay are popular agglutination tests. Among them complement depend vibriocidal antibody test is most useful .