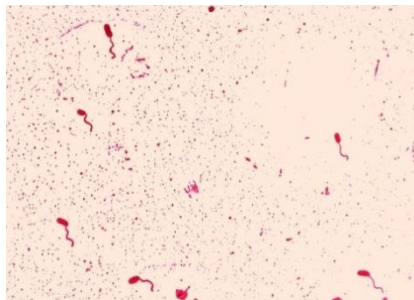


Family Vibrionaceae

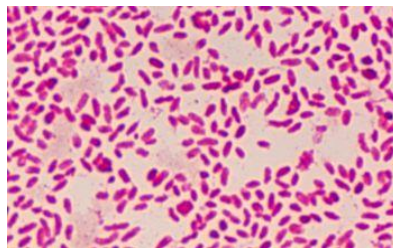
Genus Vibrio

Family Vibrionaceae includes genera *Vibrio*, *Aeromonas* and *Plesiomonas* that include species pathogenic for human. The most important is genus *Vibrio* which contains about 60 species. Type species is *Vibrio cholerae*, causative agent of cholera, especially dangerous disease of the human.

Morphology. *V.cholerae* are thin, curved, comma shaped gram-negative rods, motile (single flagellum), non-capsulated, non-sporeforming. In stained films of mucus flakes from acute cholera cases, the vibrios are seen arranged in parallel rows, as the 'fish in stream' appearance.



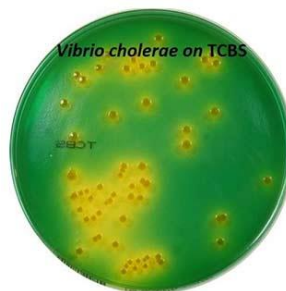
V.cholerae Leifson flagella stain



V. parahaemolyticus

Cultural properties. *V.cholerae* are strongly aerobic. Growth occurs in alkaline pH (7.5 to 9.6) between 22 and 40°C (optimum 37°C). It grows well in simple media. In alkaline peptone water *V.cholerae* grow rapidly with formation of delicate bluish pellicle. On alkaline MPA colonies are moist, translucent, circular with bluish tinge in transmitted light.

On selective Monsur's taurocholate-tellurite peptone agar (pH 9.2) colonies are translucent or semitranslucent with greyish-black colour and turbid edges. On TCBS (thiosulphate citrate bromothymol blue sucrose) medium *V.cholerae* produce large yellow convex colonies (due to the sucrose fermentation) which may become green on continued incubation.



Vibrio cholerae on TCBS Agar



Vibrio parahaemolyticus on TCBS Agar

Fermentative properties. *V. cholerae* ferment galactose, glucose, sucrose, maltose and mannose. In the first 24 hours don't ferment lactose and arabinose. They are catalase and oxidase positive, form indole, nitrate reduction is positive, liquefy gelatin, urease is negative. Heiberg (1934) classified vibrios into six groups based on the fermentation of mannose, sucrose and arabinose. True causative agents of cholera disease belong to group I (man+, suc+, ara -). They liquefy gelatin, hydrolyze casein, coagulate rabbit plasma, liquify clotted serum, peptonize milk, decomposed protein with ammonia and indole, don't form H₂S. Other groups combine vibrios, which are not the cause of cholera disease.

Differential signs of cholera causative agents

Tests	V.cholera	V.El.Tor	V.cholera O139
Hemolysis of ram erythrocytes	-	+	-
Voges-Proskauer	-	+	-
Agglutination of chick erythrocytes	-	+	+
Agglutination with O1-serum	+	+	+
Polymyxin B sensitivity	+	-	-
Group IV phage susceptibility	+	-	-
El.Tor phage susceptibility	-	+	-

Resistance. *V. cholerae* are killed by heat at 55°C in 15 minutes. They are destroyed by drying and acid. The acidity of gastric juice at once kills them. *V.cholera* resist in alkaline medium. Survival in water is influenced by its pH, temperature, salinity, presence of organic pollution and other factors.

Antigenic structure. *V. cholerae* contain O-somatic and H-flagellar antigens. Flagellar antigen is non specific, heat labile and is common to cholera and cholera like organisms. O antigen is heat stable and type specific. About 80 serogroups are identified. Two serogroups of *V. cholerae*, O1 and O139, cause outbreaks of cholera. O1 causes the majority of outbreaks, while O139 is confined to Southeast Asia. *V. cholerae* O1 has two biotypes or biovars, classical and El Tor. Both biotypes are antigenically divided into Inaba, Ogawa and Hikojima on the basis of O antigenic factors (A, B, C). Inaba contains AC, Hikojima- ABC and Ogawa - AB. Other serogroups (from 2 to 138) can cause cholera-like diarrhea and never cause epidemics. They are called «non-agglutinable vibrios» or NAG vibrios.

Virulent factors. Virulent factors include adherence factors (pili), proteolytic enzymes (gelatinase, mucinase) and toxins. *V.cholera* produce two types of toxins: endotoxin (LPS of gram-negative bacteria) and exotoxin cholero-gen. The cholero-gen consists of subunits A and B. The B (binding) units attach to the ganglioside receptors on the surface of jejunal epithelial cells. The A (active) unit causes prolonged activation of adenylate cyclases and accumulation of cAMP, leading to the outpouring into the small intestinal lumen of large quantities of water and electrolytes and the consequent watery diarrhea. Toxigenic strains O1, O139 contain vct+ gene that codes cholero-gen toxin. Only toxigenic strains have caused widespread epidemics.

Epidemiology. Cholera is classic anthroponosis, especially dangerous infection that tends to epidemic spread. Cholera is endemic in India, China, Japan and Indonesia. *V. cholerae* cause disease only in human. The natural habitat of *V.cholerae* is brackish or saltwater. The disease is transmitted from sick and convalescent persons by contaminated water, milk, fruit, vegetable, etc. Flies may disseminate organisms from feces to food.

Pathogenesis. The portal of entry is GIT. *V.cholera* cause an acute gastroenteritis. Incubation period is 24 hours to 5 days. Cholerae vibrio, after getting establishment in the intestine, multiplies producing exotoxin, absorbed onto epithelial gangliosides, which cause outpouring of fluid into the lumen. Stools are rice water containing mucus flakes, epithelial cells and vibrios. As a result, there is tremendous fluid loss, dehydration and hypochloremia.

Immunity. Immunity after infection is cellular-humoral, stable, antimicrobial and antitoxic.

Microbiological diagnosis. Bacteriological method is the most reliable to make the diagnosis. Watery stool, rectal swab, water, food, vomiting are materials for investigation. On the first stage of the disease collected samples are inoculated into alkaline peptone water (APW). In

parallel, express methods of diagnosis are used - immunofluorescence test and indirect hemagglutination test.

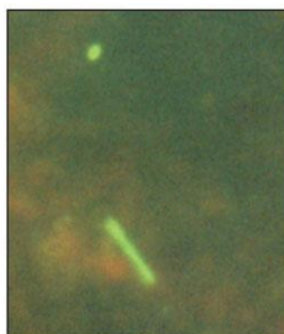


Figure 4 - Detection of viable nonculturable *Vibrio cholerae* O1 through direct immunofluorescence.

On the second stage investigated material from APW reinoculate on the alkaline peptone agar (APA) or selective media (TCBS). Microscopy of wet smears from PW for detection motility of microorganism is performed. After 16-24 hours, selection of suspicious colonies is carried. In the selection of colonies, oxidase test or indicators papers are used. Oxidase positive colonies check in agglutination test with O1 serum. If the test is positive, slide-agglutination test with Ogawa, Inaba and Hikojima serum is carried out. Prepare smears for Gram staining and for treatment with luminiscence serum. Positive agglutination test with O1, Ogawa, Inaba and Hikojima serum, positive test with luminiscence serum, morphological and culture properties allow togive previous result about the detection of *V.cholera* of O1 serogroup. Suspicious colonies on the APA or TCBS are reinoculated on the Ressler's media or alkaline agar to get pure culture. At the last stage, isolated pure culture is identified by biochemical, serological and phage typing properties.

Differential characteristics of important *Vibrio* species.

Signs	<i>V.cholera</i>	<i>V.parahaemolyticus</i>	<i>V.vulnificus</i>
Oxidase	+	+	+
Indol	+	+	+
VP	+	+	+
Citrate	+/-	+	+
Lysine	+	+	+
Ornithine	+	+	+
Sucrose	+	-	-
Lactose	slowly	-	+
Mannitol	+	+	+/-
Salicin	-	-	+
Nitrate to nitrite	+	+	+
Gelatinase	+	+	+
Growth at 0% NaCl	+	-	-
Growth at 6% NaCl	-	+	+/-

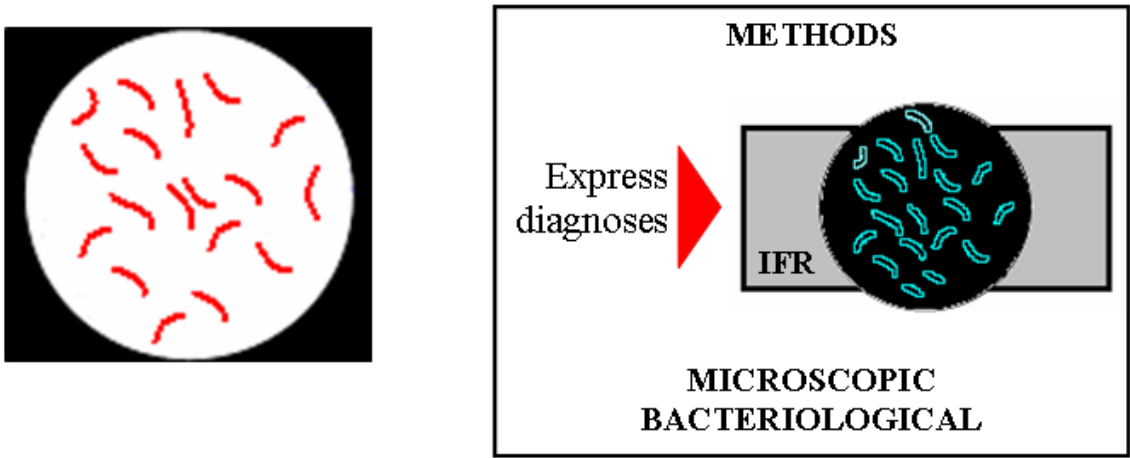
If isolated culture is not agglutinated with O1serum, agglutination with O139 serum is performed.If isolated culture doesn't agglutinate with any serum, result of non-agglutinated vibrio isolation is issued.

Treatment. Tetracycline, erythromycin, metacycline, doxycycline, chloramphenicol are used for treatment. Tetracycline is used for prophylaxis of contact persons.

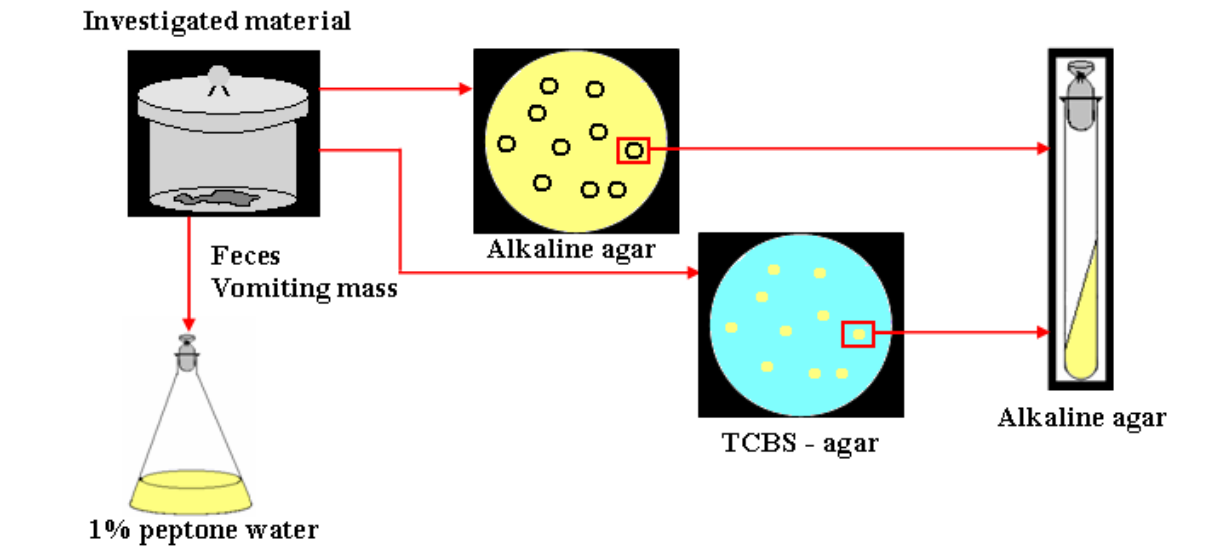
Prophylaxis. In primary prevention early detection of patients and carriers, their isolation and hospitalization, identification of contact persons are the main.The current vaccine is a complex

preparation consisting of cholera toxin (70%), chemical O-antigen (30%) of both biovar and serovars Ogawa and Inaba.

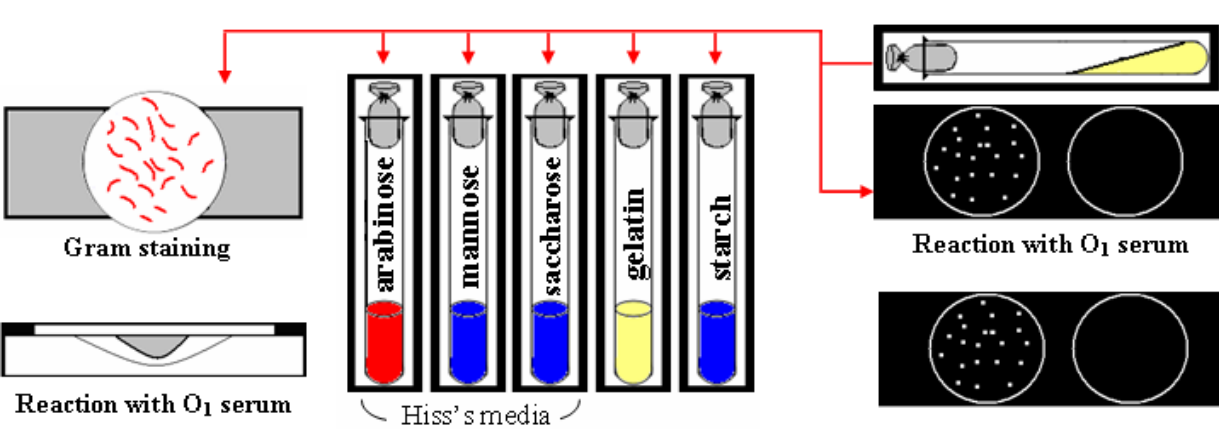
VIBRIO CHOLERA



BACTERIOLOGICAL METHOD



IDENTIFICATION



Cholera

Definition	Cholera is an acute, diarrheal illness caused by infection of the intestine with the bacterium <i>Vibrio cholerae</i> .
Etiology	Family - Vibrionaceae Genus - <i>Vibrio</i> Species: - <i>Vibrio cholerae</i> , biovar El Tor & biovar Cholerae
Morphology	Gram-negative Comma-shaped bacterium Motile Non capsulated Non spore forming
Epidemiology	Source of infection: sick people, carriers. <i>V. cholerae</i> is usually transmitted via the ingestion of food or water contaminated (directly or indirectly) with feces or vomitus of infected persons. Mode of transmission: Cholera is transmitted by the fecal-oral route. Ingestion of contaminated food or water, contact with infected objects.
Virulence factors	Endotoxin and Exotoxin (Cholera toxin) Hyaluronidase; fibrinolysin; lecithinase; hemolysins, coagulase, collagenase.
Methods of laboratory diagnostics	Bacterioscopy - direct Gram stained smear and hanging-drop test. Bacteriological method: - isolation on culture media: alkaline peptone water and alkaline agar - Identification: based on morphological, and biochemical properties; Antigenic structure (Agglutination reaction). Serological tests: Agglutination reaction, ELISA, Immunofluorescence reaction
Treatment	Oral or intravenous rehydration therapy (fluid replacement) Antimicrobial therapy: Tetracycline, Chloramphenicol.
Specific prophylaxis	Specific prophylaxis of cholera is performed by corpuscular vaccine and cholera toxin-anatoxin