

VIBRIO

General Properties of Vibrio

- Curved Gram-negative bacilli
- Actively motile by single polar flagellum
- Fermentative, strongly aerobic, oxidase positive,
- Non-sporing & non-capsulated
- Growth stimulated by salt (NaCl)
- Ubiquitously found worldwide in marine environments, surface waters, river & sewage

VIBRIO CHOLERAЕ - CLASSIFICATION

❖ Based on Salt Requirement

- **Nonhalophilic vibrios** - grow without salt, 1% salt is optimum & cannot grow at higher concentrations
 - Examples *V. Cholerae* & *V. mimicus*
- **Halophilic vibrios** - Cannot grow in absence of salt, grow at higher salt concentration (7–10%)
 - Examples – *V. parahaemolyticus*, *V. alginolyticus* & *V. vulnificus*.

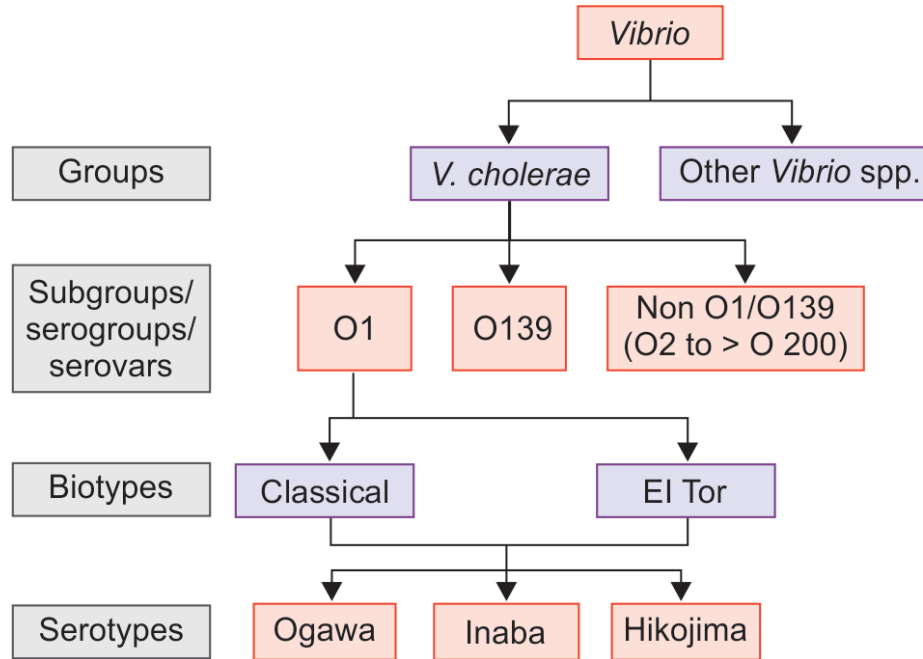
VIBRIO CHOLERAЕ - CLASSIFICATION

❖ Heiberg Classification (1934)

- **Eight groups** based on fermentation of three sugars
 1. Mannose
 2. Arabinose
 3. Sucrose
- *V. cholerae* was placed in Group I

VIBRIO CHOLERAE – CLASSIFICATION

Gardner and Venkatraman Classification



Gardner and Venkatraman Classification

- **O1 serogroup**
 - Agglutinated by O1 antisera
 - Responsible for all pandemics & most of the epidemics of cholera
- **Nonagglutinable (NAG) vibrios**
 - Not agglutinated by O1 antiserum
 - Initially thought to be non-pathogenic (**non-cholera vibrios –NCV**)

Gardner and Venkatraman Classification

- **O139 serogroup**
 - Since 1992 has caused several epidemics and outbreaks - coastal India & Bangladesh.
- **Non O1/O139 serogroups - occasional sporadic outbreaks of diarrhea & extraintestinal manifestations, but never epidemic cholera**

Differences between classical & El Tor *V.cholerae*

Biotypes of <i>V.cholerae</i> O1	Classical biotype	El Tor biotype
β hemolysis on sheep blood agar	Negative	Positive
Chick erythrocyte agglutination	Negative	Positive
Polymyxin B (50 IU)	Sensitive	Resistant
Group IV phage susceptibility	Susceptible	Resistant
El Tor Phage V susceptibility	Resistant	Susceptible
VP (Voges Proskauer) test	Negative	Positive
CAMP test	Negative	Positive
Cholera toxin gene	CTX-1	CTX-2

Serotypes of *V.cholerae* O1

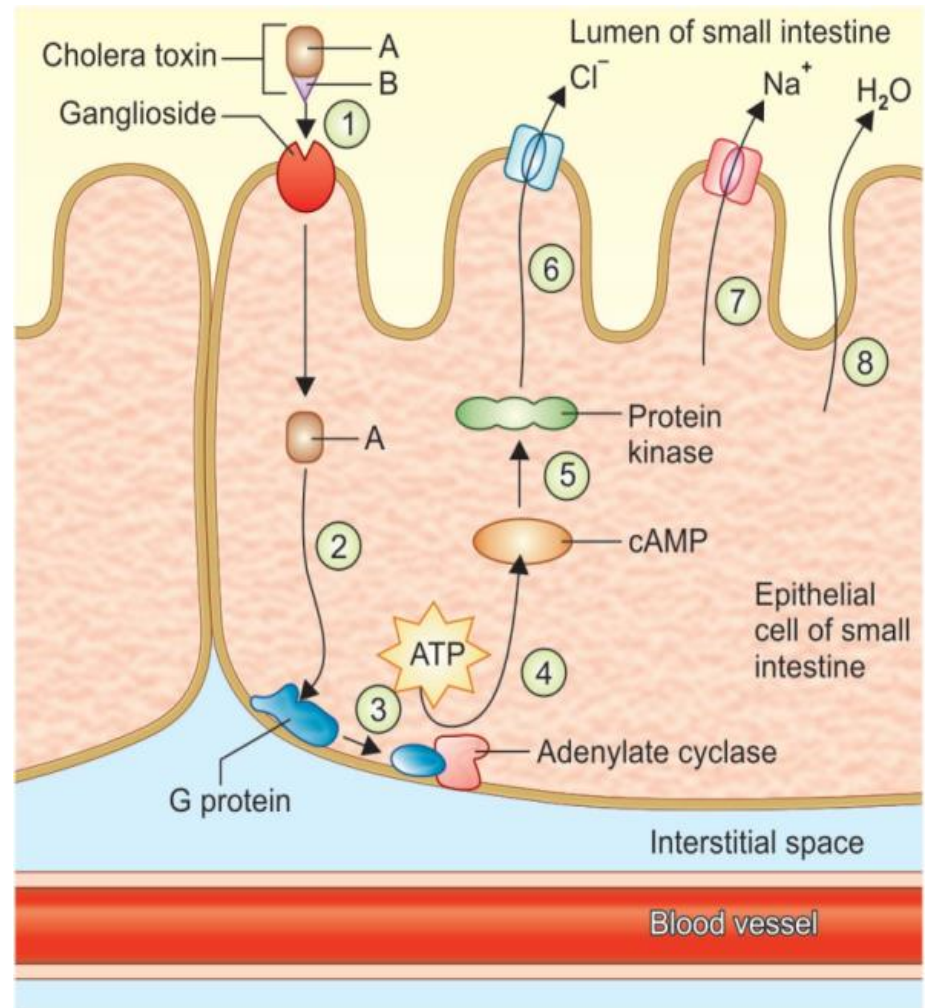
Serotype	O antigen types
Ogawa	A,B
Inaba	A,C
Hikojima	A,B,C

- **Ogawa** - most common serotype isolated followed by Inaba
- During epidemics, shifting between serotypes can take place
- **Hikojima** - unstable transitional state, both Inaba and Ogawa Ags expressed

Pathogenesis of Cholera

- **Transmission** - ingestion of contaminated water or food
- **Infective dose** - acid-labile, High infective dose
- **Factors promoting transmission** – hypochlorhydria
- **Crossing of protective layer of mucus** – active motility
 - Secreting mucinase and other proteolytic enzymes
 - Secreting hemagglutinin protease (cholera lectin)
- **Adhesion and colonization**- facilitated by a special type IV fimbria called **toxin coregulated pilus (TCP)**

- **Fragment B** - binds to GM1 ganglioside receptors
- **Fragment A2** - tethering A and B subunits together
- **Fragment A1** – active fragment - adenylate cyclase \rightarrow cAMP



Pathogenesis of Cholera

- **Increase in cyclic AMP** - accumulation of sodium chloride in intestinal lumen → Water moves passively into the bowel lumen → accumulation of isotonic fluid (watery diarrhea)
- **Loss of fluid and electrolytes** → shock (due to profound dehydration) and acidosis (due to loss of bicarbonate)

Other virulence factors

- **Zona occludens toxin** - disrupts the tight junctions between mucosal cells
- **Accessory cholera enterotoxin**- phage packaging and secretion
- **Verotoxin** - analogous to *Shigella toxin*
- **Accessory colonization factors** - adhesion & colonization
- **Siderophore** - required for iron acquisition
- **Bacterial endotoxin (LPS)** - does not contribute to the pathogenesis of cholera. Immunogenic – included in killed vaccines

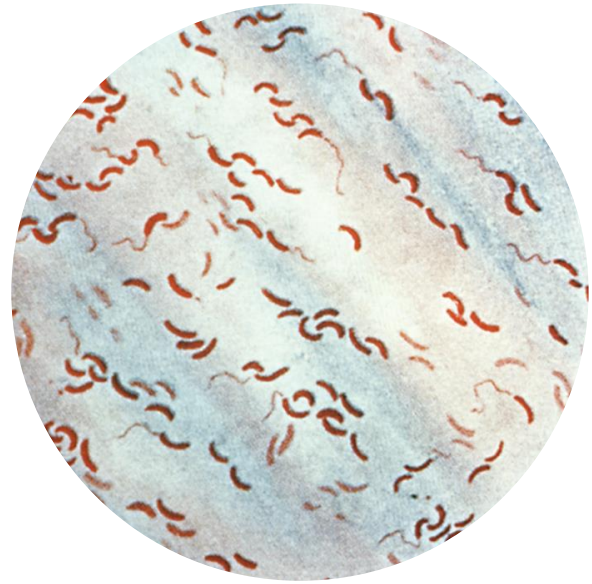
Clinical Manifestations of Cholera

1. Asymptomatic infection (75% of cases)
2. Mild diarrhea or cholera (20% of cases)
3. Sudden onset of explosive and life-threatening diarrhea (cholera gravis – 5%)
 - IP - 24 to 48 hours
 - **Watery diarrhea** - sudden onset of painless watery diarrhea
 - **Rice water stool** - watery with mucus flakes & inoffensive odor
 - Vomiting may be present but fever is usually absent

Laboratory Diagnosis

- **Specimens:** Freshly collected watery stool - cases
 - Rectal swab - convalescent patients or carriers
- **Transport/Holding Media**
 - **Venkatraman-Ramakrishnan (VR) medium** - crude sea salt & peptone water (pH 8.6–8.8)
 - **Alkaline salt transport medium** - VR medium + boric acid, NaOH and KCl (pH 9.2)
 - **Cary-Blair medium** - buffered solution of sodium chloride, sodium thioglycollate, disodium phosphate and calcium chloride (pH 8.4)
 - **Autoclaved sea water**

- **Gram-staining of mucus flakes of feces** - short curved comma-shaped gram-negative rods, arranged in parallel rows - **fish in stream appearance**

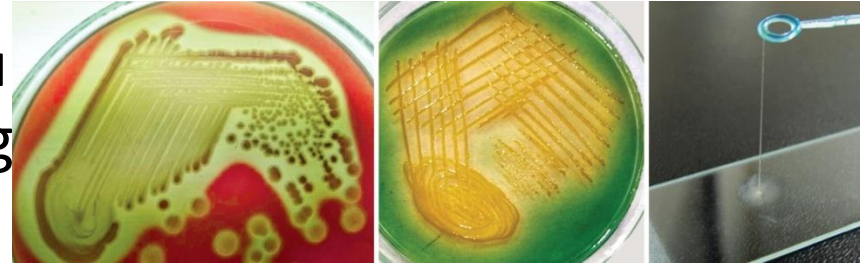


Laboratory Diagnosis

- **Motility testing (hanging drop)** - darting motility /shooting star/ swarming gnats motility
- **Motility testing after adding H-antiserum** - *V.Cholerae* becomes non-motile when a drop of the watery stool specimen is added with flagellar (H) antiserum

- **Nutrient agar** - Translucent colonies

- **Peptone water** - Uniform turbidity with surface pellicle - strong aerobic



- **Blood agar** – Hemodigestion →
- Optimum Temp 37°C
- Optimum pH 8.2 (range 7.4–9.6)
- NaCl (0.5–1%) stimulates growth

Culture Medium

- **Enrichment broths** - incubated for 4–6 hours → subculture onto selective medium
- **Alkaline peptone water (APW)**- peptone, NaCl in distilled water (pH 8.6)
- **Monsur's taurocholate tellurite peptone water (pH 9.0)**
- Both can also be used as transport media.

Selective media

- **Alkaline bile salt agar (pH 8.2):** glistening, oil drop, translucent colonies
- **Monsur's gelatin taurocholate trypticase tellurite agar (pH 8.5)-** translucent colonies with a greyish black Center and a turbid halo
- **MacConkey agar:**
 - Late lactose fermentation
 - Mildly selective, also supports *Shigella* and *Salmonella*.

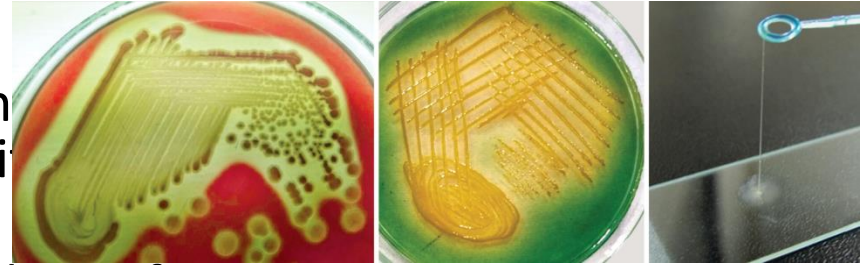
- **TCBS agar:**
 - Thiosulfate, citrate, bile salts (as inhibitor), sucrose
 - pH of 8.6
 - yellow colored colonies



Biochemical Tests

- **Catalase** and **oxidase** positive
- **Indole** test—positive, **Citrate** test—variable
- **Urease** test—negative
- **Triple sugar iron** agar test— acid/acid, gas absent, H₂S absent
- **MR** (methyl red) test—positive
- **VP** (Voges-Proskauer) test—positive for El Tor, negative for classical biotype

- **Nitrate reduction test** is positive
- **Cholera red reaction:** Positive
- **Sugar fermentation test:** Ferment glucose, sucrose and mannitol with production of acid but no gas.
- **String test:** colony mixed with a drop of 0.5% sodium deoxycholate on a slide
- **Salt tolerance test:** to differentiate from halophilic vibrios
- **Biotyping**



Biochemical Tests

- **Decarboxylase tests:**
 - *Vibrio* utilizes lysine and ornithine
 - *Aeromonas* utilizes only arginine
 - *Plesiomonas* utilizes all, i.e. lysine, arginine and ornithine.
- **Susceptible to O/129 (vibriostatic agent):** *Vibrio* species are susceptible to 10 µg of O/129 disk while *Aeromonas* and *Plesiomonas* are resistant

- **Serogrouping** - Species identification confirmed by agglutination with *V.cholerae* polyvalent O antisera:
- **Using group-specific antisera.** First the colony is tested with O1 antisera → If found negative, then tested with O139 antisera

Serotyping		
Serotype	Ogawa antisera	Inaba antisera
Ogawa	+	-
Inaba	-	+
Hikojima	+	+