

Vibrio

- Gram negative, rigid, curved rods
- Actively motile by polar flagella
- Vibratory motility (vibrae, to vibrate)
- Positive oxidase reaction
- Present in marine environment & surface water

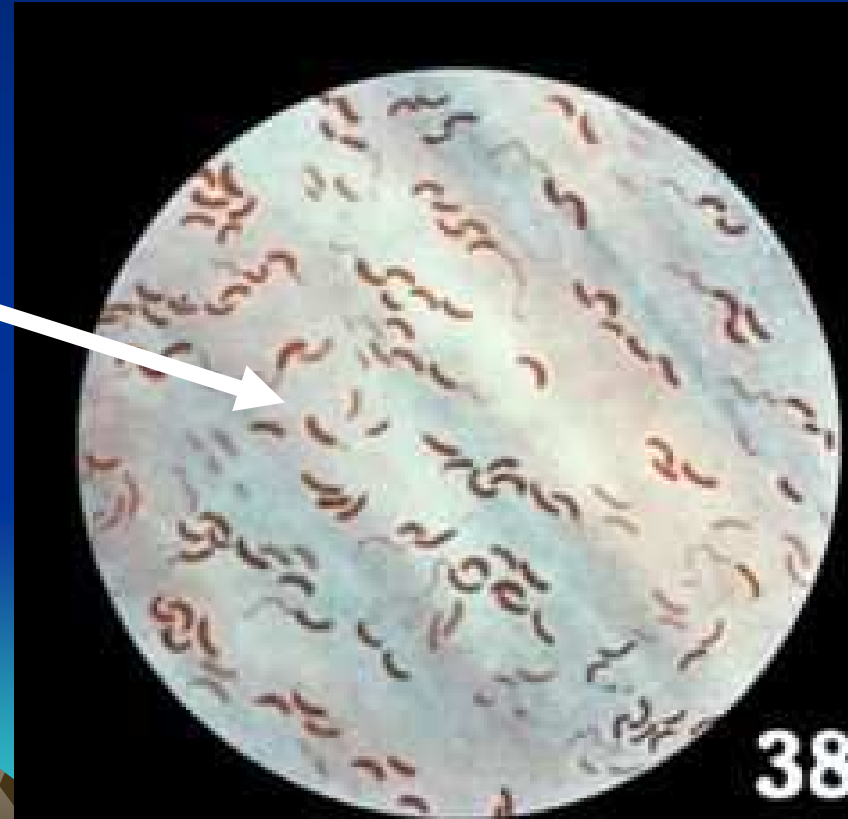


- Similarities to Enterobacteriaceae
 - Gram-negative
 - Facultative anaerobes
 - Fermentative bacilli
- Differences from Enterobacteriaceae
 - **Polar flagella**
 - **Oxidase positive**
- **classified under family of Vibrionaceae**
 - **Primarily found in water sources**
 - **Cause gastrointestinal disease**



Vibrio cholerae

- 1st isolated by Koch(1883) from cholera patients in Egypt.
- Morphology : gram negative short, curved, cylindrical rod,
- about 1.5 x 0.2-0.4 μ m
- Typical comma shaped



- S shaped or spiral forms may be seen
- Pleomorphism is frequent in old culture
- Darting motility
- In stained film of mucous flakes they are seen arranged in parallel rows, described by Koch's as the **fish in stream**.



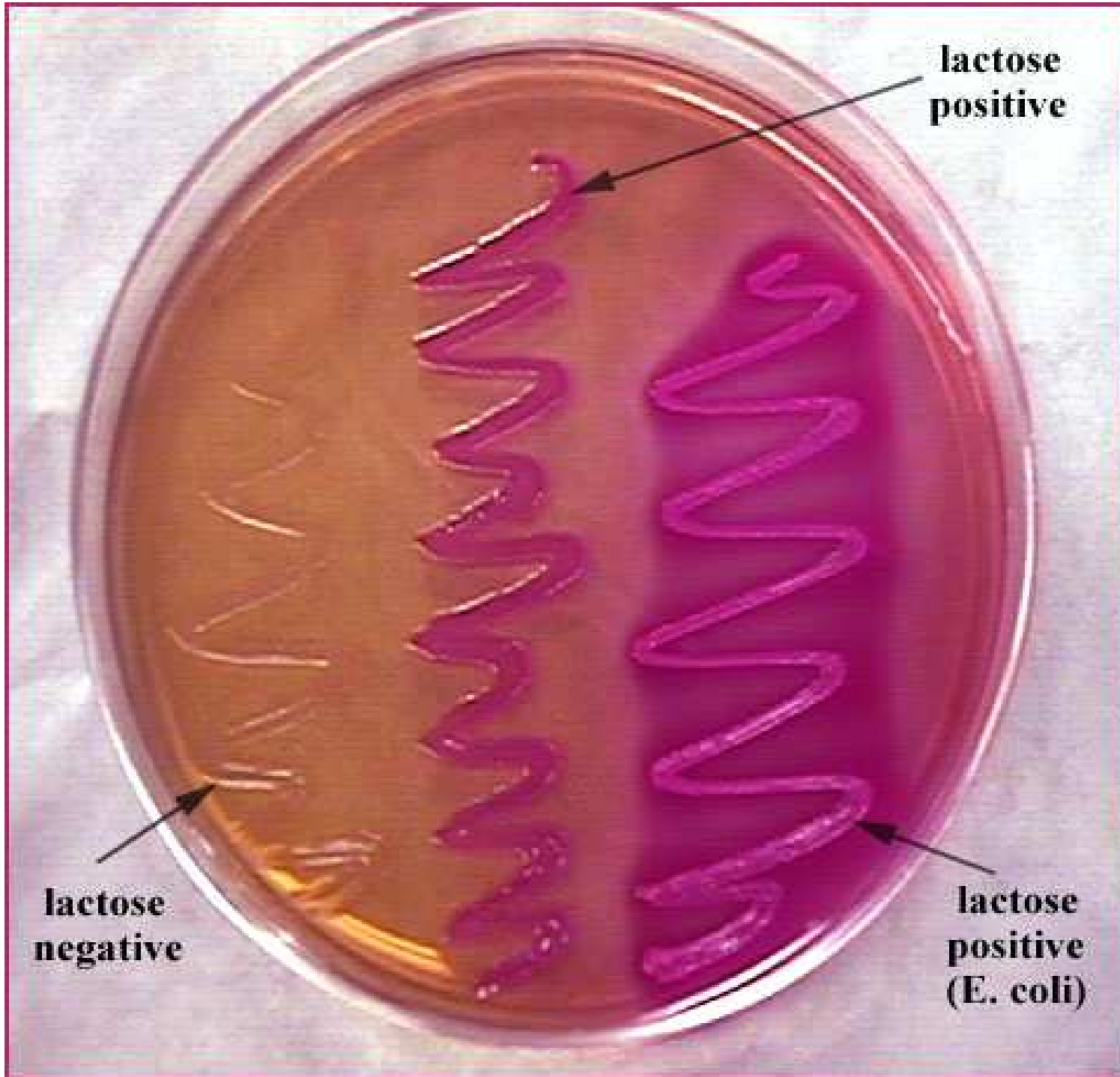
Culture Character

- Aerobic & facultative anaerobes.
- Grow well in ordinary media with a wide range of temperature.
- Grow best in strongly alkaline media
- On NA colonies are moist, translucent, round disk, 1-2 mm in diameter with a bluish tinge in transmitted light.



- On MacConkey agar colonies are pale, on prolonged incubation turn into pink.
- On BA greenish discoloration, later become clear due to haemodigestion.
- Special media : Transport media, Enrichment media, plating media
- Holding or transport media : 1)
Venkatraman-Ramkrishnan medium:





lactose positive

lactose negative

lactose positive (E. coli)

Colony Characteristics On MacConkey agar

- Contains 20gm common salt, 5gms peptone & 1000cc water, PH 8.6 to 8.8.
- It preserves *V.cholerae* & prevent the overgrowth.
- 2) Cary – Blair medium : buffered solution of Nacl, sodium thioglycollate, disodium phosphate & Calcium chloride at PH 8.4.



- Enrichment media :
- Alkaline peptone water at PH 8.6
- Monsur's taurocholate tellurite peptone water,PH 9.2.
- Both are good transport as well as enrichment media.



- Plating media :
- 1) Alkaline bile salt agar (BSA) PH 8.2: modified nutrient agar medium containing 0.5% sodium taurocholate.colonies are similar to those on NA.
- 2)TCBS medium : contains thiosulphate, citrate, bile salts & sucrose .
- Available commercially & at present very widely used. Cholera vibrio produce large yellow convex colonies which becomes green on continued incubation.





YELLOW
COLONY
ON
TCBS AGAR
OF
V.Cholerae

String test :

- A loopful of the growth is mixed with a drop of 0.5% sodium deoxycholate in saline.
- If the test is positive, the suspension loses its turbidity, becomes mucoid & forms a string when the loop is drawn away from the suspension.



Biochemical reaction :

- Cholera vibrios ferment glucose, mannitol, sucrose, maltose & mannose with production of acid without gas.
- Do not ferment inositol, arabinose, late lactose fermenter, Urease & MR –ve.
- Catalase & oxidase +ve.
- Indole +ve, reduces nitrate to nitrites.



- Cholera red reaction :when a few drops of sulphuric acid is added to 24hour peptone water culture, reddish color is developed due to the formation of nitrosoindole.
- VP test : +ve in EL Tor vibrio but negative in classical cholera vibrio.
- Hemolytic reaction: El Tor is hemolytic, classical is nonhemolytic



Resistance

- Susceptible to heat, drying & acids.
- Destroyed at 55° C in 15 minutes.
- El Tor vibrio survives longer than the classical cholera vibrio.
- Susceptible to the common disinfectants.
- Killed in a few minutes in gastric juice of normal acidity.



Classification

- Heiberg classified vibrios into six groups based on the fermentation of mannose, sucrose & arabinose.
- Two more groups were added later.
- Cholera vibrios belong to Group 1.



Classification

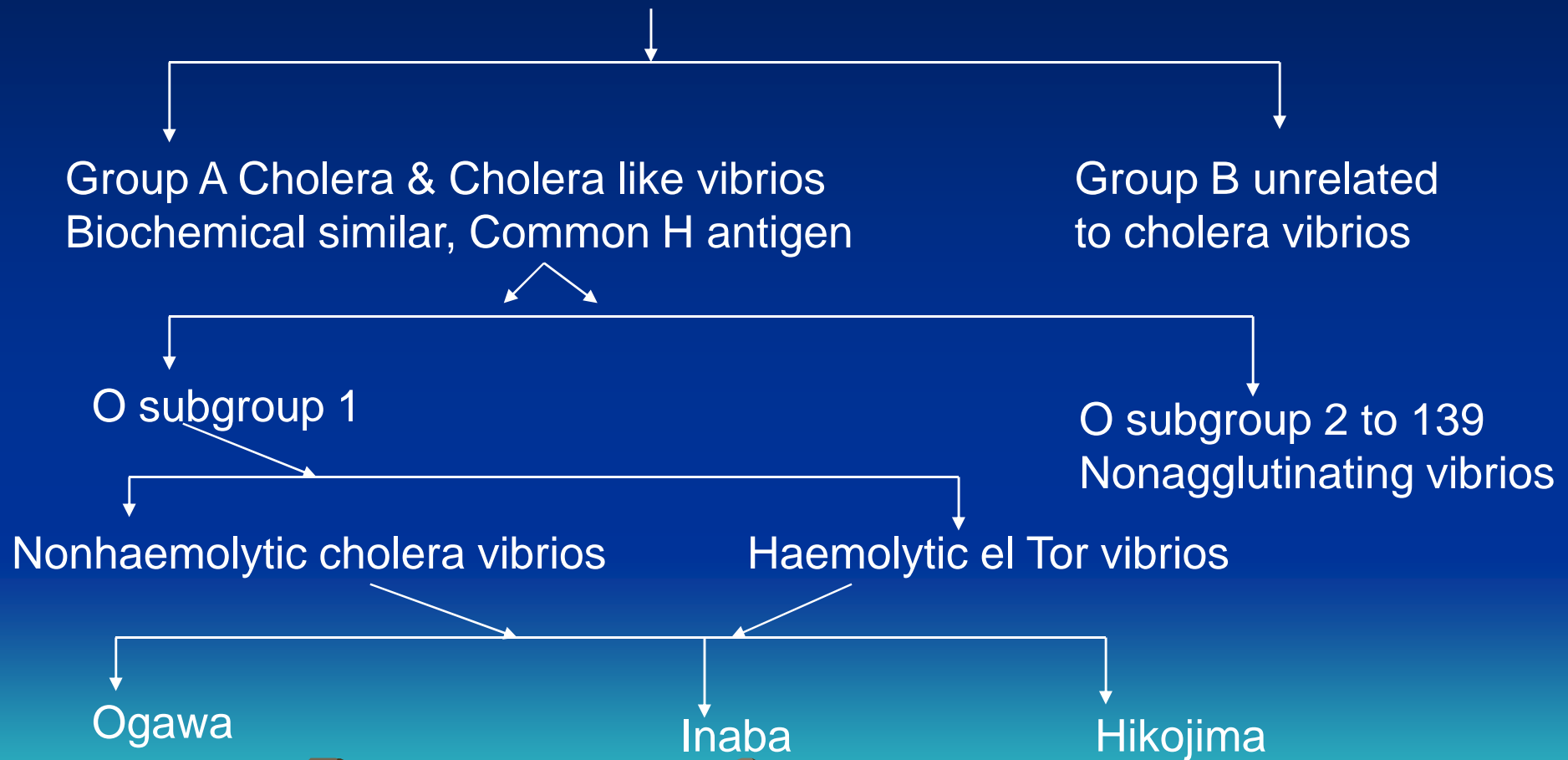
- Gardner & Venkataraman classified vibrios antigenically, divided into two groups
- Group A consisting of cholera vibrios & Biochemically similar vibrios possessing a common H antigen.
- Group B : heterogeneous collection of vibrios distinct from Group A biochemically & antigenically.



- Group A vibrios were divided into subgroups, 139 subgroup based on the O antigen. (O serogroup) , all sharing the common H antigen.
- All isolates from epidemic cholera(agglutinable vibrios) belonged to serogroup O -1.
- Nonagglutinable vibrios are not agglutinated by O -1 antiserum.



Antigenic classification of vibrios vibrio



- *V cholerae* O1 : both classical & El Tor cholera biotypes, antigenically indistinguishable .
- On the basis of minor differences in O antigen *V.cholerae* O1 subdivided into 3 subtypes- Ogawa, Inaba,Hikojima.
- El tor vibrio : Gotschlich isolated a vibrio from six Haj pilgrims at the El Tor quarantine station on the Sinai Peninsula in Arabia who had died of dysentery or gangrene of the colon.
- El tor vibrio differentiate with classical vibrio with biochemical reaction.



Difference b/w classical & el Tor vibrio

Test	Classical	El Tor
Haemolysis	-	+
Voges- proskauer	-	+
Chick erythrocyte agglutination	-	+
Polymyxin B sensitivity	+	-
Group IV phage susceptibility	+	-

- Non O1 vibrios appear to survive & multiply better than the *V.cholerae* O1 in a wide range of food, food borne outbreak are common with these organisms.
- *V.cholerae* O139 : a new serogroup reported in Oct 1992 during epidemic in Madras, producing epidemic of cholera.



- **Bacteriophage typing** : Phase typing schemes have been standardized for classical & El Tor vibrio as well as O – 139 vibrios.
- Classical strain of *V cholerae* classified into 5 types by using Mukherjee's phages. 4th phage lysed all the classical & non of the El Tor Strain.
- 5th phage was introduced later By Basu & Mukherjee which lysed all El Tor but no classical strain.



Phage type of classical V.Cholerae

Phage type of organism	1	2	3	4
1	+	+	+	+
2	-	+	+	+
3	+	-	+	+
4	-	-	+	+
5	+	+	-	+

Phage type of El Tor vibrios

Phage type of organisms	Sensitivity to phage group				
	1	2	3	4	5
1	+	+	+	+	+
2	+	+	+	-	+
3	+	+	-	+	+
4	+	+	-	-	+
5	+	-	-	-	+
6	-	+	-	-	+

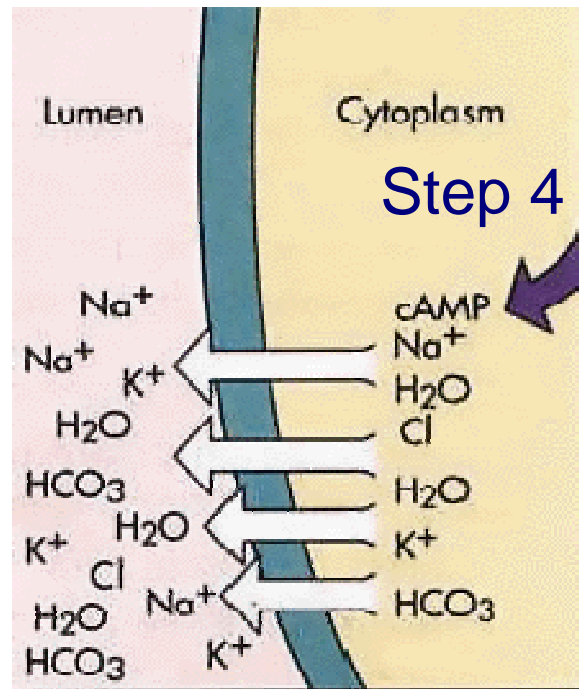
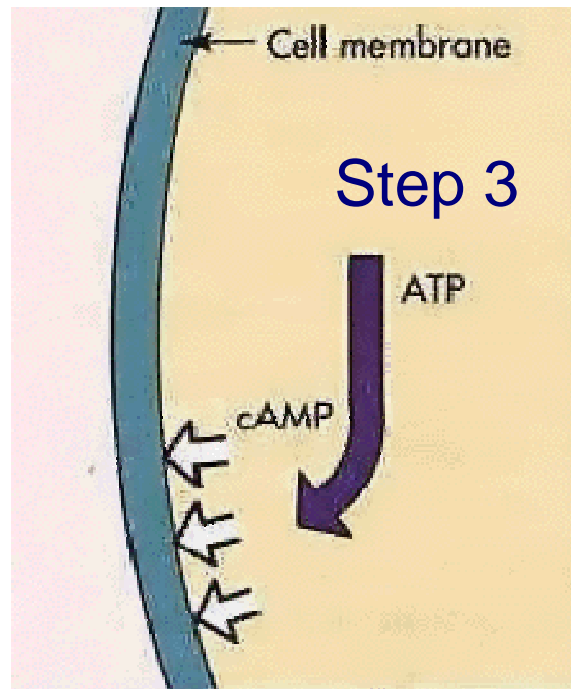
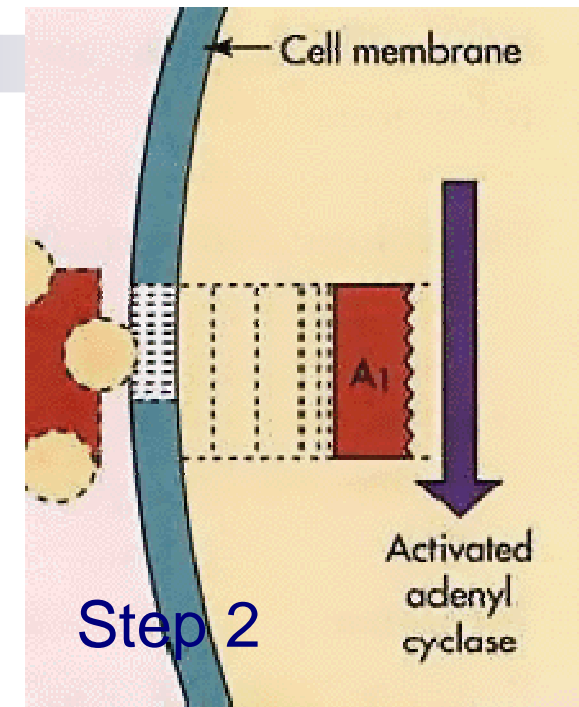
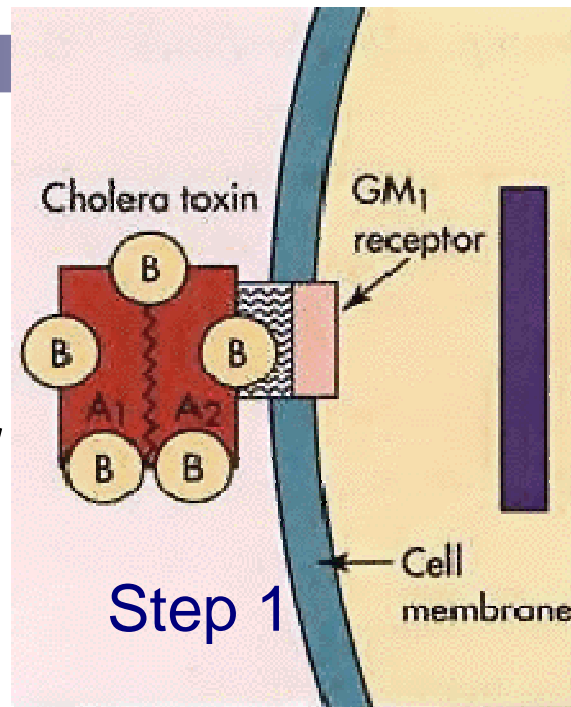
Pathogenesis

- Faeces & vomitus of human cases or carriers are the main source of infection.
- Human infection occurs by ingestion of contaminated water or food.
- Adherence & colonization : attachment to epithelium of microvilli at the brush border & multiply.
- Secretion of cholera toxin :

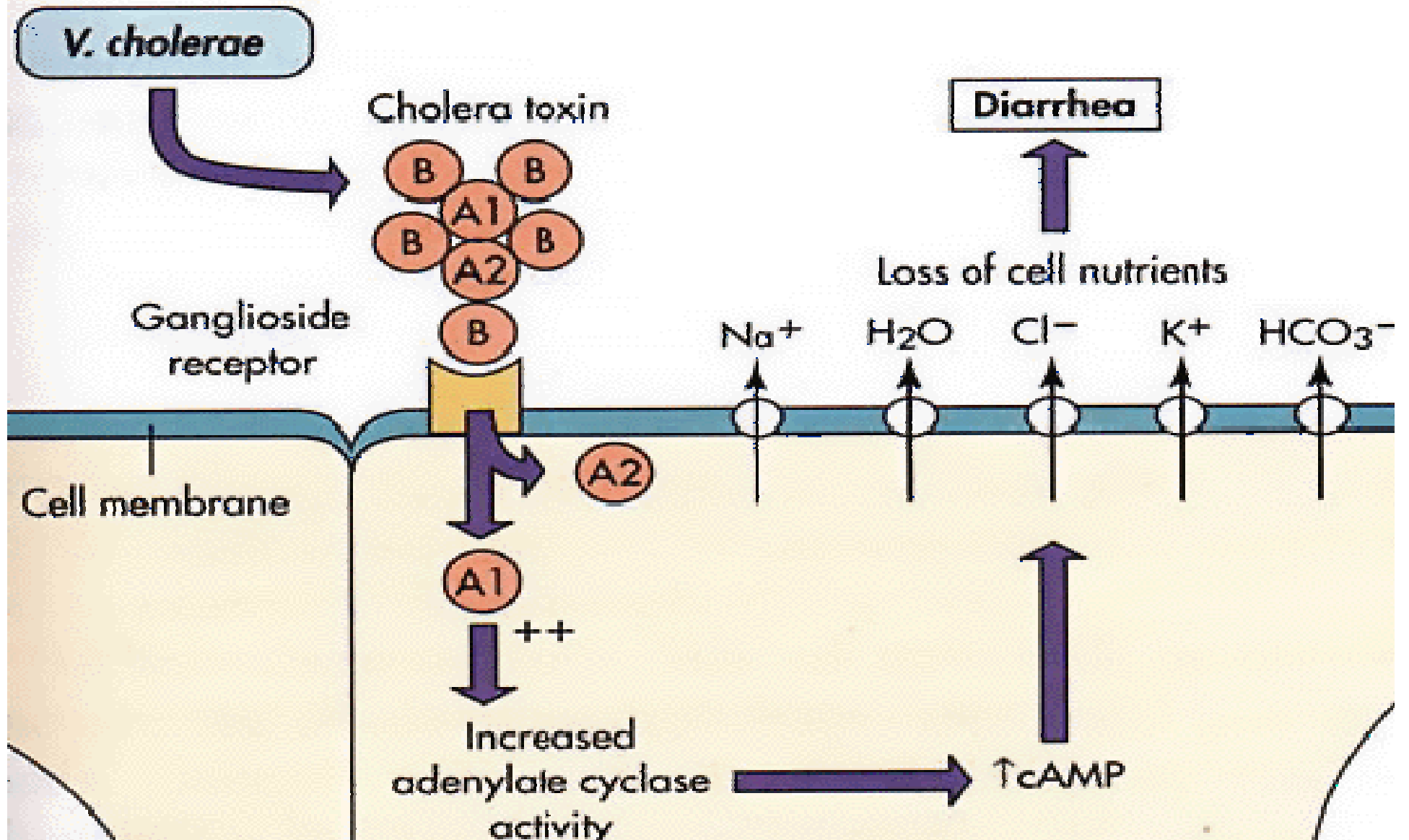


Mechanism of Action of Cholera Toxin

NOTE: In step #4, uptake of Na^+ and Cl^- from the lumen is also blocked. HCO_3^- = bicarbonate which provides buffering capacity



Mechanism of action of cholera toxin



Virulence Factor	Biologic Effect
Cholera toxin	Hypersecretion of electrolytes and water
Coregulated pilus	Adherence to mucosal cells
Accessory colonization	Adhesin factor
Hemagglutination-protease (mucinase)	Induces intestinal inflammation and degradation of tight junctions
Siderophores	Iron sequestration
Neuraminidase	Increase toxin receptors

Clinical syndrome: cholera is an acute diarrhoeal disease caused by *V.cholerae*.

- Severity of the disease of *V cholerae* 01 ranges from asymptomatic or inapparent to the most severe form.
- Symptoms results from the action of cholera toxin.
- Clinical features include vomiting, profuse rice water stools, followed by rapid dehydration & hypovolaemic shock.



Epidemiology

- Can occur as sporadic, endemic, epidemic or pandemic.
- Man is the only natural host of cholera vibrio.
- Faecal – oral transmission principally water borne.



– **7th pandemic:**

- ***V. cholerae* O1 biotype El Tor**
- Began in Asia in 1961
- Spread to other continents in 1970s and 1980s
- Spread to **Peru in 1991** and then to most of South & Central America and to U.S. & Canada
- By 1995 in the Americas, >106 cases; 104 dead

– **8th pandemic (??)**

- ***V. cholerae* O139 Bengal** is first non-O1 strain capable of causing epidemic cholera
- Began in India in 1992 and spread to Asia, Europe and U.S.
- Disease in humans previously infected with O1 strain, thus **no cross-protective immunity**



Laboratory Diagnosis

- Specimens: watery stool & mucous flakes from stool, rectal swab from contact or carriers
- Transport media : V R medium, Alkaline peptone water, Cary Blair medium.
- Microscopy : rapid Diagnosis by direct fluorescent antibody staining of smears from liquid stool, by demonstrating darting motility



- Culture :stool sample is directly plated to one or more selective media.
- Specimen enriched in enrichment media & then inoculated into plating media.
- TCBS : yellow sucrose fermenting colony,
- Identification by biochemical test
- Agglutination test : colonies are tested with V cholerae 01 antiserum. then with monospecific Ogawa & Inaba.
- NICED :National Institute of Cholera & Enteric Disease at Kolkatta.



- Serological tests : little use in the diagnosis of cholera, use in assessing the incidence of endemic foci.
- Detection of carriers : repeated stool culture is necessary as the vibrio is excreted intermittently.
- Gives better result after administration of a purgative or bile after duodenal intubation
- Serological test is useful in detecting chronic carriers.



Prophylaxis

General measures : purification of water & better provision for sanitary methods

Specific measures :

- Killed vaccines: classic killed parenteral vaccine contains 80million *V.cholerae*/ml with equal number of Inaba & Ogawa strain. Immunity is short lived.
- Nonliving oral vaccine :contain oral cholera toxin B subunit, classical *V.cholera* : Ogawa& Inaba,El Tor vibrio :Ogawa &Inaba,gives overall protection of about 80% of vaccinated persons for 2 years.




- Live oral vaccine:
- Recombinant DNA vaccine with expression of V.Cholerae 01 in attenuated strain of S Typhi Ty21a.
- Ty21 act as a carrier ,it does not cause significant side effects.
- Colonises Peyer's patches & induces IgA response by local immune system.



Treatment :

- Replacement therapy
- Antibiotics

Vibrio mimicus:

- Resembles cholera vibrio in biochemical features, sucrose negative.
 - Responsible for many sporadic cases of diarrhoeal disease on the Gulf coast of the USA.
 - Self limited disease, acquired from eating seafood especially oysters.
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Halophilic Vibrios

- Vibrios that have a high requirement of sodium chloride.
- Natural habitat is sea water & marine life
- *V.parahaemolyticus*, *V.alginolyticus* & *V.vulnificus*.
- *Vibrio parahaemolyticus* : It is an enteropathogenic vibrio 1st isolated in Japan in 1951 that causes food poisoning characterized by diarrhea.



- Morphologically resembles cholera vibrios except that it is capsulated, shows bipolar staining.
- Grows only in media containing NaCl.
- ON TCBS the colonies are green raised centre & Flat translucent periphery. The string test is positive.
- Oxidase, catalase, nitrate, indole, citrate positive.



Determinants of Pathogenicity:

- Kanagawa phenomenon :
- Strain from human patient are almost always hemolytic due to heat stable hemolysin.
- *V.parahaemolyticus* causes food poisoning associated with marine food.



- Diarrhea is self limiting.
- Cases are common in summer.& in adults than in children.

Vibrio Alginolyticus :

- Resembles *V.parahaemolyticus*,formerly considered a biotype of it.
- Ferments sucrose, has been associated with infection of eyes, ears & wounds in human beings exposed to sea water.



V. vulnificus:

- VP negative, ferments lactose but not sucrose.
- It produces wound infection .
- It also produces septicemia in compromised hosts particularly those with liver disease.

