Course: Clinical Microbiology

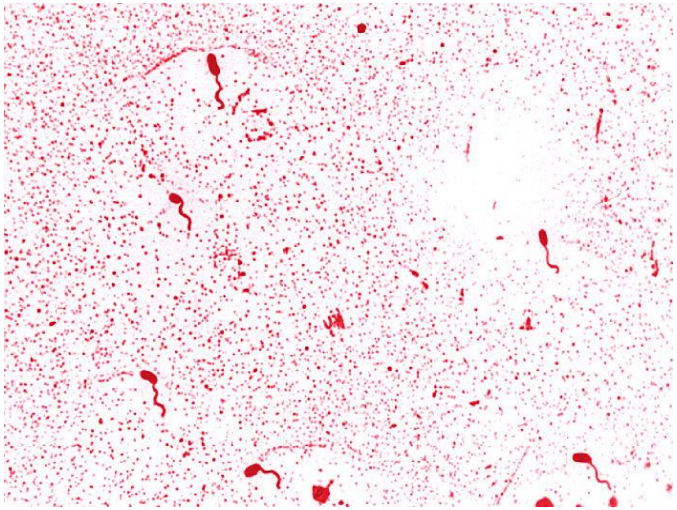
Introduction to vibrios

Dr.K. Sathiyamurthy
Assistant Professor
Department of Biomedical Science
Bharathidasan University

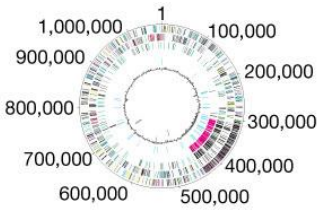
Vibrios

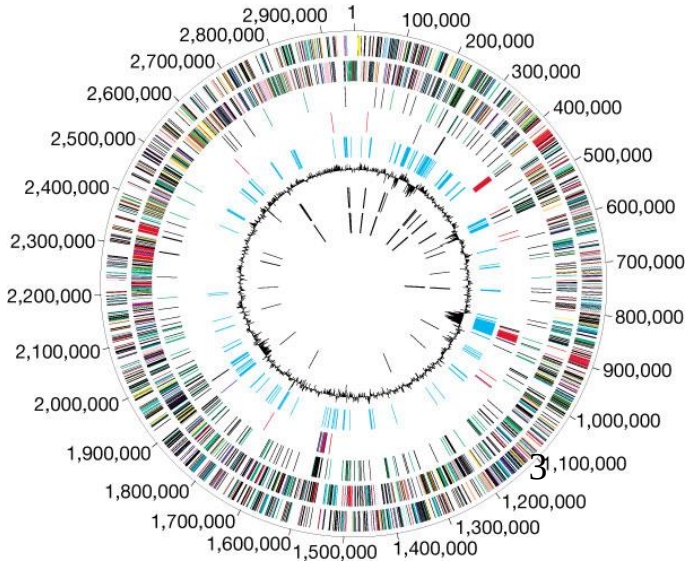
- **“Vibrio” is derived from vibrare, meaning to vibrate**
- ***Vibrio cholerae* -gastroenteritis**
- ***Vibrio parahaemolyticus* -gastroenteritis, wound infection, bacteremia**
- ***Vibrio vulnificus* -wound infection, bacteremia**

Vibrio sp.



- Gram-negative rods
- Curves or comma shaped
- Non-spore forming
- Highly motile-single polar flagella
- Associated with salt water
- Oxidase positive
- Facultative anaerobe
- Tolerate alkaline conditions to pH9.0
- Readily cultivated, Simple nutritional requirements





Vibrio cholerae

- **Antigenic structure**
  - **Common heat-labile flagellar "H" antigen**
  - **"O" lipopolysaccharide confers serologic specificity**
    - **More than 206 "O" antigen serogroups**
    - **Only O-1 and O139 serogroups cause Asiatic cholera**
    - **Three serotypes - Ogawa, Inaba and Hikojima**
    - **Two biovars - Classic and El Tor**

Classification Scheme

Toxigenic *V. cholerae*

Division into 2 epidemic serotypes

O1

Division into 2 biotypes

O139

Classical ***El Tor***

Each O1 biotype can have 3 serotypes

inaba **ogawa** **hikojima**

Division into ribotypes

A & C

A & B
(A little C) Antigens

A, B, C

Designed using information presented in review by NS Crowcroft. 1994. Cholera: Current Epidemiology. The Communicable Disease Report. 4(13): R158-R163.

VIBRIOS

Gp A

Common ‘H’

Different ‘O’

Gp B

Unrelated to Cholera vibrios

Serologically

O subgroup I

(*V. cholerae* 01)

O subgroups II-VI

(NAG vibrios or *V. cholerae* non-01[NCV])

Non-haemolytic cholerae vibrios (**Classical type**)

Voges-Proskauer -ve (VP)

Based on minor differences in 01

Ogawa Inaba Hikogima

Haemolytic El Tor vibrios

Voges-Proskauer +ve (VP)

Based on minor differences in 01

Ogawa Inaba Hikogima

V. cholerae - Transmission

feces

↓

water

– fresh

– salt

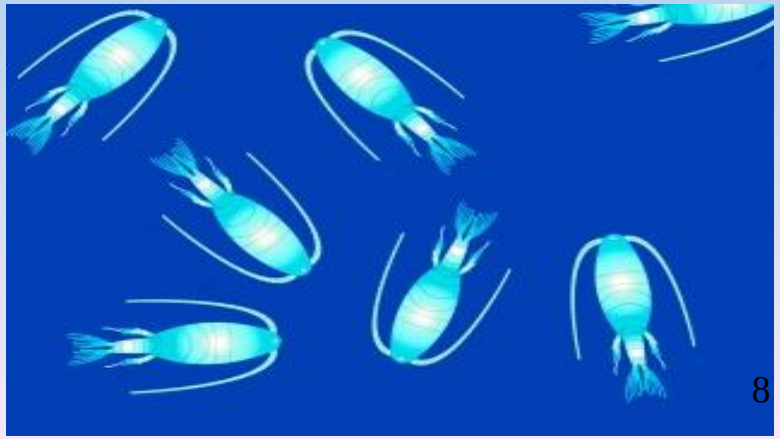
↓

food

Water (infectious dose = 10^9)
Food (infectious dose = 10^3)
Person-to-person

Vibrio cholerae

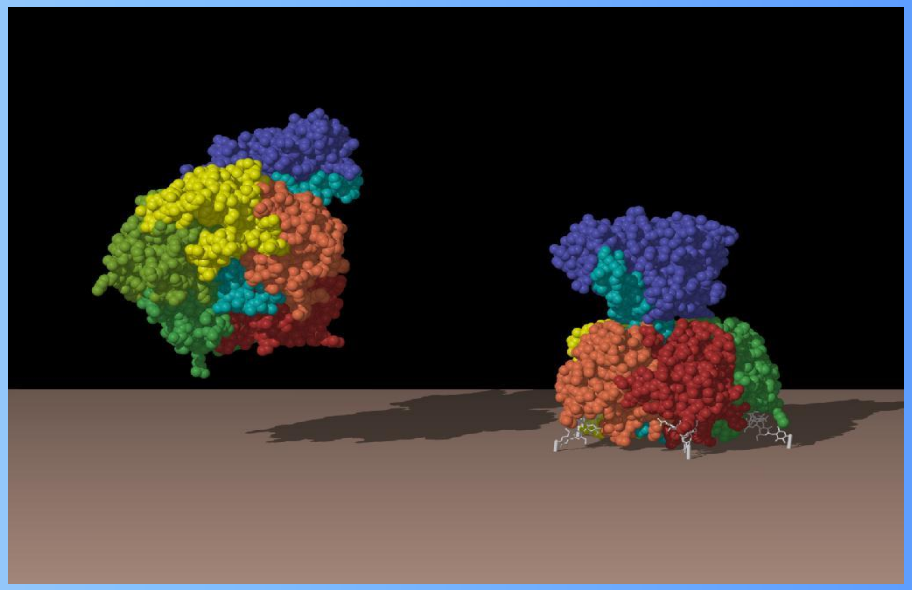
- **Epidemiology**
  - **Epidemic cholera-spread by contaminated water under conditions of poor sanitation**
  - **Endemic-consumption of raw seafood**
  - **Copepods**



Vibrio cholerae

- **Pathogenesis**
  - **Ingest 10^8-10^{10} organisms**
  - **Non invasive infection of small intestine**
  - **Organisms secrete enterotoxin**
  - **Watery diarrhea**

Cholera toxin

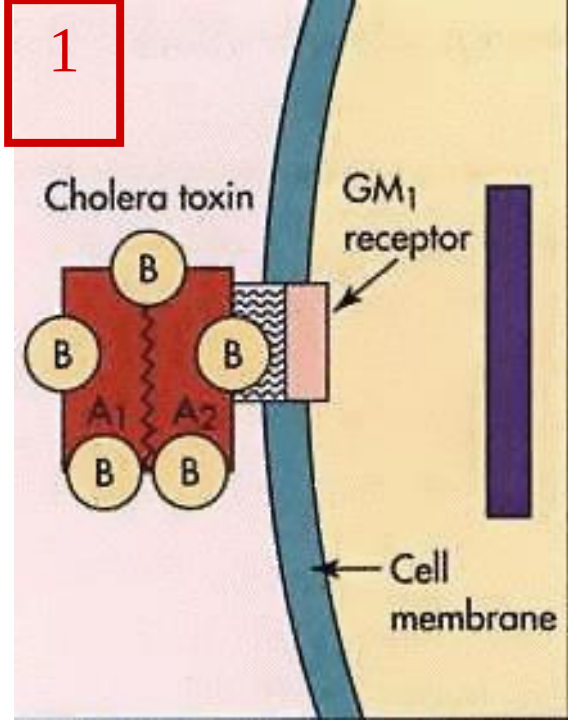


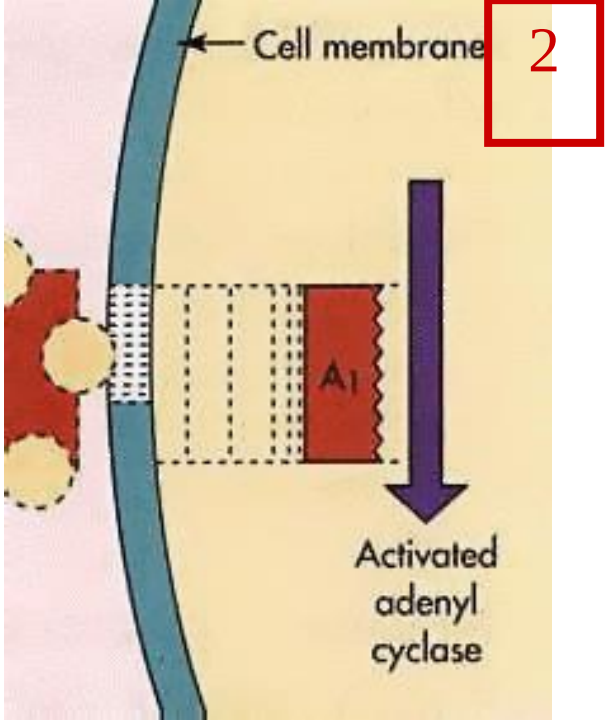
- **Enterotoxin-cholera toxin-CtxAB**
  - **Encoded by a prophage**
  - **Molecular mass of 84,000 daltons**
  - **A subunit-ADP-ribosylating toxin**
  - **B subunit-bind GM1-gangliosides on enterocytes**
  - **A subunit ADP ribosylates Gs-alpha which regulates activation of adenlyate cyclase**
  - **Result is persistent increase in cAMP levels**
  - **Hyper secretion of Na, Cl, K, bicarbonate and H_20**

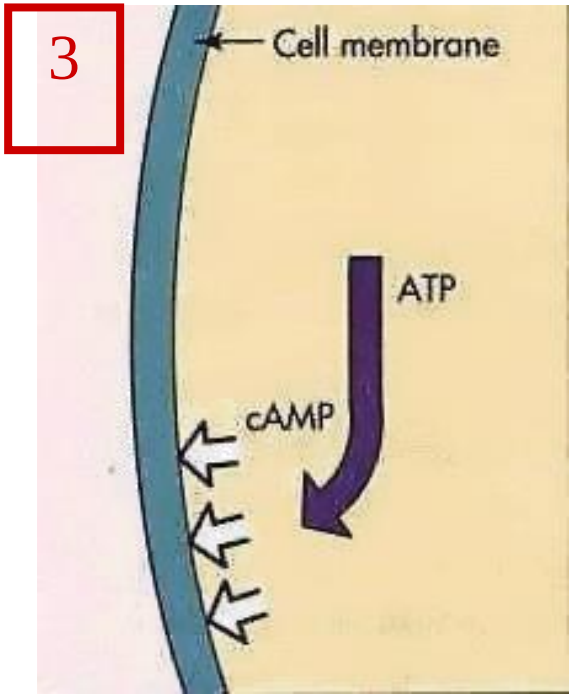
Cholera Toxin (A2-5B)(Vibrio cholerae)

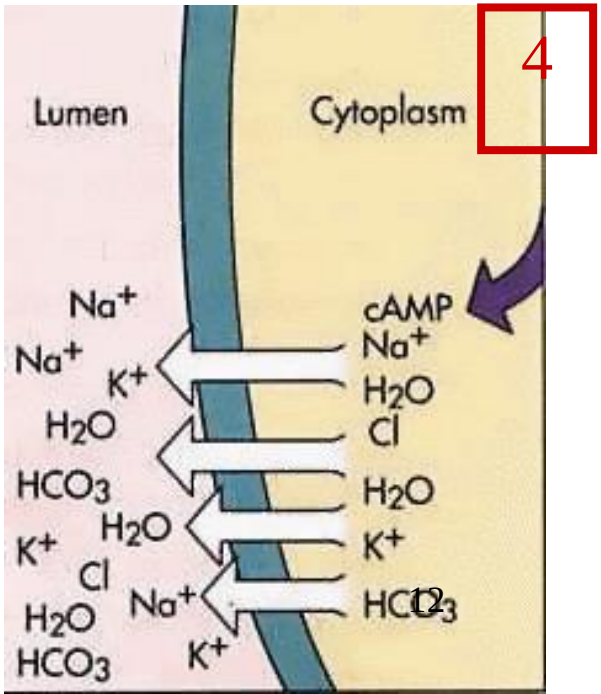
- Chromosomally-encoded; Lysogenic phage conversion; Highly conserved genetic sequence
- Structurally & functionally similar to ETEC LT
- B-subunit binds to GM_1 ganglioside receptors in small intestine
- Reduction of disulfide bond in A-subunit activates A_1 fragment that ADP-ribosylates guanosine triphosphate (GTP)-binding protein (G_s) by transferring ADP-ribose from nicotinamide adenine dinucleotide (NAD)
- ADP-ribosylated GTP-binding protein activates adenyl cyclase leading to an increased cyclic AMP (cAMP) level and hypersecretion of fluids and electrolytes

Mechanism of Action of Cholera Toxin





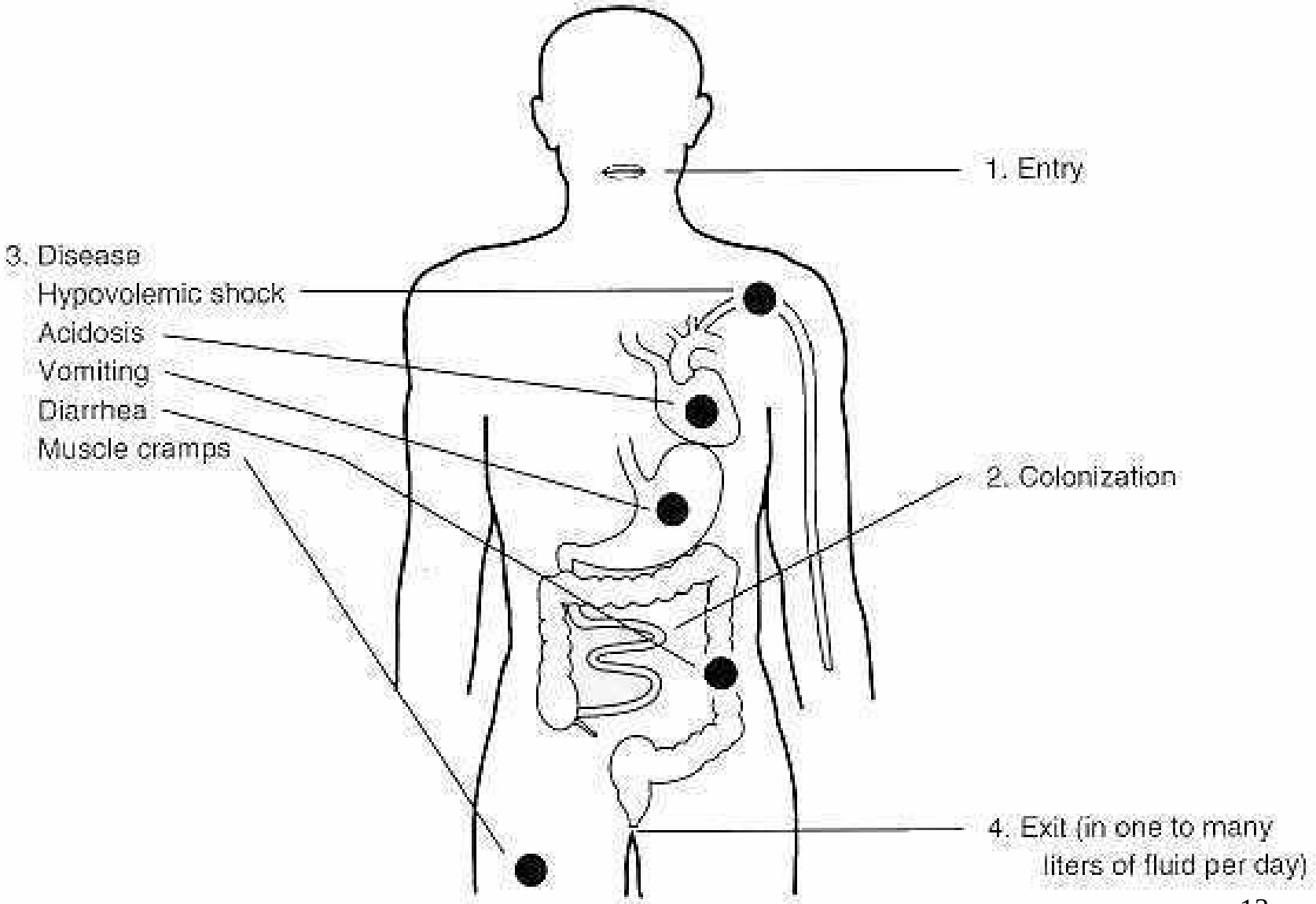




NOTE: In step #4, uptake of Na^+ and Cl^- from the lumen is also blocked.

HCO_3^- = bicarbonate which provides buffering capacity.

Clinical manifestations



Pathogenicity of V. cholera

- **Dehydration and death**
- **Massive secretion of ions/water into gut lumen**

Immunity

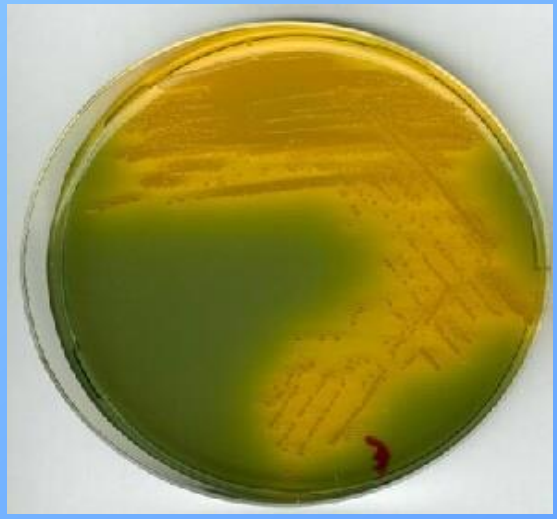
- **Strong immunity after recovery, SIgA**

Cultural characteristics

- **Aerobic; optimum temp(37°C)**
- **pH 6.4-9.6**
- **Colonies are moist, translucent, round disk, about 1-2mm in diameter on TCBS**
- **Holding /transport media: Venkatraman-Ramakrishnan(VR)medium &Cary-Blair medium**
- **Enrichment media: Alkaline peptone water**
- **Monsur's taurocholate tellurite peptone water**

Cultural characteristics

- **Selective media: Monsur's gelatin taurocholate trypticase tellurite agar (TTGA) –small translucent with greyish black centred**
- **Thiosulphate citrate bile salt sucrose (TCBS) –yellow convex colonies**

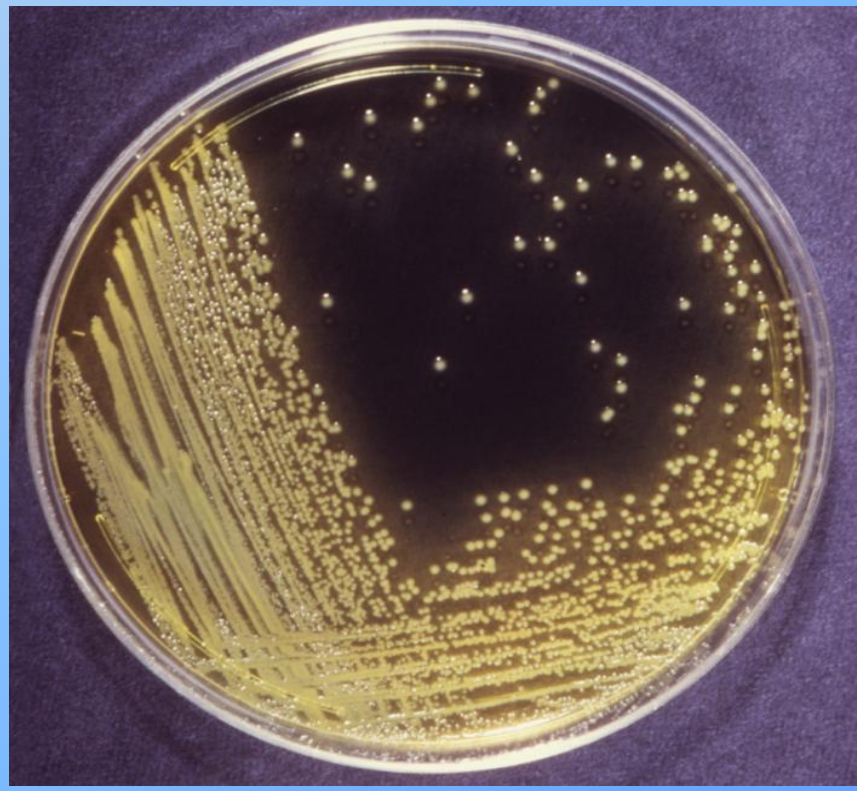


Biochemical reactions

- **Biochemical reactions:** ferment glucose, mannitol, maltose, mannose and sucrose but not inositol, arabinose, or lactose
- Catalse & oxidase +ve
- IMViC = (+ - - -);Urease –ve; TSI = acid butt but no gas; Gelatin is liquefied
- LAO = Lysine,Arginine, ornithine (+ - +)

Bacteriological Diagnosis

- **Specimens: stool, vomitus.**
- **Stained smear**
- **Culture: alkaline peptone water of agar plate, and TCBS agar plate.**
  - **Quick immunological methods: immunofluorescent “ball” test; Molecular methods – PCR & DNA probes**



V. parahaemolyticus

- Between 1973 and 1998, a total of 40 outbreaks of *V. parahaemolyticus* infection in the U.S.A. were reported to the CDC with >1000 persons involved
- *V. parahaemolyticus* are presently 12 O (LPS) antigens and 59 K (capsular polysaccharide) antigen recognized

V. parahaemolyticus

- A apecial consideration in the taxonomy of *V. parahaemolyticus* is the ability of certain strains to produce a hemolysis, called the TDH, or Kanagawa positive hemolysin, which is linked to virulence in the species
- *V. parahaemolyticus* occurs naturally in estuarine waters throughout the world

V. parahaemolyticus

- The last source includes at least 30 different species, among them clams, oysters, lobster, scallops, shrimp, and crab
- Gastroenteritis from *V. parahaemolyticus* is almost exclusively associated with seafood that is consumed raw, inadequately cooked, or cooked but recontaminated

V. parahaemolyticus

- *V. parahaemolyticus* has generation times of 8 to 9 min at 37°C and 12 to 18 min in seafood; hence, *V. parahaemolyticus* has the ability to grow rapidly, both in vitrio and in vivo, contributing to the infection dose required for illness
- Sympto`ms may begin 4 to 30 h after the ingestion of contaminated food, with a mean onset time of 23.6 h.

V. parahaemolyticus

- Primary symptoms include diarrhea and abdominal cramps, along with nausea, vomiting, and fever
- The symptoms subside in 3 to 5 days in most individuals
- Approximately 10^5 to 10^7 CFU is required for illness
- The number of *V. parahaemolyticus* organisms present in fish and shellfish are usually no greater than 10^4 cells, suggesting that temperature abuse of contaminated food occurs prior to consumption

V. parahaemolyticus

- *V. parahaemolyticus* possesses at least three hemolytic components; a thermolabile hemolysin gene (*tlh*), a TDH gene (*tdh*), and a TDH-related gene (*trh*), linked to disease
- Several adhesive factors have been proposed, including the outer membrane, lateral flagella, and pili and a mannose-resistant, cell-associated hemagglutinin

V. vulnificus

- *V. vulnificus* is the most serious of the pathogenic vibrios in the United States; it is responsible for 95% of all seafood-borne deaths
- Among the susceptible population at risk for infection by this bacterium, primary septicemia cases resulting from raw oyster consumption typically have fatality rates of 60%; it is the highest death rate for any foodbrone disease

V. vulnificus

- The bacterium can produce wound infections in addition to gastroenteritis and primary septicemias
- Wound infections carry a 20 to 25% fatality rate and are also seawater and/or shellfish associated
- Surgery is usually required to clean the infected tissue The isolation of *V. vulnificus* from blood samples is straightforwards, as the bacterium grows readily on TCBS, MaConkey, and Blood agars
- Isolation from the environment is much more difficult.

V. vulnificus

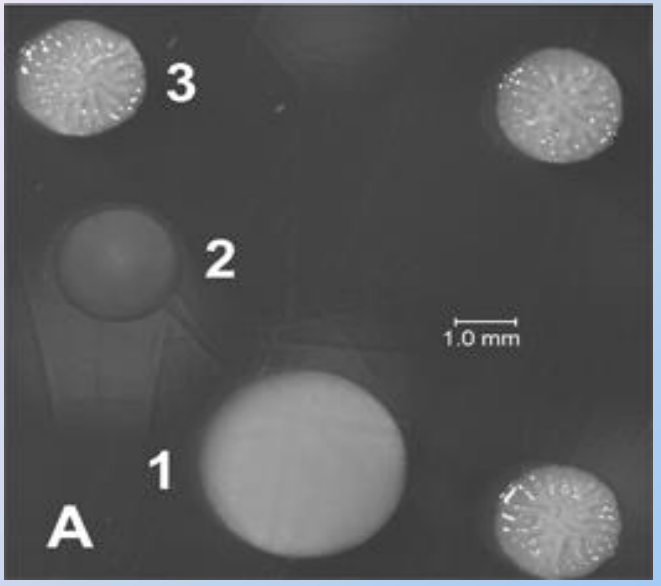
- *V. vulnificus* is widespread in estuarine environments to location around the world
- The presence of *V. vulnificus* in water is not associated with the present of fecal coliforms
- *V. vulnificus* is seldom isolated from water or oysters when water temperature are low
- In most cases *V. vulnificus* illness occurs during the months of April through October, when the water is warmer

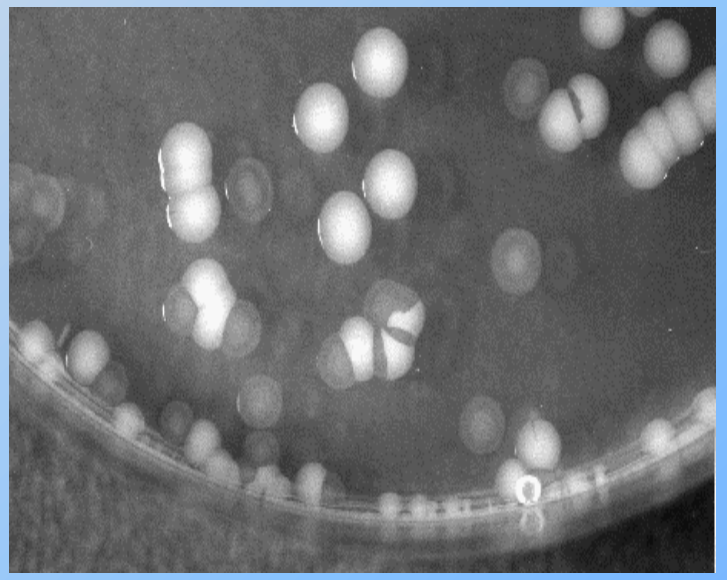
V. vulnificus

- Symptoms typical of gastroenteritis-abdominal pain, vomiting, and diarrhea-are not common
- The infectious dose of *V. vulnificus* is not known
- *V. vulnificus* is susceptible to most antibiotics
- The polysaccharide capsule, LPS, and a large number of extracellular compounds contribute to virulence

V. vulnificus

- Avirulence strains produce “translucent” acapsular colonies
- Symptoms which occur during *V. vulnificus* septicemia, including fever, tissue edema, hemorrhage, and especially the significant hypertension, are those classically associated with endotoxic shock caused by this gram-negative bacteria





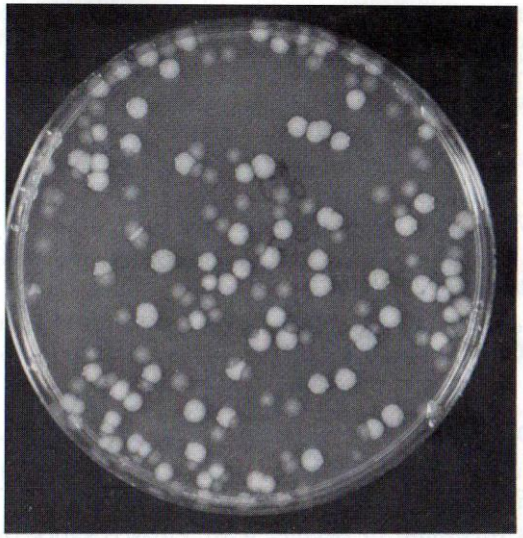


Figure 12.2 Opaque (encapsulated) and translucent (acapsular) colonies of *V. vulnificus*. Virulent strains are generally encapsulated, whereas nonencapsulated cells are usually avirulent. Reprinted from L. M. Simpson, V. K. White, S. F. Zane, and J. D. Oliver, *Infect. Immun.* **55:**269–272, 1987, with permission.

Visible Symptoms

- These include:
  - Sunken eyes and cheeks
  - Decreased skin suppleness
  - Dry mucous membranes
  - Urine production is sharply decreased or stopped altogether
  - Renal failure is the most common

Treating Cholera

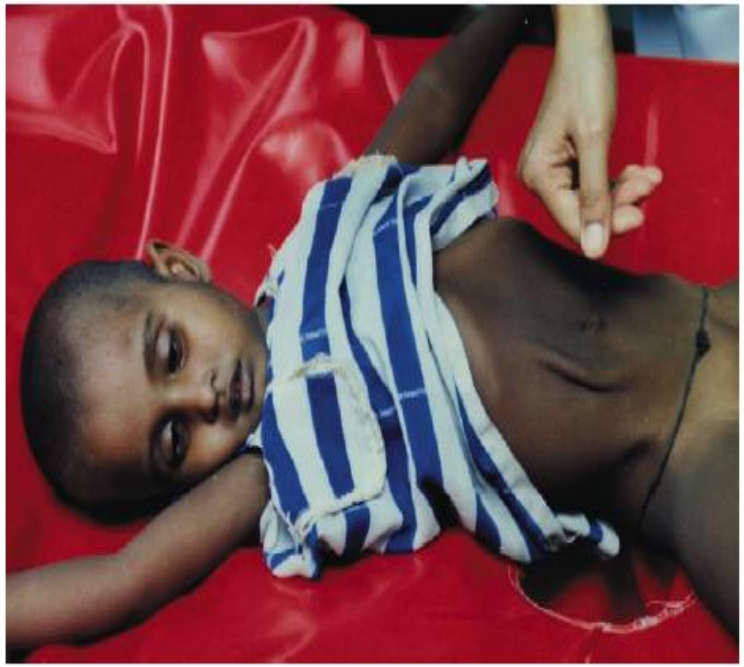


Figure 2: **A child, lying on a cholera cot, showing typical signs of severe dehydration from cholera**
The patient has sunken eyes, lethargic appearance, and poor skin turgor, but within 2 h was sitting up, alert, and eating normally.

Management of patients with suspected cholera

Assess for dehydration.

Rapidly rehydrate the patient with intravenous Ringer's solution for severely dehydrated patients or ORS for those with less severe dehydration; use rice-based ORS if possible.

Severely dehydrated patients require replacement of 10% of their bodyweight within 2–4 h.

Use cholera cot (if possible) to monitor stool output; monitor status of hydration and monitor severity of purging frequently.

Maintain hydration by replacing continuing fluid losses until diarrhoea stops.

Give an oral antibiotic (eg, doxycycline) to dehydrated patients as soon as vomiting stops.

Provide food as soon as patient is able to eat (within a few hours).

Sack, David, *et al*. 2004. Seminar: Cholera. The Lancet. 363: 223-233.

Preventing Cholera: Vaccines

- Orochol
  - Contains $2x10^8$ viable cells of attenuated strain CVD 103-HgR in a lyophilized form
  - Oral immunization of children older than 2
  - Subunit A of the cholera toxin (CT) has been removed
- Dukoral
  - Protects against O1 Inaba and Ogawa, Classical & El Tor strains
  - Contains 1x10 heat/formalin killed cells of strain WC/rBS

Image from: http://www.pharmeragroup.com/dukoralb.htm

Epidemic Control Measures

- Hygienic disposal of human waste
- Adequate supply of water
- Good food hygiene
  - Thoroughly cooking food
  - Eating food while it's hot
  - Preventing cooked foods from contacting raw foods (including water or ice)
  - Avoiding raw fruits or vegetables
  - Washing hands after defecation & before cooking

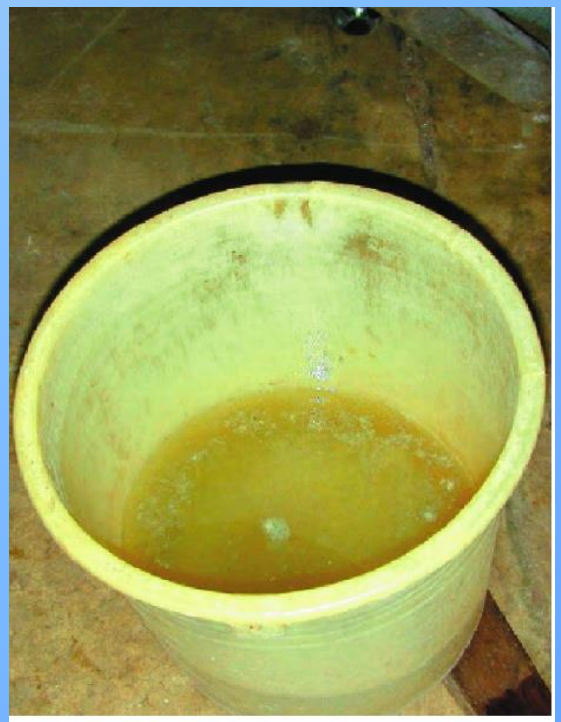
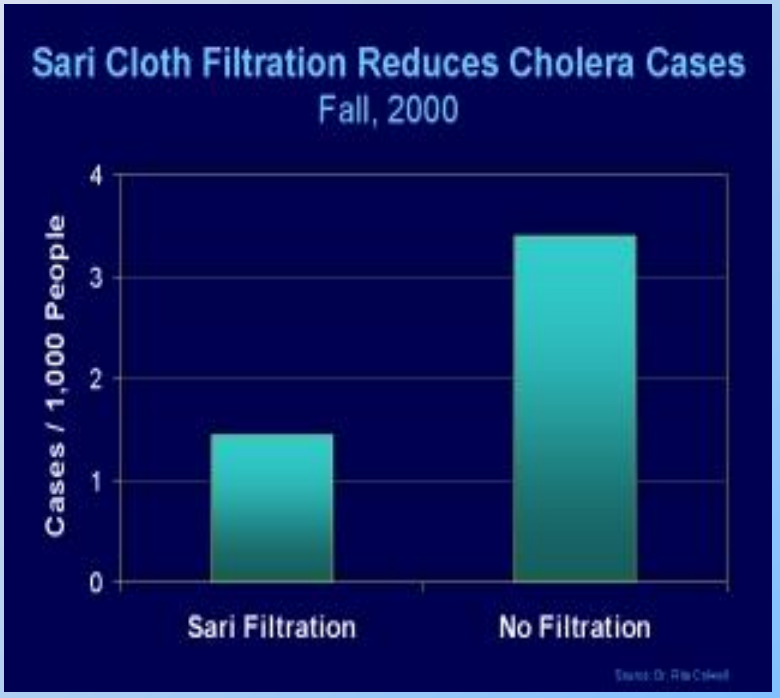


Figure 1: **Bucket with typical rice-water stool from a patient with cholera**

Sack, David, *et al.* 2004. Seminar: Cholera. The Lancet. 363: 223-233.

Sari Cloth Filtration: Preventive Measure



Using Sari cloth to filter Water

Thank you