



دانشگاه اصفهان

دانشکده علوم و فناوری‌های زیستی، گروه زیست‌شناسی
سلولی و مولکولی، آزمایشگاه میکروبیولوژی

آزمایشگاه باکتری شناسی ۲

آشنایی با خصوصیات کلی، رنگ آمیزی و مشاهده باکتری ویبریو

رنگ آمیزی گرم و آشنایی با خصوصیات میکروسکوپی و ماکروسکوپی باکتریهای جنس اسپیتوباکتر
رنگ آمیزی

VIBRIO



- Gram-negative, rigid
- Curved rods or comma shaped
- Highly motile-single polar flagella
- They are asporogenous & noncapsulated
- Vibrios are present in marine environments & surface waters worldwide.
- The most important member of the genus is *Vibrio cholerae*



تپسه کننده: سهیلا عباسی



- Filippo Pacini discovered cholera in 1854.
- He discovered and studied the bacteria with a microscope.





Discovery



- **Filippo Pacini (1812-1883)**
 - 1854: Cholera reaches Florence, Italy. Pacini discovers causative agent
 - Publishes “Microscopical Observations and Pathological Deductions on Cholera”
 - 1965: Bacterium named *Vibrio cholerae Pacini 1854*



Robert Koch Isolates *V.cholrae* 1883



تپسه کننده: سهیلا عباسی

- It was first isolated by Koch (1883) from cholera patients in Egypt.

Medically important species



- Vibrio cholerae*
- Vibrio parahaemolyticus*
- Vibrio vulnificus*



Vibrio cholerae

Morphology

- Gram negative
- rigid, curved rods that are actively motile
- Comma shaped
- Sheathed, polar flagellum
- About $1.5 \times 0.2-0.4 \mu\text{m}$ in size



Physiology

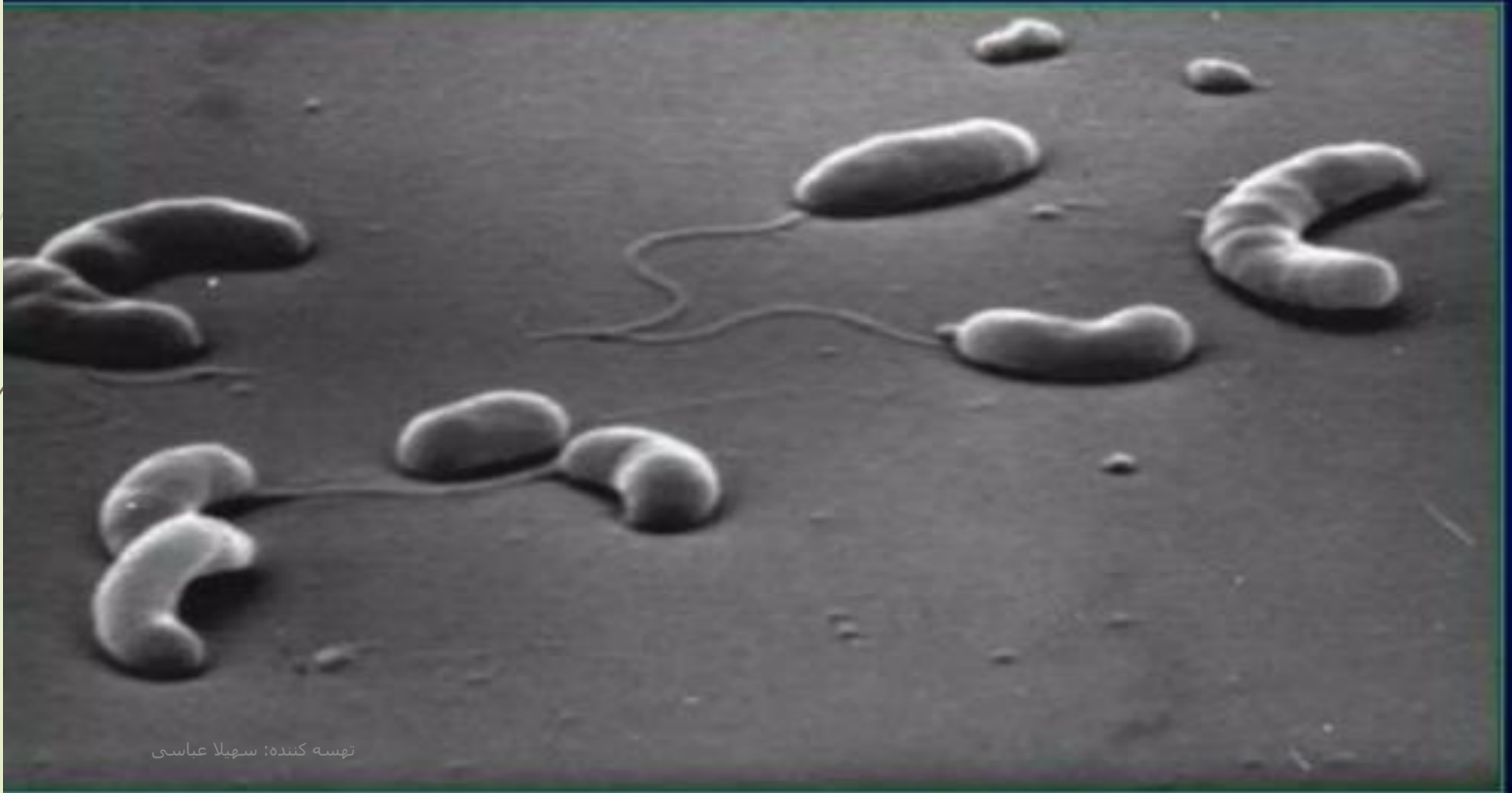
- Facultative anaerobic
- Asporogenous & noncapsulated.
- Growth stimulated by NaCl
- pH 6.4 – 9.6 (optimum 8.2).
Acid labile
- Temperature 16 - 40°C
(Optimum 37°C)



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Vibrio cholerae

curved rods with polar flagellum (EM)



Vibrio cholerae

Gram-negative, curved rods

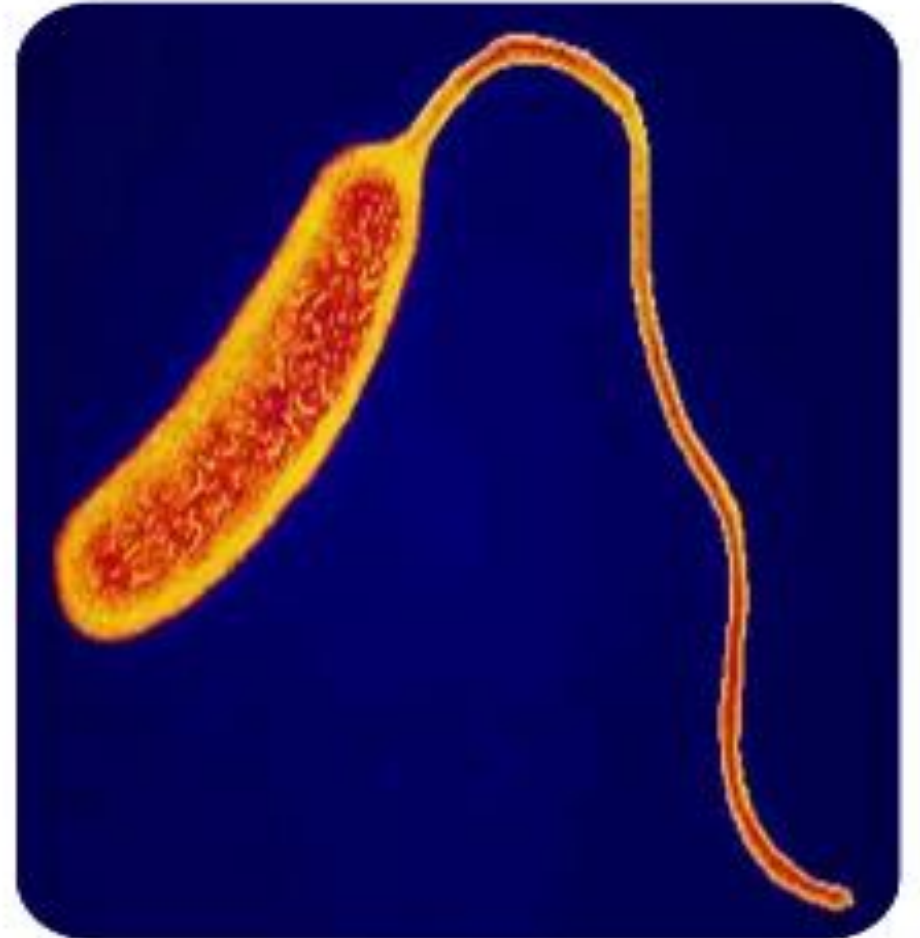


توسعه کننده: سهیلا عباسی

Morphology



- The *V. cholerae* is a **short, curved, cylindrical, rod**, about $1.5 * 0.2-0.4 \mu\text{m}$ in size, with rounded or slightly pointed ends.
- It is actively motile, with a single sheathed polar flagellum.



Cultural characteristics



- *V. cholerae* is a facultative anaerobic organism.
- It grows within a temperature range of 16-40⁰c (optimum 37⁰c)
- Growth is better in an alkaline medium, the range of pH being 6.4 – 9.6(optimum 8.2).
- It grows well on ordinary media.

Selective Medium – TCBS



V.cholrae grows well on Thiosulphate citrate bile sucrose (TCBS) agar, on which it produces yellow colonies that are readily visible against the dark green background of the agar.



Biochemical reactions

- Carbohydrates metabolism is fermentive, producing acid, but no gas.
- Cholera vibrio **ferment** glucose, mannitol, maltose, mannose, & sucrose **but not** inositol, arabinose or lactose.
- Indole is formed & nitrates are reduced to nitrites. These two properties contribute to the '*cholera red reaction*'
- Catalase & oxidase tests are +ive.
- Methyl red & urease tests are -ive

Resistance

- *C. vibrio* are susceptible to heat, drying & acids, but resist high alkalinity.
- They are destroyed at 55 degree C in 15 minutes.
- They survive in clean water for 30 days.
- On fruits, they survive for 1-5 days at room temperature & for a week in the refrigerator.

Cholera



- **Cholera** is an infection of the small intestine caused by the bacterium *Vibrio cholerae*.
- The main symptoms are watery diarrhea and vomiting. This may result in dehydration and in severe cases grayish-bluish skin.
- **Transmission** occurs primarily by drinking water or eating food that has been contaminated by the feces (waste product) of an infected person, including one with no apparent symptoms.

- The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance, and death in some cases.
- The primary treatment is oral rehydration therapy, typically with oral rehydration solution, to replace water and electrolytes.
- Worldwide, it affects 3–5 million people and causes 100,000–130,000 deaths a year as of 2010.

- Diarrhea
- Vomiting
- Dehydration
- Irritability
- Drowsiness
- Droopy eyes
- Having a dry mouth with extreme thirst
- Dry/shriveled skin
- Decrease in urination
- A very low blood pressure
- An irregular heart beat



Pathogenesis

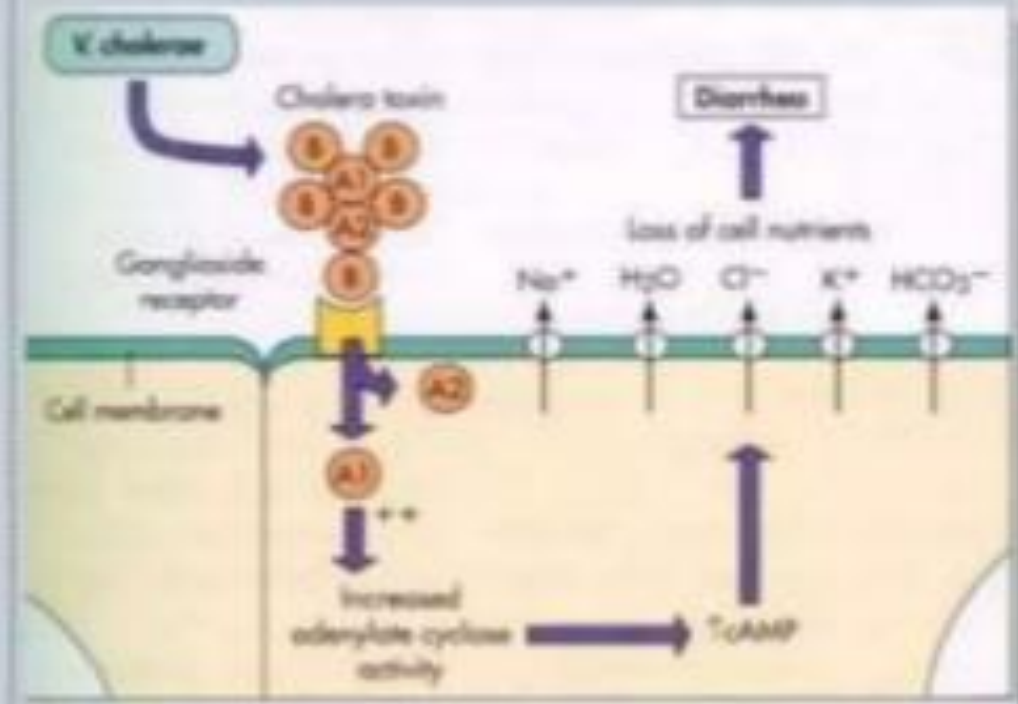


- Natural infection with cholera occurs only in humans & not in animals.
- When bacteria are consumed with food few organism as much as $10^2 - 10^4$ organisms are adequate to cause an attack because of the buffering capacity of the food.
- Any medication or conditions that decreases stomach acidity makes a person more susceptible to infection with V.cholrae.



Vibrio Cholerae

- **Vibrio Cholerae** enterotoxin activates the stimulatory Gs protein via ADP-ribosylation. This stimulates secretion of chloride ions and water from enterocytes into the small intestines, and causing watery diarrhea.





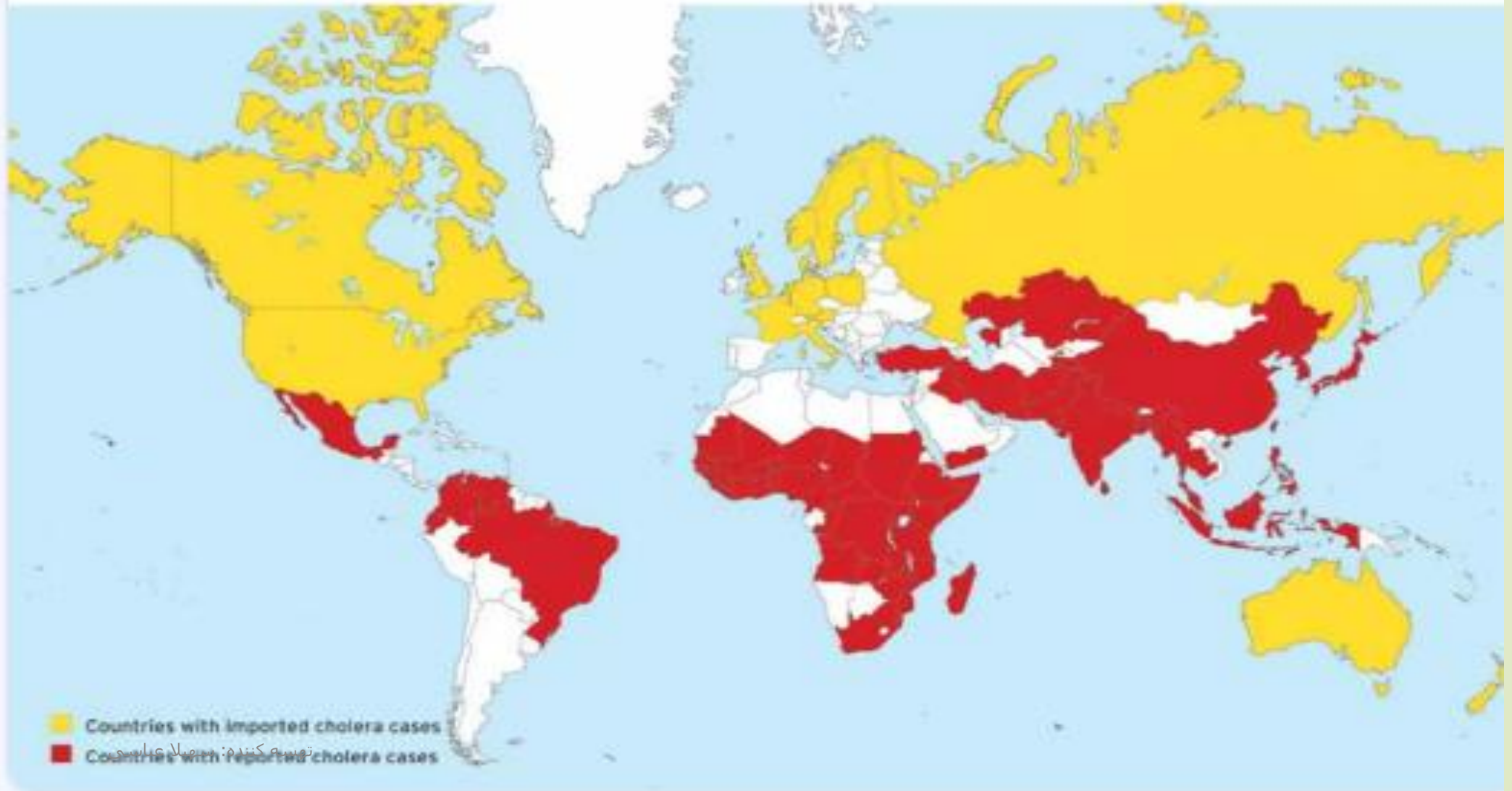
Epidemiology



Ingestion of *V. cholera* contaminated water is a typical mode of pathogen transmission. Provider CDC

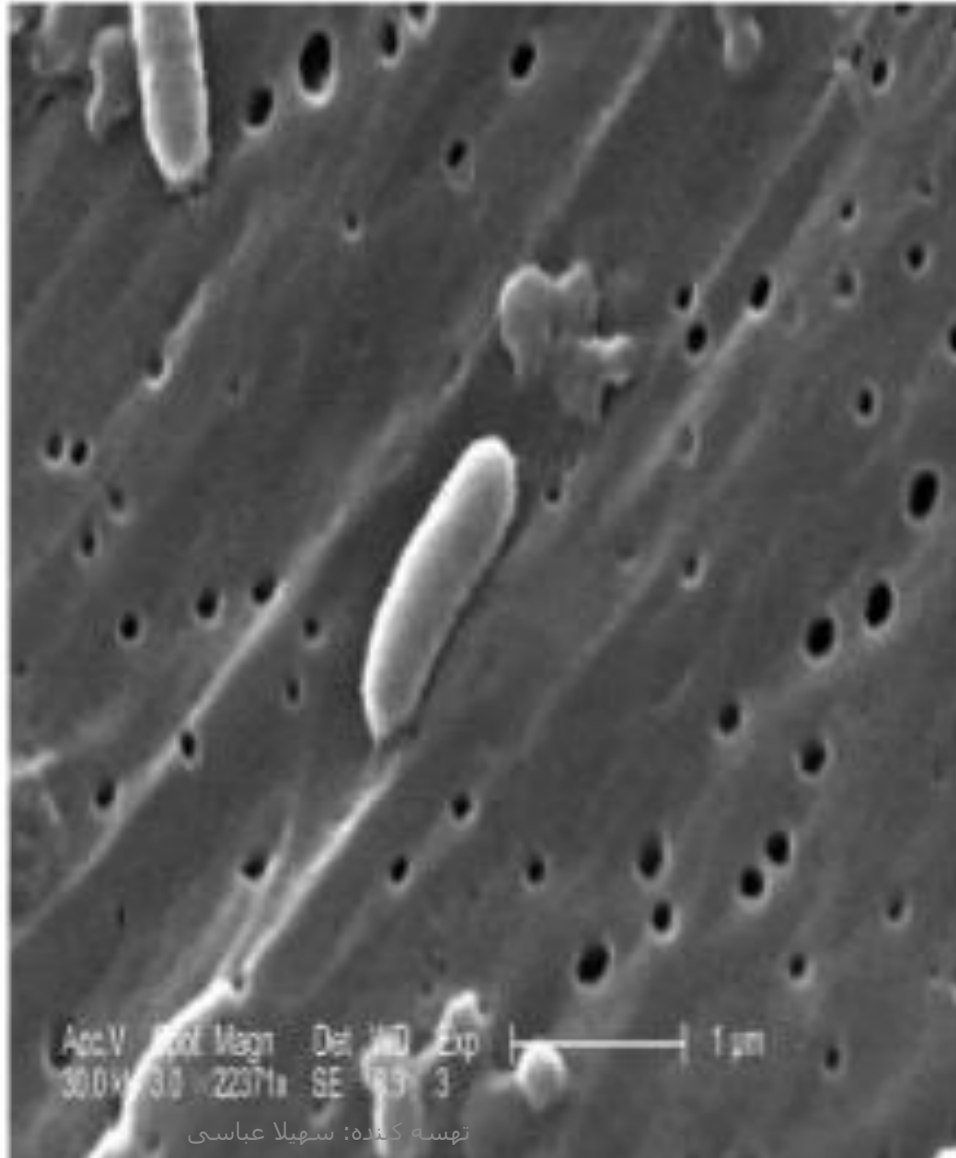
Any infected water and any foods washed in the water, as well as shellfish living in the affected waterway, can cause an infection. Cholera is rarely spread directly from person to person. If ingested with water, the infectious dose is 100000 organisms.

Cholerae Outbreaks



Diagnosis

- Stool culture: Toxigenic *Vibrio cholerae* O1
- Use Cary Blair Transport media if available
 - Viable for many days at room temperature
- Use TCBS media for culture
- Use *V. cholerae* serogroup O1 antisera
- Confirm presence of cholera toxin
- Cholera Rapid Test Dipsticks



- Mucus flecks from stool are cultured.
- Smears are not useful for diagnosis.
- Dark field microscopy shows rapidly motile vibrio's.

Laboratory Diagnosis



- Stools collected in the acute stage of the diseases,
- Growth is rapid on Blood agar,
- On TCBS medium typical colonies can be picked in 18 hours.
- The stool specimens can be transported in ***Venkataraman Ramakrishnan medium***
- Alkaline peptone water is ideal enrichment medium

Vibrio parahaemolyticus



- Halophilic vibrio
- Associated with seafood
- Gastroenteritis:
 - Outbreaks of Food Poisoning
- Extraintestinal infections > wounds
- Virulence Factors:
 - Haemolysin >
 - heat-stable cytotoxin
 - Heat-labile enterotoxin
 - Adherence to human intestinal cells

Thank you for
your attention!

