

# Cholera

## Lecture objectives:

- Know the epidemiology of cholera and history of cholera
- Know the microbiological characteristic of cholera
- Describe the pathogenesis of cholera
- Describe the clinical features of cholera
- Describe the methods for laboratory diagnosis
- Know the management of cholera and control of outbreak

### Color index:

- Important
- Doctors' note
- Extra
- Found in Girls' slides
- Found in Boys' slides

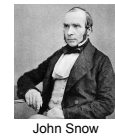
# Introduction, Discovery & Epidemiology

## ● Introduction:

Cholera	
Definition	<ul style="list-style-type: none"> <li>● A <b>waterborne</b> life threatening diarrheal disease</li> </ul>
Etiology	<ul style="list-style-type: none"> <li>● <b>Vibrio Cholerae</b></li> <li>- Comma shaped Gram negative rods<sup>1</sup> (oxidase positive)</li> </ul>
Epidemiology	<ul style="list-style-type: none"> <li>● Found in <b>salt and fresh water</b>.</li> <li>● leads to outbreak and epidemic.</li> </ul>
Characteristics <sup>3</sup>	<ul style="list-style-type: none"> <li>● Has many serotypes based on O-antigen</li> <li>● <b>O 1 and O 139<sup>2</sup></b>.</li> <li>● Produce a <b>non-invasive enterotoxin</b>.</li> <li>● Can be prevented by good sanitation system.</li> </ul>

## ● Discovery:

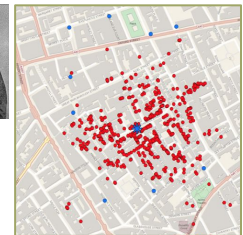
- John Snow discovered an outbreak in London 1854.
- It was related to broad street pump sewage contamination.
- Removal of the pump handle end of the outbreak.



John Snow



Jon Snow



## Epidemiology

**V. cholera O1 and O139** serogroup organisms are the causes of epidemic cholera.

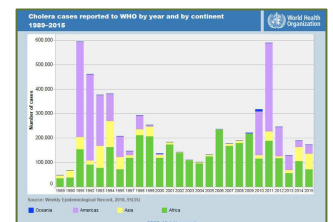
O1 (from 1817 till now):

- Classical: 1 case per 30-100 infections
- El Tor<sup>4</sup>: 1 case per 2-4 infections (Seventh pandemic)

O139 (recently in 1992 in Asia only):

- Contained in India, Bangladesh.

- Seven major outbreaks.
- Majority in India, Sub-Saharan Africa, Southern Asia.
- Endemic in > 50 countries.
- Each year 3-5 millions cases result in 100,000 deaths.



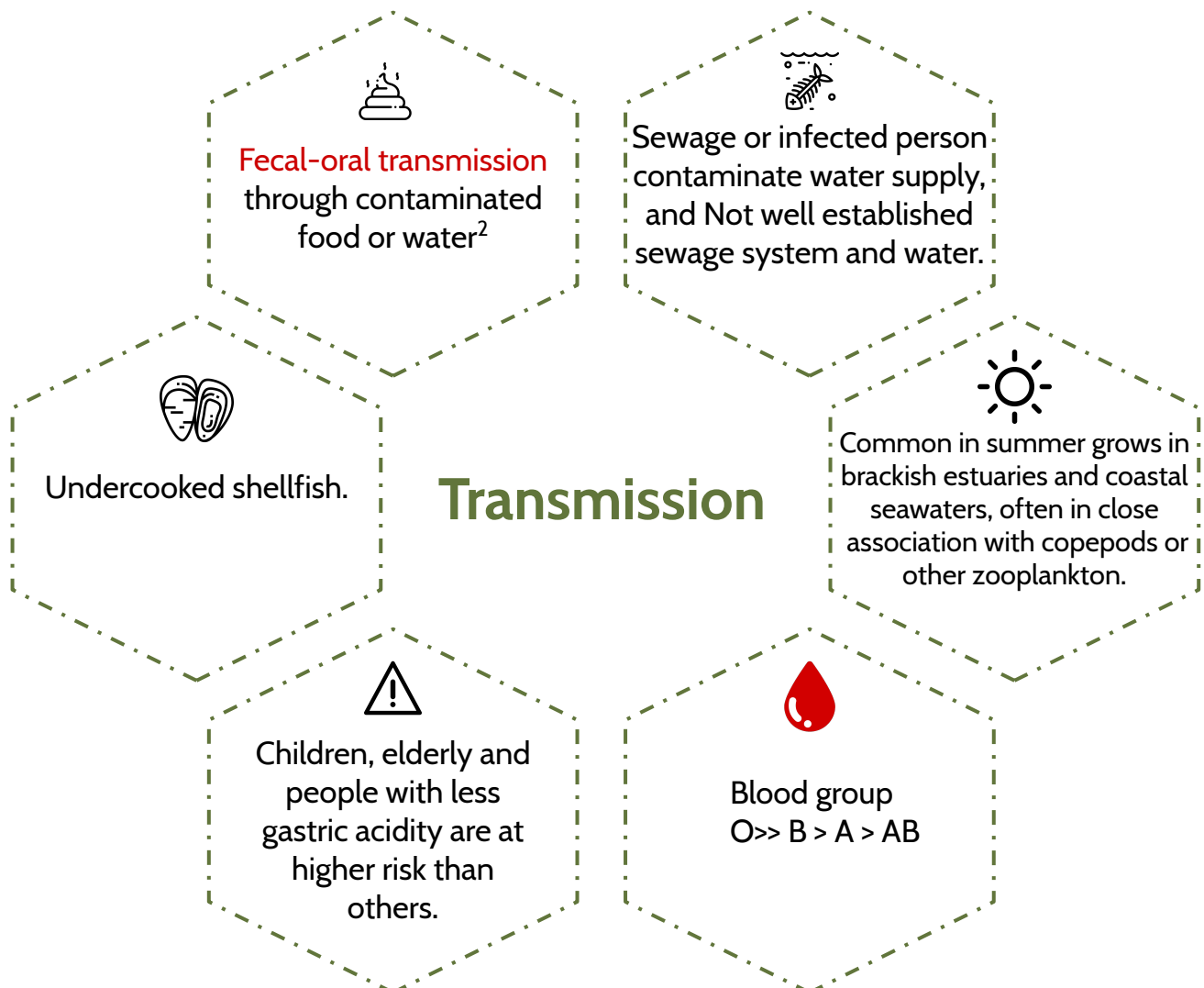
In 2016 in Haiti after Hurricane Matthew, in South Sudan & Yemen and many other African countries

1- flagellated  
 2- other serotypes do not produce the toxin that causes the disease  
 3- mainly found in small intestine  
 4- less severe

# Infectivity & Transmission


## ● Infectivity

- Period of infectivity during acute stage till recovery ( end one to three weeks )
- Infected person can produce up to 20 L of  $10^9$  CFU/ml /day<sup>1</sup>
- **Has high infectious dose NOT like Shigella**, typhoidal Salmonella and Enterhemorrhagic E.coli
- Infectious dose  $10^6$ - $10^{11}$  colony-forming units, Due to harsh environment of the intestine i.e. temperature and stomach acidity, Bile salts and organic acids in the intestine



1- Very easy to contaminate the water  
 2- In polluted water the following organisms are common to find :  
 Cholera , poliovirus , hepatitis A and salmonella typhi

# Pathogenesis & Clinical manifestations

Pathogenesis		
Toxin	<ul style="list-style-type: none"> <li>◦ <b>Vibrio cholerae uses toxin-coregulated pili (TCP)</b> to colonize the human intestine.</li> <li>◦ Cholera results from secretory diarrhea caused by the actions of cholera toxin (CT) on intestinal epithelial cells.</li> <li>◦ CT is an adenosine diphosphate – ribosylating enzyme that leads to chloride, sodium, and <b>water loss</b> from intestinal epithelial cells</li> </ul>	
Mechanism	<ol style="list-style-type: none"> <li>1. Cholera toxin binds to Monosialoganglioside (GM1) which is a glycosphingolipid on the surface of epithelial cells.</li> <li>2. The toxin must undergo cleavage of the active, A1 component(CTA1), which goes on to constitutively activate the Gs protein</li> <li>3. Nicotinamide adenine dinucleotide (NAD), mediated by CTA1 becomes Adenosine diphosphate (ADP)-ribose &amp; binds to G protein</li> <li>4. G protein regulates adenylyl (adenylate) cyclase activity (AC).</li> <li>5. <b>Elevation in the intracellular cyclic adenosine monophosphate (cAMP) concentration.</b></li> <li>6. Water and electrolyte shift from the cell to the intestinal lumen, This results in extremely watery diarrhea accompanied by electrolyte imbalances</li> </ol>	
Clinical Manifestations <sup>1</sup>		
<ul style="list-style-type: none"> <li>◦ Ranges from a few hours to 5 days( range 1-3 days).</li> <li>◦ Depending on gastric acidity and initial infectious dose.</li> <li>◦ Majority have mild, or no symptoms at all:               <ul style="list-style-type: none"> <li>- 75% asymptomatic</li> <li>- 20% mild disease</li> <li>- 2-5% severe</li> </ul> </li> </ul>		
Mild disease		Cholera gravis <sup>1</sup>
Symptoms	<ul style="list-style-type: none"> <li>🤢 Vomiting.</li> <li>🤰 Cramps.</li> <li>🚽 Watery diarrhea (1L/hour):           <ul style="list-style-type: none"> <li>- with flecks of white mucus (<b>rice water stool</b>) &amp; a fishy odor.</li> </ul> </li> <li>🔥 ↓ Ca<sup>++</sup> and K can lead to ileus, muscle pain, spasm, &amp; even tetany.</li> </ul>	<p>More severe symptoms due to Rapid loss of body fluids:</p> <ul style="list-style-type: none"> <li>📦 Rapidly lose more than 10% of body weight.</li> <li>💧 <b>Dehydration and shock<sup>1</sup>.</b></li> <li>👁️ Sunken eyes, and ↓skin turgor (tenting), cold and clammy.</li> <li>🌀 Anuric &amp; lactic acidosis (Kussmaul breathing).</li> <li>👉 Hypoglycemia → seizure or comma.</li> <li>🫀 Cardiac and Renal failure.</li> <li>🫁 Aspiration pneumonia.</li> </ul>
Water Loss	1 liter/hour.	6 liters/hour (10 <sup>7-9</sup> vibrios CFU/mL).
Mortality	<ul style="list-style-type: none"> <li>◦ Death occurred in (18 hours - several days) if not treated due to dehydration.</li> </ul>	<ul style="list-style-type: none"> <li>◦ Death within 2-12 hours or less.</li> <li>◦ Mortality 50-60% without treatment.</li> <li>◦ Mortality &lt;1% with rehydration.</li> </ul>

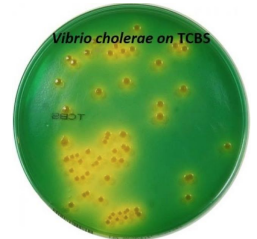
1-severe form of disease

2-what is the main cause of death in cholera? hypovolemic shock.

# Diagnosis

## ● Diagnosis:

- Suspected in severe diarrhea with dehydration.
    - 1- Complete history and physical examination.
    - 2- Insert central line for IV fluid<sup>1</sup>, collect blood<sup>2</sup> for basic routine tests (chemistry and hematology).
    - 3- **Stool:**
      - Recovery of organisms can be enhanced by enrichment of stool in alkaline peptone water. (60-100%)
      - Send stool for smear and culture on special media.
  - **Microscopy:**
    - Dark field microscopy (shooting stars).
    - **Gram stain (curve Gram Negative bacilli)**
  - **Culture<sup>3</sup>:**
    - **Culture on thiosulfate citrate bile sucrose (TCBS) agar-yellow colonies<sup>4</sup>**
    - Culture not routinely performed, you have to request it.
- Other non-invasive bacterial, ETEC and viral gastroenteritis might have similar presentation.



## ● Diagnosis / Microbiology

**Vibrio cholera is highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum** (oxidase positive)



Biotype O1 antigen	Serotype	Antigen
Classical	Ogawa	A,B
	Inaba	A,C
	Hikojima	A,B,C
El Tor	Ogawa	A,B
	Inaba	A,C
	Hikojima	A,B,C

O 139 serogroup antigen appeared in Bangladesh in 1992

Has polysaccharide capsule but does not have O1 antigen

Non-O1, Non-O139 Serogroup

Most are CT (cholera toxin) negative and are not associated with epidemic disease.

1- rehydration is the main treatment  
 2- To check creatinine for renal failure  
 3- Culture is not enough cause even if the culture is positive it's not necessary that it will cause the disease  
 4- Indicates sucrose fermentation

# Treatment & prevention

## Treatment

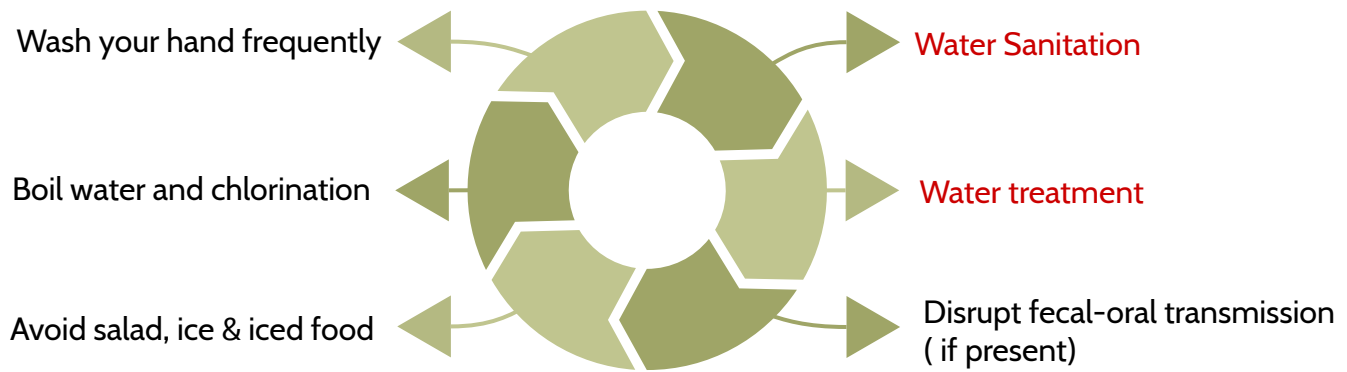
◦ Basically **rehydration** and antimicrobial therapy

Rehydration	Antibiotics <sup>1</sup>
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- Should be started immediately before confirming the diagnosis.
- Either:
  - **Oral rehydration if the patient can tolerate (not vomiting)**
  - **IV rehydration.**
- Patients recovered within 3-6 days.
- Decrease mortality from 50% to 1%.
- Give 1.5 time the amount lost
- Start when 10% of total body weight lost.
- Oral Rehydration Salt (ORS) by WHO & UNICEF: One pack in 1 liter contain NaCl, KCl, NaHCO<sub>3</sub>, glucose.
- IV use either Ringer's lactate, Saline or Sugar and water.

- **Reduce the recovery time to 2-3 days.**
- Decrease infectivity
- **Azithromycin:** Single-dose (preferred, especially in children)
- or **Ciprofloxacin,**
- or **Tetracycline, Doxycycline**

## ● Prevention



	Killed Whole-cell Vaccines	Live Attenuated Vaccines
Adult	50% protection for 6 months	60% protection for 2 years
Children (aged 2-5)	<25% protection	protection rapidly declines after 6 months
Doses	Multiple doses	-
Side effects	-	Mild diarrhea, abdominal cramping

1- The doctor said that you can use any of the mentioned drugs

# Possible use in bioterrorism & International efforts

- **Can be a bioterrorism agent:**

- Ease of procurement

- Silent dissemination



- Simplicity of production in large quantities at minimal expense

- Silence and Ease of dissemination with low technology.

- **International Efforts:**

- WHO: Global Task Force on Cholera Control:
  - Reduce mortality and morbidity
  - Provide aid for social and economic consequences of Cholera
- CDC
- U.N.: GEMS/Water
- Global Water Quality Monitoring Project:
  - Addresses global issues of water quality with monitoring stations on all continents

## Dr.Khalifa's notes:

### Vibrio cholera

- **Curved Gram Negative bacilli**
- Campylobacter and vibrio cholera are highly motile organism.
- **Water-borne (Natural reservoir), infection is mainly through water supply contamination**
- Has a lot of serotypes based on O antigen but our concern are O1 and O139 which causes the disease in human. (Other serotypes don't produce cholera toxin thus has no clinical significance)
- Leads to **outbreak** and epidemic.
- **High infectious dose**
- Fecal-oral route
- Children have higher risk for cholera.
  
- **Pathogenesis:**  
Through **cholera toxin(enterotoxin)** → activate the Gs protein → increase cAMP → which activate the CFTR (cystic fibrosis transmembrane conductance regulator) channel, leading to a **large efflux** of chlorine and other ions and water into the GI lumen → Watery diarrhea
  
- **Symptoms:**
  - **Watery diarrhea(rice water stool)** because the small white flecks of mucus resemble grains of rice, vomiting.
  - Most are asymptomatic but some may develop symptoms depending on patient status and risk factors.
  
- **Cholera gravis:**
  - Very severe disease, with a severe loss of water (Dehydration → shock) which requires immediate rehydration therapy or the patient may die.
  - **Severe dehydration** will affect cardiac, renal and different organs functions.
  - With vomiting they may develop aspiration pneumonia.
  
- **Diagnosis:**
  - Selective media: **Culture on thiosulfate citrate bile sucrose (TCBS) agar-yellow colonies (sucrose fermenter)**
  
- **Prevention:**
  - Water sanitation, وأمور البلدية الباقية
  - Vaccines(Used in cases of outbreaks not used routinely due to its protective rate):
    - A-Killed Whole-cell Vaccines
    - B- Live Attenuated Vaccines
  
- **Treatment: Rehydration** with antimicrobial therapy( Ciprofloxacin or Azithromycin or Tetracycline and Doxycycline)



# Quiz:

## MCQ:

Q1:B, Q2:C, Q3:D, Q4:B, Q5:C

**Q1:** A 24-year-old American man is traveling in rural India during the monsoon season. Over the course of a few hours, he develops severe watery diarrhea. In the next 30 hours, he has approximately one episode per hour of liquid stools that appear clear with small white flecks of mucus. He also has occasional episodes of vomiting. He quickly becomes lethargic and generally ill with crampy abdominal pain but is afebrile. He attempts to rehydrate himself during the illness, and the symptoms resolve within approximately 48 hours. He was diagnosed with cholera. Which of the following *Vibrio cholera* serotypes has the highest potential causing the disease?

- A- O1 & O138
- B- O1 & O139
- C- O40 & O130
- D- O139 & O2

**Q2:** What is the mode of transmission of *V. cholera*?

- A- Fecal - oral transmission through contaminated Animal & water
- B- Oral- oral transmission through contaminated food & water
- C- Fecal - oral transmission through contaminated food & water
- D- Oral - oral transmission through contaminated Animal & water

**Q3:** Which of the following have high infectious dose?

- A- Typhoidal salmonella
- B- Shigella
- C- Enterohemorrhagic *E. coli*
- D- *Vibrio cholera*

**Q4:** What is the receptor that cholera toxin binds to on the surface of the epithelial cells leading to activation of Gs protein which leads to an increase in cAMP which will activate CFTR channel leading to a large efflux of chlorine and other ions into the GI lumen?

- A- CTA-2
- B- GM1
- C- CTB
- D- CTA-1

**Q5:** A 50-year-old man develops profuse, non-bloody, watery diarrhea while working as an aid worker in Bangladesh. He arrived in the area two days ago. A stool smear shows no WBCs. His mucous membranes are dry, and his skin shows signs of tenting. He subsequently develops electrolyte abnormalities that lead to cardiac and renal failure. What is the pathogenesis mediated by the most likely causative organism?

- A- Cytotoxin
- B- Invasion
- C- Enterotoxin
- D- None of the above

## SAQ:

**CASE:** A fourth-year medical student is working in a medical relief group in Haiti for several months. Several parents bring their children to the clinic and explain that the children have had profuse, watery stool with flecks of white mucus and fishy odor along with watery vomiting. All of the children are afebrile, slightly hypotensive, and tachycardic but have a normal respiratory rate. Urine output is reduced. Biopsy is performed and the culture and microscopy showed gram negative curved or comma-shaped rods with single polar flagellum.

**Q1:** What is the most likely diagnosis?

A: Cholera

**Q2:** What is the most likely causative agent?

A: *Vibrio cholera*

**Q4:** What media is used for this organism?

A: Culture on thiosulfate citrate bile sucrose (TCBS) agar (*Vibrio cholerae* is sucrose fermenter)

**Q5:** What is the appropriate treatment for this patient?

A: Oral rehydration (if the patient can tolerate it. If not use IV) + Antimicrobial therapy (Azithromycin is often the preferred therapy especially in children. Or Ciprofloxacin Or Tetracycline, Doxycycline)

**Q6:** What is the main clinical manifestation of this condition?

A: the hallmark of cholera is rice-water stools, because the small white flecks of mucus resemble grains of rice

# Members board:

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