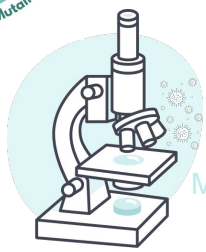

TEAM443
MICROBIOLOGY

Cholera

Prof Ali & Fawzia

Objectives

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

Any future corrections will be in the editing file, so please check it frequently

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Main text
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Cholera

Cholera

A **water borne** live threatening diarrheal disease

Cholera is not a zoonotic disease, and it has nothing to do with animals (source of infection is human feces).

Caused by

- Vibrio Cholerae which is Short curved, Comma shaped Gram -ve rods (oxidase positive)

Epidemiology

- Found in salt and freshwater.
- leads to outbreak and epidemic.

Characteristics {7}

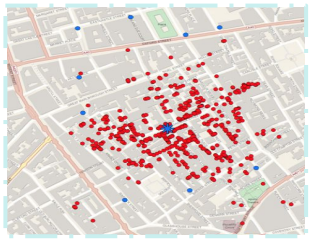
- Has many serotypes based on O-antigen
- O 1 and O 139.
- Produce a non-invasive enterotoxin.
- Can be prevented by good sanitation system.

Discovery {1}

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



Epidemiology {2},{3},{4}

A. O139 (recently in 1992 in Asia only): - Contained in India, Bangladesh.

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

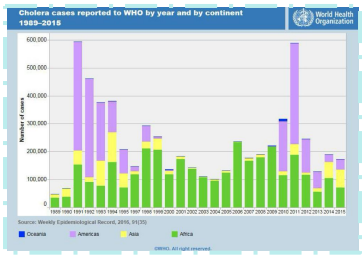
C. Seven major outbreaks.

D. O1 (from 1817 till now):
- Classical: 1 case per 30-100 infections
- El Tor: 1 case per 2-4 infections (Seventh pandemic)

E. Majority in India, Sub-Saharan Africa, Southern Asia.

F. Endemic in > 50 countries.

G. Each year 3-5 millions cases result in 100,000 deaths.



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

Has high infectious dose NOT like Shigella

Infectious dose 10^6-10^{11} colony-forming units, Due to harsh environment of the intestine(ie. temperature and stomach acidity and Bile salts, organic acid in intestine)

Transmission & Clinical manifestation

Transmission

Sewage or infected person contaminate water supply, and Not well established sewage system and water treatment. **{8} -{12}**

Blood group O>> B > A > AB (There's No strong evidence)

Children, elderly and people with less gastric acidity are at higher risk than others.**{11}**

Undercooked shellfish

Common in summer grows in brackish estuaries and coastal seawaters, often in close association with copepods or other zooplankton.

Fecal-oral transmission through contaminated food or water From human to human through stool by his contaminated hands

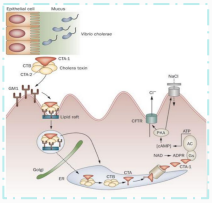
Clinical manifestation

- Ranges from a few hours to 5 days (range 1-3 days)

○ Depending on gastric acidity and initial infectious dose.
○ Majority have mild, or no symptoms at all

- 75% asymptomatic
- 20% mild disease
- 2-5% severe

Pathogenesis

Pathogenesis		
Toxin	○ Vibrio cholerae uses toxin-coregulated pili (TCP) to colonize the human intestine. ○ Cholera results from secretory diarrhea caused by the actions of cholera toxin (CT) on intestinal epithelial cells. ○ CT is an adenosine diphosphate – ribosylating enzyme that leads to chloride, sodium, and water loss from intestinal epithelial cells	
Mechanism {10} 	1. Cholera toxin binds to Monosialoganglioside (GM1) which is a glycosphingolipid on the surface of epithelial cells. 2. The toxin must undergo cleavage of the active, A1 component(CTA1), which goes on to constitutively activate the Gs protein 3. Nicotinamide adenine dinucleotide (NAD), mediated by CTA1 becomes Adenosine diphosphate (ADP)-ribose & binds to G protein 4. G protein regulates adenylyl (adenylate) cyclase activity (AC). 5. Elevation in the intracellular cyclic adenosine monophosphate (cAMP) concentration. 6. water and electrolyte shift from the cell to the intestinal lumen, This results in extremely watery diarrhea accompanied by electrolyte imbalances	
	Mild disease	**Cholera gravis**
Symptoms {9}	**Vomiting**. **Cramps**. **Watery diarrhea**[1] (1L/hour): **with flecks of white mucus (rice watery stool)** & a fishy odor.{3} ↓ **Ca++ and K** can lead to ileus, muscle pain, spasm, & even tetany.	**More severe symptoms due to Rapid loss of body fluids:** Rapidly lose more than 10% of body weight. **Dehydration and shock.** **Sunken eyes**[2], **and ↓skin turgor**[3] (tenting), cold and clammy. **Anuric & lactic acidosis** (Kussmaul breathing)[4]. **Hypoglycemia** ➔ seizure or comma. **Cardiac and Renal failure.** **Aspiration pneumonia.**
Water Loss	1 liter/hour.	6 liters/hour (10^{7-9} vibrios CFU/mL).
Mortality	Death occurred in (18 hours - several days) if not treated due to dehydration.	○ Death within 2-12 hours or less. ○ Mortality 50-60% without treatment. ○ Mortality <1% with rehydration.

Diagnosis {5},{6}

- Suspect in severe diarrhea with dehydration.
- Other non-invasive bacterial, ETEC and viral gastroenteritis might have similar presentation.
- Complete history and physical examination.
- Insert central line for IV fluid, collect blood for basic routine tests (chemistry and hematology).
- Send stool for smear and culture on special media.
- Culture not routinely performed, you have to request it
- Dark field microscopy (motile shooting stars)
- Gram stain : curve Gram Negative bacilli
- Culture on thiosulfate citrate bile sucrose **(TCBS) agar-yellow colonies**


- Recovery of organisms can be enhanced by enrichment of stool in alkaline peptone water (60-100%)

Vibrio cholera is highly **motile**, **Gram-negative, curved or comma shaped rods/bacilli** with a single polar flagellum.

Types of cholera

- **Biotype O 1 antigen**
  - **Classical**
  - **El Tor**
- **O 139 serogroup appeared in Bangladesh 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.

Biotype O 1 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

Cholera Treatment

Rehydration and antimicrobial therapy {13}	
Rehydration	**Antibiotics**
- Rehydration should be started immediately before confirming the diagnosis - Either **oral rehydration** if the patient can tolerate it (not vomiting) or **start IV rehydration.** - Decrease mortality from 50% to 1 % - Give 1.5 time the amount lost. - Start when 10% of total body weight lost. - Patients recovered within 3-6 days. - Oral Rehydration Salt (ORS) by WHO and UNICEF one pack in 1 liter contain NaCl, KCl, NaHCO3, 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 is often the preferred therapy especially in **children**, - or Ciprofloxacin - or **Tetracycline** , Doxycycline

Bioterrorism agents

- **Ease of dissemination with low technology**
- **Silent dissemination**
- **Simplicity of production in large quantities at minimal expense**
- **Ease of procurement**

Prevention {14}

Wash your hand frequently

Boil water and chlorination.

Cook all types of food very well.

Disrupt fecal-oral transmission if present

Water Sanitation/ treatment

Avoid salad, ice and iced food

Vaccination {15}		
	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

International Effort

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 Notes

1. History of cholera, known from 1800 by the epidemiologist John snow in "Thames/times river" in London, 2 different companies distribute water from there, John did an epidemiological study he collected all these cases(people who died/ and with no symptoms/ and with diarrhoea) he collected all necessary info from them and marks them on a map (London map around the river or -broad way-) He also did a calculations for the relative risk (it's relative because it's -cohort study-number of people who drunk from the pump in the top of river and whose drunk from the bottom (according to water companies that distribute water), so then he reported to close broadways and then the outbreak stopped!
2. Most outbreak have been seen in Haiti, Katrina in America, Also in **Yemen** (No available safe drinking water, so one case can infect all the population!
3. Why do we fear cholera? First, vibrio is very rare in Saudi Arabia, BUT when foreigners come from (**Bangladesh**, Africa, Kenya) they could have cholera.
   ◎ **for example:** someone came from outside Saudi Arabia, from (**devolving country/ war/ Earthquakes/ Floods/ hurricanes**) And he's having diarrhoea (this diarrhoea characterised by the following:
   1. **Large** Amount diarrhoea
   2. Mucus (**flex white** mucus) /**Rice** like
      (These are very **important clues** that tell you **this case is cholera**)

4. In Saudi Arabia (yes we don't have cholera cases) but we have lots of foreigners almost 50% of the population! And they go and come back again, there's also "حجاج ومعتمرين " that come every year, that's why we have to have all tools and laps ready to identify cholera.
5. In laps When there's vibrio in media, does this mean it's cholera only? Or could it be other thing? There's **lots of Vibrio bacteria other than** (vibrio **cholera**). Some laps make mistakes when they see "vibrio" in media they report it as vibrio "cholera" and it turns out that it's not cholera! This is why in vibrio we always make sure it's "cholera" (How? There're some tests to confirm that, check point 6..)
6. As we mentioned when **vibrio appears in media** (as **yellow** colonies) how to know it's vibrio "cholera"? Some **important tests** can be used:
   - **string** test (appears like thread/string colonies)
   - **Shooting star** (when you put the colonies on slide and add normal saline it move fast just like shooting star!) so lap is important

7. Characteristics of Vibrio cholera:
   - Gram negative, Non lactose fermentative, curved, motile, comma shaped
   - Grow on **TBCA media (yellow)**, **Positive string test**, **Oxidase positive**

8. What is the **source**?
   Contaminated drinking ***water*** Not from hands because it has high infectivity unlike shigella.
9. clinical presentation: Short Incubation period, (Large Amount/ flex mucus watery diarrhoea)
   - It must be mentioned in the question that it's (Large/flex) and the patient is coming from outside Saudi Arabia (Travelled)
10. Pathogenesis: Produce mucus so has (**Cytotoxin** and **enterotoxin**) there will be destruction in mucosa because of binding of organism so nutrients (**Na**, **K**) will not go inside cell and will stay in lumen, water will follows (osmolarity) -> diarrhoea.
11. Risk factors: In general, anyone living in (countries with disasters) one of the important risk factors also, people taking **antacids** get sick with **lower** Infectious dose.
12. Why we don't have cholera? Why it's in developing/wars countries? Because they "drink contaminated water" from "المجاري" there will be sewage then it goes with the drinking water.
13. Treatment:
    - We treat to kill organism and decrease symptoms and prevent from infection
    - Doxycycline but اهم شي "Fluids ":

    - **If there's available IV line:** low mortality rate (specially that not all people get sever disease only 2-5%).
    - **If there's NO available IV line:** High mortality rate, when patient has the diarrhoea → hypovolemic shock (in wars/ poor countries) (there's no IV lines) ,even when you give him fluid orally he cannot he will vomit → these cases needs fast intervention unlike Salmonella typhi 'week', here it's only hours leads to→ multi Organ failure, it's irreversible so No good survival (cardiac, Renal, Liver failure and they will have lactic acidosis. They might also have decreased mentation and loss of consciousness and they die. and this case we call it *gravis cholera*, (in outbreaks populations get infected so 2-5% would be very high number of severe cases)

14. Prevention is for :
    - People who expose to the pathogen: get vaccine.
    - People in the outbreak: has to take safe drinking water.
15. we have two types of Vaccine
    A. **Dead** one which is characterized by having **less** symptoms but **less** immunogenicity
    B. **Life** attenuated which is characterized by having **symptoms** but with **longer** immunity (no need to take it every year)
    - Who take the vaccine?
      Not for everybody, only for People who **travel** there eg.campaigns
      People **exposed** to sewage.

Summary

Extra

Overview	○ A **water borne** live threatening diarrheal disease ○ Majority have mild, or no symptoms at all (75% asymptomatic, 20% mild disease , 2-5% severe)	
Etiology	**Vibrio Cholerae** Characteristics**:** ○ Has many serotypes based on O-antigen (O 1 and O 139) ○ Produce enterotoxin. ○ which is highly **motile,** Short curved, Comma shaped Gram -ve rods (oxidase positive)	
Epidemiology	○ Found in salt and freshwater. ○ leads to outbreak and epidemic. ○ **Bangladesh**, Africa, Kenya, Haiti, Katrina in America, **Yemen**	
Infectivity	Has high infectious dose (10^6-10^{11}) NOT like Shigella,	
Transmission	○ Contaminated drinking **water** ○ Not well established sewage system and water treatment	
Risk factors	○ people with less gastric acidity ○ anyone living in (countries with disasters)	
Pathogensis	Cholera will produce **Cytotoxin** and **enterotoxin, and it will disrupts the normal functioning of the intestine by destruction the mucusa** →**that will lead to triggering the release of electrolytes (Na, K)** → electrolyte shift from the cell to the intestinal lumen followed by water (due to osmolarity)→ extremely watery diarrhea accompanied by electrolyte imbalances	
Symptoms Depends on severity	Mild disease	○ **Watery diarrhea with flecks of white mucus (rice watery stool)** ○ ↓ **Ca++ and K**
	Cholera gravis	○ **Dehydration and shock, Sunken eyes,** ○ **Cardiac and Renal failure.** ○ **lactic acidosis**
Diagnosis	○ Suspect in severe diarrhea with dehydration. ○ Dark field microscopy (motile shooting stars) ○ Culture on thiosulfate citrate bile sucrose **(TCBS) agar-yellow colonies**	
Management	**Rehydration**	○ should be started immediately before confirming the diagnosis ○ Either **oral rehydration** if the patient can tolerate it (not vomiting) or **start IV rehydration.**
	Antibiotics	**Azithromycin** (in children) or Ciprofloxacin or **Tetracycline** , Doxycycline
Prevention	○ Water Sanitation/ treatment ○ **Vaccination** **Dead** one which is characterized by having **less** symptoms but **less** immunogenicity **Life** attenuated which is characterized by having **symptoms** but with **longer** immunity	

MCQs

Q1 - Name the type of diarrheal infection associated with V.cholerae?			
A) Acute watery bloody diarrhea	B) Acute watery rice water diarrhea	C) Acute watery diarrhea with no blood	D) None
Q2 - Which blood group is most susceptible to get cholera?			
A) AB	B) A	C) B	D) O
Q3 - What is the mode of transmission of V. cholera?			
A) Oral - oral transmission through contaminated Animal & water	B) Fecal - oral transmission through contaminated Animal & water	C) Oral- oral transmission through contaminated food & water	D) Fecal - oral transmission through contaminated food & water
Q4 - A watery stool sample was collected f rom the 5 year old boy who is suffering f rom diarrhea for 2 days. After the incubation in high pH media containing NaCl, smooth and round colonies appeared. Gram-negative, motile, comma-shaped bacteria were observed after the microscopic examination. Name the possible bacteria?			
A) Aeromonas spp	B) E.coli	C) H.pylori	D) Vibrio cholerae
Q5 - All the following statements about vibrio cholerae are true EXCEPT:			
A) Multiple flagella	B) Motile	C) Comma shaped rods	D) Gram negative
Q6 - Which of the following is NOT a symptom of cholera gravis?			
A) Hypoglycemia	B) Shock	C) Alkalosis	D) Renal failure
Q7 -Which of the following medium are used to differentiate the colonies of Vibrio cholerae?			
A) Alkaline bile salt	B) MacConkey	C) Thiosulphate-citrat e-bile-sucrose	D) XLD

Answers: 1.B 2.D 3. D 4. D 5.A 6.C 7.C

SAQ

Case 1

A 25 year old man presented in emergency department (ER) complaining of severe watery diarrhea, muscle cramps and dehydration after eating uncooked shellfish

Q1: What is the most likely causative organism ?
Q2: what are the points(factors) that indicate to the cause ?
Q3: Describe the culture in TCBS media
Q4: what is the pathogenesis ?
Q5: describe the microbiology characteristic
Q6: how do we treat it ?
Q7: what are the clinical features ?

Answers

A1: Vibrio cholera
A2: Shellfish, watery diarrhea and dehydration
A3: A yellow colonies
A4: by enterotoxin (cholera toxin (CT))
A5: Gram-negative, curved or comma-shaped rods/bacilli
A6: Mainly by Rehydration Antibiotics in severe cases
A7 : Vomiting, watery diarrhea ,abdominal cramps

TEAM 443
MICROBIOLOGY

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