V. CHOLERAE – BACTERIAL PATHOGENESIS

PATH 417A 2017W2

VIVIAN HUANG

CASE

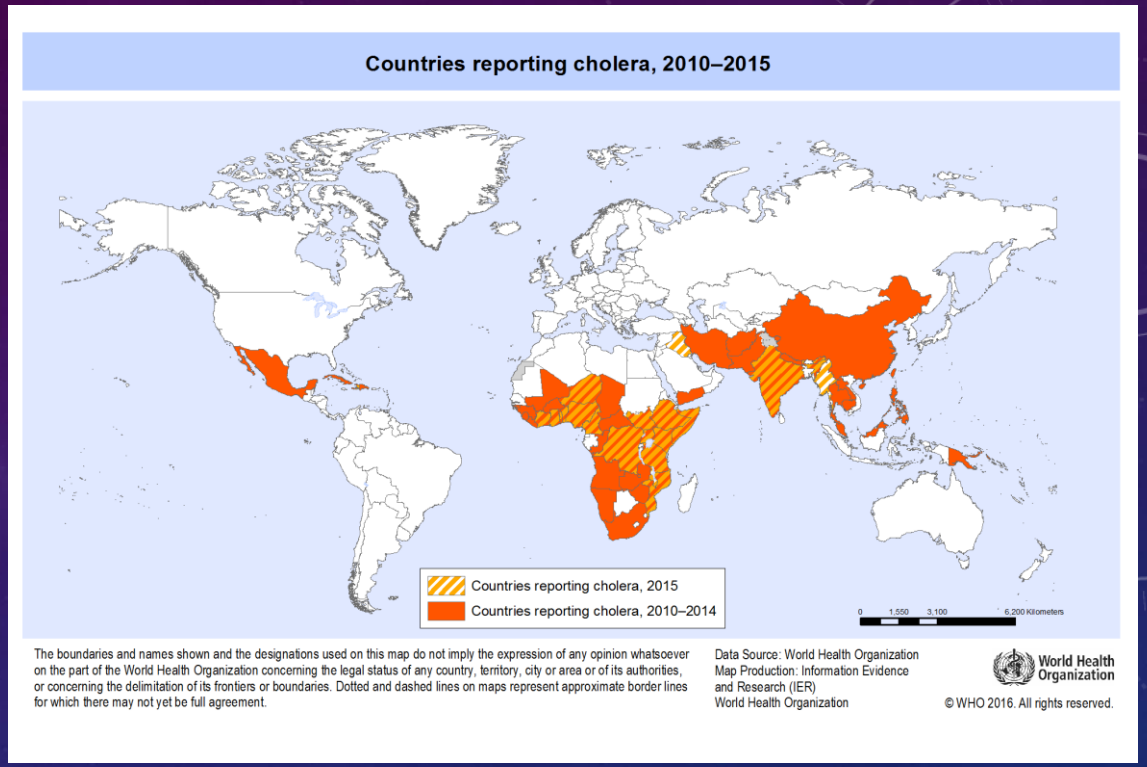
Fulfilling a long held travel dream, Robert has taken six months off work and is making his way through India taking in the sights, experiencing local festivals and making time to get to know the people. He is cautious in his hygiene, eating and drinking habits but despite this he contracts a diarrhea with voluminous outpouring of fluid accompanied by vomiting. He suspects cholera and with the help of a fellow traveler gets himself to a local hospital where a stool sample is examined and his presumptive diagnosis is confirmed. He stocks up on appropriate fluids and stays put at the hostel he has booked into for a few days, experiencing some minor leg cramping along with the diarrhea. His curiosity about his illness has him reading up on the organisms when he returns to North America and he is left wondering what serotype of Vibrio cholerae he might have contracted, should he have been prescribed antibiotics, was there anything more he could have done to prevent contracting the organism and might he now be a carrier?

1. ENCOUNTER

GEOGRAPHIC RESIDENCE, HOST RESIDENCE, RELATED V. CHOLERAE CHARACTERISTICS, PATIENT CONTACT

GEOGRAPHICAL RESIDENCE

- Found extensively in ecosystem:
  - Invertebrates: zooplankton, crustaceans, shellfish
  - Vertebrates: fish, humans
  - Microorganisms: protozoa
- Aquatic environments, such as rivers, brackish waters, estuaries, coastal waters
- Prevalent in developing countries with low income, poor sanitation, clean drinking water, insufficient infrastructure. Endemic in Asia, East Africa, some parts of Central and South America, and worsens in war-torn areas or in the aftermath of a natural disaster.



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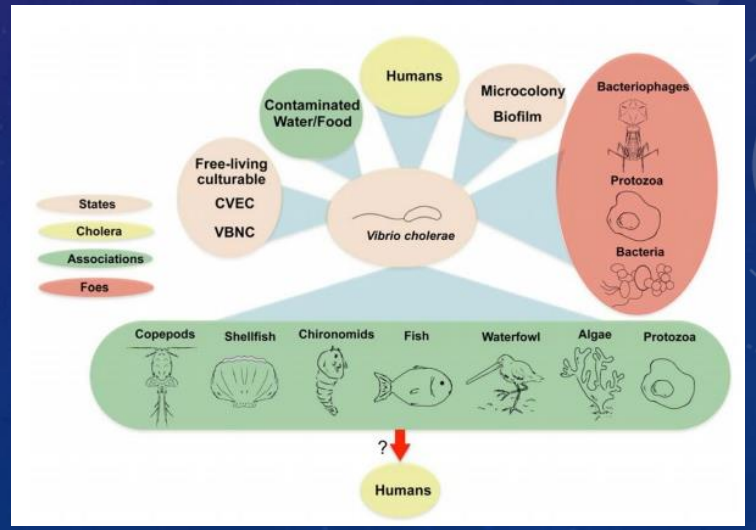

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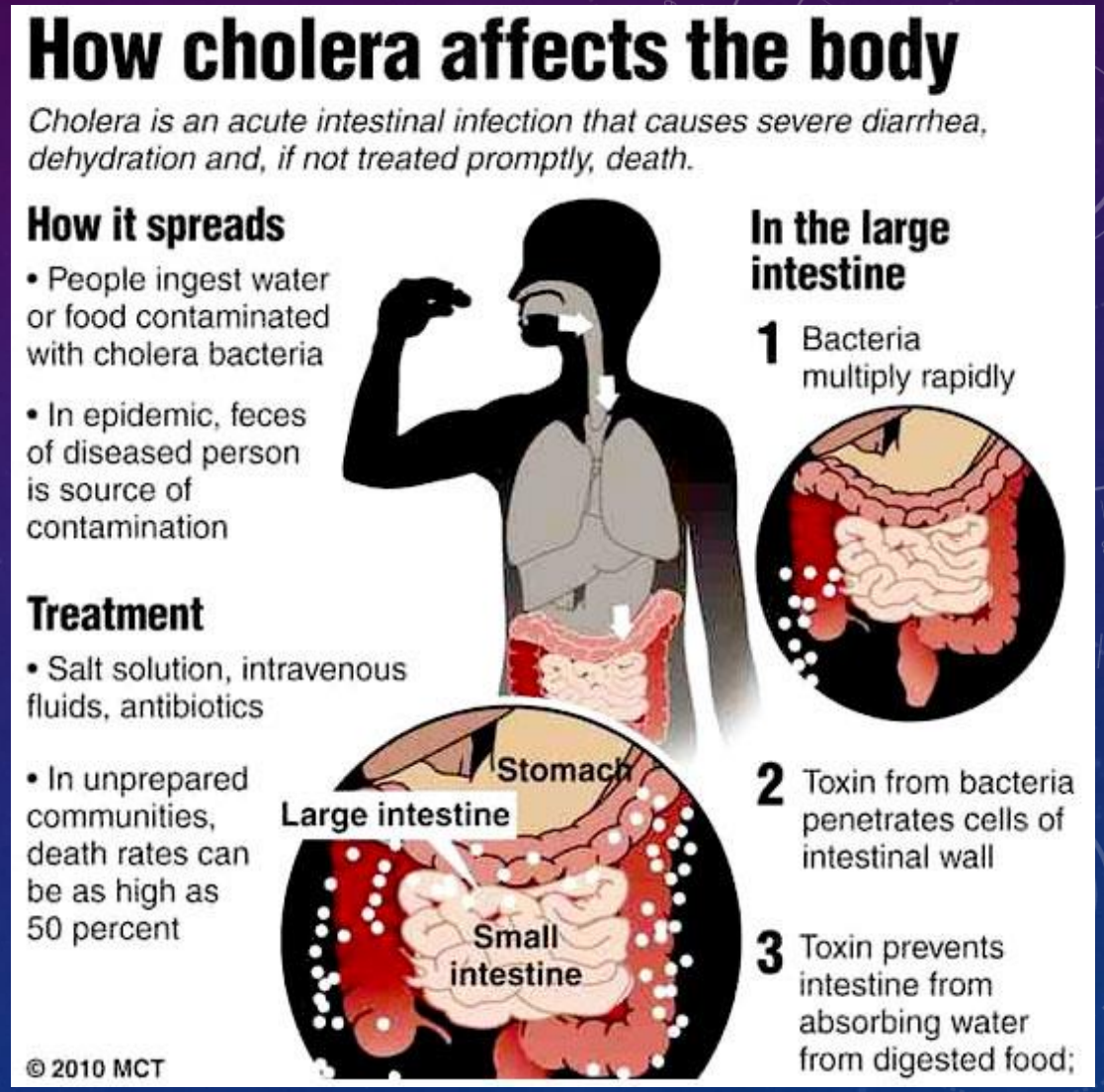
HOST RESIDENCE

- In the human host, *V. cholerae* colonizes the intestines, attaches to the intestinal epithelium where it releases toxins into the epithelial cells
  - O1 and O139 serogroups only have human reservoirs
- Colonization factors allow bacterial residence in chitinous copepod (crustacean) exoskeletons, providing an environment with a carbon source for growth while the bacteria undergo horizontal gene transfer of pathogenic genes



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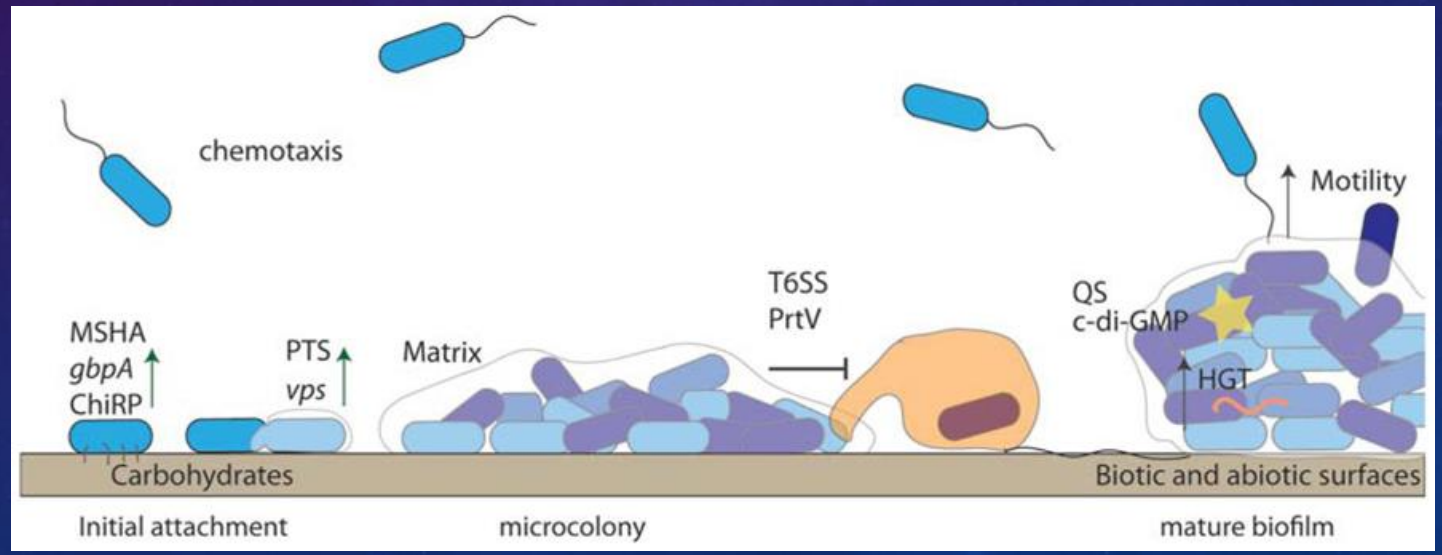
BACTERIAL CHARACTERISTICS AIDING RESIDENCE

- **Biofilms:** polysaccharide matrices protective against low pH and antimicrobials; helps growth and persistence
- **Toxin-coregulated pilus (TCP):** TCP induces bacterial clusters, through autoagglutination, called **microcolonies** that resist low pH, bile, antimicrobials, and prevent bacterial shedding. TCP is crucial for colonization



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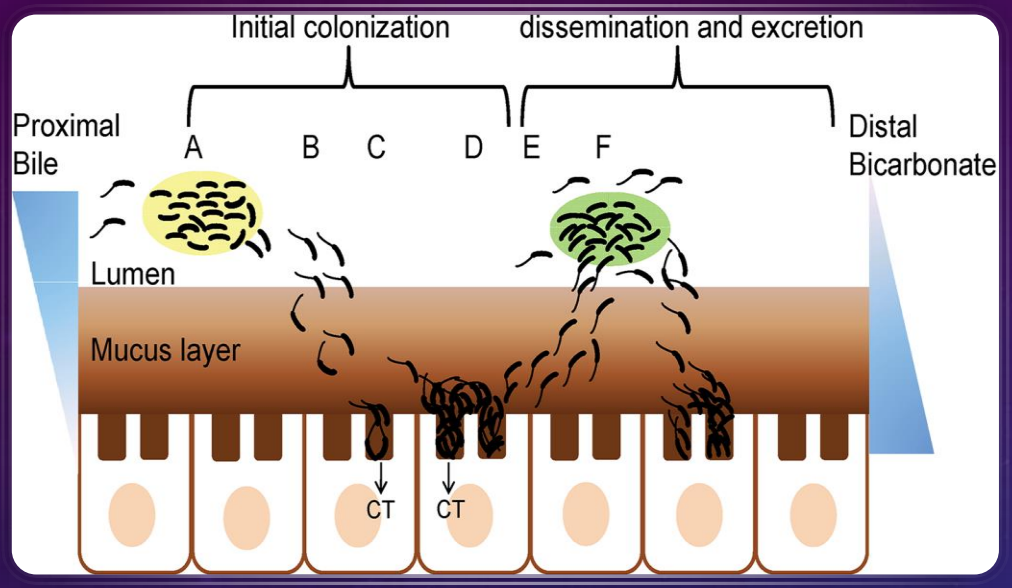
BACTERIAL CHARACTERISTICS AIDING RESIDENCE

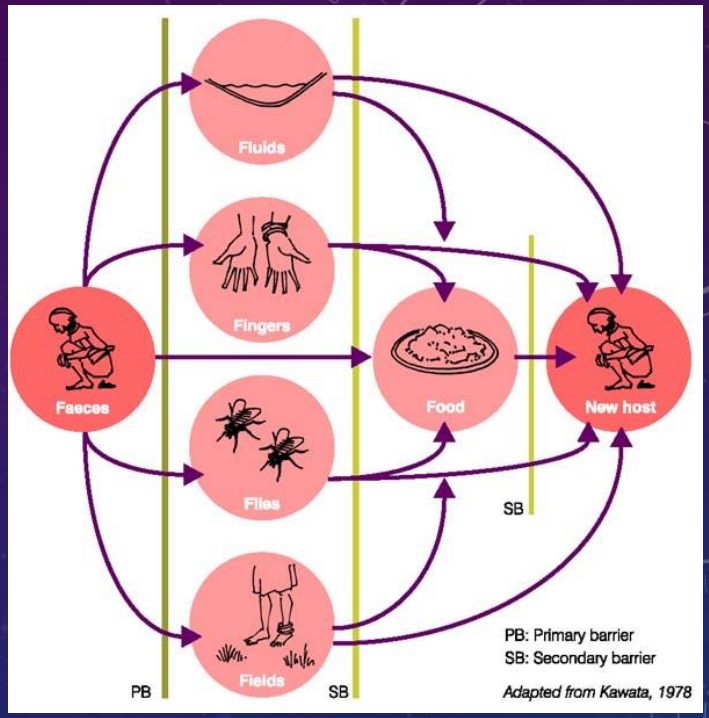


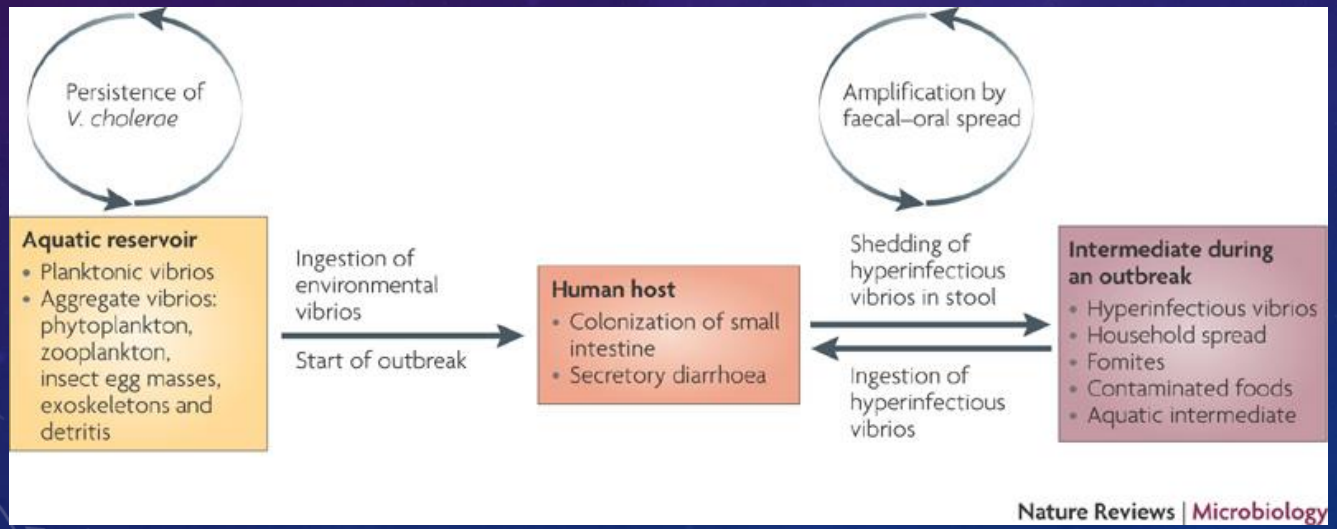
- Evolved to regard bile as a chemorepellent as it can be a bactericide, this drives movement from intestinal lumen and towards the mucus layer. Motility involving the **flagellum** and **chemotaxis** helps penetration of the mucus layer surrounding the epithelium
- **Mucinases HapA, TagA, NanH**, etc. help movement through mucus, modify cell surface glycoproteins, reveal cell receptors

- **Non-specific adhesin GlcNAc-binding protein (GbpA)** binds GlcNAc on glycoproteins and lipids on mucus and intestinal epithelial cells, then other adhesins are produced for committed attachment
- Evolved ways to utilize sialic acid and GlcNAc as carbon sources in gut mucosa

PATIENT CONTACT

Patients contact *V. cholerae* through ingesting fecally contaminated water or food. The risk is higher in areas with low sanitation, poor preparation and disinfection of water and food. Objects coming into contact with contaminated water, soil, etc. carry pathogenic Vibrio cholerae O1 or O139.





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http://www.nbc.na/sites/default/files/styles/large/public/Health%20Cholera.png?itok=L-gcWYEO

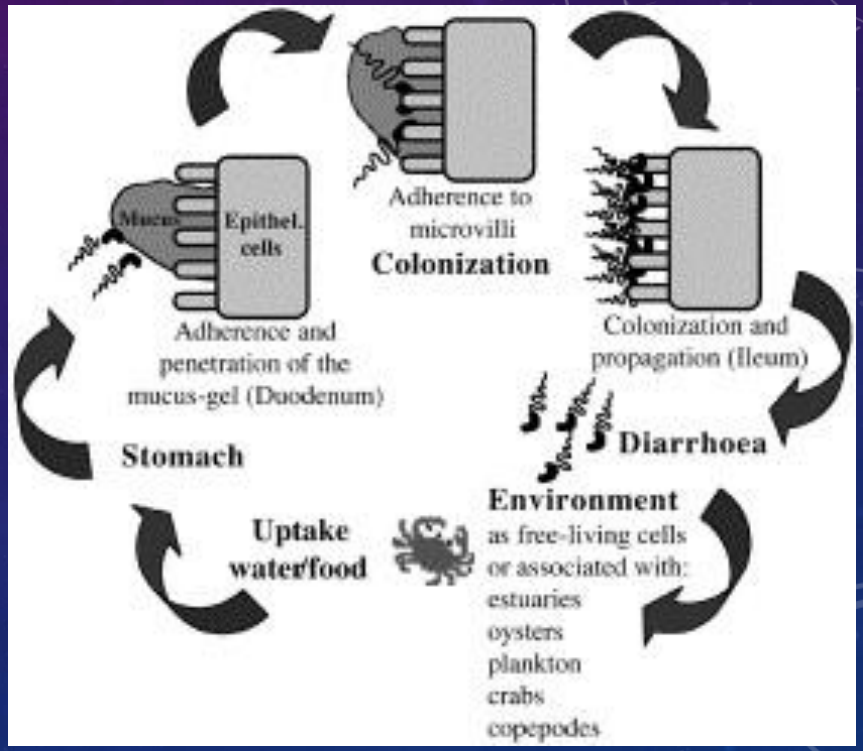
https://www.researchgate.net/profile/Vishal_Singh8/post/Is_cholera_spread_by_drinking_water/attachment/59d624f0c49f478072e9a0a5/AS:272154669584390@1441898137225/image/p058.jpg

2. ENTRY

FACILITATION OF ENTRY, BACTERIAL AND HOST MOLECULAR, CELLULAR, PHYSIOLOGICAL FACTORS AT ENTRY/ADHERENCE

ENTRY/ADHERENCE

- Entry into the human body orally and through the gastrointestinal tract into the small intestines, colonization occurs with flagellar movement, mucinases, and establishment of microcolonies by Toxin-coregulated pilus (TCP)
- Adherence is dependent on:
  - Factors enhancing colonization, eg. Motility
  - Factors necessary for colonization, eg. TCP, GbpA



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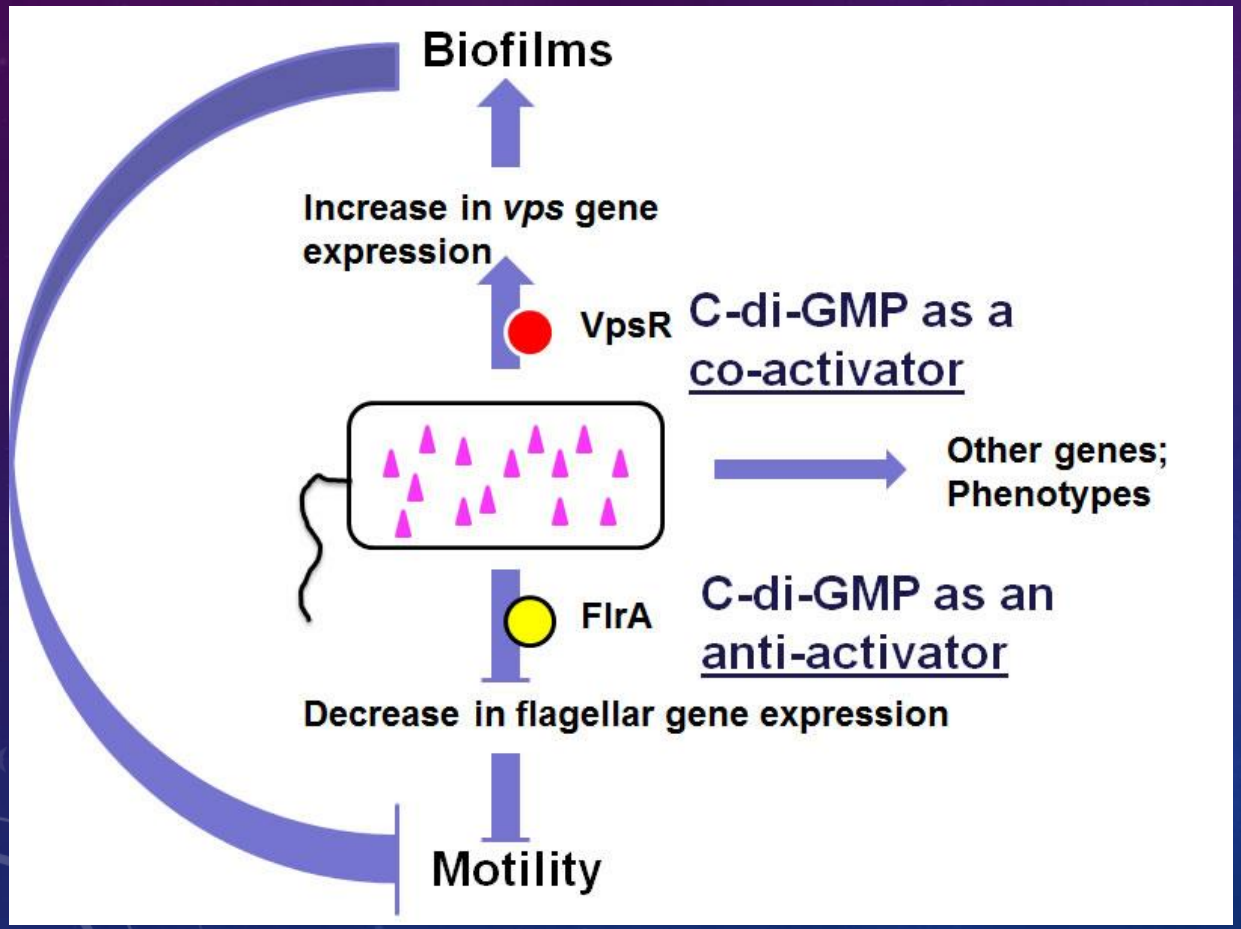
ADHERENCE



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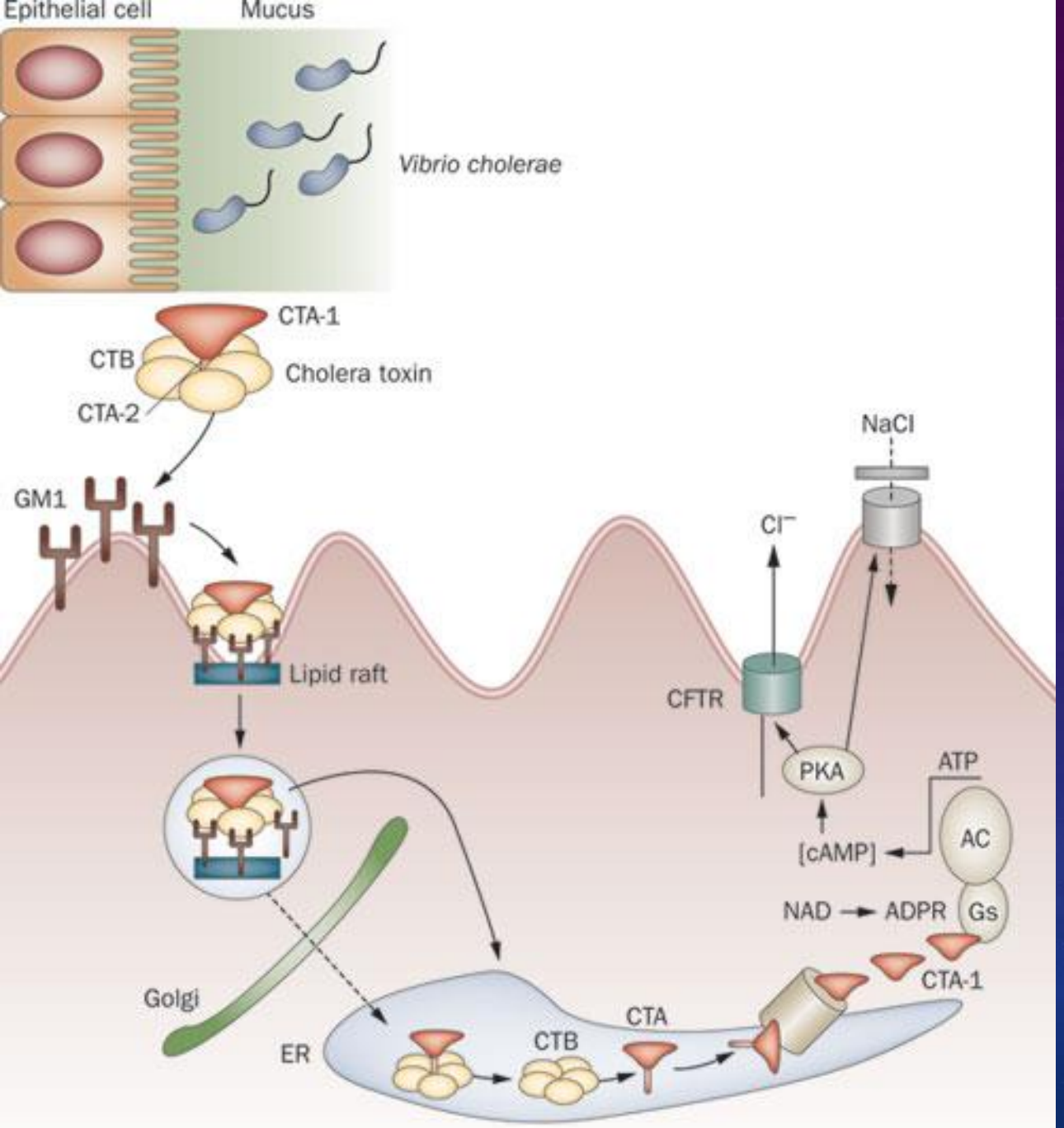
- Biofilm formation is important for colonization and adherence, providing protection against bile, antimicrobial peptides, mechanical clearance, and increases infectivity
- Forms along villi and crypts
- High c-di-GMP (secondary cyclic diguanylic acid) concentrations increases expression of VpsR and VpsT biofilm activators, while FlrA decreases flagellar expression



ADHERENCE/ PENETRATION

- Production of GbpA initially increases mucin production in the small intestine, which in turn increases GbpA expression = cooperative upregulation to facilitate mucin attachment by GbpA
- Penetration through mucus by flagellar motility is dependent on mucus viscosity. High viscosity mucus involve other methods such as mucinases, eg. Zn-dependent metalloprotease hemagglutinin (HA)/protease
- **Neuraminidase** degrades ganglioside to monosialosyl forms to reveal receptors

https://www.researchgate.net/profile/Sunheang_Shin/publication/51782058/figure/fig2/AS:267467838717978@1440780710846/Figure-2-Cholera-pathogenesis-and-cholera-toxin-actionAfter-ingestion-V-cholerae.png

HOST DEFENSE MECHANISMS AGAINST ENTRY AND ADHERENCE

- IgA antibodies in the small intestines can neutralize *V. cholerae* from attachment to mucus layer and also prevent binding of enterotoxins
- *V. cholerae* are sensitive to stomach acid, keeping infectious dose low, about 10^{11} bacteria for infection

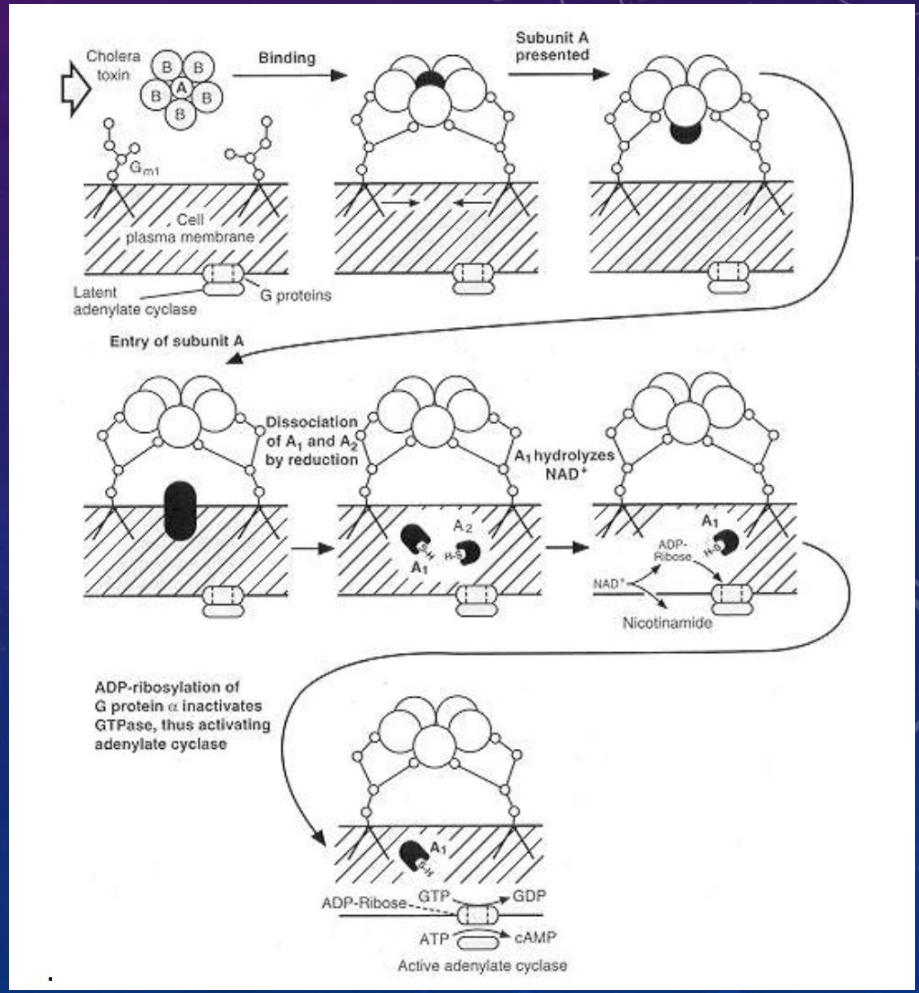
https://wonderopolis.org/_img?img=/wp-content/uploads/2014/12/1374_f.jpg&transform=resizeCrop,720,450

3. MULTIPLICATION AND SPREAD

DETERMINANTS OF BACTERIAL ENTRY INTO CELLS, BACTERIAL SPREAD AND SECONDARY INFECTION SITES

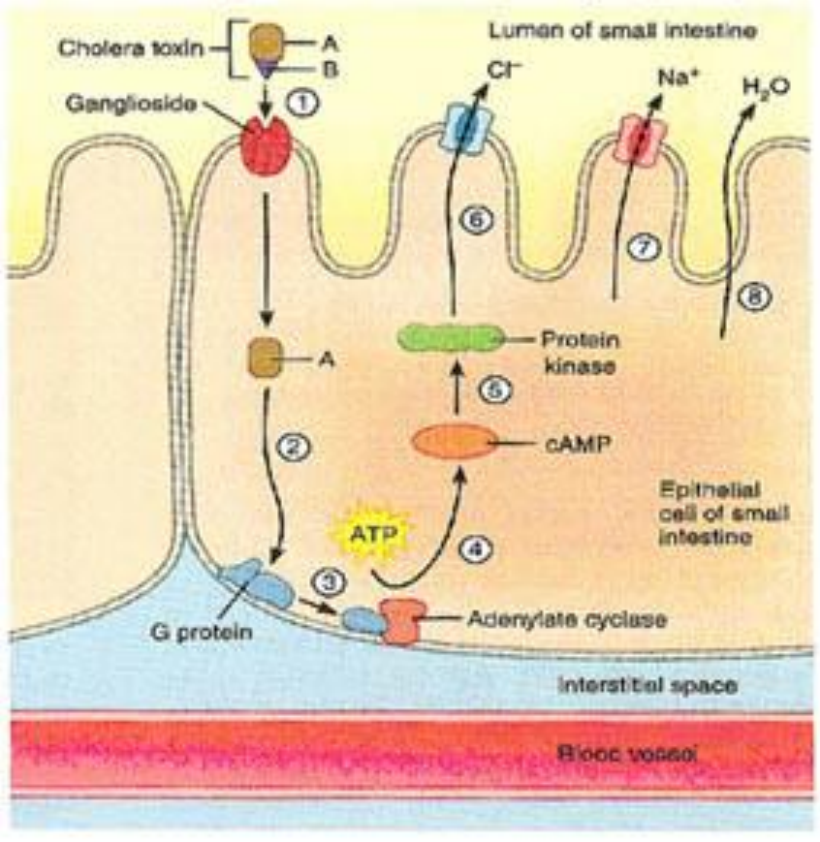
AN EXTRACELLULAR PATHOGEN

- *V. cholerae* is non-invasive and extracellular
- The TCP, allowing colonization, is encoded by the Vibrio pathogenicity island (VPI) on the bacteriophage, CTXΦ
- Only the **Cholera Toxin (CT)** enterotoxin enters epithelial cells and causes the disease (CT1 for O1 serogroups, CT2for O139 and other serogroups)
- CT is an AB5 ribosyltransferase: 5 B binding subunits, 1 A enzymatic subunit



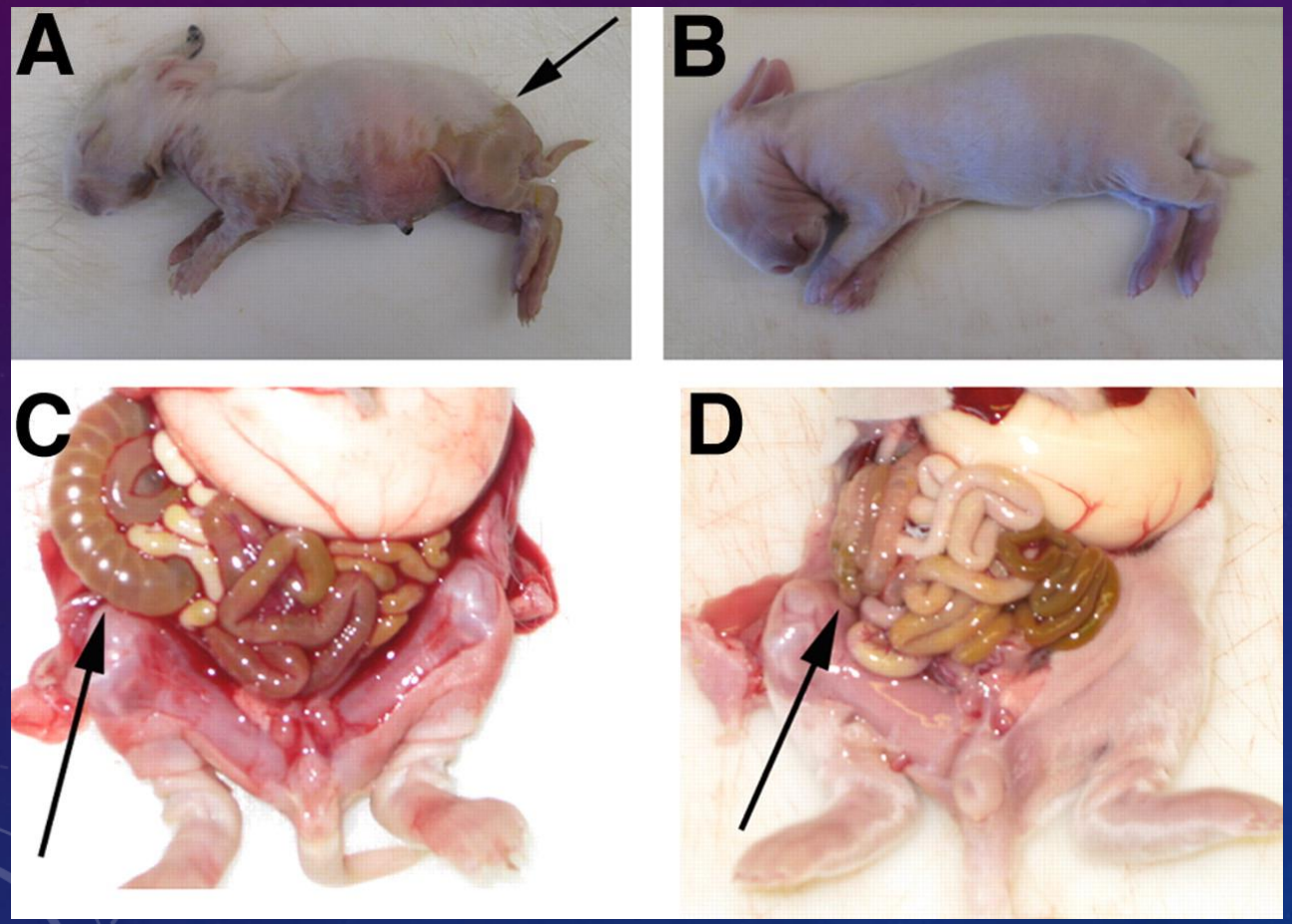
http://textbookofbacteriology.net/cholera_3.html

AN EXTRACELLULAR PATHOGEN



- B subunit binds to **monosialotetrahexosylganglioside (GM1)** on intestinal epithelial cells
- A subunit is endocytosed, ADP-ribosylates Gs_{α}, and the GTP-bound G protein continuously activates Adenylate Cyclase to produce cyclic AMP
- High [cAMP] activate the cystic fibrosis transmembrane conductance regulator (CFTR), resulting in excess secretion of electrolytes and ions, and water loss into the lumen
- Patient suffers from dehydration and watery diarrhea

LOCALIZATION



Infant rabbits inoculated with V. cholerae (left) or buffer (right)

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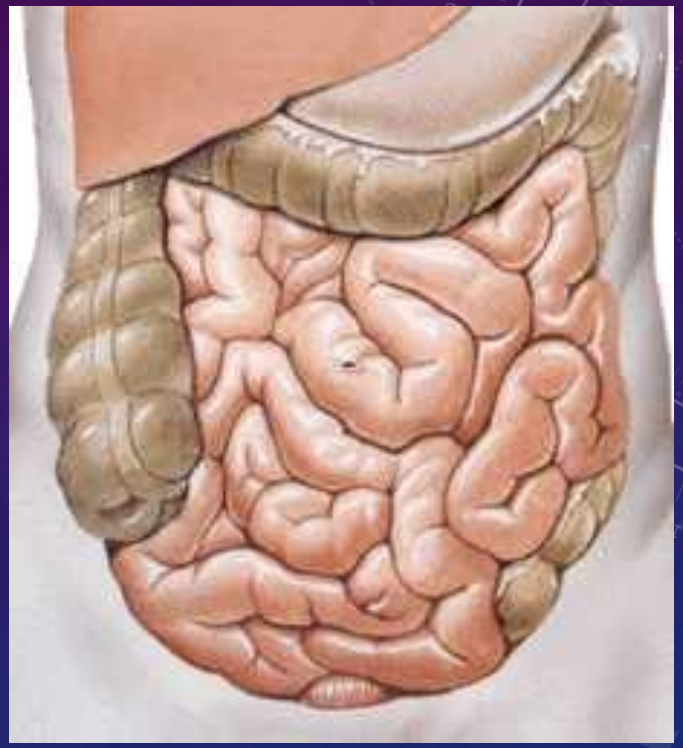


https://www.nobelprize.org/nobel_prizes/medicine/laureates/1994/illpres/cho-intestine.jpg

- *V. cholerae* preferentially colonize and localize in the small intestine, especially the distal region where mucin levels are lower

4. BACTERIAL DAMAGE

BACTERIAL DAMAGE AND HOST RESPONSE, CONNECTION TO SIGNS AND SYMPTOMS

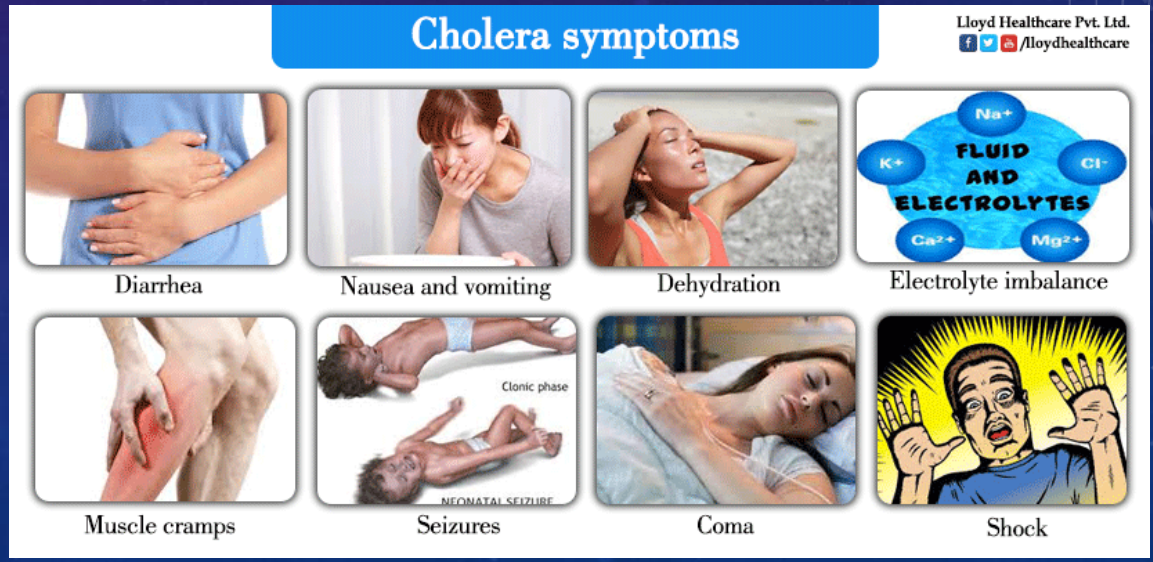
DAMAGE

- Host damage comes from Cholera Toxin's effect on intestinal epithelial cells, disrupting normal cellular function and regulation of electrolyte and water balance

https://image.shutterstock.com/z/stock-photo-severe-bacterial-shigella-salmonella-cholera-infection-of-the-intestines-resulting-in-121998433.jpg

LINK TO SIGNS AND SYMPTOMS

- Water loss into the lumen leads to dehydration, anuria, acidosis and shock. Other manifestations include low skin elasticity and dry mucous membranes.
- Electrolyte loss (sodium, potassium, calcium, and chloride, etc.) disrupts neuronal signaling to the muscles, causing muscle cramps. Loss of potassium ions may result in cardiac complications and circulatory system failure.
- Cholera Toxin damages mucus and epithelial cells, excreted in diarrhea
- The patient may additionally feel thirsty, restless, and irritable



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ADDITIONAL REFERENCES

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