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Title:

Vibrio cholerae: an opportunist of human crises

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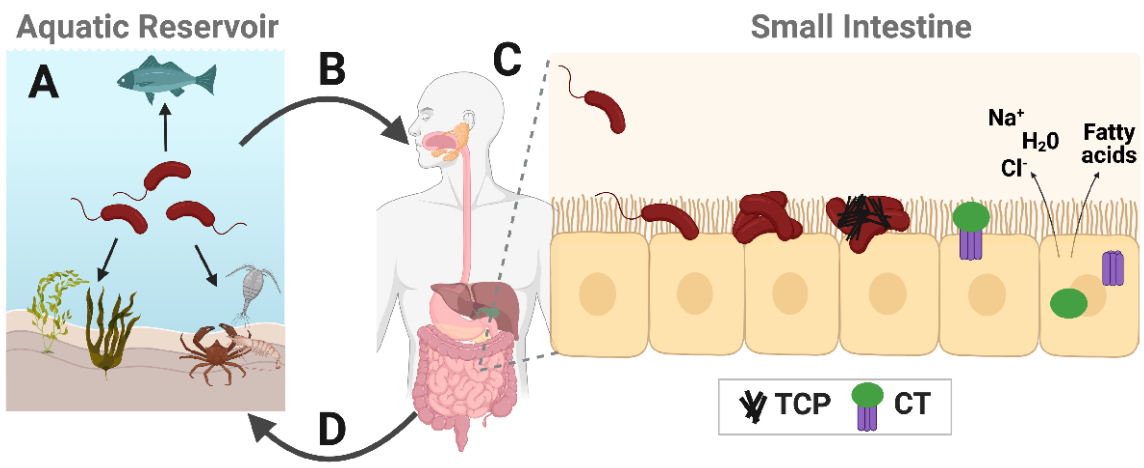
Abbreviations: TCP, toxin-coregulated pilus; CT, cholera toxin; VPI, *Vibrio* pathogenicity island; VSP, *Vibrio* Seventh pandemic island; ICE, integrative and conjugative element; SXT, ICE encoding resistance to sulfamethoxazole and trimethoprim.

SECONDARY TITLE/TAGLINE DESCRIBING THE MEDICALLY IMPORTANT MICROBE
Cholera: Humanity's scourge
GRAPHICAL ABSTRACT



Graphical abstract

Infection cycle of *Vibrio cholerae* O1. A) *V. cholerae* O1, the causative agent of the severe diarrheal disease cholera, is a natural inhabitant of aquatic ecosystems where it can be found associated with numerous reservoirs such as fish or crustaceans. **B)** Cholera can be contracted upon ingestion of food or water contaminated with the bacterium. **C)** Once in the small intestine, flagella-mediated motility propels *V. cholerae* O1 through the mucus layer towards the epithelium where it attaches, proliferates, and produce toxin-coregulated pilus (TCP) and cholera toxin (CT). Cholera toxin leads to increased concentrations of fatty acids in the lumen and causes an electrolyte imbalance that results in the profuse rice-water diarrhea associated with cholera. **D)** The diarrhea facilitates the dispersal of hyperinfectious *V. cholerae* O1, and the continuation of the infection cycle.

ABSTRACT

Vibrio cholerae O1 is the etiological agent of the severe diarrheal disease cholera. Annually, there are an estimated 1-4 million cholera cases worldwide and over 140,000 deaths. The primary mode of disease transmission is through the consumption of water or food contaminated with the bacterium. Although cholera patients can be effectively treated using rehydration therapy, the disease remains a major scourge in areas with limited access to clean water and proper sanitation. Its continued prevalence highlights the failures of socioeconomic policies leading to wealth disparities, fragile and dated public infrastructure, and lack of appropriate health surveillance.

HISTORICAL PERSPECTIVE

Cholera is an ancient disease dating back to the 5th century BC [1]. There have been seven recorded pandemics to date. The first pandemic originated in India and quickly spread across Asia and Europe. The seventh and current pandemic continues to wreak havoc around the globe.

CLINICAL PRESENTATION

Infections have an incubation time of 18 hours to 5 days and a mortality rate of over 50 %, if left untreated [1,2]. Cholera symptoms include prolonged and profuse 'rice-water' diarrhea, and vomiting, leading to rapid fluid and electrolyte loss and dehydration. Further complications that may occur include hypovolemic shock resulting in death within hours. Mild cholera cases often present with diarrhea lasting a few days.

MICROBIAL CHARACTERISTICS: PHENOTYPIC AND GENOTYPIC FEATURES

V. cholerae O1 is a halophilic, comma-shaped Gram-negative bacterium with a polar flagellum. The bacterium favors growth at alkaline pH, is oxidase positive, and able to ferment sucrose. From more than 200 serogroups, only the O1 and O139 serogroups can cause epidemic cholera. To date, only serogroup O1 has caused pandemic cholera and O139 is now virtually extinct. *V. cholerae* O1 strains can be further classified into two biotypes: classical, and El Tor, which caused the first six pandemics and the seventh, respectively [2]. Serogroup O1 is further divided into three serotypes, Ogawa, the most prominent, Inaba, and Hikojima, a rarer serotype. Serotype switching can occur between Ogawa and Inaba.

CLINICAL DIAGNOSIS, LABORATORY CONFIRMATION AND SAFETY

Clinical Diagnosis

Stool or rectal swabs are the primary specimens used for clinical diagnosis of cholera. Point-of-care diagnosis is achieved using the Crystal VC rapid dipstick test especially in places with limited or no laboratory testing facilities. Nonetheless, it is recommended to follow up these tests with traditional culture-based or PCR methods.

Laboratory confirmation

Specimens are transported in Cary Blair medium to ensure bacterial viability and diagnostic accuracy. The primary diagnostic test involves enrichment of specimens at alkaline pH and plating on selective media such as thiosulfate citrate bile salts sucrose agar on which *V. cholerae* forms yellow colonies. PCR-based detection of the *ompW* gene, which encodes an outer membrane protein, also confirms diagnosis. Subsequent biochemical and immunological assays are performed to further characterize *V. cholerae* isolates. Biochemical tests often investigate enzymatic activity and include tests for arginine dihydrolase and esculin hydrolysis for which *V. cholerae* is negative. Immunoassays to determine the serogroup, serotype, and presence of the cholera toxin include slide agglutination and immunoassays such as ELISA [1,2]. In contrast to endemic regions, where cholera patients are often quickly diagnosed, in non-endemic regions, cholera symptoms are often confused with other enteric pathogens. Nevertheless, persons presenting symptoms such as profuse ‘rice water’ diarrhea and recent travel to cholera endemic regions should be assessed for potential *V. cholerae* O1 infection.

Laboratory safety

V. cholerae O1 exists in a hyperinfectious state in the fecal matter of infected individuals [3]. Healthcare professionals should ensure proper disposal of fecal matter and handwashing to prevent transmission. *V. cholerae* O1 is classified as a Hazard Group 2 biological agent and laboratory procedures are recommended to be performed in a Biosafety level II facility. Handling of the bacterium requires the use of basic personal protective equipment such as gloves, laboratory coat and eyewear. Furthermore, any potentially aerosol-generating procedures should be contained within a microbiological safety cabinet. In rare cases, laboratory associated infections due to

ingestion or parenteral inoculation have been reported and treatment requires the use of antibiotics.

TREATMENT AND RESISTANCE

Treatment

Cholera patients with mild diarrhea, fluid loss, and electrolyte imbalance are primarily treated with oral rehydration salts. However, in severe cases, intravenous fluids are required to quickly restore fluid and electrolyte balance [1,2]. Zinc supplements reduce disease severity in affected children. In addition, antibiotics including doxycycline, erythromycin, and ciprofloxacin, are administered to shorten disease symptoms.

Resistance

V. cholerae O1 has become increasingly resistant to commonly used antibiotics, as observed in recent outbreaks in Mozambique. *V. cholerae* has acquired numerous integrative conjugative elements and plasmids, which, in conjunction with accumulated spontaneous mutations in target proteins, facilitate its antibiotic resistance [4]. Indiscriminate misuse of antibiotics for non-medical applications further exacerbates the problem of resistance.

PATHOGENIC STRATEGIES: HOST RANGE, TRANSMISSION, INFECTION AND VIRULENCE FACTORS

Host range and Infection

V. cholerae O1 transitions between two vastly different environments: the aquatic reservoir (mainly brackish waters) where it preferentially attaches to shellfish and the human gastrointestinal tract (Fig. 1). The infectious dose of *V. cholerae* O1 ranges from 10^2-$10^{6.}$ Upon entry into the human host, *V. cholerae* O1 must survive the acidic environment of the stomach, swim through the intestinal mucus layer, and proliferate after attaching to the intestinal epithelium, where it forms microcolonies that produce cholera toxin.

Transmission

Primary transmission of cholera occurs through consumption of food or water contaminated with *V. cholerae* O1 (Fig. 1). Cholera occurs in both endemic and epidemic patterns. In cholera-endemic regions, such as the Bay of Bengal, outbreaks occur annually and follow a seasonal pattern [1]. However, in non-endemic areas, cholera can be imported and cause local outbreaks that develop into epidemics. As seen in Yemen and Haiti, civil unrest, or natural disasters exacerbate the likelihood of an “opportunistic” disease like cholera [5]. In addition, asymptomatic carriers as well as community transmission within and among households facilitate amplification and persistence of the disease [5,6]**.**

Virulence factors

At the intestinal epithelium, two major virulence factors are expressed: the toxin-coregulated pilus (TCP), an essential intestinal colonization factor that mediates microcolony formation, and the cholera toxin (CT), which leads to nutrient release and fluid accumulation resulting in diarrhea [7,8] (Fig. 1). Several regulatory proteins exert transcriptional control over expression of the virulence cascade [9]. Central to this cascade is the cooperation between two regulator pairs: TcpPH and ToxRS. Both TcpP and ToxR are required for the maximal transcriptional activation of the downstream master virulence regulator ToxT, which directly activates the expression *of tcp* and *ctx* genes. Both TCP and CT are encoded within horizontally acquired genetic elements: the *Vibrio* pathogenicity island-1 (VPI-1) and the CTXΦ phage, respectively. The *zonula occludens* toxin (Zot) and the accessory enterotoxin (ACE), which increases fluid secretion, are also encoded within CTXΦ. *V. cholerae* O1 strains (classical and El Tor) also encode the *Vibrio* pathogenicity island-2 (VPI-2), which confers a fitness advantage during intestinal colonization. Finally, El Tor strains uniquely encode the *Vibrio* seventh pandemic island-1 and 2 (VSP-1 and VSP-2) and the integrative and conjugative element SXT.

EPIDEMIOLOGY, PREVENTION AND RISK GROUPS

Prevention

Preventative measures of cholera transmission include access to clean water, proper disposal of fecal matter, chlorination of drinking water and proper hygienic practices such as hand washing. In addition, public awareness and preparedness helps reduce disease burdens. Several recombinant and whole-cell vaccines have been developed, such as Dukoral and Shanchol that are 76 % effective [2]. Incorporation of climate dynamics into disease surveillance systems can help increase disease preparedness in endemic regions [10].

Risk groups

Several risk factors enhance transmission of cholera such as traveling to or from cholera endemic regions or consumption of uncooked/undercooked contaminated shellfish. Other host-related risk factors include blood type O or individuals on medications such antacids and proton pump inhibitors. Cholera outbreaks are often driven by weakened public infrastructure and severe wealth disparities, which, to date, continue to highlight the millions worldwide that struggle to obtain access to clean water.

OPEN QUESTIONS

1. What effects will global travel and climate change have on the frequency and severity of cholera outbreaks?

2. Why have only two serogroups emerged to be choleragenic, and what led to their emergence?

3. What host factors result in some people being asymptomatic carriers of *V. cholerae* O1?

4. Why does cholera follow seasonal patterns in endemic areas?

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

1. **Harris JB, LaRocque RC, Qadri F, Ryan ET & Calderwood SB.** Cholera. *Lancet*. 2012;379(9835):2466-2476 doi:10.1016/S0140-6736(12)60436-X
2. **Clemens JD, Nair GB, Ahmed T, Qadri F & Holmgren J.** Cholera. *Lancet* 2017;390:1539–1549 doi: 10.1016/S0140-6736(17)30559-7
3. **Merrell DS, Butler SM, Qadri F, Dolganov NA, Alam A *et al.*** Host-induced epidemic spread of the cholera bacterium. *Nature*. 2002;417(6889):642-645 doi:10.1038/nature00778
4. **Das B, Verma J, Kumar P, Ghosh A, & Ramamurthy T.** Antibiotic resistance in Vibrio cholerae: Understanding the ecology of resistance genes and mechanisms. *Vaccine*. 2020;38 Suppl 1:A83-A92. doi:10.1016/j.vaccine.2019.06.031
5. **Qadri F, Islam T, Clemens JD.** Cholera in Yemen - An Old Foe Rearing Its Ugly Head. *N Engl J Med*. 2017;377(21):2005-2007 doi:10.1056/NEJMp1712099
6. **Sugimoto JD, Koepke AA, Kenah EE, Halloran ME, Chowdhury F *et al.*** Household Transmission of *Vibrio cholerae* in Bangladesh. *PLOS Negl Trop Dis*. 2014;8(11):e3314 doi:10.1371/journal.pntd.0003314
7. **Almagro-Moreno S, Pruss K, & Taylor RK.** Intestinal Colonization Dynamics of *Vibrio cholerae*. *PLOS Pathogens* 2015;11(5) doi: 10.1371/journal.ppat.1004787
8. **Rivera-Chávez F, & Mekalanos JJ.** Cholera toxin promotes pathogen acquisition of host-derived nutrients. *Nature.* 2019;572, 244–248 doi: 10.1038/s41586-019-1453-3
9. **Matson JS, Withey JH, DiRita VJ.** Regulatory networks controlling Vibrio cholerae virulence gene expression. *Infect Immun*. 2007;75(12):5542-5549 doi:10.1128/IAI.01094-07
10. **Akanda AS, Jutla AS, Colwell RR.** Global diarrhoea action plan needs integrated climate-based surveillance. *Lancet Glob Health*. 2014;2(2):e69-e70 doi: 10.1016/S2214-109X(13)70155-4