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Vibrio cholerae: Epidemiology, Surveillance and Occurrence in Iraq

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INTRODUCTION

Cholera is caused by the gram-negative bacteria *Vibrio (V.) cholerae*, which in turn causes diarrhea. *V. cholerae* occurs in a habitat of fresh and salt water and is endemic to hot countries such as Latin America, South of Asia, and some areas of Africa. (Baker-Austin et al. 2013; Ali et al. 2015). Approximately three to five million people are infected with cholera each year, resulting in about 140,000 fatalities, half of which are children younger than five years old (Ali et al. 2015). As the global climatic modification and rising temperatures, *V. cholerae* is spreading to a new areas, which is requiring the creation of an emergency research to better understand this bacterium (Vezzulli et al. 2016; Deeb et al. 2018). Most *V. cholerae* are "non-O1/O139" environmental isolates which may or may not cause gastroenteritis, but only strains classified as O1 or O139 can cause pandemic cholera (Baker-Austin et al. 2018). The genome of *V. cholerae* includes 2 circular DNA. Cholera toxin (CT), a protein that leads to serious and unique watery diarrhea (known as "rice-water stool"). The toxin is produced by coding genes present in CTXphi (CTXφ) which is a temperate bacteriophage. The Insertion of the viral gene into the DNA of a bacteria is necessary for the production of the toxin (Al-Obaidi 2006; Arif et al. 2010).
Due to poor sanitation and hygiene procedures and slow treatment and prevention as per inadequate healthcare system, cholera is endemic in many developing countries. A report by the Islamic Relief Project indicated 1,500 deaths in Iraq in 2015 due to Cholera outbreak. There has been a new outbreak of cholera in Iraq on 20th of June 2022. The Iraqi Ministry of Health recorded 13 cases, most of which have been found in the Sulaymaniyah governorate/Kurdistan region (Qamar et al. 2022a).

The Causative Agent

the most important characteristics of *V. cholerae* are; comma-shaped, Gram-negative and facultative anaerobic bacteria. The bacteria can survive in salt water environments and readily attach to the chitin of shells of shrimps, crabs, and other crustaceans. Cholera is a potentially fatal disease spread by eating raw or uncooked marine life (Tarsi and Pruzzo 1999).
The bacterium is supplemented with a flagellum at one end and numerous pili all over its exterior. Their metabolic processes involve; aerobic respiration and anaerobic fermentation. Cholera outbreaks usually caused by two different serotypes or serogroups: O1 and O139. (Ramamurthy et al. 2020).

Clinical Symptoms

Cholera is a highly virulent disease that can trigger severe cases of vomiting and watery diarrhea. An individual could develop symptoms 12 hours to 5 days after consuming contaminated water or food. Cholera is a disease that can kill both children and adults within hours if they are not treated (Azman et al. 2013). Most infected people with *V. cholera* are asymptomatic, but the bacteria can live in their stool between 1 to 10 days after infection to be sheded into the environment, where they can infect others. Most people who get sick have only mild to moderate symptoms, while a few get extremely sick with acute watery diarrhea and severe dehydration, shock and finally death If left without treatment (Sack et al. 2004; Legros 2018).

Pathogenesis

Cholera specificity is to the small intestine only and nowhere else in the body. *V. cholerae* possess one or more adhesion

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factors that permit them to stick to the micro-villi and allowing them to take up residence and grow in the human small intestine (which is normally relatively free of bacteria due to the effective clearance mechanisms of peristalsis and mucus secretion). It has been hypothesized that a number of toxin-co-regulated pili and hemagglutinins play an important role in adherence, but the underlying mechanism has not yet been understood. It's possible that several mechanisms may be involved. Vibrio motility may contribute to pathogenicity by allowing the organisms to destroy the mucus barrier. Proteases, mucinolytic and neuraminidase enzymes are also produced by the bacteria (Ojeda Rodriguez and Kahwaji 2022).

Pathogenic genes in *V. cholerae* express proteins that play a role in the bacteria's virulence either directly or indirectly. Outer membrane vesicles (OMVs) which is a protective membrane modifications is released by *V. cholera* and help the bacterium adaptation to the intestines of a host to evade the host's defenses, such as bile acids and antimicrobial peptides (Ramamurthy et al. 2020).

By entering the small intestine of a human host, the genes encoding virulence factors expression starts to produce toxin-co-regulated pilus (Tcp) and cholera toxin. Cholera toxin, which is made up of two different parts called CtxA and CtxB, binds to the ganglioside (asyalylated glycosphingolipid) GM1 on the cell membrane of intestinal epithelium. Once cholera toxin is bound, it is endocytosed and retrogradely transported to the endoplasmic reticulum (ER), where the toxin's subunits are released. CtxA subunit allosteric activation by ADP ribosylation factor 6 (ARF6) requires its release from the endoplasmic reticulum (ER) into the cytosol (Almagro-Moreno et al. 2015). Adenylyl cyclase is stimulated when the ARF6-bound, active CtxA subunit catalyzes the ADP ribosylation of a G protein-coupled receptor. Intense diarrhea is caused by an elevation in cellular cAMP levels, which in turn stimulates the protein kinase A (PKA)-dependent phosphorylation (P) of the cystic fibrosis transmembrane receptor (CFTR) (Baker-Austin et al. 2018; Chen et al. 2022).

Immunity To *V. cholerae*

Pathogenic *V. cholerae* strains are distinguished from non-pathogenic strains by their ability to produce cholera toxin (CT) and a toxin coregulated pilus (TCP) (Baek et al. 2020). Bacteria, intestinal epithelial cells and mucus are all floating within watery diarrhea. The mucus layer that normally covers intestinal epithelial cells performs numerous tasks, such as acting as an energetic protective barrier against flora and foreign microbes. This mucus gel layer is secreted by goblet cells which are the specialized cells that line mucosal tissue (Cornick et al. 2015). It is postulated that mucin manufacturing can be altered by 2 methods; either by microbial factors that modify the release and production of mucin, or by host factors that are produced by epithelial cells or immune cells in reaction to intestinal bacteria (Grondin et al. 2020). The effects of CT of the bacteria triggers the production of vast quantities of mucin through a cAMP-dependent mechanism, which contributes to the diarrhoea creation. It has been shown that *V. cholerae* can use its flagellum to invade mucus membranes, while non-motile Vibrios are either unable to colonize or are avirulent (Herath et al. 2020). Recent research has demonstrated that exposure to *V. cholerae* outer membrane vesicles (OMVs) can prime CD4+ T cells for an inflammatory T helper 2 (Th2) response and increase expression of the IL-17, IL-13 and IL-4 cytokines, all of which have been linked to an increase in mucin output (Melhem et al. 2021).

CD4+ T cells appear to be influenced by *V. cholerae* and CT in a way that promotes their differentiation to both Th1 and Th2 cell lineages, with a possible bias toward the latter. When T cells are activated by intracellular bacteria, they usually develop into the Th1 cell ancestry and fasten a cellular response, by the activation of phagocytes like macrophages and cytotoxic T cells (CD8+ T cells) rather than antibody production. A humoral immune response, include B cell activation and immunoglobulin generation, is triggered by excitation of Th2 cells in response to extracellular pathogens (Grondin et al. 2020).

In spite of the significant effort put into studying the immunology of cholera and the pathogenesis of *V. cholerae*, many un-answered questions persist. The most pressing issue is developing more effective cholera vaccines, as the existing one only protect 60% against the disease (Shaikh et al. 2020).

Diagnosis

Nevertheless, the criterion for a cholera diagnosis in the lab is the isolation and identification of *V. cholerae* serogroup O1 or O139 from a fecal sample through culture. The Cary Blair media and thiosulfate-citrate-bile salts agar (TCBS), are ideal for transporting isolation and identification of pathogenic bacteria in addition to serogrouping *V. cholerae* isolates by specific reagents. Rapid diagnostic tests available for purchase are helpful in times of epidemic but should not be used for routine diagnosis because they do not produce an isolate for antimicrobial susceptibility testing and subtyping (CDC 2022).

The standard for confirming a cholera diagnosis, is the isolation of pathogen from a stool or rectal swab culture. Culture should be on alkaline peptone water then streak on the media TCBS, or taurocholate- tellurite gelatin agar to grow the bacteria taken by the swabs. After initial culture in non-selective media, *V. cholerae* O1 and O139 detection must be confirmed by slide agglutination using specific monoclonal or polyclonal antibodies (Somboonwit et al. 2017; Falconer et al. 2022). However, the distinguishable darting motility of *V. cholerae*, which is inhibited by *V. cholerae* specific antibody, allows for rapid detection using dark field microscopy, which is only about 50% as sensitive as culture (Martinez et al. 2010). Rapid and low-cost tests are crucial for the diagnosis of cholera in both clinical and public health settings. Unfortunately, there are currently a few tests available, including the the Sensitive Membrane Antigen Rapid Test, co-agglutination test, Medicos and the Institute Pasteur and Crystal VC rapid dipsticks. The use of PCR assays is restricted, and they are only performed in specialized labs (Falconer et al. 2022).

Treatment

The cholera can be treated with simple medication. When given quickly, oral rehydration solution (ORS) can effectively treat the vast majority of patients. One sachet of the WHO/UNICEF recommended oral rehydration salts (ORS) is mixed with 1 L of purified water. On day one, moderately dehydrated adults may need as much as 6 L of ORS (Williams and Berkley 2018). Patients with diarrhea receive antibiotics to reduce the severity of their symptoms, the amount of rehydration fluids required, and the time spent excreting *V. cholerae* in their feces (Hsueh and Waters 2019). As long as patients are diagnosed and treated promptly, the case mortality rate can be kept well below 1%. In children younger than 5 years, zinc is an essential supportive therapy that not only shortens the length of diarrhoea but also protects against forthcoming episodes of severe watery diarrhoea due to other reasons. Encouragement of breastfeeding is also important (Lazzerini and Wanzira 2016). Patients are typically advised to take doxycycline first, while children and pregnant women should take azithromycin. In South Asia new strains of bacteria are appearing that are multiple drug resistance, and they are also resistant to trimethoprim-sulfamethoxazole, tetracycline and quinolones. Although the Hispaniola strain is multiple drug resistance , it can be treated with tetracycline and doxycycline (N'cho et al. 2022). To compete the MDR of *V. cholerae*, a non-traditional antimicrobial strategy should be used in the treatment and management of *V. cholerae* infection such as Nanoparticles (Das et al. 2018)**.** Nanotechnology has recently assumed greater significance in the biomedical and pharmaceutical sectors as an alternative antimicrobial plan (Desselberger 2000). When compared to chemical and physical synthesis methods, the biosynthesis of green nanoparticles using natural extracts of different organisms offers significant economic and environmental benefits (Samuel et al. 2022). It is hypothesized that their biocidal efficacy results from the close interactions with microbial membranes made possible by their small size (100 nm) and high surface-to-volume ratio (Morones et al. 2005; Allaker 2010). Another perk of inorganic antibacterial agents like metals and metal oxides is that they are more stable than organic compounds, which are more susceptible to degradation (Sawai 2003; Sondi and Salopek-Sondi 2004). Zinc oxide, in particular, has garnered interest as a potential antimicrobial agent among these metal oxides. ZnO prevents the attachment and endocytosis of enterotoxigenic *V. cholerae* (Sarwar et al. 2017).

In addition, ZnO's nanoparticle size makes it a potential bactericidal agent by inducing the generation of harmful oxygen radicals that can cause damage to DNA, cell membranes, and proteins (Salem et al. 2015).

Epidemiology, Risk Factors and Disease Burden

There are two types of cholera outbreaks: endemic and epidemic. In an endemic area, the disease is constantly present and has been there for at least three years (Ecarnot et al. 2021). In addition to endemic countries, cholera free countries can also undergo an epidemic outbreak. However, in endemic countries, cholera outbreaks can be seasonal or intermittent (Jutla et al. 2013). Having at least one cholera case confirmed in *cholerae* free country, means there is an outbreak in a country where cholera is not commonly found (Muzembo et al. 2022). Poor sanitation and water supply are major contributing factors in the spread of cholera. Places like slums on the outskirts of cities and refugee camps often fall into this category because they lack basic services like clean water and toilets. If cholera bacteria are already available or are introduced, the chance of spreading is increased due to the humanitarian crisis's effects, such as the destruction of water and sanitation facilities or the emigration of people to insufficient and overcrowded camps. Epidemics have never been linked to non-infected carcasses (Nsagha et al. 2015).

Prevention and Control

Ending Cholera: a global roadmap to 2030, is a strategic plan for cholera control, released in 2017 with the goal of reducing cholera disease mortalities to only 10% by 2030. It is predicted by scientists that about 21,000 to 143,000 individuals die yearly due to cholera infections around the world (GTFCC 2022). Cholera outbreaks can be contained and prevented with the help of oral cholera vaccines in affected area where this disease is common. Cholera continues to be a major health concern around the world and is a reflection of social inequality and stagnation (Kanungo et al. 2022).

Cholera control requires multiple strategies working in tandem. Oral cholera vaccines and therapies are used alongside other measures such as surveillance and WASH (water, sanitation, and hygiene) initiatives and community outreach (Kanungo et al. 2022).

Surveillance

Surveillance for cholera must be a part of a larger system for monitoring the spread of disease, for international collaboration (Ngwa et al. 2016). Cholera can be detected more easily with the help of rapid diagnostic tests (RDTs), where the presence of even one positive sample will authorize a cholera warning (Chowdhury et al. 2021). In order to confirm the results, the specimens are sent to a lab where they can be cultured or tested with polymerase chain reaction. The effectiveness of a surveillance system and the preparation of control legislation depend critically on the local capacity to detect (diagnose) and monitor (collect, compile, and analyze data) cholera occurrence. In order to better identify and react to cholera outbreaks, it is recommended that countries at risk improve their disease surveillance and national readiness. When deciding whether or not to issue an official alert, public health officials must always compare the circumstances surrounding a cholera outbreak to the standards listed in the International Health Regulations (Ganesan et al. 2020).

Water and Sanitation Interventions

To effectively combat cholera over the long period of time, there is need of an economical investment and ensurance that everyone has access to clean water and proper sanitation. Implementing long-term sustainable WASH solutions to ensure the use of safe water, basic sanitation, and good hygiene standards in cholera zones is the primary action promoting environmental conditions. In addition to preventing cholera, these sorts of intervention strategies help in the fight against poverty, malnutrition, and the lack of education. Water, Sanitation, and Hygiene (WASH) measures to combat cholera are aligned with Sustainable Development Goals (SDG) targets (Challa et al. 2022).

Community Engagement

To be "community engaged" is to have people and communities take part in planning and executing projects. The adaptation of preventative hygiene measures, such as hand washing with soap, safe food preparation and storage, and safe disposal of children's stool, is greatly facilitated by local cultural practices and beliefs, as are funeral rituals for cholera victims that reduce the likelihood of the disease spreading to mourners (WHO 2020).

Oral Cholera Vaccines

Dukoral®, ShancholTM, and Euvichol-Plus® are the three currently available WHO-approved oral cholera vaccines (OCV). All three vaccines require 2 doses for full protection (WHO 2022a).
WHO Position Paper on Vaccines against Cholera in 2017 stated that "oral cholera vaccines must be used in locations with endemic cholera, in humanitarian disasters with a greater risk of cholera, and during cholera outbreaks; always in combination with other cholera prevention and control strategies; vaccination should not hinder the requirement of other urgent healthcare systems to govern or inhibit cholera outbreaks" (WHO 2022a).

Non-Cholera Infections

The most common types of human disease caused by Vibrio bacteria are cholera and non-cholera infections (Baker-Austin et al. 2018).
Non-cholera Vibrio spp. other than *V. cholerae*, such as *V. parahaemolyticus* and *V. vulnificus*, cause a group of infections with varying clinical features based on the bacterium species, route of infection, and host susceptibility. Mild gastroenteritis or primary septicemia can result from ingesting non-cholera bacteria, while exposing skin wounds to contaminated water can lead to a wound infection and, potentially, secondary septicemia. Non-cholera Vibrio spp. occur naturally in sea water and seafood, and prefer environments with a medium to high salinity. These bacteria thrive in aquatic and marine settings (Baker-Austin et al. 2018).

V. cholerae in Veterinary Medicine

One of the very important zoonotic bacterium is *V. cholerae* which has been found to infect marine mammals in a variety of environments, including fresh and salt water (Chen et al. 2022). Dogs can become infected with cholera if they consume a large quantity of food or water contaminated with *V. cholerae*. Epidemics in bison, dogs and cattle have already been observed (CFSPH 2006).
Though restricted discharge standards have been constructed for aquatic wastewater because of the industry's rapid growth, lax monitoring inevitably leads to environmental pollution and the subsequent spread of pathogens (Chen et al. 2022).
Bacterial infections were a major issue in the onset of intensive Atlantic salmon farming, specifically cold water vibriosis (*V. salmonicida*, *V. anguillarum*), and furunculosis (Aeromonas salmonicida ssp. salmonicida). The use of antibiotics to treat these diseases was only moderately successful at first, but vaccination eventually proved to be the most effective method for eradicating them (Novotny et al. 2004).
External and internal normal flora of fish typically include vibrios, which are common, facultative bacteria. Salmonids and other fish and aquatic animals kept in marine and brackish environments are susceptible to vibriosis. Vibriosis, also known as saltwater furunculosis, has been linked to MAS and furunculosis. *V. anguillarum* is the most prevalent pathogenic species in salmonids (Webster and Lim 2001).
However, fish are the vector for the *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*, all of which are found in the marine ecosystem (Novotny et al. 2004).

V. cholerae in Iraq

Cholera epidemics could break out in Iraq because the country is on the path of pilgrims take to reach Mecca and houses several important religious sites. During the cholera outbreak of 1820, many people in Basra, Iraq, died. In Baghdad, the disease had a similar impact. After that, the disease vanished from Iraq altogether, only to re - emerge in August 1966 as part of the seventh pandemic wave. Since then, the occasional outbreaks have continued in Iraq. After the Gulf War in 1991, cholera spread to every governorate in Iraq. The rural areas suffered the most during the months of April through November, when the danger level was the highest (Khwaif et al. 2010).

Baghdad city records represented one-third of all reported cases in Iraq during the 1991 epidemic, but for significantly lower percentage of the total cases infected during the years between the two epidemics of 1991 and 1998. In 1999, however, Baghdad once again contributed approximately one third of all reported cases. From 1999 to 2005, only 35 cases were reported and in the three years prior to 2007, there were no September-December cholera recorded cases in Baghdad. In 2007, however, cholera broke out across Iraq (Al-Obaidi 2006 Jameel et al. 2016).

There were 4667 confirmed cases of cholera in Iraq in 2007, with 136 (2.9% of the total) being recorded in the capital city of Baghdad. In Baghdad, the cases were reported in the period from September to December. Five asymptomatic cholera cases were found to be carriers (0.5%). There were three cholera deaths in Baghdad, to comprise a case fatality rate (CFR) of 2.2% (Khwaif et al. 2010).

The frequency of cholera outbreaks in Iraq appears to repeat every four to five years. After the peak in 1998–1999, there were four more peaks in 2003, 2007, 2012, and 2015; the most recent epidemic impacted people of all ages and had a case count of around 3000. This long-term pattern may be attributable to the period of time needed for the microorganism to reproduce and reorganize itself in a large population, or to the buildup of a vulnerable cluster prior to the outbreak of an epidemic. Potential contributors to these pandemics include a lack of public knowledge about the diseases' transmission mechanisms, a dearth of medical professionals, and the destabilization of health infrastructure (including the availability of clean water) (Hussain and Lafta 2019).

Over the recent years, WHO has received a disproportionately large number of reports of cholera. As of March, 2020, there had been reported cases in 24 countries comprising of 323 369 cases, with 857 deaths. Many cases are not recorded due to gaps in surveillance systems and fear of impact on trade and tourism, which accounts for the difference between these statistics and the estimated burden of the disease (WHO 2022b).

On June , 2022, the Central Public Health Laboratory (CPHL) confirmed 13 cases of cholera in Sulaymaniyah, Kirkuk, and Muthanna (WHO 2022d).

As of August, 2022, 865 cases of cholera had been confirmed in Iraq, resulting in 4 deaths. Thi -Qar, Baghdad-Rasafa, and Kirkuk are the governorates with the highest numbers of cases and fatalities: 55, 224 and 480 cases respectively. The Early Warning, Alert and Response Network (EWARN) system has also received reports of acute diarrhea cases in camps (WHO 2022c).

If the case fatality rate (CFR) for cholera is less than 1%, as recommended by the World Health Organization (proper cholera case management should keep CFR below 1%), then the case management is successful. Many water sources in the Sulaymaniyah governorate have been treated, and the water trucking industry has been enlisted to help to get clean water to the people who need it. Cholera cases decreased in the Sulaymaniyah province as a result of this and effective crisis intervention (WHO 2022c) .

Social and economic instability in Iraq has persisted for decades, creating problems with access to healthcare, labor availability, and the availability of necessary supplies. From 2014 to 2017, the Islamic State of Iraq and the Levant (ISIL) employed a strategy of aggression in which it deliberately destroyed water organization and delivery systems in order to flood cities, disturb agriculture, and withdraw residents of access to Water, Sanitation, and Hygiene (WASH) resources. (Qamar et al. 2022b).

Iraq is particularly susceptible to waterborne and infectious diseases due to its site lengthways the Tigris and Euphrates, whose polluted water provide the greater part of the country's water needs, and its proximity to adjacent countries that experience frequent disease outbreaks. The result was a devastating cholera epidemic in 2015, with the maximum number of informed cases appearing in October after unusually heavy precipitation (WHO 2022d). With the importance of these risk factors for Cholera clearly established, strategies to aid the Iraqi public, such as early detection, management, and treatment, become crucial (Chen et al. 2022). As of the 20th of June, 2022, the Iraqi Ministry of Health is dealing with 13 cases of Cholera, most of which have been reported in the Sulaymaniyah province of the Kurdistan region. The recent shipment of medical supplies to the Kurdistan region attests to the United Nations continuing to cooperate with the Ministry of Health of Iraq to improve healthcare outcomes during the Cholera outbreak as of June 2022 (WHO 2022d) Local health officials are committed to preventing the disease's spread and have been conducting awareness campaigns, monitoring and treating water sources, and conducting routine food inspections to demonstrate their resolve. The local health authorities' encouraging response is consistent with a U.N. initiative to encourage governments to be more pro-active in providing basic necessities to the poor and vulnerable (Qamar et al. 2022a).

Conclusion

Infection with the bacterium *V. cholerae*, (Cholera) continues to be a major health problem in many parts of the world. After experiencing an outbreak of cholera in 2015, Iraq is still fighting with the disease today. Inadequate infrastructure, ineffective healthcare system, inadequate sanitation and hygiene, malnutrition, and a military conflict state; all play a role in the spread of cholera. Rehydration treatments, antidiarrheals, vaccinations, food, and monetary aid are still being distributed as part of massive-scale relief efforts. Long-term health gains can only be achieved through the application of education campaigns and the development of better facilities and public healthcare facilities. More information and study are needed to devise methods for controlling Cholera's spread and bringing down its worldwide prevalence.

REFERENCES

Allaker RP, 2010. The use of nanoparticles to control oral biofilm formation. Journal of Dental Research 89(11): 1175-1186.

Ali MN et al., 2015. Updated Global Burden of Cholera in Endemic Countries. PLOS Neglected Tropical Diseases 9(6): e0003832.

Almagro-Moreno S et al., 2015. Intestinal Colonization Dynamics of *Vibrio cholerae*. PLoS Pathogens 11(5): e1004787.

Al-Obaidi RM, 2006. A Comparison Study Between Clinical *Vibrio cholerae* Isolated from Patients and those Isolated from Surface Water in Iraq by Pulsed Feild Gel Electrophoresis [MSC]. University of Baghdad.

Arif SK et al., 2010. Application of PCR In Rapid Detection of Toxigenic *Vibrio Cholerae* in Water Samples in Sulaimania-Iraq. The Egyptian Journal of Experimental Biology (Botany), 6(1): 65–65.

Azman AS et al., 2013. The incubation period of *cholera*: A systematic review. Journal of Infection 66(5): 432–438.

Baek Y et al., 2020. Cholera Toxin Production in *Vibrio cholerae* O1 El Tor Biotype Strains in Single-Phase Culture. Frontiers in Microbiology 11(10): 3389.

Baker-Austin C et al., 2018. Vibrio *spp*. Infections. Nature Reviews Disease Primers 4(1): 1–19.

Baker-Austin C et al., 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. Nature Climate Change 3(1): 73–77.

CDC, 2022. Diagnosis and Detection | *Cholera* | CDC. https://www.cdc.gov/cholera/diagnosis.html

CFSPH, 2006. *Cholera*. CFSPH. https://www.cfsph.iastate.edu/search_gcse/

Challa JM et al., 2022. Inadequate Hand Washing, Lack of Clean Drinking Water and Latrines as Major Determinants of *Cholera* Outbreak in Somali Region, Ethiopia in 2019. Frontiers in Public Health 10: 845057.

Chen Y et al., 2022. *Vibrio cholerae*: A pathogen shared by human and aquatic animals. The Lancet Microbe 3(6): e402.

Chowdhury G et al., 2021. Laboratory evaluation of the rapid diagnostic tests for the detection of *Vibrio cholerae* O1 using diarrheal samples. PLoS Neglected Tropical Diseases 15(6): e0009521.

Cornick S et al., 2015. Roles and regulation of the mucus barrier in the gut. Tissue Barriers 3(1–2): e982426.

Das S et al., 2018. Neutralization of cholera toxin with nanoparticle decoys for treatment of *cholera*. PLoS Neglected Tropical Diseases 12(2): e0006266.

Deeb R et al., 2018. Impact of Climate Change on *Vibrio vulnificus* Abundance and Exposure Risk. Estuaries and Coasts 41(8): 2289–2303.

Desselberger U, 2000. Rotaviruses: basic facts. Methods in Molecular Medicine 34: 1–8.

Ecarnot F et al., 2021. Vaccines and Senior Travellers. Frontiers in Aging 2: 677907.

Falconer J et al., 2022. Cholera diagnosis in human stool and detection in water: A systematic review and meta-analysis. PLoS ONE 17(7): e0270860.

Ganesan D et al., 2020. Cholera surveillance and estimation of burden of cholera. Vaccine 38: 13–17.

Grondin JA et al., 2020. Mucins in Intestinal Mucosal Defense and Inflammation: Learning from Clinical and Experimental Studies. Frontiers in Immunology 11: 2054.

GTFCC, 2022. Roadmap 2030 – Global Task Force on Cholera Control. https://www.gtfcc.org/about-gtfcc/roadmap-2030/

Herath M et al., 2020. The Role of the Gastrointestinal Mucus System in Intestinal Homeostasis: Implications for Neurological Disorders. Frontiers in Cellular and Infection Microbiology 10: 248.

Hsueh BY and Waters CM, 2019. Combating Cholera. F1000Research 2019: 8.

Hussain AMA and Lafta RK, 2019. Trend of cholera in Iraq in the time of unrest. Mustansiriya Medical Journal 18(1): 1.

Jameel SK et al., 2016. The Isolation of *Vibrio cholera* and Other Enteric Bacteria with Molecular Characterization of *Vibrio cholera* during the Outbreak of Baghdad/Iraq in 2015. Advances in Microbiology 6(9): 699–715.

Jutla A et al., 2013. Environmental Factors Influencing Epidemic Cholera. The American Journal of Tropical Medicine and Hygiene 89(3): 597–607.

Kanungo S et al., 2022. Cholera. The Lancet 399(10333): 1429–1440.

Khwaif JM et al., 2010. Cholera outbreak in Baghdad in 2007: An epidemiological study. Eastern Mediterranean Health Journal 16(6): 584–589.

Lazzerini M and Wanzira H, 2016. Oral zinc for treating diarrhoea in children. The Cochrane Database of Systematic Reviews 2016(12): CD005436.

Legros D, 2018. Global Cholera Epidemiology: Opportunities to Reduce the Burden of Cholera by 2030. The Journal of Infectious Diseases 218(3): 137–140.

Martinez RM et al., 2010. Growth and Laboratory Maintenance of *Vibrio cholerae*. Current Protocols in Microbiology 17(1): 6A-1.

Melhem H et al., 2021. Mucins Dynamics in Physiological and Pathological Conditions. International Journal of Molecular Sciences 22(24): 13642.

Muzembo BA et al., 2022. Cholera Outbreaks in India, 2011–2020: A Systematic Review. International Journal of Environmental Research and Public Health 19(9): 5738.

Morones JR et al., 2005. The bactericidal effect of silver anoparticles. Nanotechnology 16(10): 2346–2353.

N'cho HS et al., 2022. Cholera. Yellow Book: Travelers' Health, CDC. https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/cholera

Ngwa MC et al., 2016. Cholera public health surveillance in the Republic of Cameroon-opportunities and challenges. The Pan African Medical Journal 24: 222.

Novotny L et al., 2004. Fish: A potential source of bacterial pathogens for human beings. Veterinární Medicína 49: 343-358.

Nsagha DS et al., 2015. Assessing the risk factors of cholera epidemic in the Buea Health District of Cameroon. BMC Public Health 15: 1128.

Ojeda Rodriguez JA and Kahwaji CI, 2022. *Vibrio Cholerae*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK526099/

Qamar K et al., 2022a. Rise of cholera in Iraq: A rising concern. Annals of Medicine and Surgery 81: 104355.

Qamar K et al., 2022b. Rise of cholera in Iraq: A rising concern. Annals of Medicine and Surgery 81: 104355.

Ramamurthy T et al., 2020. Virulence Regulation and Innate Host Response in the Pathogenicity of *Vibrio cholerae*. Frontiers in Cellular and Infection Microbiology 10: 572096.

Sack DA et al., 2004. Cholera. The Lancet 363(9404): 223–233.

Shaikh H et al., 2020. Current and future cholera vaccines. Vaccine 38: 118–126.

Salem W et al., 2015. Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*. International journal of medical microbiology (IJMM) 305(1): 85–95.

Samuel M S et al. 2022. A Review on Green Synthesis of Nanoparticles and Their Diverse Biomedical and Environmental Applications. Catalysts (MDPI), 12(5), 459.

Sarwar N et al., 2017. Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives. Chemosphere 171: 710–721.

Sawai J, 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of Microbiological Methods 54(2): 177–182.

Somboonwit C et al., 2017. Current views and challenges on clinical cholera. Bioinformation 13(12): 405–409.

Sondi I and Salopek-Sondi B, 2004. Silver Nanoparticles as Antimicrobial Agent: A Case Study on *E. coli* as a Model for Gram-Negative Bacteria. Journal of Colloid and Interface Science 275: 177-182.

Tarsi R and Pruzzo C, 1999. Role of Surface Proteins in *Vibrio cholerae* Attachment to Chitin. Applied and Environmental Microbiology 65(3): 1348–1351.

Vezzulli L et al., 2016. Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. Proceedings of the National Academy of Sciences 113(34): 5062–5071.

Webster CD and Lim C, 2001. Nutrition and Fish Health (Illustrated edition). CRC Press Inc.

WHO, 2020. Community Engagement: A health promotion guide for universal health coverage in the hands of the people. World Health Organization.

WHO, 2022a. Cholera. https://www.who.int/news-room/fact-sheets/detail/cholera

WHO, 2022b. Cholera. https://www.who.int/news-room/fact-sheets/detail/cholera

WHO, 2022c. WHO | Situation Report Iraq | Week 31 | United Nations in Iraq. https://iraq.un.org/en/194395-who-situation-report-iraq-week-31.

WHO, 2022d. WHO provides Sulaymaniyah with urgent medical supplies to respond to cholera outbreak. World Health Organization - Regional Office for the Eastern Mediterranean. http://www.emro.who.int/iraq/news/who-provides-sulaymaniyah-with-urgent-medical-supplies-to-prepare-for-and-respond-to-cholera-outbreak.html

Williams PCM and Berkley JA, 2018. Guidelines for the management of paediatric cholera infection: A systematic review of the evidence. Paediatrics and International Child Health 38(1): 16–31.