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Blood group O and risk of infection with Vibrio cholerae

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BLOOD GROUP O AND RISK OF INFECTION WITH VIBRIO CHOLERAE

by

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ABSTRACT

Cholera is an acute diarrheal illness burdening several developing countries caused by toxigenic *Vibrio cholerae*, where endemics maintain a seasonal pattern and occur more than once a year. Cholera is endemic in certain regions of Africa and South America, and southern Asia, where outbreaks are associated with poor hygiene and sanitary conditions. Every year, 3–5 million cases of cholera are diagnosed, and it causes more than 100,000 deaths worldwide. Cholera toxin is secreted by the *V. cholerae* bacteria and causes extreme secretory diarrhea, most commonly in poor hygiene environment. Watery diarrhea, vomiting, and abdominal cramps characterize the illness and approximately 5–10% of patients die of severe fluid loss if left untreated. The structure and function of the cholera toxin, its subunits, receptor, and impact on hyperactivation of cyclic adenosine monophosphate (cAMP) were sufficiently described in the 1970s. These findings fit with epidemiologic observations, which determined that the cholera toxin must first enter intestinal cells by binding to monosialoganglioside (GM1) on the host's epithelial surface.

The correlation between increase risk of *V. cholerae* infection and individuals with a particular ABO blood group type is unclear because of the scarce information and few studies conducted. Thus, this study reviews published research articles to better

understanding the association between the blood group O and susceptibility to developing severe cholera symptoms.

Several large studies have recorded an association between ABO blood groups and different infectious agents). Anthropological surveys suggest that the racial and geographic distribution of human blood types reflects tendencies towards specific erythrocyte types susceptible to infectious disease, such as cholera and malaria. Experimentally, the *V. cholerae* toxin has been extensively used as an experimental adjuvant, and its association with ABO groups is of practical importance for the development of an oral cholera vaccination. The results of previous studies provided strong evidence that individuals with blood type O are more vulnerable than other persons to severe cholera symptoms, even though the biologic basis for this association remains unknown.

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LIST OF ABBREVIATIONS

Ace ..... Accessory cholera enterotoxin

Arf6 ..... ADP-ribosylation factor 6

cAMP ..... Cyclic Adenosine 5'-Monophosphate

CDC ..... Centers of Disease Control

Cep ..... Core-encoded pilin

CFTR ..... cystic fibrosis transmembrane conductance regulator

CT ..... Cholera Toxin

CTA ..... Cholera toxin A subunit

CTB ..... Cholera toxin B subunit

CTX ..... Phage-encoded cholera toxin

CTXphi ..... Filamentous bacteriophage

ELISA ..... Enzyme-Linked Immunosorbent Assay

ER ..... Endoplasmic Reticulum

ERAD ..... Endoplasmic Reticulum Associated Degradation

GalNAc ..... N-acetylegalactose

Glc ..... Glucose

GM1 ..... Monosialoganglioside

LPS ..... Lipopolysaccharide

MSPP ..... Ministry of Public Health and Population

NANA ..... N-acetylneuraminic Acid

OCVs ..... Oral Cholera Vaccines

ORF ..... Open Reading Frame

PCR ..... Polymerase Chain Reactions

SMART .....Sensitive Membrane Antigens Rapid Test

TCBS..... Thiosulphate Citrate Bile Salts

TCP ..... Toxin-coregulated pilus

WC .....Whole-Cell

WHO .....World Heal Organization

Zot .....Zonula Occludens Tox

INTRODUCTION

1. VIBRIO CHOLERAE

1.1 *Vibrio Cholerae* History and Discovery

During London's *Vibrio Cholerae* (*V. cholerae*) epidemic in 1854, a scientist named John Snow demonstrated that all cholera cases in London could be traced to a single well. He believed this well to be the source of the epidemic, making this one of the earliest epidemiological studies (1). *V. cholerae* was officially described in 1854 by Picini, and Robert Koch, who collected a pure sample from Egyptian stool 30 years later, in 1883. In Calcutta in 1959, Dutta and colleagues used infant rabbits to study the production of diarrhea by a crude protein isolate from a *V. cholerae* culture filtrate. They showed how a cell-free culture filtrate could cause massive bacterial accumulation, forming what is known as rice-water fluid in the ligated ileal loops of an adult rabbit.

The *Vibrio* genus of bacteria is pathogenic to humans and *V. cholerae* is the most widespread among all human pathogenic species. It contains a wide variety of biotypes, colonization factors, antibiotic resistance, receiving and transferring genes for toxin, capsular polysaccharides that help in chlorine resistance, and unique antigens on the bacteria's surface (1). *V. cholerae* (family*: Vibrionaceae,* genus: *Vibrio*) is a facultative anaerobic, highly motile, gram-negative bacterium with curved or comma-shaped rods and a single polar flagellum. It is primarily found in undercooked seafood and untreated seawater, where the bacteria freely multiply (Figure 1) (2).

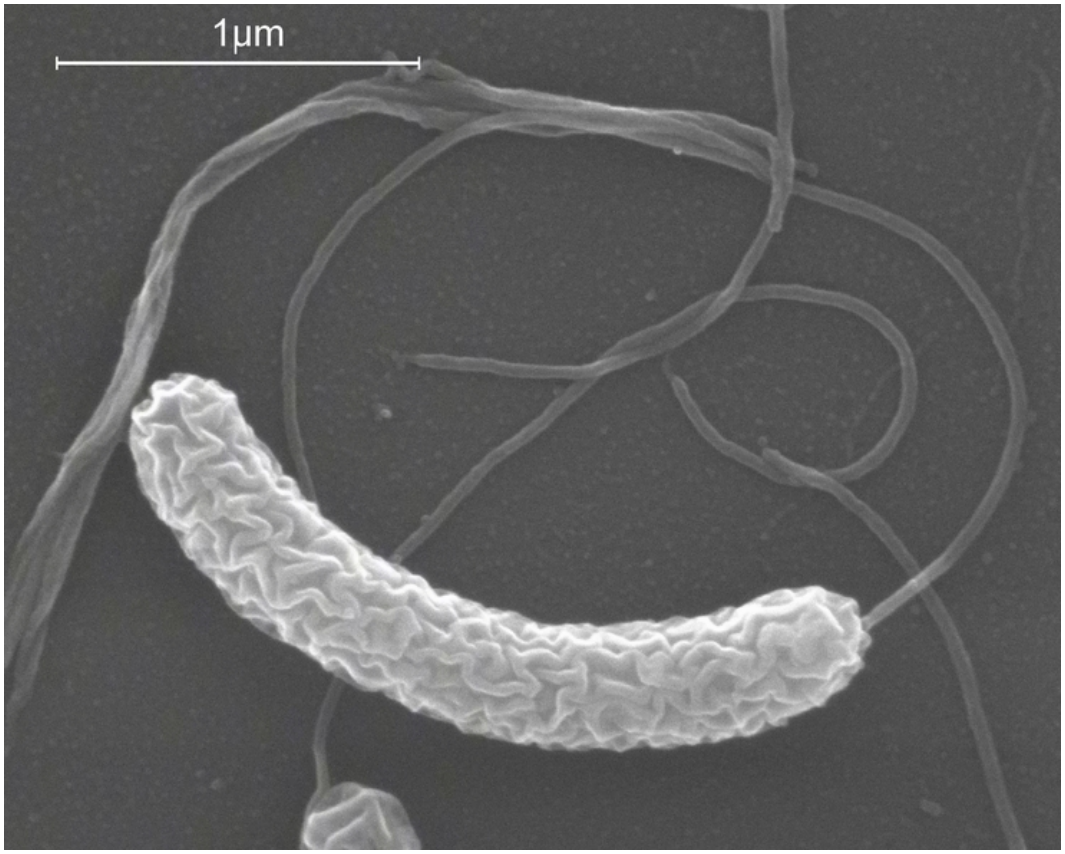

Figure 1. *V. cholerae* cell morphology imaged using an electron micrograph. The curve-shaped single cell is visible with a central polar flagellum (12).

1.2 Nomenclature

In the past, gram-negative, curved and rod-shaped bacteria that possess a polar flagellum were classified as bacteria of the *Vibrio* genus. In the mid-1960s, new criteria was developed to identify *Vibrio* genus taxonomy by the International Subcommittee on the Taxonomy of Vibrios, which excluded many species previously classified as *Vibrio*. Taxonomic studies used biochemical characteristics to separate true species of *Vibrio* from closely related genera, such as *Aeromona* and *Plesiomonas* (3).

1.3 Most Common Pathogenic strain of *Vibrio*

The *Vibrio* genus contains species that are both pathogenic and non-pathogenic to humans, and currently contains 76 species that have been involved in human infections. The most common pathogenic species are *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* (Table 1). *V. cholerae* and *V. parahaemolyticus* are causative agents for diarrheal illness, while *V. vulnificus* infection can vary from self-limiting gastroenteritis to serious soft tissue necrotization. *V. mimicus* excretes cholera toxin (CT), resulting in the development of *V. cholerae*-like symptoms, and *V. furnissii* usually causes gastroenteritis, skin lesions, and septicemia (3).

Table 1: Most Significant Pathogenic *Vibrio* Species in Humans

Vibrio species	Clinical presentation			No. of cases (no. of deaths)[a]
	Gastroenteritis	Wound or ear infection	Septicemia	
V. cholerae				
Epidemic (O1, O139)	++	(+)	—	5 (0)[b]
Nonepidemic	++	+	+	45 (0)
V. mimicus	++	+	—	10 (0)
V. parahaemolyticus	++	+	(+)	116 (1)
V. fluvialis	++	+	+	19 (0)
V. furnissii	++	—	—	1 (0)
V. hollisae	++	+	(+)	13 (0)
V. vulnificus	+	++	++	83 (31)[c]
V. alginolyticus	—	++	—	28 (0)
V. damsela	—	++	—	2 (0)
V. cincinnatiensis	—	—	(+)	0 (0)
V. carchariae	—	(+)	—	0 (0)
V. metschnikovii	(+)	—	(+)	1 (0)

++, Usually with clinical presentation, +, neither uncommon nor most normal clinical presentation, (+) rare.
[a] Data illustrate *Vibrio* infections reported to the Centers of Disease Control (CDC) in the US (1999).
[b] Data incorporate four cases related with remote travel.
[c] The 31 announced cases are from a gathering of 75 cases for which information were accessible (2).

1.4 Epidemiology of *V. cholerae* Infections

The *V. cholerae* O1 serotype is the most common most serotype worldwide, contributing to significant mortality rates in developing countries (Figure 2). Serotype O1 is responsible for major pandemics, whereas serotype O139 causes a clinically similar disease, epidemics, and minor pandemics. The bacteria are usually associated with marine shellfish, algae, plankton, and chitinous of brackish estuaries and coastal salt water (2–3% NaCl), including areas along the coast of the United States (US).

Aggregates of bacteria surrounded by a protective biofilm prevents chlorine and other disinfectants from sanitizing such water.

V. cholerae epidemiology includes (i) a high degree of case clustering associated with location and season, (ii) infection rates in endemic areas that are higher among children aged 1–5 years than in adults, (iii) increasing rates of antibiotic resistance, (iv) clonal differing qualities of epidemic strains, and (v) increased awareness and protection against the disease (4). During the last century, cholera cases and deaths have steadily decreased due to improved sanitation and hygiene measures. During the summer season, minor outbreaks occur in afflicted developing countries as a consequence of overcrowding, poor living conditions, and insufficient clean drinking water.

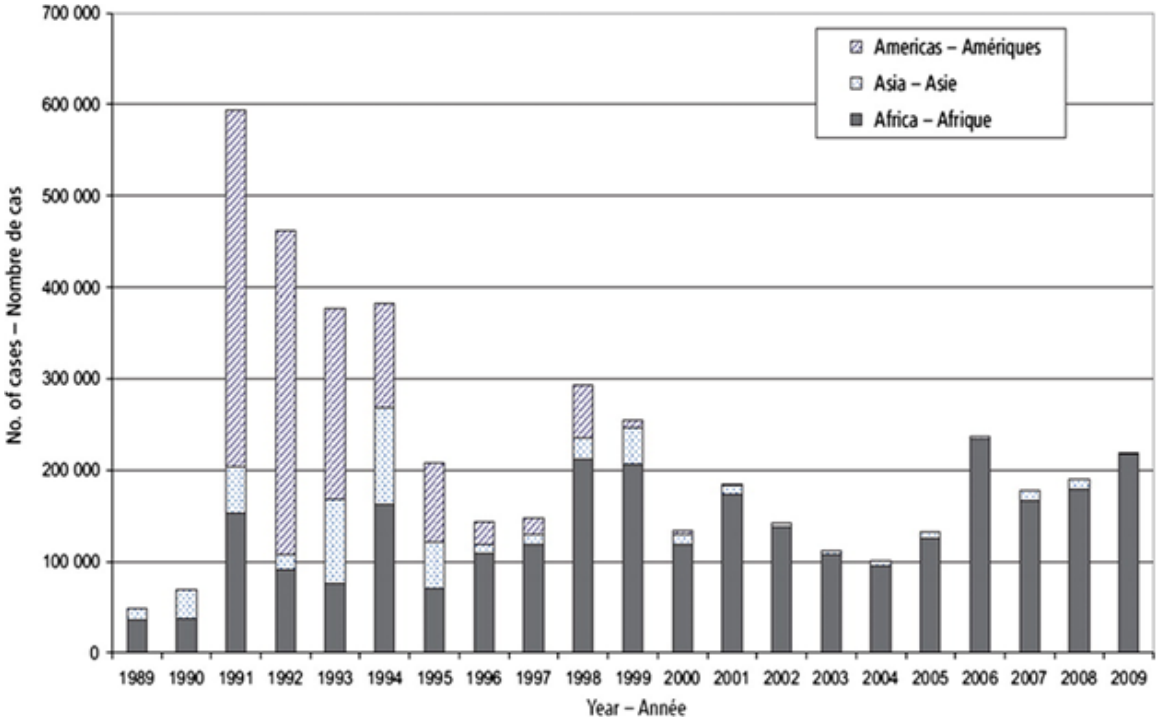

Figure 2. The number of cholera cases between 1989 and 2009 worldwide. Cholera cases reached 44,083 in 1989 as reported by the World Health Organization (35,606 cases were in Africa and 8,477 cases were in Asia). In 1990, 48,403 cases were reported worldwide. In 1991, the disease appeared in Latin America (Peru) for the first time in 100 years, with 75,000 cases and 6,500 deaths, which increased until 1993. In 1994, a cholera outbreak in a Rwandan refugee camp near Goma, Zairw caused tens of thousands of deaths within a month. The 1995–1997 outbreak resulted in 19,810 cases, but in 1996, Senegal reported the largest cholera outbreak among all African countries: 16,107 cases with 765 deaths. From 1998–2007, the number of cases was 322,532 in various African countries, and from 2008–2009, an estimated 4,200 people died in Zimbabwe (20).

1.4.1 *V. cholerae* pandemics

The first pandemic emerged in the Indian subcontinent (the Ganges delta) and extended to other continents over many years. The second pandemic occurred during the 1830s in the British Isles, which was fully described by the epidemiologist John Snow. Canada was also affected during the second pandemic via ships from Ireland carrying infected immigrants. Between 1847 and 1854, the third and fourth pandemics took place in the United State, especially in cities and town along the Mississippi, Ohio, and

Missouri rivers. South America was affected during the fifth pandemic, with high mortality rates in Argentina, Peru, and Chile, at which time, it also spread throughout Germany, where 8,600 people died and the residents held the government responsible for the tragedy (5). In 1883, Robert Koch was able to isolate samples from the rice-water stool of infected patients in Egypt. The sixth pandemic started in India, where it killed more than 800,000 people and moved across the Balkan peninsula, the Middle East, North Africa, and Eastern Europe between 1899 and 1923.

The seventh cholera pandemic was a major outbreak that occurred from 1961 to the 1970s and is still ongoing (6). It began on the Indonesia Island of Sulawesi, spread to further islands, including Java, Borneo, and Sarawak, and after that to the Philippines before reaching Bangladesh by 1963. The pandemic causative agent was *V. cholerae* O1 of El Tor biotype, which caused 155,000 reported cases of cholera worldwide in 1971. By 1991, the number of cases reached 570,000 with the help of modern transportation, migration, and inadequate healthcare infrastructure (7). The World Health Organization estimated that during 1991, reported cases reached 750,000 with a mortality rate of 50% (6,500 deaths) in the Americas, while 12,000 died in poverty-stricken Rwandan refugees camps. The seventh pandemic keeps on creating occasional outbreaks in nations, particularly India and Bangladesh (Figure 3). The mortality rates significantly dropped when governments began to take more serious action against the disease through modern curative and preventive measures, which decreased the death rate to 3% by 1992 (8).

In late December 1992 in Bangladesh and India, the eighth pandemic started. All reported cases had similar cholera-like symptoms, and the causative agent was determined to be the *V. cholerae* non-O1 strain in 40–50% of the cases, which was later serogrouped as the O139 strain (9). By March 1993, over 100,000 cases and 1,400 deaths were reported in India, Pakistan, Nepal, and Bangladesh. Infected immigrants imported the disease to the US and Europe, but it remains confined to Asian countries (10).

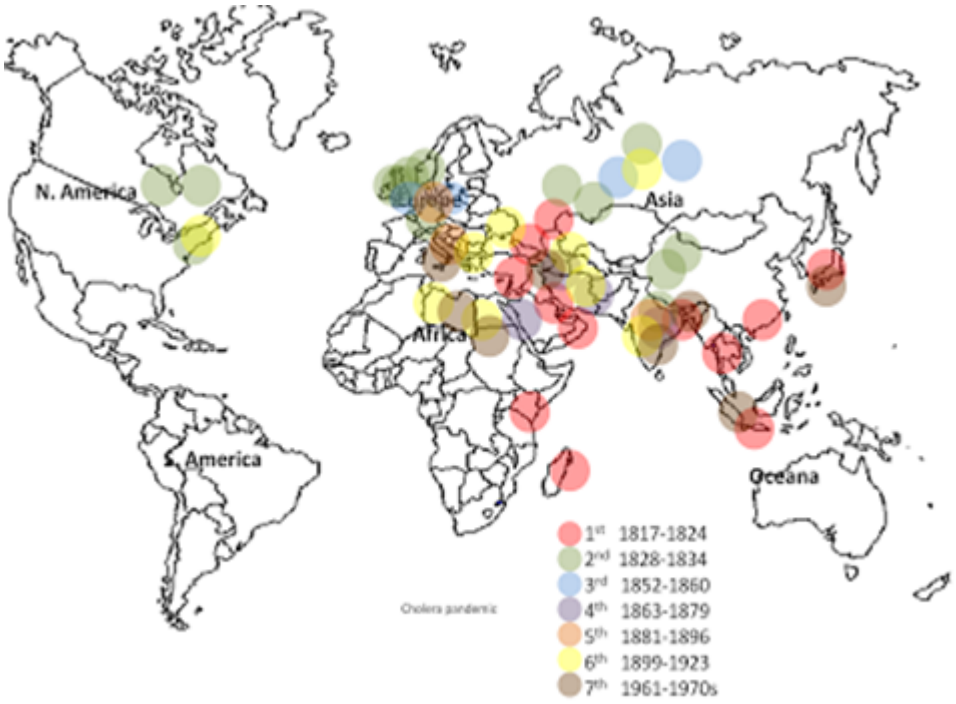

Figure 3. Locations of the seven cholera pandemics. The first pandemic remained predominantly in Asia, starting in Indonesia. The second pandemic also started in Asia and moved to North America. The third and fourth pandemics began in Asian countries and moved to towns the Mississippi River. The fifth pandemic affected South America and reached Germany. The sixth pandemic started in India and moved across the Balkan Peninsula, Middle East, North Africa, and Eastern Europe. The seventh pandemic continues in India and Bangladesh (41).

1.5 Toxigenic Serogroups

Surface antigens of *Vibrio* species are used to differentiate them, and variations in sugar structure on the heat-stable surface somatic O antigen allow serological classification of *V. cholerae* (Figure 4). There are 206 O serogroups divided into O1 and non-O1 serogroups based on agglutination with the O antiserum (4). The O1 serogroup exists as two biotypes: El Tor and classical. For further differentiation, biotypes have three serotypes (two major and one minor) that express different antigenic factors. The first major serotype is Ogawa, which has strains that express the A and B antigens and a trace amount of the C antigen. The second major serotype is Inaba, which expresses the A and C antigens. A minor serotype is Hikojima, which expresses all three (A, B, and C) antigens but is unstable and not as common as the two major serotypes. *V. cholerae* has non-pathogenic stains that generally do not produce cholera toxins and do not lead to epidemics, but in 1993, a new epidemic in Bangladesh and India was the result of a cholera-like disease referred to it as *V. cholerae* non-O1 because it did not agglutinate with the O1 antiserum. After further examination, this bacteria did not belong to any of the O serogroups, but rather to another and never described *V. cholerae* serotype, which was named the O139 Bengal serogroup (11).

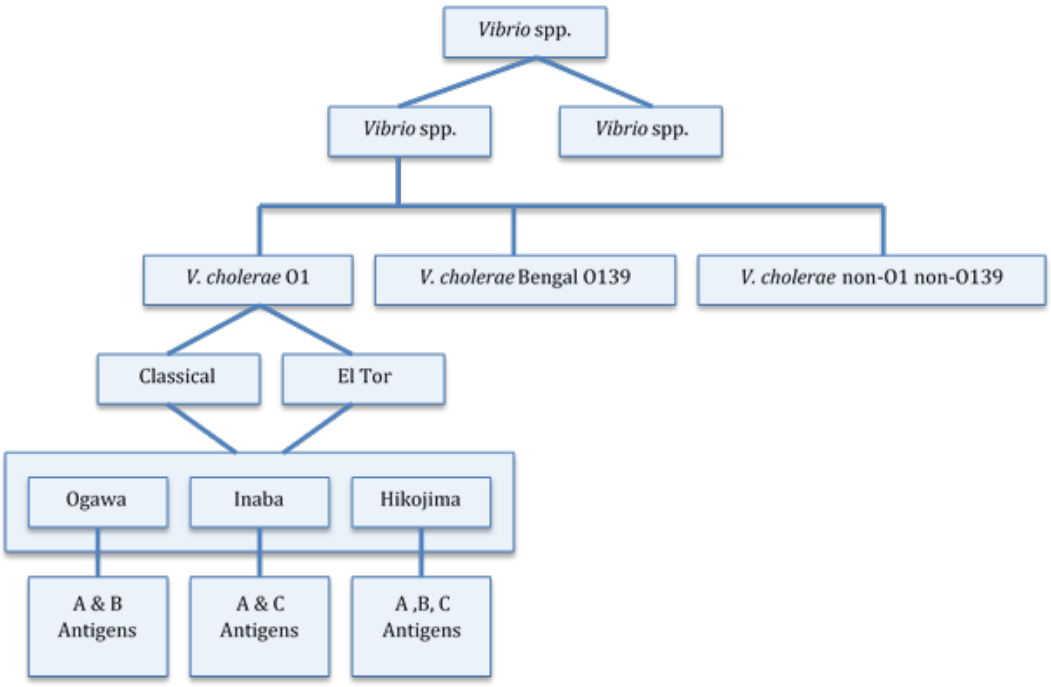

Figure 4. *V. cholerae* serological classification begins with three serogroups: *V. cholerae* O1, *V. cholerae* Bengal O139, and *V. cholerae* non-O1 non-O139. It has two biotypes (classical and El Tor), which have three serotypes: Ogawa, Inaba, and Hikojima. The Ogawa serotype presents A and B antigens, the Inaba presents A and C antigens, and the Hikojima present the A, B, and C antigens.

1.6 Genome Structure

The genomic structure of *V. cholerae* consists of two circular chromosomes. The first large chromosome has 2,961,149 base pairs with 2,770 open reading frames (ORF) and carries main genes for toxicity, regulation, and vital cell functions, for example, translation and transcription. The second smaller chromosome is not similar to plasmid, because it contains essential genes, such as housekeeping genes that included bacterial metabolism, heat-shock proteins, and ribosomal-RNA genes (Figure 5). It shapes 40% of the complete genome. Unlike plasmids, the two chromosomes are not self-transmissible. A viral genome with pathogenic genomic islands is responsible for the bacteria's

pathogenicity, which becomes integrated into its genome and lysogenized with phage DNA and change its natural state (12).

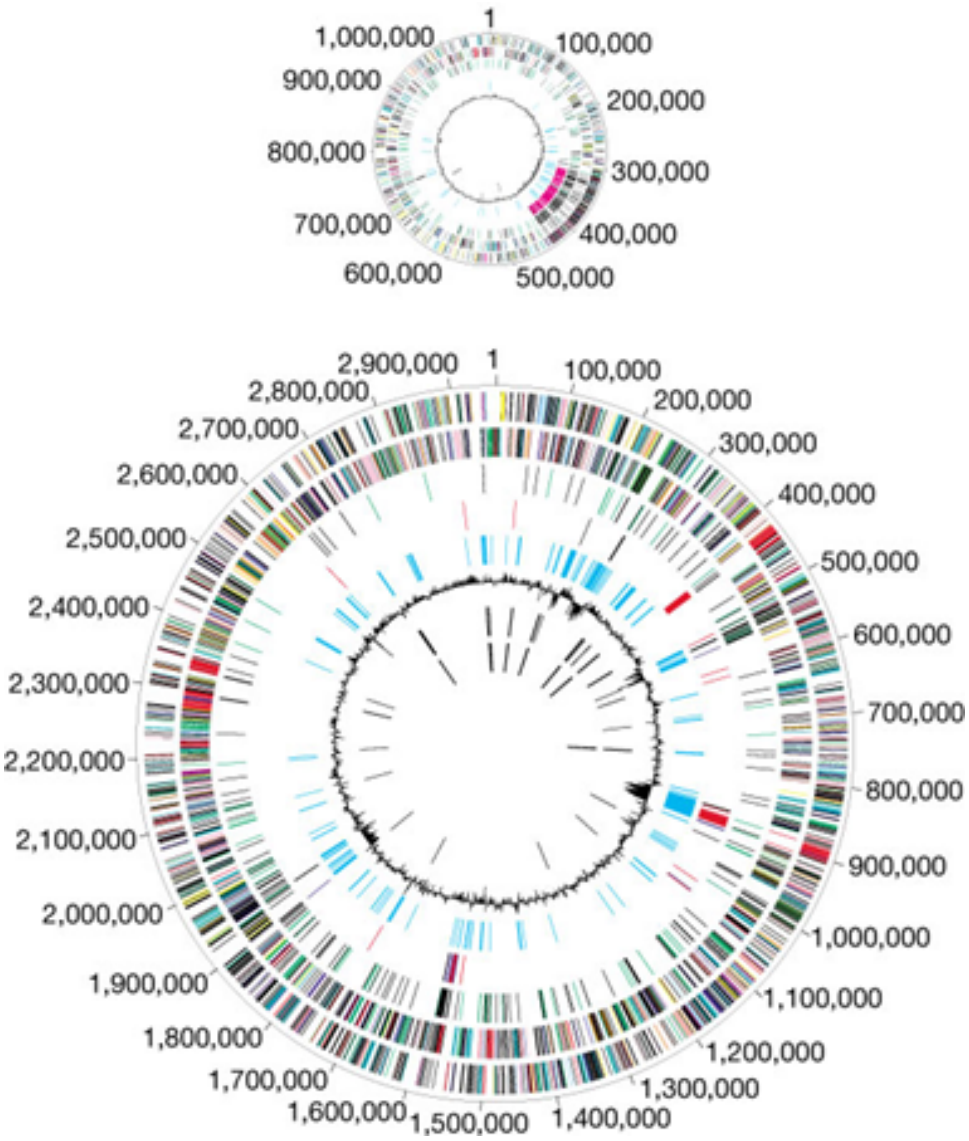

Figure 5. *V. cholerae* genome structure consists of two circular chromosomes. The first large chromosome (at the bottom) has 2,961,149 base pairs with 2,770 ORFs and carries the main genes for toxicity, regulation, and critical cell functions, such as translation and transcription. The second smaller chromosome (at the top) is distinct from the plasmid, because it contains essential housekeeping genes, including bacterial metabolism, heat-shock proteins, and ribosomal-RNA genes (9).

1.7 *V. Cholerae* Toxin and Virulence Factors

V. cholerae toxin (CTX) is a phage-encoded A-B toxin, which belongs to the AB5 toxins family, and it is a heteromeric enzyme of one toxigenic active catalytic A (CTA) subunit anchored to a second adhesive B subunit (CTB); Figure 6). The genes encoding the cholera toxin found on the CTX genetic element: a 7–9.7 kb segment of the DNA present on the chromosome of the toxigenic strains (usually in multiple tandemly arrayed copies) but missing in nontoxigenic species (13). The DNA of the CTC may have CTXphi (CTXφ), a filamentous bacteriophage either integrated as a part of the *V. cholerae* genome or present extra-chromosomally. It also carries at least six genes referred to it as virulence cassette, including CTX-AB (encodes the A and B subunit), zot (encodes the zonula occludens toxin), ace (encodes the accessory cholera enterotoxin), cep (encodes the core-encoded pilin), and orfU (encodes a product of unknown function) (4). The five B subunits form a ring and is responsible for attaching to the receptors of the small intestine's epithelial cells, while the A subunit produces toxic effects. In vivo, the CTA subunit is hydrolyzed by enzymes to CTA1 and CTA2. CTA1 is responsible for the cholera disease, whereas CTA2 acts as a bonder to the CTB subunit. When CTB (monosialoganglioside (GM1)-binding pentameric) binds to GM1 on intestinal cells, it promotes the absorption of cholera toxin through retrograde endocytosis from the Golgi apparatus to the endoplasmic reticulum (14). This requires two coordinating regulatory factors for complete virulence: the cholera toxin (enterotoxin) and toxin-coregulated pilus (TCP), which are surface organelles essential for intestinal colonization. The CTX

structural genes are encoded by a filamentous bacteriophage, CTX (Phi), that uses TCP as its receptor. Different types of proteases (*V. cholerae* HA/protease) are produced to facilitate activation of cholera toxin and other factors, such as fimbriae. The toxR gene encodes the ToxR protein to stimulate the regulatory cascade in Vibrios species that is responsible for controlling more than 17 virulence genes, the CTX-AB gene being one of these (15).

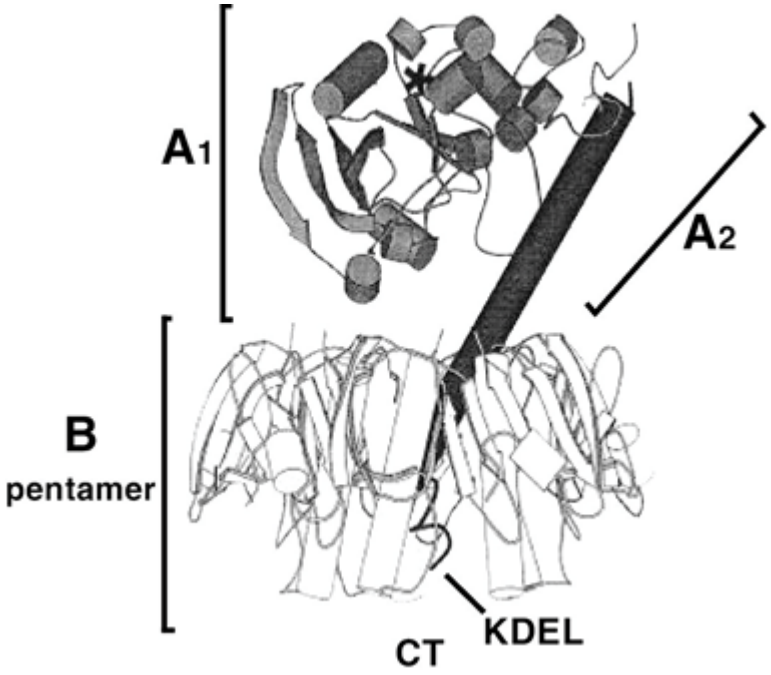

Figure 6: The tertiary structure of the cholera toxin is a subunit composed of the toxigenic A1 peptide and A2 peptide (bonder to B subunit). A subunit is anchored with five B subunits in the center of the ring, which are bound to the GM1 receptors on the host's intestinal cell membranes via KDEL motif (15).

2. CHOLERA

2.1 Disease Description And Clinical Symptoms

Since antiquity, cholera has referred to any type to diarrhea or dysentery, originating from the Greek word *cholē,* meaning bile. In American medical records during the 19th century, three types of cholera were reported: cholera infantum, cholera morbus (summer cholera, which occurred from July to September), and cholera Asiatic (another name for *V. cholerae*) (17). Cholera infantum and cholera morbus were terms for non- specific diarrhea or dysentery in children and adults, respectively. Cholera morbus was attributed to contaminated food, while cholera infantum caused the deaths of children under the age of five and was linked to teething and finger foods, for example, it was responsible for the deaths of many children in 19th century in Illinois (18).

Cholera is an acute, diarrheal intestinal infection caused by the bacteria, *V. cholerae*. It is nicknamed the "blue death" because infected individual's skin often turns bluish from sever loss of water and electrolytes. Among infected people who show symptoms, the majorities have minor to moderate symptoms, with a smaller number developing acute watery diarrhea with severe dehydration, which can prompt to death if left untreated. The classic symptoms usually begin between two hours to five days after exposure and are characterized by massive amounts of watery diarrhea that lasts for several days. Abdominal pain and cramps, vomiting of clear fluid, nausea, excessive sweating, and blue-tinged skin due to uncontrolled dehydration are other common symptoms. Diarrhea can be severe, leading to rapid dehydration and lose of electrolytes,

creating an imbalance. Infected individuals with cholera produce10–20 liters of rice-water diarrhea per day. If no treatment is provided and diarrhea persists, infected individuals can die by the end of the first day. Fever is not common and should raise suspicion for secondary infections (19).

2.2 Laboratory Diagnosis

Routinely, a panel of laboratory tests is conducted to confirm that the causative agent of an infection is *V. cholerae*. The laboratory tests to diagnose cholera involve (i) using culture plates (the gold-standard method), (ii) molecular testing for *V. cholerae* O1 and O139 strains using rapid immunochromatographic dipstick testing in endemic areas, and (iii) test for antibodies against *V. cholerae* using the enzyme-linked immunosorbent assay (ELISA) technique (20).

2.2.1 Culture Method

A stool sample is taken from an infected patient using a sterile cotton bud and placed on a special culture plate called a thiosulphate citrate bile salts (TCBS) agar, which is a selective medium plate used to isolate bacteria, especially *V. cholerae*, *V. parahaemolyticus*, and other Vibrio species, from diarrheal deposits. The TCBS agar contains high concentrations of sodium thiosulphate and sodium citrate to prevent the growth of *Enterobacteriacea*e, whereas bile salts and sodium cholate inhibits the growth of gram-positive bacteria. It also contains a fermentable sucrose to metabolize the Vibrio species. The medium has an alkaline pH to enhance growth of *V. cholerae* and inhibits

growth of intestinal flora. Other additives are indicators of pH changes, used to show the limits of colony growth, such as thymol blue and bromothymol blue. After applying the proper streaking on the agar using a cotton bud, the plate is placed in an aerobic incubator at 35ºC for 18–24 hours. Immediately after removal from the incubator, the plate is examined for characteristic yellow of *V. cholerae* colonies. Other Vibrio species may grow with different shades of green, yellow, or even blue, such as *V. parahaemolyticus*, *V. fluvialis*, and *V. vulnificus* (Table 2). Using the TCBS agar provides a definitive diagnosis because it distinguishes Vibrios from other bacteria, viruses, or protozoa that cause diarrhea (21).

Table 2. Colonial morphology on a TCBS agar

Vibrio Species	Colonial Morphology
V. cholerae	Yellow colonies
V. parahaemolyticus	Blue- to green-centered colonies
V. vulnificus	Green (85%) or yellow (15%)
V. hollisae	Green (very poor growth)
V. metschnikovii	Yellow (low growth)
V. mimicus, V. demsela	Green colonies
V. alginolyticus, V. fluvialis, V. furnissii	Yellow colonies

2.2.2 Molecular Testing for O1 and O139 Strains

Molecular assays are usually used as secondary diagnostic tests for *V. cholerae* detection. Polymerase chain reactions (PCRs) are the most well-known and well-documented molecular procedure for detecting different DNA sequences of the O1 and O139 strains. PCRs can detect multiple genes, such as the regulatory ToxR (found in all *V. cholerae* species), tcpA genes of both classic and El Tor biotypes (to distinguish between them), the wbe gene sequence (specific to O1 and O139 antigens), and the CTXA gene that encodes the A subunit (21).

2.2.3 Rapid Immunochromatographic Dipstick Testing

Various rapid diagnostic immunochromatographic tests have been described to prove the presence of *V. cholerae* by detecting the cholera toxin or lipopolysaccharide (LPS) antigen found in the O1 or O139 strains. The most recently developed colorimetric immunoassay is the multistep Sensitive Membrane Antigen Rapid Test (SMART), which has a 95% sensitivity and 100% specificity for the O1 *V. cholerae* strain and 100% sensitivity and 97% specificity for the O139 strain. Stool samples should be collected through out the acute phase of the sickness, when pathogens are present in the highest numbers, prior to antibiotic therapy. A strip from the rapid dipstick laboratory kit can be placed in the stool for fast biochemical reactions to allow immediate Vibrio confirmation. Cholera is confirmed after 2–15 minutes, when two red lines appear on the dipstick; it should be ruled out if only one red line appears. The first red line is the control, whereas the second is coated with conjugated antibodies that are specific to *V. cholerae* antigens.

When the antibodies capture the antigens, the reddish purple line starts to develop. Patient samples can be stored in cases for which repeating the test is necessary (22).

2.2.4 Testing for Antibodies Against *V. Cholerae* Using an Enzyme-Linked Immunosorbent Assay

GM1 ganglioside is the natural receptor for the cholera toxin in the small intestine of humans; thus, a ganglioside enzyme-linked immunosorbent assay (GM1-ELISA) was developed to detect this. To perform this test, several colonies from the TCBS agar are diluted into distilled water to create culture supernatants, which are added to microtiter plate wells coated with GM1 ganglioside. With time, cholera toxin will bind to the GM1 receptors to form a complex that can be detected by adding antiserum, followed by the enzyme-conjugated antiglobulin antibody. The well is then inserted in the ELISA machine to measure antibody titer in each well (23).

2.3 Pathogenesis

Evolution and emerging bacterial pathogens from new strains depend on horizontal and vertical transfer of virulence genes by phage, plasmids, chromosomal (pathogenicity) islands, transposons, and other genetic elements through the bacterial population, allowing excess evolutionary fitness for pathogenic host cells and, therefor, to associated nucleic acids. Now and again, these components can be manipulated to study transmissions between strains in well-constructed laboratory environment, but little is

thought about the conditions required to excite genetic exchange between clones and strains.

V. cholerae transmitted through the fecal–oral route is responsible for a spectrum of clinical manifestations and a range of disease severity from asymptomatic to lethal. Most infected individuals who do not show symptoms still harbor the bacteria, which are found in their feces for 1–10 days post-infection. These bacteria are shed into the environment until they infect other individuals. Most cholera cases involve acute diarrhea that sometimes progresses to dehydration resulting in death within hours, which is known as "cholera gravis" or fatal diarrhea. Usually, the bacteria are sensitive to gastric acid and most die in the stomach, but some of the surviving virulent organisms may adhere to the small bowel and multiply (24). While colonizing, they secrete potent cholera enterotoxin (also called "choleragen") that binds to the plasma membranes of intestinal epithelial cells and releases enzymatically super-active subunits responsible for over production of cyclic adenosine 51-monophosphate (cAMP).

The B subunit (CTB) of the toxin targets GM1 gangliosides and binds to the intestinal surface and other types of glycans to facilitate toxin entrance. GM1 contains a pentasaccharide moiety with two galactose residues: N-acetyl galactose (GalNAc) and glucose (Glc) and N-acetylneuraminic acid (NANA; Figure 7). The toxin complex is ready to seize endogenous pathways of the host cell once it has been endocytosed (the active-transport process) by the cell; then, the A subunit is activated by the hemagglutinin

metalloprotease (HA Protease) into CTA1 and CTA2 chains that are linked by a single disulfide bond. The CTA1 peptide chain is released and recognized by the endoplasmic reticulum chaperon before it begins to unfolding. Then, CTA1 leaves the endoplasmic reticulum through the Sec61 channel to the cytoplasm, where it refolds and evades deactivation as a result of ubiquitination. The CTA1 chain is now free in the cytoplasm to bind to host proteins, called ADP-ribosylation factor 6 (Arf6). This changes the shape of CTA1 to enable catalytic activity. Subsequently, adenylate cyclase is increased and stimulates the production of cAMP to more than 100-folds the normal rate. The increased intracellular cAMP phosphorylates the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel proteins, which lead up to these constantly opening and the efflux of chloride ions, resulting in massive secretion of H2O and electrolytes (Na+, K+, and HCO3) into the lumen. This creates the characteristic watery diarrhea of cholera (Figure 8) (25).

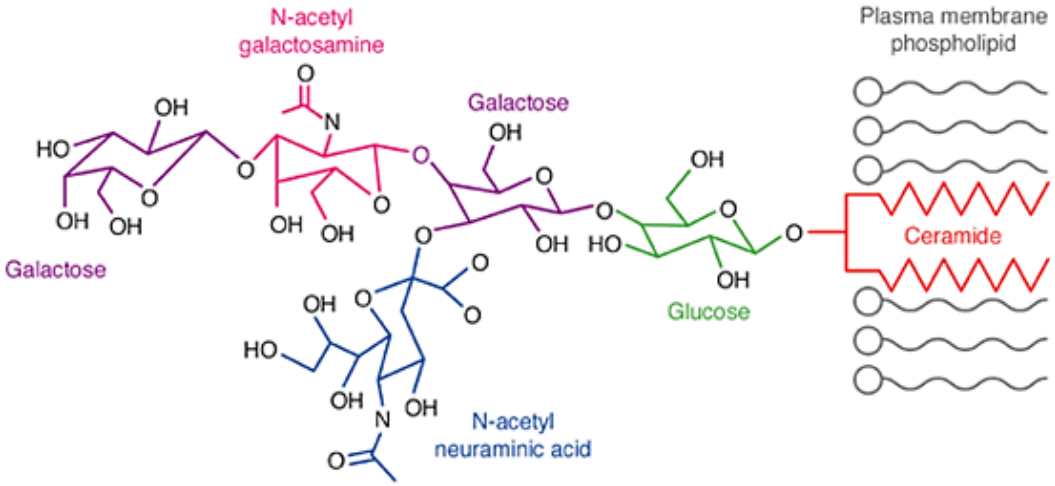

Figure 7. Structure of the membrane glycolipid GM1, which composed of a pentasaccharide moiety [Gal(β1-3)GalNac(β1-4)(NeuAc(α2-3)Gal(β1-4)Glc(β1-1)] uncovered at the surface of the cell membrane and tightly anchored by a ceramide tail. The ceramide endings are embedded into the external leaflet of the plasma membrane (17).

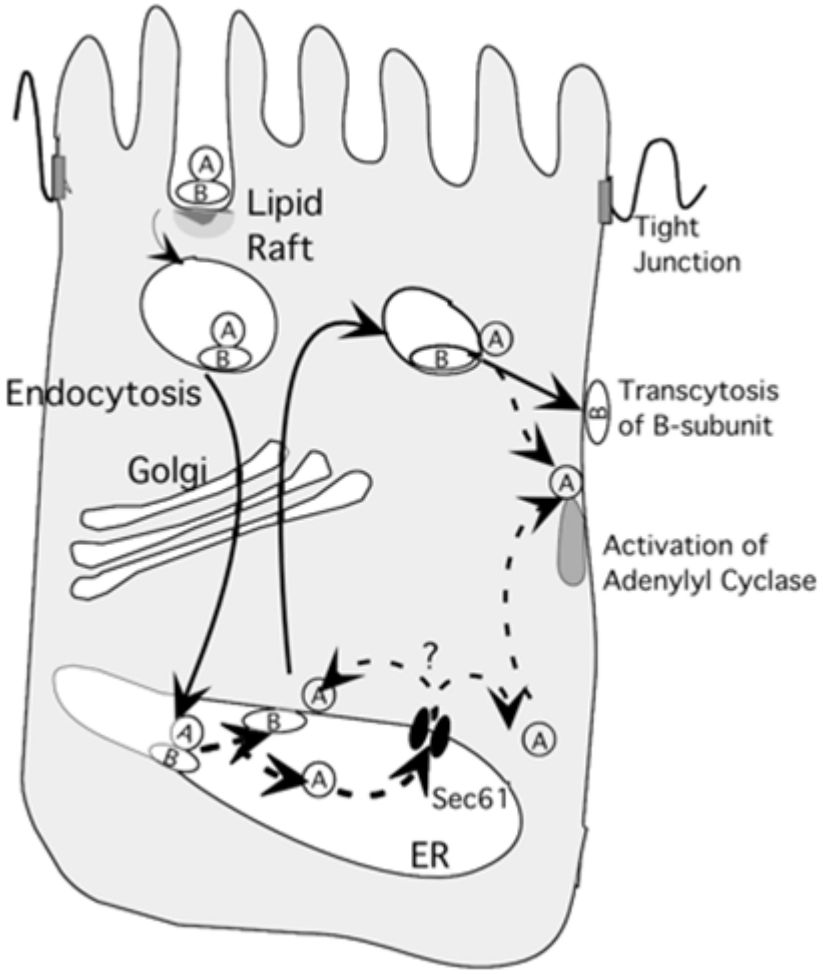

Figure 8. A working model for trafficking cholera toxin into intestinal epithelial cells. Cholera toxin subunit B binds to the GM1 receptor. After endocytosis, the CT-GM1 complex retrotranslocates through the Golgi apparatus into the lumen of the endoplasmic reticulum (ER), where the A subunit detaches from the B subunit and the A1 peptide chain activates the ER Associated Degradation pathway (ERAD). A1 released into the lumen of the ER through the sec16 translocon before entering the cytosol. The B subunit doesn't get unfold in the ER and proceed to the basolateral membrane by trafficking back out of the secretory pathway (15).

2.4 Treatment

Dehydration can lead to death if fluid loss is more than one liter per hour, unless promptly treated by aggressive fluid repletion and electrolyte replacement therapy to restore hydration. Severe cases may require intravenous fluid replacement therapy. Cholera therapy also includes oral administration of a cholera vaccine consisting of dead *V. cholerae*. The risk of contracting the disease varies from one area to another based on precautions that are taken. To avoid infection, residents and visitors to an infected area should always drink bottled, boiled, or chemically-treated water with unbroken seals and never drink directly from tap water or a fountain. Boiling is a widely used procedure to disinfect water in endemic areas. Even dishes should be washed with hot, chemically treated water or 60% alcohol-based hand cleaner, if water is not available (19). Only packaged food or freshly cooked food should be consumed and raw or undercooked meats, unpeeled fruits and vegetables, and undercooked seafood should be avoided. Sewer systems must be established in endemic areas, following proper standards for feces disposal in a sanitary manner and defecation should never occur in a natural water source to prevent contamination of both water and food. Visitors can use chemoprophylaxis along with antibodies to lower their chances of contracting cholera (27).

2.4.1 Vaccines

Since the discovery of cholera in the nineteenth century, researches have sought to perfect a vaccine. Different types of vaccines have been developed, such as oral cholera vaccines (OCVs) and parenteral vaccines. OCVs are efficient methods of stimulate intestinal mucosal immune responses to produce antibodies against *V. cholerae*.

In comparison to parenteral delivered vaccines, oral vaccines have low risk for blood-transmitted diseases, are easier to administer, and are more convenient for patients (28). There are two types of OCVs: killed whole-cell (WC) and live attenuated vaccines. The killed WC vaccines include killed cholera cells from both the El Tor and classical biotypes, the Ogawa and Inaba serotypes of the O1, and the recombinant B subunit toxin. These vaccines are suitable for individuals who are two years old or more and are administered through multiple doses (i.e., two doses for both children (more than 6 years old) and adults and three doses for children less than 5 years old) (29). There are four main OCVs with different trade names that target different biotypes (Table 3) (20).

Table 3. Licensed Oral Cholera Vaccines (20)

Variable	WC-rBS	WC-only	Modified WC-only	CVD 103-HgR
Trade name	Dukoral	ORC-Vax	Shanchol or mORC-Vax	Orochol or Mutacol
Live or killed	Killed	Killed	Killed	Live
Target	O1 classical and El Tor	O1 classical and El-Tor; possibly O139 (no clinical evaluation to date)	O1 classical and El-Tor; possibly O139 (no clinical evaluation to date)	O1 classical and El Tor
Regimen	2 doses given 7–42 days apart (3 doses for children 2–5 years of age)	2 doses given at least 14 days apart	2 doses given 14 days apart	1 dose
Duration of protection	2 years (6 months for children 2–5 years of age)	≥3 years	≥3 years	≥ 6 months (established only in North American volunteers)
Booster dose requirements	Every 2 years (every 6 months for children, 2–5 years of age)	Every 2 years	Every 3 years (may be longer after further evaluation of Kolkata trial)	Unknown
Age range for vaccination	>2 years	≥1 year	Shanchol: ≥1 year; mORC-Vax: ≥2 years	>2 years
Requirement for oral buffer	Yes	No	No	Yes
Storage temperature	2–8°C	2–8°C	2–8°C	2–8°C
Shelf life	3 years	2 years	2 years	2 years
International acceptance	WHO prequalified	Not prequalified by WHO	Pending WHO prequalification	Not prequalified by WHO
Price to the public sector per dose	~$5.25	$0.75	Shanchol: $1.85 or less depending on volume mORC-Vax: ~$0.75	Vaccine not currently available

NOTE. WC, whole cell. Adapted from [18]. WHO, World Health Organization.

2.5 Government Efforts

Assistance is provided to affected countries from various international agencies. Public health authorities have always put massive efforts into enhancing surveillance for cholera outbreaks by investigating every cholera-like case across the globe (27). The Center for Disease Control and Prevention (CDC) monitors these cases, keeps accurate records of the affected countries, and encourages the continuous training of laboratory workers to ensure professional identification techniques for *V. cholerae* are used. The CDC also provides informative materials about cholera diagnosis and treatment to educate the public about how to reduce the chances of contracting the disease. For example, the US agency for International Development provides medicine and medical supplies, bottled water, and sanitation supplies, such as cleaning detergents and hand sanitizers to affected areas. The Food and Drug Administration tests locally caught and imported sea animals, such as shellfish, for *V. cholerae* based on the "Shellfish Sanitation Program". Haiti is at the top of the list of countries associated with the epidemic and recently suffered from an outbreak of *V. cholerae* in its capital city and villages along the Artibonite River. The Haitian Ministry of Public Health and Population (MSPP) immediately notified the National Public Health Laboratory, which identified the causative bacteria as *V. cholerae* serogroup O1, serotype Ogawa. The MSPP, with the help of governmental and nongovernmental partners, established a preventive protocol to (i) provide better water sources and access to drink water, (ii) educate residents on the proper food preparation practices, (iii) advise patients to start rehydration at the onset of

the watery diarrhea, (iv) and increase the number of healthcare institutions and their capacity to treat as many patients as possible (30).

3. ABO BLOOD GROUPS

3.1 History

The first blood transfusion in history was applied by Richard Lower, a member of the Royal Society (a prestigious scientists' organization) in 1665 on animals (31). His first experiment was a blood transfusion from a mastiff (a large breed of dog) to an injured medium-size dog. Lower's noticed that the recipient dog regained wellness and did not develop any signs of discomfort. After this experiment, he transfused animal blood to humans, but the recipients were not affected, even though animals' blood was incompatible with the humans', because he only transfused small amounts. In 1668, the Royal Society prohibited practicing blood transfusion to prevent unpredictable consequences (32).

3.2 Blood Groups Discovery

Karl Landsteiner discovered the principle of blood types in 1900 at the University of Vienna while attempting to understand why some blood transfusions saved patients and others caused death (31). His work was influenced by Paul Ehrlich's article about goats' blood typing that was published in 1900 (31). In 1901, Landsteiner discovered blood types A, B, and O, which he called A, B, and C (Figure 9). Two scientists working

under Landsteiner, Adriano Sturli and Alfred von Decastello, discovered the AB blood type a year later. Landsteiner received the Nobel Prize for his discovery in 1930 (32).

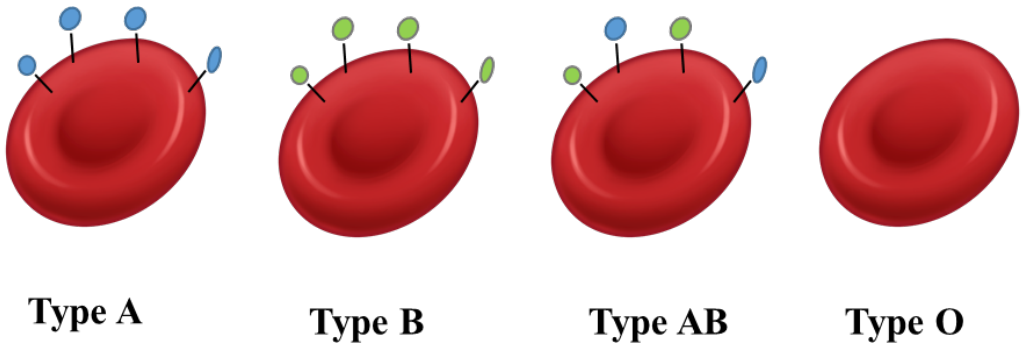

Figure 9. Blood types and their surface antigens, from left to right: blood group A has antigen A on its surface, blood group B has antigen B on its surface, blood group AB has both antigens A and B on its surface, blood group O has no antigens on its surface.

3.3 Blood Groups and Susceptibility to Infections

Many studies have focused on the possible relationship between blood type and infections (33). This field of interest deals with four main aspects: the antigenic relatedness and similarities of microbe and erythrocytes membranes, adhesion to cells through specific receptors or blood group substances, modulation of the antibody response, and anthropologic surveys that link the geographic and racial variables among humans to blood type susceptibility to infectious diseases (Tables 4-6) (33).

Table 4. Associations between human blood type and bacterial infections (33)

Disease or organism	Blood group or substance	Clinical observation
Bacterial		
Plague	0	increased disease incidence
Cholera	0	increased disease severity
Tuberculosis	0	increased disease incidence
		decreased disease incidence
	B	increased disease incidence
	AB0	no relationship to incidence
Leprosy	A	increased incidence of lepromatous form
	B	increased incidence of lepromatous form
	0	increased incidence of tuberculoid form
	AB0, Rh	no relationship to incidence
Yaws	M	increased disease among homozygotes
Syphilis	AB0, Rh	no relationship to incidence
Streptococcus pneumoniae	B	decreased disease incidence
	AB0	increased incidence in nonsecretors
Neisseria meningitidis	AB0	increased incidence in nonsecretors
Haemophilus influenzae		increased incidence in nonsecretors
Gonorrhea	B	increased disease incidence
	AB0	no relationship to incidence
Staphylococcal infection	AB0	no relationship to incidence
Staphylococcus aureus	AB0 Rh	no relationship to incidence
Dental caries	M	decreased disease incidence
	N	increased disease incidence
	AB0, MN	no relationship to incidence
Rheumatic fever	ABH	increased disease in nonsecretors
	A	increased disease in incidence
	A, AB	increased disease incidence
Hematogenous osteomyelitis	0	increased disease incidence
Escherichia coli	P	increased incidence of urinary tract infection
		no increase in renal scarring
		increased incidence of bacteremia
	A, Kell	stimulation of production of anti-Kell and anti-A antibody
	B	increased incidence of septicemia
Enterobacteriaceae	AB0	increased virulence of strains with antigens related to blood group substances
	B	increased incidence of upper urinary tract infection

Table 5. Associations between human blood type and viral infections (33)

Disease or organism	Blood group or substance	Clinical observation
Viral		
Epstein Barr virus	N	anti-N antibody, hemolytic anemia
	i	anti-i cold agglutinin, hemolysis
Influenza	0	increased viral antibody present
		increased disease incidence
	A	increased disease severity
	ABO	no relationship to incidence
	Rh, MN	no relationship to incidence
Smallpox	A, AB	increased disease incidence
Adenovirus	A	conflicting findings
Kuru	ABO, MN, Rh	no relationship to incidence
Mumps	0	increased disease incidence
Hepatitis B	0, Rh negative	increased prevalence of carriage
	A	increased prevalence of carriage
	AB	increased prevalence of carriage
		no relationship to carriage

Table 6: Associations between human blood types and parasitical infestations (33)

Disease or organism	Blood group or substance	Clinical observation
Filariasis	AB	increased disease incidence
	A	increased disease incidence
	ABO	no relationship to incidence
Schistosoma haematobium	ABO	no relationship to incidence
Schistosoma japonicum	A, AB	increased disease incidence
Fasciola hepatica	P1	anti-P1 causes false positive
		indirect hemagglutination test for echinococcosis
Clonorchis sinensis	P2	anti-P1 antibody elevated
Opisthorchis viverrini	P2	anti-P1 antibody elevated
Echinococcus granulosis	P2	anti-P1 antibody elevated
	P1	anti-P1 antibody elevated by Casoni test
Torocara spp.	AB	elevated isohemagglutinin titres
Giardia lamblia	A	increased reinfection rate
Leishmania donovani	ABO	no relationship to incidence
Trypanosoma brucei	ABO	no relationship to incidence

4. PUBLISHED STUDIES

4.1 Correlations between Blood Type and *V. Cholerae*

In 1977, Barua and Paguio suggested an association between ABO blood groups and cholera infection after examining a group of patients presenting with diarrheal illness and taking the cholera treatment at San Lazaro Hospital. Among these patients, the severity of the disease varied even though they were infected with the same *V. cholera*e serotype. After further investigation, the patients were found to be of blood group O, which was associated with their more severe symptoms compared to other patients.

Infection with cholera is associated with different factors, such as immunity, gastric acidity, bacterial inoculum size, and blood group. Different blood groups have different surface proteins. The surface proteins on red blood cells of an individual determine the blood type or group and are known as antigens. These antigens are not restricted to red blood cells; they are also present on tissues, which are referred to as histo-blood-group antigens. Individuals in blood group O are more vulnerable to cholera infection compared to individuals with different blood types, but the mechanism of this vulnerability is not fully understood. Type O red blood cells have no proteins (antigens) on their surface, in contrast to blood types A, B, or AB. After drinking contaminated water (or consuming contaminated undercooked food), colonization, and secretion of cholera toxin, the B subunit of the *V. cholerae* toxin binds to the small intestine epithelial cells' receptor GM1, followed by penetration of host cells by the A subunit, which

interrupts the cAMP pathway. For individuals with blood types other than the O, surface proteins distract the B subunit of the cholera toxin and reduce its chances of binding to the GM1, thus reducing the severity of infection. Because blood type O erythrocytes lack these surface proteins, they cannot serve as distractions; thus, they cannot interfere with the B subunit binding to the GM1, which eventually leads to severe diarrheal illness (34-37).

4.2 Blood Group O Individuals Are More Susceptible to Cholera

The human body naturally secretes antibacterial and antitoxic antibodies, which work together cooperatively to defend the host against *V. cholerae*. Various types of oral vaccines have been developed to support the body's natural defense mechanisms (40). In one study in Bangladesh, a group of scientists constructed a cohort study proposing a speculation for understanding the relationship between blood type O and the risk of contracting *V. cholerae* O1 and O139 along with developing severe illness. They enrolled 300 patients and 912 household contacts between 2001 and 2004. Of these, only 269 patients and 793 contacts completed the required day of follow-up (21 days) and were included in their analysis (40). They included the statistic, microbiologic, clinical, and immunologic characteristics of both patients and household contacts (Table 7). 34% of the patients and 33% of the households were blood type O. They found; fifty-seven (21%) of the afflicted persons with *V. cholerae* O139, while 212 (79%) were infected with O1 El Tor biotype (83 were infected with Ogawa serotype and 129 with Inaba

serotype). More that 90% of patients had massive dehydration and were given intravenous fluid and antibiotics. They gave vibriocidal to both patients and household contacts and the results were; patients with blood type O had higher vibriocidal titers on the 21-day of follow-up after the onset of symptoms than those with different blood types (Figure 10). Of the household contacts, individuals with blood type O had much more severe symptoms comparing to persons with other blood types (Table 8) (40).

Table 7. Statistic, microbiologic, clinical, and immunologic components of cholera patients and their household contacts (40)

Characteristic	Patients (N=269)	Household contacts		
		Infected (N=260)	Not Infected (N= 533)	P (for infected versus uninfected household contacts)
Demographic data				
Age	25 (±14)	18 (±15)	21 (±15)	0.03
Female	155 (58%)	132 (51%)	267 (50%)	0.8
Blood group				
O	115 (43%)	74 (28%)	190 (36%)	0.03
A	60 (22%)	67 (26%)	136 (26%)	0.9
B	72 (27%)	85 (33%)	155 (29%)	0.4
AB	22 (8%)	34 (13%)	52 (10%)	0.1
Stool or rectal swab culture result				
Any	269 (100%)	185 (72%)	NA	
O1 Inaba	129 (48%)	83 (32%)	NA	
O1 Ogawa	83 (31%)	53 (20%)	NA	
O139	57 (21%)	49 (19%)	NA	
Severe dehydration	241 (90%)	0 (0%)	NA	
Immunologic data				
Baseline log10 vibriocidal antibody titer	1.8 (±0.7)	1.6 (±0.9)	1.9 (±0.9)	0.001
Day 21 log10 vibriocidal antibody titer	3.3 (±0.6)	2.6 (±0.9)	1.9 (±0.9)	<0.001
Baseline log10 anti-CT IgG	2.0 (±0.3)	1.9 (±0.3)	2.0 (±0.4)	0.5
Day 21 log10 anti-CT IgG	2.4 (±2.4)	2.2 (±0.3)	2.0 (±0.3)	<0.001
Fourfold rise in vibriocidal antibody titer	265 (99%)	190 (73%)	NA	

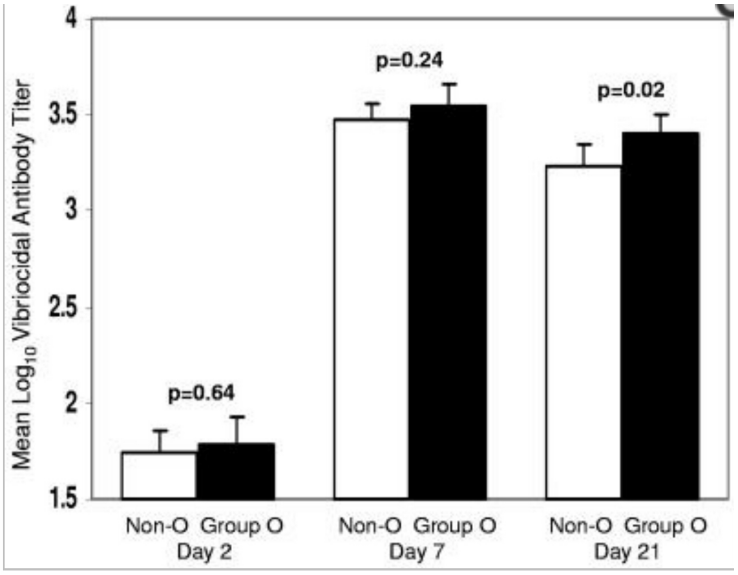

Figure 10. Vibriocidal counter acting agent reactions in patients with cholera by blood aggregate. P values allude to the distinctions in mean titer between blood group O and non-blood group O patients on days 2, 7, and 21 (40).

Table 8. Danger of disease and serious loose bowels on both patients and households with *Vibrio cholerae* O1 and O139 infection (40)

V. cholerae **serogroup in index patient**	**Blood group of household contact**	**Infection in household contact**	**OR for infection (95% CI)**	***P***	**Severe diarrhea in household contact, if infected**	**OR for severe diarrhea (95% CI)**	***P***
O1 & O139 combined	O	74/264 (28%)	0.80 (0.65-0.98)	0.03	14/66 (21%)	2.4 (1.2-4.7)	0.01
	Non-O	186/529 (35%)			15/173 (9%)		
O1	O	49/208 (24%)	0.67 (0.53-0.85)	0.0008	8/43 (19%)	2.3 (0.98-5.6)	0.055
	Non-O	151/423 (36%)			11/139 (8%)		
O139	O	25/56 (45%)	1.2 (0.80-1.9)	0.32	6/23 (26%)	2.3 (0.86-6.2)	0.10
	Non-O	35/106 (33%)			4/34 (12%)		

To further study the association between blood groups and the severity of cholera, Clemens and colleagues performed a study in Bangladesh to study the risk of severe cholera, blood group type, and the efficiency of oral vaccination (25). They randomly selected patients of different ages to take three doses of either cholera B subunit-whole killed vaccine (BS-WC), whole-killed cell only vaccine (WC), or an *Escherichia coli K12* strain placebo (K12). They had 3,647 patients who finished the three-doses trial of 895 (24.5%) with blood type A, 1,247 (34.2%) with blood type B, 324 (8.9%) with blood type AB, and 1.181 (32.4%) with blood type O. They found that the risk to El Tor cholera among blood type O is markedly significant than the risk from groups A, B, and AB combined in children (Table 9) (25).

Table 9. Placebo Observation of El Tor (25)

Feature	No. of cholera patients in blood group (risk per thousand)			
	AB	A	B	O
Age (in years)*				
2-5[a]	0	4 (4.3)	4 (3.1)	21 (16.9)
>5	2 (1.3)	12 (2.9)	14 (2.4)	21 (3.8)
Severity				
Severe[b]	1 (0.5)	13 (2.5)	8 (1.1)	27 (4.0)
Nonsevere[c]	1 (0.5)	3 (0.6)	10 (1.4)	15 (2.2)

* At introductory vaccination in January 1985.
[a] P < .005 (two-tailed) for pattern of increasing risk for groups AB, A and B combined, and O; and P < .0001 (two-tailed) for difference of risk between group O and other ABO groups.
[b] P < 005 (two-tailed) for pattern of increasing risk for groups AB, A and B combined, and O; and P < .001 (two-tailed) for difference of risk between group O and other ABO groups.
[c] P < .05 (two-tailed) for pattern of increasing risk for groups AB, A and B combined, and O, and P < .01 (two-tailed) for difference of risk between group O and other ABO groups.

The three medications had almost indistinguishable dispersions among members. Protective efficacy was 60% for BS-WK, and 55% for WC vaccine. Concluding that the protective efficacy against every single treated scene of cholera was lower among blood group O people and indicated low level of protection against El Tor than other blood types (Table 10). Their data confirmed that persons with blood group O are at higher hazard for El Tor cholera than other ABO blood groups individuals (25).

Table 10. Protection efficacy (PE) for the three medications among various blood classifications (25)

Outcome	No. of cholera episodes per treatment group (% Protective Efficacy)		
	BS-WC	WC	K12
Severe			
Group O	31 (40)*[a]	28 (46)[d]	52
All other groups	20 (76)[b]	31 (63)[b]	84
Total	51 (62)[b]	59 (56)[b]	136
Nonsevere			
Group O	9 (72)[c]	16 (50)*	32
All other groups	22 (45)*	19 (52)[d]	40
Total	31 (57)[b]	35 (51)[c]	72
Overall			
Group O	40 (52)[b]	44 (47)[c]	84
All other groups	42 (66)[b]	50 (59)[b]	124
Total	82(60)[b]	94 (55)[b]	208

* $P < .05$ (one-tailed) for PE

[a] $P < .01$ (two-tailed) for difference of PE for group O versus other beneficiaries of BS_WC

[b] $P < .0001$ (one-tailed) for PE

[c] $P < .001$ (one-tailed) for PE

[d] $P < .01$ (one-tailed) for PE

In a recent study, Kuhlmann and colleagues hypothesized that binding of cholera toxin to the gastrointestinal glycans of A, B, or AB blood groups may impede interaction of the toxin with GM1 receptors (31). To explore the immediate effect of blood group expression on cholera toxin activation of target cells, they picked a novel enteric model framework. First, they analyzed the capacity of toxin to activate cAMP in enteroids extracted from gastrointestinal stem cells acquired from persons with blood group O or blood group A. They found that enteroids summarize many characteristics of human intestinal epithelium, and erythrocytes are one of them. In addition, they express antigens of blood group on erythrocytes surface (Figure 11) (31).

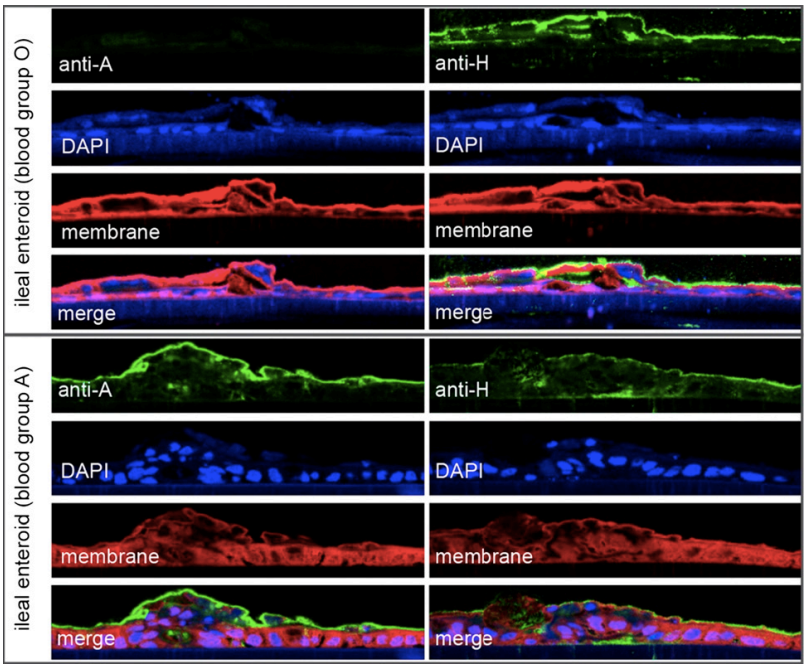

Figure 11. Immunofluorescence pictures of the developed enteroids exhibiting the presence of blood group O antigen H in enteroids extracted from blood group O persons (top), or blood group A persons (bottom) (Kuhlmann study; 31).

Therefore, they utilized enteroids isolated from ileal and colonic biopsies extracted from different people of different blood type to study CT-mediated initiation of cAMP. Strangely, intestinal cells of blood group O persons showed reliably elevation in cAMP levels comparing to cells of blood group A individuals (Figure 12) (31).

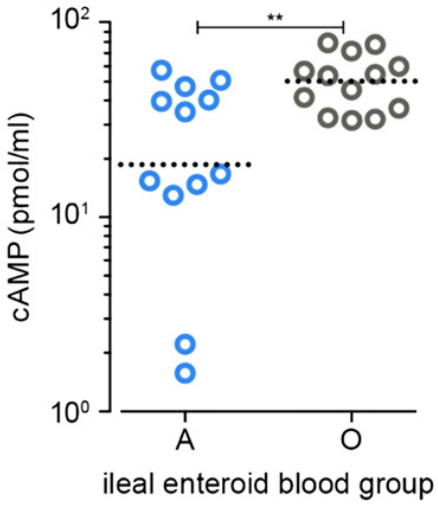

Figure 12. cAMP production results after a overnight incubation of ileal enteroids with cholera (CT, 0.1 µg/mL). Data of both blood groups from two distinct subjects (Kuhlmann study; 31).

Despite the fact that cholera is thought to be an ileal infection, they found that colonic biopsies cells from blood group O persons showed more strong cAMP reaction when exposed to cholera toxin (Figure 13) (31).

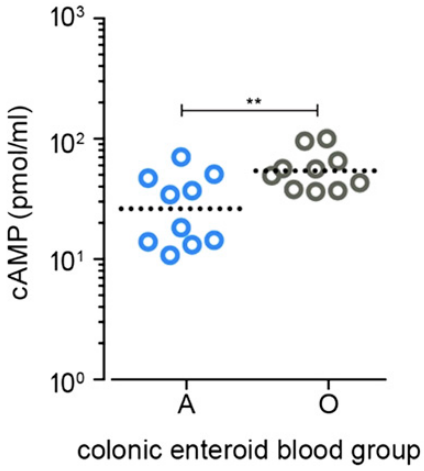

Figure 13. cAMP production results after a overnight incubation of colonic enteroids from both blood groups A and blood group O subjects (31).

DISCUSSION

Despite numerous studies having been conducted, the study of disease transmission and biology of cholera stay testing to explore because scientists cannot always determine its origin and where to begin investigation. Cholera remains a global health challenge, especially in developing countries in different parts of Asia and Africa. Recent efforts in this field have provided a motive for further investigation of the life form to comprehend the molecular basis for the rise of the sickness and natural marvels controlling the preservation of some hereditary attributes. *V. cholerae* gives a characteristic framework to concentrate the coevolution of microscopic organisms and the hereditary materials related with its virulence variables.

Earlier research (28-30, 35, and 38) has pointed out that persons with blood group O are at risk for more severe symptoms due to higher concentrations of free toxin being available to bind GM1 on small intestinal epithelial cells, compared to other blood groups. It has been suggested (31) that blood types A and B secrete substances that reduce antibodies and cross-react with the cholera toxin. Blood types other than O may be able to change intestinal microflora in a way that affects *V. cholerae* multiplication and binding (41). Toxigenic serogroups O1 and O139 of *V. cholerae* are responsible for worldwide epidemics, whereas non-O1 and non-O139 serogroups cause occasional diarrhea or irregular extra-intestinal infections. So far, the World Health Organization supports administration of the whole-killed cholera vaccine WC-rBS to protect against

V. cholerae O1 classical and El Tor and control epidemics (39). The vaccines elevate serum antibodies in all recipients, whether they receive the whole *Vibrio* alone or combined with genetically recombinant B subunits. Additionally, half of the individuals who receive the combination vaccine show significant increases in IgA antibodies, even though the mechanisms of protective immunity are still not fully understood.

New approaches are needed to help design the most effective vaccine or drugs to inhibit the binding process of the *V. cholerae* toxin to the GM1 ganglioside presented on the intestinal squamous cells. Computational biology and phage therapy are options to help for future therapies. Phage therapy involves the targeted application of bacteriophages that can infect and kill them and could be used as a prophylaxis to prevent the spread of cholera and sometimes cures the infection.

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