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Salmonella typhi and Vibrio cholerae

Classification

Higher order taxa

Bacteria; Proteobacteria; Gammaproteobacteria; Enteriobacteriales;
Enterobacteriaceae

Species

Salmonella Typhi

Description and significance

There are over 2,000 various groupings (serovars) that comprise *S. enterica*, each very closely related to each other making *Salmonella typhi* a prime example of a serovar. *Salmonella typhi* is a gram negative bacterium that causes systemic infections and typhoid fever in humans. This rod-shaped, flagellated organism's sole reservoir is humans. It has caused many deaths in developing countries where sanitation is poor and is spread through contamination of water and undercooked food. Eradication seems highly unlikely due to recent emergence of multi drug resistance strains. *Salmonella Typhi* strain Ct18 was originally isolated from a patient in a hospital in Vietnam. The chromosome sequence is 4,809,037 bp in length with a G+C content of 52.09%. The chromosome was sequenced though the method of shotgun sequencing with 97,000 shotgun reads. Since then, *Salmonella typhi* has undergone evolutionary change and has become resistant to antibiotics .

Genome structure

The genome for *Salmonella typhi* has been completely sequenced. There are about 204 pseudogenes encoded in *Salmonella typhi*. A majority of these genes have been inactivated by a stop codon, which shows that the genes were recently modified due to evolutionary changes. Of the 204 genes, twenty seven are remnants of insert sequences and genes of bacteriophage origin. Seventy five are involved in house keeping functions and 46 of the gene mutations have to do with host interaction.

There are two commonly used strains of *Salmonella typhi*, CT18 and Ty2. *Salmonella typhi* CT18 has a large circular chromosome consisting of 4.8 Mb and two plasmids, pHCM1 and pHCM2, one of which has multiple drug resistance (pHCM1). *Salmonella typhi* Ty2 has one large chromosome that is 4.7 Mb and unlike CT18, it does not have plasmids

and can be affected by antibiotics. In fact, the current vaccine was developed using *S. typhi* Ty2. Out of the 204 pseudogenes in *Salmonella*, 195 genes are the same in both strains CT18 and Ty2, making them 98% identical .

Cell structure and metabolism

Salmonella typhi is a rod-shaped, gram negative bacteria that contain features that separates itself from other types of bacteria which include: having 2 membranes (and outer and an inner), periplasm, and a Lipopolysaccharide chain that consists of -d-galactosyl-(1 2)- -d-mannosyl-(1 4)-l-rhamnosyl-(1 3)-repeating units, and has short branches of single 3,6-dideoxyhexose residues .

Salmonella typhi has a complex regulatory system, which mediates its response to the changes in its external environment. Sigma factors, which are global regulators that alter the specificity of RNA polymerase, are examples of such regulation. Some sigma factors direct transcription to produce stress proteins, which increases the chances of the bacteria surviving environmental changes. RNA polymerase S is produced in response to starvation and changes in pH and temperature. It also regulates the expression of up to 50 other proteins and is also involved in the regulation of virulence plasmids.

In order to survive in the intestinal organs of its hosts where there are low levels of oxygen, *Salmonella typhi* has to be able to learn to use other sources other than oxygen as an electron acceptor. Therefore, *Salmonella* has adapted to grow under both an aerobic and anaerobic conditions. *Salmonella*'s most common source of electron acceptors is nitrogen. Examples of other electron acceptors are: nitrate, nitrite, fumarate, and dimethylsulphoxide. Global and specific regulatory systems of anaerobic gene expression, like the ones mentioned above, are implemented to make sure that the most energetically favorable metabolic process is used. Evidence shows that the availability of oxygen is an environmental signal that controls *Salmonella*'s virulence .

Ecology

Salmonella typhi is a food born pathogen and that is increasingly difficult to control. *Salmonella*'s ability to change its phenotype and genotype in response to environmental changes make it almost impossible to eradicate from the food chain. When a culture of *Salmonella* was transferred to higher temperatures (60 C), it took 60 minutes to maximize heat resistance. When the pH was lowered, acid resistance increased. The time taken to kill 90% was 4-14 minutes. *Salmonella* cells experience gradual changes which is why *Salmonella* thrives in undercooked meat. It is able

to adapt to survive the cooking process and also has the ability to cross the gastric acid barrier (this is how they enter the human intestine). A high-fat matrix is protects Salmonella against these stressful environments .

Pathology

Salmonella typhi has killed over 600,000 people annually all over the world. It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water. It has become an epidemic in South Asian countries where sanitation is lacking. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver, and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, a headache, and nausea. Other common symptoms include loss of appetite, diarrhea, and enlargement of the spleen (depending on where it is located).

Salmonella typhi involves colonization of the Reticuloendothelial system. Some individuals who are infected with *S. typhi* become life-long carriers that serve as the reservoir for these pathogens. *S typhi* has an endotoxin (which is typical of Gram negative organisms), as well as the Vi antigen, which increases virulence. It also produces a protein called invasins that allows non-phagocytic cells to take up the bacterium and allows it to live intracellularly. Salmonella typhi is a strong pathogen for humans due to its resistance to the innate immune response system .

Recently, strains of MDR (multi-drug resistant) Salmonella have been identified and grouped together in a single haplotype named H58. It has been found that these strains are now resistant to nalidixic acid and have reduced susceptibility to fluoroquinolones. This strain has been recently found in Morocco, which shows that the MDR strain has reached as far as Africa .

Structure, Classification, and Antigenic Types

Salmonellae are Gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars). H antigen may occur in either or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. Vi antigen is a superficial antigen overlying the O antigen; it is present in a few serovars, the most important being *S typhi*.

Antigenic analysis of salmonellae by using specific antisera offers clinical and epidemiological advantages. Determination of antigenic structure permits one to identify the organisms clinically and assign them to one of nine serogroups (A-I), each containing many serovars. H antigen also provides a useful epidemiologic tool with which to determine the source of infection and its mode of spread.

Ecologic Classification of Salmonellae.

As with other Gram-negative bacilli, the cell envelope of salmonellae contains a complex lipopolysaccharide (LPS) structure that is liberated on lysis of the cell and, to some extent, during culture. The lipopolysaccharide moiety may function as an endotoxin, and may be important in determining virulence of the organisms. This macromolecular endotoxin complex consists of three components, an outer O-polysaccharide coat, a middle portion (the R core), and an inner lipid A coat. Lipopolysaccharide structure is important for several reasons. First, the nature of the repeating sugar units in the outer O-polysaccharide chains is responsible for O antigen specificity; it may also help determine the virulence of the organism. Salmonellae lacking the complete sequence of O-sugar repeat units are called rough because of the rough appearance of the colonies; they are usually avirulent or less virulent than the smooth strains which possess a full complement of O-sugar repeat units. Second, antibodies directed against the R core (common enterobacterial antigen) may protect against infection by a wide variety of Gram-negative bacteria sharing a common core structure or may moderate their lethal effects. Third, the endotoxin component of the cell wall may play an important role in the pathogenesis of many clinical manifestations of Gram-negative infections. Endotoxins evoke fever, activate the serum complement, kinin, and clotting systems, depress myocardial function, and alter lymphocyte function. Circulating endotoxin may be responsible in part for many of the manifestations of septic shock that can occur in systemic infections.

Diagnosis

The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens. Laboratory identification of the genus *Salmonella* is done by biochemical tests; the serologic type is confirmed by serologic testing. Feces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, *Salmonella-Shigella*, and brilliant green agars) as well as into enrichment broth such as selenite

or tetrathionate. Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and a presumptive identification is made. Biochemical identification of salmonellae has been simplified by systems that permit the rapid testing of 10–20 different biochemical parameters simultaneously. The presumptive biochemical identification of *Salmonella* then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. *Salmonella* isolates then should be sent to a central or reference laboratory for more comprehensive serologic testing and confirmation.

Treatment

General salmonellosis treatment measures include replacing fluid loss by oral and intravenous routes, and controlling pain, nausea, and vomiting. Specific therapy consists of antibiotic administration. Typhoid fever and enteric fevers should be treated with antibiotics. Antibiotic therapy of non-typhoidal salmonellosis should be reserved for the septicemic, enteric fever, and focal infection syndromes. Antibiotics are not recommended for uncomplicated *Salmonella* gastroenteritis because they do not shorten the illness and they significantly prolong the fecal excretion of the organisms and increase the number of antibiotic-resistant strains. Antibiotic therapy for enteric fever; determine appropriate antibiotic with drug susceptibility testing

Nontyphoidal Salmonella

Infection with nontyphoidal serovars of *Salmonella* will generally result in food poisoning. Infection usually occurs when a person ingests foods that contain a high concentration of the bacteria. Infants and young children are much more susceptible to infection, easily achieved by ingesting a small number of bacteria. In infants, infection through inhalation of bacteria-laden dust is possible.

The organism enters through the digestive tract and must be ingested in large numbers to cause disease in healthy adults. An infection can only begin after living salmonellae (not merely *Salmonella*-produced toxins) reach the gastrointestinal tract. Some of the microorganisms are killed in the stomach, while the surviving salmonellae enter the small intestine and

multiply in tissues. Gastric acidity is responsible for the destruction of the majority of ingested bacteria, however *Salmonella* has evolved a degree of tolerance to acidic environments that allows a subset of ingested bacteria to survive. Bacterial colonies may also become trapped in mucus produced in the oesophagus. By the end of the incubation period, the nearby host cells are poisoned by endotoxins released from the dead salmonellae. The local response to the endotoxins is enteritis and gastrointestinal disorder.

There are approximately 2,000 serotypes of non-typhoidal *Salmonella*, which may be responsible for as many as 1.4 million illnesses in the United States each year. People who are at risk for severe illness include infants, elderly, organ-transplant recipients, and the immunocompromised.

Invasive nontyphoidal salmonella disease

While in developed countries, nontyphoidal serovars present mostly as gastrointestinal disease, in sub-Saharan Africa these serovars can create a major problem in bloodstream infections, and are the most commonly isolated bacteria from the blood of those presenting with fever. Bloodstream infections caused by nontyphoidal salmonellae in Africa were reported in 2012 to have a case fatality rate of 20–25%. Most cases of invasive nontyphoidal salmonella infection (iNTS) are caused by *S Typhimurium* or *S Enteritidis*. A new form of *Salmonella Typhimurium* (ST313) emerged in the southeast of the African continent 75 years ago, followed by a second wave which came out of central Africa 18 years later. This second wave of iNTS possibly originated in the Congo Basin, and early in the event picked up a gene that made it resistant to the antibiotic chloramphenicol. This created the need to use expensive antimicrobial drugs in areas of Africa that were very poor, making treatment difficult. The increased prevalence of iNTS in sub-Saharan Africa compared to other regions is thought to be due to the large proportion of the African population with some degree of immune suppression or impairment due to the burden of HIV, malaria and malnutrition, especially in children. The genetic makeup of iNTS is evolving into a more typhoid-like bacteria, able to efficiently spread around the human body. Symptoms are reported to be diverse, including fever, hepatosplenomegaly, and respiratory symptoms, often with an absence of gastrointestinal symptoms.

Typhoidal *Salmonella*

Typhoid fever is caused by *Salmonella* serotypes which are strictly adapted to humans or higher primates—these include Salmonella Typhi, Paratyphi A, Paratyphi B and Paratyphi C. In the systemic form of the disease, salmonellae pass through the lymphatic system of the intestine into the blood of the patients (typhoid form) and are carried to various organs (liver, spleen, kidneys) to form secondary foci (septic form). Endotoxins first act on the vascular and nervous apparatus, resulting in increased permeability and decreased tone of the vessels, upset of thermal regulation, vomiting and diarrhoea. In severe forms of the disease, enough liquid and electrolytes are lost to upset the water-salt metabolism, decrease the circulating blood volume and arterial pressure, and cause hypovolemic shock. Septic shock may also develop. Shock of mixed character (with signs of both hypovolemic and septic shock) is more common in severe salmonellosis. Oliguria and azotemia may develop in severe cases as a result of renal involvement due to hypoxia and toxemia.

Molecular mechanisms of infection

Mechanisms of infection differ between typhoidal and nontyphoidal serovars, owing to their different targets in the body and the different symptoms that they cause. Both groups must enter by crossing the barrier created by the intestinal cell wall, but once they have passed this barrier they use different strategies to cause infection.

Nontyphoidal serovars preferentially enter M cells on the intestinal wall by bacterial-mediated endocytosis, a process associated with intestinal inflammation and diarrhoea. They are also able to disrupt tight junctions between the cells of the intestinal wall, impairing the cells' ability to stop the flow of ions, water and immune cells into and out of the intestine. The combination of the inflammation caused by bacterial-mediated endocytosis and the disruption of tight junctions is thought to contribute significantly to the induction of diarrhoea.

Salmonellae are also able to breach the intestinal barrier via phagocytosis and trafficking by CD18-positive immune cells, which may be a mechanism key to typhoidal *Salmonella* infection. This is thought to be a more stealthy way of passing the intestinal barrier, and may therefore contribute to the fact that lower numbers of typhoidal *Salmonella* are required for infection than nontyphoidal *Salmonella*. *Salmonella* are able to enter macrophages via macropinocytosis. Typhoidal serovars can use this to achieve dissemination throughout the body via the mononuclear phagocyte

system, a network of connective tissue that contains immune cells, and surrounds tissue associated with the immune system throughout the body.

Much of the success of *Salmonella* in causing infection is attributed to two type three secretion systems which function at different times during infection. One is required for the invasion of non-phagocytic cells, colonization of the intestine and induction of intestinal inflammatory responses and diarrhoea. The other is important for survival in macrophages and establishment of systemic disease. These systems contain many genes which must work co-operatively to achieve infection.

The AvrA toxin injected by the SPI1 type three secretion system of *Salmonella* Typhimurium works to inhibit the innate immune system by virtue of its serine/threonine acetyltransferase activity, and requires binding to eukaryotic target cell phytic acid (IP6). This leaves the host more susceptible to infection.

Treatment

Electrolytes may be replenished with oral rehydration supplements (typically containing salts sodium chloride and potassium chloride). Appropriate antibiotics, such as ceftriaxone, are given to kill the bacteria. Azithromycin has been suggested to be better at treating typhoid in resistant populations than both fluoroquinolone drugs and ceftriaxone. Antibiotic resistance rates are increasing throughout the world, so health care providers should check current recommendations before choosing an antibiotic.

Vi antigen

The presence of the Vi antigen also increases the infectivity of *Salmonella* serovar Typhi and the severity of disease in volunteers. The Vi capsule is, however, not essential for infection, as Vi-negative mutant of *Salmonella* serovar Typhi are able to establish infection and cause a typhoid fever-like illness in human volunteers. Furthermore, there have been reports of outbreaks of typhoid fever caused by Vi-negative *Salmonella* serovar Typhi.

Salmonella H antigen

Ã Flagellin, the flagellar filament

- A protein antigen

- Variation in the middle surfaceexposed portion of the protein Å Salmonella is unique in having 2 different H antigens: y

Phase 1/Phase 2

- Phase 1 has a homolog in other enteric
- Phase 2 gene is in a Salmonella-specific region of the genome
- The 2 flagellins are coordinately expressed— one is off when other is on H Antigens Designations

Salmonella O Antigen

A **somatic antigen** is an antigen located in the cell wall of a gram-positive or gram-negative bacterium.

Lipopolysaccharides (LPS), also known as lipoglycans and **endotoxin**, are large molecules consisting of a lipid and a polysaccharide decomposed of O-antigen, outer core and inner core joined by a covalent bond; they are found in the outer membrane of Gram-negative bacteria, and elicit strong immune responses in animals.

The term lipooligosaccharide ("LOS") is used to refer to a low molecular weight form of bacterial lipopolysaccharides. LPS is the major component of the outer membrane of Gram-negative bacteria, contributing greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps stabilize the overall membrane structure. It is of crucial importance to gram-negative bacteria, whose death results if it is mutated or removed. LPS induces a strong response from normal animal immune systems. It has also been implicated in non-pathogenic aspects of bacterial ecology, including surface adhesion, bacteriophage sensitivity, and interactions with predators such as amoebae.

LPS is required for the proper conformation of OmpT activity; however, smooth LPS will sterically hinder ompTins.

Composition

It comprises three parts:

1. O antigen (or O polysaccharide)
2. Core oligosaccharide
3. Lipid A

O-antigen

A repetitive glycan polymer contained within an LPS is referred to as the O antigen, O polysaccharide, or O side-chain of the bacteria. The O antigen is attached to the core oligosaccharide, and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different *E. coli* strains. The presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O-chains would render the LPS smooth, whereas the absence or reduction of O-chains would make the LPS rough. Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more hydrophobic.^[6] O antigen is exposed on the very outer surface of the bacterial cell, and, as a consequence, is a target for recognition by host antibodies.

Core

The Core domain always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO, keto-deoxyoctulosonate). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids, and ethanolamine substituents.

Lipid A

Lipid A is, in normal circumstances, a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of Gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea, and possible fatal endotoxic shock (also called septic shock). The Lipid A moiety is a very conserved component of the LPS

Two types of O Group antigens

1- “Ancillary” O antigens

2- O Group antigens

a) Most important for determining serotype

b) rfb region contains genes responsible for O group

c) Found in all Enterobacteriaceae

Ancillary O antigens

a) Typically encoded by extra-chromosomal elements (bacteriophages, plasmids)

b) Found in specific O groups

c) Most can vary within a given serotype, so are less important for serotype determination.

Vibrio cholerae

is a Gram-negative, comma-shaped bacterium. Some strains of *V. cholerae* cause the disease cholera. *V. cholerae* is a facultative anaerobic organism and has a flagellum at one cell pole. *V. cholerae* was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854, but his discovery was not widely known until Robert Koch, working independently 30 years later, publicized the knowledge and the means of fighting the disease

Pathogenesis

V. cholerae pathogenicity genes code for proteins directly or indirectly involved in the virulence of the bacteria. During infection, *V. cholerae* secretes cholera toxin, a protein that causes profuse, watery diarrhea. Colonization of the small intestine also requires the toxin coregulated pilus (TCP), a thin, flexible, filamentous appendage on the surface of bacterial cells. *V. cholerae* can cause syndromes ranging from asymptomatic to cholera gravis. In endemic areas, 75% of cases are asymptomatic, 20% are mild to moderate, and 2-5% are severe forms such as cholera gravis. Symptoms include abrupt onset of watery diarrhea (a grey and cloudy liquid), occasional vomiting, and abdominal cramps. Dehydration ensues, with symptoms and signs such as thirst, dry mucous membranes, decreased skin turgor, sunken eyes, hypotension, weak or absent radial pulse, tachycardia, tachypnea, hoarse voice, oliguria, cramps, renal failure, seizures, somnolence, coma, and death.^[1] Death due to dehydration can occur in a few hours to days in untreated children. The disease is also particularly dangerous for pregnant women and their fetuses during late pregnancy, as it may cause premature labor and fetal death. In cases of cholera gravis involving severe dehydration, up to 60% of patients can die; however, less than 1% of cases treated with rehydration therapy are fatal. The disease typically lasts 4–6 days. Worldwide, diarrhoeal disease, caused by cholera and

many other pathogens, is the second-leading cause of death for children under the age of 5 and at least 120,000 deaths are estimated to be caused by cholera each year. In 2002, the WHO deemed that the case fatality ratio for cholera was about 3.95%.

Genome

V. cholerae has two circular chromosomes, together totalling 4 million base pairs of DNA sequence and 3,885 predicted genes. The genes for cholera toxin are carried by CTXphi (CTX), a temperate bacteriophage inserted into the *V. cholerae* genome. CTX can transmit cholera toxin genes from one *V. cholerae* strain to another, one form of horizontal gene transfer. The genes for toxin coregulated pilus are coded by the VPI pathogenicity island (VPI). The entire genome of the virulent strain *V. cholerae* El Tor N16961 has been sequenced, and contains two circular chromosomes. Chromosome 1 has 2,961,149 base pairs with 2,770 open reading frames (ORF's) and chromosome 2 has 1,072,315 base pairs, 1,115 ORF's. The larger first chromosome contains the crucial genes for toxicity, regulation of toxicity, and important cellular functions, such as transcription and translation.

The second chromosome is determined to be different from a plasmid or megaplasmid due to the inclusion of housekeeping and other essential genes in the genome, including essential genes for metabolism, heat-shock proteins, and 16S rRNA genes, which are ribosomal subunit genes used to track evolutionary relationships between bacteria. Also relevant in determining if the replicon is a chromosome is whether it represents a significant percentage of the genome, and chromosome 2 is 40% by size of the entire genome. And, unlike plasmids, chromosomes are not self-transmissible. However, the second chromosome may have once been a megaplasmid because it contains some genes usually found on plasmids.

V. cholerae contains a genomic island of pathogenicity and is lysogenized with phage DNA. That means that the genes of a virus were integrated into the bacterial genome and made the bacteria pathogenic.

Bacteriophage CTX

CTX (also called CTXphi) is a filamentous phage that contains the genes for cholera toxin. Infectious CTX particles are produced when *V. cholerae* infects humans. Phage particles are secreted from bacterial cells without lysis. When CTX infects *V. cholerae* cells, it integrates into specific sites on either chromosome. These sites often contain tandem arrays of integrated CTX prophage. In addition to the *ctxA* and *ctxB* genes encoding cholera toxin, CTX contains eight genes involved in

phage reproduction, packaging, secretion, integration, and regulation. The CTX genome is 6.9 kb long.

***Vibrio* pathogenicity island**

The *Vibrio* pathogenicity island (VPI) contains genes primarily involved in the production of toxin coregulated pilus (TCP). It is a large genetic element (about 40 kb) flanked by two repetitive regions (*att*-like sites), resembling a phage genome in structure. The VPI contains two gene clusters, the TCP cluster, and the ACF cluster, along with several other genes. The *acf* cluster is composed of four genes: *acfABCD*. The *tcp* cluster is composed of 15 genes: *tcpABCDEFHIJPQRST* and regulatory gene *toxT*.

Ecology and epidemiology

The main reservoirs of *V. cholerae* are people and aquatic sources such as brackish water and estuaries, often in association with copepods or other zooplankton, shellfish, and aquatic plants.

Cholera infections are most commonly acquired from drinking water in which *V. cholerae* is found naturally or into which it has been introduced from the feces of an infected person. Other common vehicles include contaminated fish and shellfish, produce, or leftover cooked grains that have not been properly reheated. Transmission from person to person, even to health care workers during epidemics, is rarely documented. *V. cholerae* thrives in a aquatic environment, particularly in surface water. The primary connection between humans and pathogenic strains is through water, particularly in economically reduced areas that do not have good water purification systems.

Nonpathogenic strains are also present in water ecologies. The wide variety of strains of pathogenic and nonpathogenic strains that co-exist in aquatic environments are thought to allow for so many genetic varieties. Gene transfer is fairly common amongst bacteria, and recombination of different *V. cholerae* genes can lead to new virulent strains.

Gram-negative •

Curved rod •

.5-.8 μm width •

1.4-2.6 μm length •

- Facultative anaerobe •
- Single polar flagellum •
- Chemoorganotroph •
- Optimal growth 20-30 degrees •

Treatment of cholera,

An oral or intravenously administered solution containing glucose, sodium chloride, potassium chloride and trisodium citrate can save a patient from dehydration. The antibiotics tetracycline and quinolones have been widely used to reduce the symptoms of cholera, but the emergence of *V. cholerae* strains resistant to antibiotics has restricted their use to patients with severe dehydration. In severe cases, a single dose of doxycycline (a member of the tetracycline antibiotics group) co-administered with fluid replacement therapy is usually sufficient to stabilize the patient. Alternatively, a multidose treatment of tetracycline can be administered; in the case of young children, liquid erythromycin is preferred. In a randomized clinical trial, erythromycin yielded the best clinical recovery rates in children. Though there are obvious benefits to individuals who are treated with antibiotics, the World Health Organization does not recommend their general use because antibiotics contribute to increasing antimicrobial resistance, making cholera and other bacterial infections more difficult to treat. Use of antibiotics to treat cholera should be strictly relegated to patients suffering from severe dehydration.

Mechanisms of antibiotic resistance

V. cholerae becomes drug resistant by exporting drugs through efflux pumps, chromosomal mutations or developing genetic resistance via the exchange of conjugative plasmids, conjugative transposons, integrons or selftransmissible chromosomally integrating SXT elements

Bacterial efflux pumps

V. cholerae uses multidrug efflux pumps to export a broad range of antibiotics, detergents and dyes that are chemically and structurally

unrelated (Paulsen et al., 1996). The two major groups of *V. cholerae* efflux pumps are distinguished by their energy sources: ATP hydrolysis, or the proton-motive force (PMF) of transmembrane H⁺ or Na⁺ gradients (Putman et al., 2000). PMF pump families include MATE (multidrug and toxic compound extrusion), MFS (major facilitator superfamily), RND (resistance–nodulation–cell division) and SMR (small multidrug resistance)

Cholera toxin

Cholera toxin (also known as **cholera toxin** and sometimes abbreviated to **CTX**, **Ctx** or **CT**) is a protein complex secreted by the bacterium *Vibrio cholerae*. CTX is responsible for the massive, watery diarrhea characteristic of cholera infection.

Structure

The cholera toxin is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A, enzymatic), and five copies of the B subunit (part B, receptor binding), denoted as AB₅. Subunit B binds while subunit A activates the G protein which activates adenylate cyclase. The three-dimensional structure of the toxin was determined using X-ray crystallography

The five B subunits—each weighing 11 kDa, form a five-membered ring. The A subunit which is 28 kDa, has two important segments. The A1 portion of the chain (CTA1) is a globular enzyme payload that ADP-ribosylates G proteins, while the A2 chain (CTA2) forms an extended alpha helix which sits snugly in the central pore of the B subunit ring.

This structure is similar in shape, mechanism, and sequence to the heat-labile enterotoxin secreted by some strains of the *Escherichia coli* bacterium

Pathogenesis

Cholera toxin acts by the following mechanism: First, the B subunit ring of the cholera toxin binds to GM1 gangliosides on the surface of target cells. Once bound, the entire toxin complex is endocytosed by the cell and the cholera toxin A1 (CTA1) chain is released by the reduction of a disulfide bridge. The endosome is moved to the Golgi apparatus, where the A1 protein is recognized by the endoplasmic reticulum chaperon, protein disulfide isomerase. The A1 chain is then unfolded and delivered to the membrane, where the ER- α -oxidase - ER

oxidoreductin triggers the release of the A1 protein by oxidation of protein disulfide isomerase complex. As the A1 protein moves from the ER into the cytoplasm by the Sec61 channel, it refolds and avoids deactivation as a result of ubiquitination.

CTA1 is then free to bind with a human partner protein called ADP-ribosylation factor 6 (Arf6); binding to Arf6 drives a change in the shape of CTA1 which exposes its active site and enables its catalytic activity.^[5] The CTA1 fragment catalyses ADP-ribosylation of the Gs alpha subunit (G_s) proteins using NAD. The ADP-ribosylation causes the G_s subunit to lose its catalytic activity in hydrolyzing GTP to GDP + P_i; so it remains activated longer than normal. Increased G_s activation leads to increased adenylyl cyclase activity, which increases the intracellular concentration of 3',5'-cyclic AMP (cAMP) to more than 100-fold over normal and over-activates cytosolic PKA. These active PKA then phosphorylate the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel proteins, which leads to ATP-mediated efflux of chloride ions and leads to secretion of H₂O, Na⁺, K⁺, and HCO₃⁻ into the intestinal lumen. In addition, the entry of Na⁺ and consequently the entry of water into enterocytes are diminished. The combined effects result in rapid fluid loss from the intestine, up to 2 liters per hour, leading to severe dehydration and other factors associated with cholera, including a rice-water stool.

Interestingly, the pertussis toxin (also an AB₅ protein) produced by *Bordetella pertussis* acts in a similar manner with the exception that it ADP-ribosylates the G_i subunit, rendering it inactive and unable to inhibit adenylyl cyclase production of cAMP (leading to constitutive production).