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Epidemiology and Molecular Biology of Vibrio Cholerae O139

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Abstract

Cholera is one of the most feared epidemic infectious diseases that can affect human beings. Seven pandemics of cholera have been recorded since 1817. Each one of these pandemics caused significant morbidity and mortality, had a negative impact on the economy, trade, and commerce of the affected countries, and caused panic amongst the general public.Cholera is a highly contagious acute dehydrating diarrheal disease caused by Vibrio cholerae. There are over 200 serogroups of V. cholerae known to date; however, only two (O1 and 139 serotypes) are responsible for the vast majority of outbreaks.

Cholera is an intestinal infection caused by Vibrio cholerae .The hallmark of the disease is profuse secretory diarrhea. Cholera can be endemic, epidemic, or pandemic. Despite all the major advances in research, the condition still remains a challenge to the modern medical world. Although the disease may be asymptomatic or mild, severe cholera can cause dehydration and death within hours of onset. The objective of this study was to review prevalence and molecular characterization of vibrio cholerae O139 to help control and prevention of the spread of this highly-transmissible diseases in in the world and also provides a detailed literature review on molecular biology drivers of cholera patterns.

Keywords: Cholera, V. cholerae O139, Epidemiology, Molecular characterization

Introduction

Background

Cholera is one of the most feared epidemic infectious diseases that can affect human beings. Seven pandemics of cholera have been recorded since 1817. Each one of these pandemics caused significant morbidity and mortality, had a negative impact on the economy, trade, and commerce of the affected countries, and caused panic amongst the general public [1]. Cholera is a highly contagious acute dehydrating diarrheal disease caused by Vibrio cholerae. Cholera is an intestinal infection caused by Vibrio cholerae .The hallmark of the disease is profuse secretory diarrhea. Cholera can be endemic, epidemic, or pandemic. Despite all the major advances in research, the condition still remains a challenge to the modern medical world. Although the disease may be asymptomatic or mild, severe cholera can cause dehydration and death within hours of onset.

There are over 200 serogroups of V. cholerae known to date; however, only two (O1 and 139 serotypes) are responsible for the vast majority of outbreaks [2, 3].

In late 1992, epidemics of severe acute watery diarrhea, clinically resembling cholera and mainly affecting adults, was reported in Madras, a southern port city of India, and in Southern Bangladesh. The epidemics later spread to other parts of both countries and to some of the neighboring countries of the region. The bacterium responsible for the epidemics resembled V. cholerae 01 in cultural and biochemical characteristics, but did not agglutinate with V. cholerae 01 antisera . Primers specific for the cholera toxin (CT) genes of V. cholerae 01 amplified sequences corresponding to CT in these strains in PCR ,and all strains tested also were positive for CT production by standard bioassays for CT. However, this bacterium did not belong to any of the 138 O serogroups for V. cholerae described until then; the conclusion was that it belonged to a new serogroup. The new epidemic strain of V. cholerae was later serogrouped as O139 and given the synonym “Bengal” in recognition of the first appearance of this serogroup in regions in the vicinity of the Bay of Bengal. Before the emergence of V. cholerae O139, non-01 serogroups of V. cholerae were not known to be associated with such large outbreaks of diarrhea. Moreover, they were known to produce CT at a very low frequency unlike the O139 serogroup, given that almost all tested isolates of this serogroup produced the toxin. Since then, V. cholerae O139 has persisted as a second etiologic agent of cholera. There are now two serogroups, O1 and O139, that have been associated with cholera epidemics, and the simple distinction between O1 and non-O1 V. cholerae regarding epidemic potential has, therefore, become obsolete [4,5].

The O139 serogroup is composed of a variety of genetically

diverse strains, both toxigenic and nontoxigenic, with at least nine different ribotypes identified [12]. This novel serogroup is genetically closer to El Tor V. cholerae, and might have been originated from it, acquiring distinctive features from a nonidentified donor, likely a non-O1 vibrio, through recombination of genetic material [4,5].

While many bacterial pathogens can cause diarrhea, only Vibrio cholerae, the causative agent of cholera, has caused repeated pandemics. Cholera has since the beginning of the 19th century caused epidemic disease on virtually every continent. Despite the fact that John Snow showed its infectious nature over a century ago, we are still unable to control cholera throughout the developing world. Even though there are safe and effective vaccines that protect against V. cholerae, cholera still extracts a death toll that measures in the thousands worldwide [6].

Some would argue that the seventh El Tor pandemic has now given way to the eighth pandemic with the appearance of the new cholera serogroup O139. Recently the number of cholera cases worldwide has been increasing, owing in large part to the arrival of the seventh pandemic in Peru in 1991 and to the emergence of O139 serogroup of V. cholerae in 1992. Between these large outbreaks of disease, cholera has remained present in the Indian subcontinent and Africa largely in endemic foci [6].

The most common symptom of cholera is a life-threatening amount of watery diarrhea, causing an extreme loss of water, up to 1 L per hour, which can lead to death within hours of the first onset of symptoms if left untreated. The diarrhea is usually painless and not accompanied by the urge to evacuate the bowels. Early in the illness, vomiting can be a common symptom as well [6].

Morphological, cultural, and biochemical characteristics.
The morphological, cultural, and biochemical characteristics of V cholerae 0139 are similar to those of V cholerae 01. It is a gram-negative, facultative, anaerobic, curved bacillus, measuring 2 to 3 by 0.5, um and having a single polar flagellum and shows the typical darting motility of V cholerae. It grows in media containing 0 to 3%, but not 8%, salt. It grows on a variety of nonselective media such as nutrient agar and sheep blood agar and on selective media for V cholerae such as thiosulfate-citrate-bile salt-sucrose agar and taurocholate-tellurite-gelatin agar (TTGA). On thiosulfate-citrate-bile saltsucrose agar, it produces typical yellow colonies, and on TTGA, it produces grayish colonies with dark centers surrounded most often by a zone of opacity (due to gelatinase production. On many media including Luria agar, gelatin agar, and TTGA, like certain other V cholera non-O1 organisms, two colony variants can be seen, translucent and opaque. Some isolates produce either form, and others produce a mixture of both. Opaque variants on gelatin-containing media such as gelatin agar and TTGA produce, in addition, a zone of opacity around the colonies as a result of gelatinase activity, whereas this characteristic is absent or minimal with translucent variants. Upon incubation for longer periods (beyond 24 h), this distinction becomes less apparent, and translucent forms become opaque. Like some V cholerae non-O1 sero groups, opaque colonies of V cholerae 0139 possess a capsule, whereas this capsular layer seems to be negligible or absent in translucent colonies [6,7].

Like all vibrios, V cholerae 0139 is positive for indophenol oxidase and ferments a variety of sugars without gas production. Especially important is that, like 01 vibrios, it ferments D-(+)-mannose and sucrose but not L-(+)-arabinose and thus belongs to Heiberg group 1 vibrios [8]. Like El Tor biotype vibrios, it is positive for the Vogues-Proskauer reaction, shows variable hemolysis of sheep erythrocytes in conventional tubetests, agglutinates chicken erythrocytes, produces kappa type phage, and is resistant to polymyxin B. However, it is not attacked by Mukherjee's phages specific for El Tor or classical vibrios. Like most current strains of V cholerae O1 in Bangladesh, V. cholerae 0139 is resistant to the vibriostatic compound 0/129 (2,4-diamino-6,7-diisopropyl pteridine). All isolates tested to date are susceptible to tetracycline (unlike most of the currently prevalent strains of V: cholerae 01 in Bangladesh), ampicillin, chloramphenicol, erythromycin, ciprofloxacin, furazolidone, doxycycline, and nalidixic acid but resistant to trimethoprim-sulfamethoxazole and streptomycin. However, no data are available comparing the MICs of theseantimicrobial agents for V cholerae 0139 and 01. Like V cholerae 01, V. cholerae 0139 thrives in an alkaline pH but is susceptible to a pH below 5.0 [9].

V. cholerae is a curved gram-negative bacillus varying in size from 1 to 3 μm in length by 0.5 to 0.8 μm in diameter that belongs to the family Vibrionaceae and shares common characteristics with the family Enterobacteriaceae. The bacterium has a single polar flagellum that confers the erratic movement on microscopy. The antigenic structure of V. cholerae is similar to that of other members of the family Enterobacteriaceae, with a flagellar H antigen and a somatic O antigen. The O antigen is used to further classify V. cholerae in serogroups, O1 and non-O1. Approximately 206 serogroups of V. cholerae have been identified to date, but only the serogroups O1 and O139 are associated with clinical cholera and have pandemic potential [9,10].

Capsular Antigen

Like a majority of V. cholerae non-01 isolates, but unlike V cholerae 01, V cholerae 0139 isolates possess a capsule . Preliminary analysis of the capsular layer suggests that it is distinct from the lipopolysaccharide (LPS) antigen (see below) and has the following sugars: 3,6-dideoxyhexose (abequose or colitose), quinovosamine and glucosamine, and traces of tetradecanoic and hexadecanoic fatty acids . It is thought that, as in other capsulated V cholerae non-01 serogroups, the presence of a capsule on V cholerae 0139 may confer increased virulence to the organism, such as resistance to serum killing and capacity to produce bacteremia. Like other encapsulated V. cholerae non-01 serogroups, V cholerae 0139 produces bacteremia and death in mice upon intradermal inoculation . It is also interesting that V cholerae 0139 caused bacteremia in an adult patient with chronic underlying liver disease . There is difficulty in developing a vibriocidal assay for V cholerae 0139 because the organism cannot be easily killed in sera even though the specific antibody content to the organism, as measured by other immunoassays, is high . It is hypothesized that this problem may be attributable to the presence of the capsule. What role this capsule plays in antigen recogni-

tion is not known. In volunteer studies with other non-01 strains, the presence of a capsule appeared to mask certain critical surface antigens, with a resulting decrease in host immune response . However, rabbits immunized with heat-killed, whole bacterial cells of V cholerae 0139 produced antibodies to bothcapsular and LPS antigen when tested by enzyme-linked immunosorbent assay [11,9].

Lipopolysaccharides Antigen

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of an LPS preparation from V cholerae 0139, followed by silver staining of the gel, suggested that LPS does not show a distinct ladder pattern corresponding to lipid A, core oligosaccharide, and high-molecular-weight 0 antigen side chain of smooth V cholerae 01. Instead, it showed a doublet pattern with an electrophoretic mobility slightly slower than that of the lipid A plus core oligosaccharide region of V cholerae 01 strains, with a lesser amount of comigrating material. Thus, V. cholerae 0139 appeared to have a modified core structure and no high-molecular-weight 0- antigen-specific side chain but probably a low-molecularweight short chain. This LPS pattern resembled a semi-roughtype LPS . One group of workers reported that this structure contains a 3,6-dideoxyhexose (identified as colitose) and claimed that this sugar is found for the first time in members of the family Vibrionaceae . However, a second group of workers claimed that the dideoxyhexose is part of the capsular polysaccharide. The LPS contains glucose, glucosamine, and heptose as component sugars and tetradecanoic acid, hexadecanoic acid, 3-hydroxy-dodecanoic acid, and 3- -hydroxy-tetradecanoic acids as component fatty acids [12].

Fimbrial Antigens

A number of different fimbrial appendages have been demonstrated in V cholerae 01 organisms, but their role in the colonization of the organism remains to be defined. The fimbriae include toxin-coregulated pilus with a subunit molecular mass of 20.5 kDa (TcpA), mannosesensitive hemagglutinin, a 16-kDa subunit structure pilus, a hydrophobic pilus with a subunit molecular mass of 18 kDa, and a pilus with a subunit structure of 20 kDa. The presence of these fimbrial antigens has also been investigated in V cholerae 0139. Under optimal AKI-SW cultural conditions (the medium contains 1.5% peptone, 0.4% yeast extract, 0.5% NaCl, and 0.3% NaHCO3 and is initially incubated for 4 h as a stationary culture and then incubated for 16 hour as a shaker culture at 37°C)) as those for El Tor vibrios, V cholerae 0139 produced TcpA . Mutant derivatives with insertional inactivation of the structural gene tcpA resulted in decreased colonization ability of the mutant for suckling mouse intestine. V. cholerae 0139 also produces the morphologically and immunologically related fimbrial antigens, other than TcpA, listed above). In addition, it produces a curved (wavy) pilus with a subunit molecular mass of 2.8 kDa (minipilus) which is shared by many species of gram-negative bacteria). In keeping with the presence of various fimbrial (colonization) antigens, the strain adheres very strongly in vitro to HEp-2 cell monolayers. It has also been recovered in high numbers from the upper small intestinal fluids of infected patients and has been demonstrated to be adherent to the upper small intestinal mucosal biopsies [12].

Molecular Characterization

Molecular analysis of V cholerae 01 strains has suggested that most of the genes that encode virulence factors are carried on a 4.5-kb virulence cassette or core region of the chromosome. The genes that have been identified in the core region are cxtAB (that encodes CT), zot (that encodes zonula occludens toxin), ace (that encodes accessory cholera enterotoxin), and cep (that encodes a pilin antigen which enhances colonization). This core region is flanked by RS1 elements (repetitive sequence 1, an insertion sequence 2.7 kb long normally found at the junction of tandem duplication of ctxAB and which is responsible for amplification of cxAB). The core region and the RS1 element constitute the CTX element. Normally, V cholerae 01 isolates carry multiple copies of the CIX element. Similarly, V cholera 0139 isolates also carried two or more copies of the CTX element at the same chromosomal site as that of the CTX element in El Tor vibrios. Moreover, they carried the tcpA gene and three iron-regulated genes, irgA (a virulence gene), viuA (the gene for the receptor for the siderophore vibriobactin), and fur (an iron regulatory gene) previously described for 01 vibrios in the same chromosomal location as in El Tor vibrios [13]. Further relatedness with El Tor vibrios was suggested by the identity of sequences for cxAB and 16S rRNA genes in V cholerae 0139 and El Tor vibrios. Furthermore, with the construction of toxR (a positive regulator gene for cxAB) null mutants, it was found that the expression of CT, TcpA, and outer membrane protein OmpU in 0139 vibrios is dependent on ToxR as it is in 01 vibrios. Similarity between 01 and 0139 vibrios was also found in the iron-regulated outer membrane protein profiles. The rfb region encoding thr 0-antigen synthesis in V cholerae 0139 was examined with V cholerae 01-specific rjb gene probes. It was found that all of the genes involved in 0-antigen synthesis and Ogawa serotype modification in V cholerae 01 are absent in V cholerae 0139. These genetic data thus complement the structural data of the LPS antigen. Molecular epidemiological studies have been carried out by using multilocus enzyme electrophoresis, ribotyping, cixA genotyping and pulsed-field gel electrophoresis. In all of these typing methods, V cholerae 0139 solates were either indistinguishable from, or similar to, the seventh pandemic strain (El Tor biotype) of cholera and were distinctly different from other non-O1 vibrios. However, by some of these molecular typing techniques, outbreak strains appeared to be heterogeneous. This may suggest that V. cholerae 0139 may be more prone to mutation and that it is mportant to monitor the strains over extended periods of time. With a molecular subtyping scheme, it may be possible to associate specific clones with certain geographical areas as in the case of V. cholera 01 [12, 14].

Epidemiology

Cholera can be endemic or epidemic. A cholera-endemic area is an area where confirmed cholera cases were detected during the last 3 years with evidence of local transmission (meaning the cases are not imported from elsewhere). A cholera outbreak/epidemic can occur in both endemic countries and in countries where cholera does not regularly occur.In cholera endemic countries an outbreak can be seasonal or sporadic and represents a greater than expected number of cases. In a country where cholera does not regularly occur, an outbreak is defined by the occurrence of at least 1 confirmed case of cholera with evidence of

local transmission in an area where there is not usually cholera [13,15].

Cholera is considered endemic in over 50 countries, but it can manifest as an epidemic, as has recently been the case in Yemen (2017–present), a country previously not exposed to cholera Cholera transmission is closely linked to inadequate access to clean water and sanitation facilities. Typical at-risk areas include peri-urban slums, and camps for internally displaced persons or refugees, where minimum requirements of clean water and sanitation are not been met.The consequences of a humanitarian crisis – such as disruption of water and sanitation systems, or the displacement of populations to inadequate and overcrowded camps – can increase the risk of cholera transmission, should the bacteria be present or introduced. Uninfected dead bodies have never been reported as the source of epidemics [16].

Since 1817, up to seven major plagues and cholera outbreaks have been reported in Asia and Africa, with minor cases in Australia and America. Sub-Saharan Africa is broadly affected by many cholera epidemics [17].

The number of cholera cases reported to WHO has continued to be high over the last few years. During 2016, 132 121 cases were notified from 38 countries, including 2420 deaths. The discrepancy between these figures and the estimated burden of the disease is due to the fact that many cases are not recorded due to limitations in surveillance systems and fear of impact on trade and tourism [16].

Epidemiological data on the emergence and prevalence of V. cholerae O139 and its coexistence with the O1 El Tor strains are available primarily from Bangladesh and India (Fig. 3) through systematic surveillance studies. In the Ganges Delta region of India and Bangladesh, epidemics of cholera occur with a regular seasonality, but temporal variation in the prevalence of the two epidemic serogroups O1 and O139 have been noticed. The emergence of V. cholerae O139 initially caused a complete displacement of the El Tor biotype strains in both these countries. However, V. cholerae O139 was again displaced in 1994 by a new genetic variant of the O1 strain, and this variant strain dominated until 1996 in India. In August, 1996, a new variant of the O139 strain emerged, and cholera caused by the new O139 genetic variant dominated for a year, until September, 1997 in Calcutta. Similarly in neighboring Bangladesh, during 1994 and till the middle of 1995, in most northern and central areas of the country, the O139 vibrios were replaced by a new clone of V. cholerae O1 of the El Tor biotype, whereas in the southern coastal regions, the O139 vibrios continued to exist . During late 1995 and through 1996, cases of cholera caused by both V. cholerae O1 and O139 were again detected in various regions of Bangladesh. However, since 1996, choleras in Bangladesh were caused mostly by V. cholerae O1 of the El Tor biotype, whereas only a few cases were caused by strains of the O139 serogroup. This changing epidemiology of cholera in Bangladesh shifted further recently, and a large outbreak of cholera caused predominantly by V. cholerae O139 occurred in the capital city of Dhaka and adjoining areas during the first half of 2002 [13,15].

Although substantial information is available on the epidemiology of V. cholerae and the emergence of new epidemic clones, it is not clear what drives the frequent emergence of new clones often associated with epidemics and the replacement of existing clones. Analyzing genetic variation in isolates of V. cholerae O1 and O139 from successive outbreaks of cholera and the determination of whether these genetic variations contribute to the emergence of new clones of V. cholerae can be an important step in understanding the evolution of new pathogenic strains. An important area that needs to be addressed is whether preexisting immunity against one clone of either O1 or O139 can provide protection completely against another emerging clone. From an epidemiological viewpoint, it certainly appears that genetic rearrangement fosters some advantage to the emerging clone. It is also crucial to understand whether the observed genetic reassortment in the O1 and O139 strains is accompanied by other undiscovered discreet changes in the organism that enables the organism to escape the immune pressure of the host population against previously existing clones of toxigenic V. cholerae. There is no cross protection between V. cholerae O1 and O139 in the animal models. The predominantly adult population infected by O139 cholera also provides evidence that O1 strains do not protect efficiently against O139 strains. Considering the extent of longevity of a clone and the frequency of appearance and reappearance of different clones in a cholera-endemic area, as well as the occurrence of epidemics of cholera with seasonal regularity, it appears that the clonal turnover has greater implications. We hypothesize that the continual emergence of new toxigenic strains and their selective enrichment during cholera outbreaks constitute an essential component of the natural ecosystem for the evolution of epidemic V. cholerae strains to ensure its continued existence. The molecular epidemiological studies indicating continuously developing genetic diversity among clones of V. cholerae O1 and O139 are likely to complicate further the development of an effective cholera vaccine.

Cholera remains a significant public health problem in many parts of the world. In 2016, 38 countries reported a total of 132 121 cases including 2420 deaths, resulting in an overall case fatality rate (CFR) of 1.8%. Although this represents a 23% decrease in the number of cases reported compared with 2015 (172 454 cases), the decline is, nonetheless, accompanied by a more than doubling of the CFR (0.8% in 2015). Cholera was reported from countries in all regions: 17 countries in Africa, 12 in Asia, 4 in Europe, 4 in the Americas, and 1 in Oceania. Five countries, the Democratic Republic of the Congo (DRC), Haiti, Somalia, the United Republic of Tanzania, Yemen, together accounted for 80% of all cases. Of cases reported globally, 54% were from Africa, 13% from Asia and 32% from Hispaniola. Imported cases were reported from 9 countries [18].

Vibrio pathogen infections remain a significant health challenge in middle-income countries, notably in Africa and Asia, endangering the basic health of weak people in the society. Vibrio species have been implicated in cases of bloody diarrhea, necrotizing fasciitis, and primary septicemia in immunecompromised individuals, especially in developing countries with inadequate sanitation, socioeconomic conditions, and water supply systems; however, this is responsible for the varying degree of ill health

and death in all age groups worldwide. Natural tragedies such as tsunami and floods also aid outbreaks by unsettling the normal balance of nature .This results in varying health challenges, making food and water supplies prone to contamination by parasites and bacteria when vital systems like those for water and sewage are destroyed. An example of such is the current outbreak of cholera in Yemen that has claimed over 1500 lives with more than 246,000 new cases and this now affects 21 out of the 22 provinces in Yemen [16].

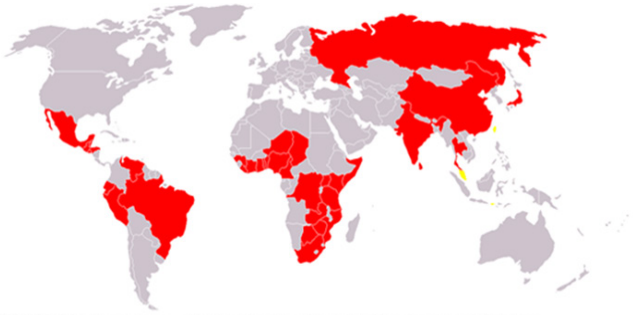

FIGURE 1: Countries affected by the Seventh Pandemic of Cholera
(compiled from World Health Organization (WHO), Center for Disease Control and Prevention (CDC), and various news sources, Countries in the red shade have reported cholera outbreaks)

Pathogeneses

The pathology of cholera results from V. cholera colonization in the small intestine and subsequent production of the cholera toxin(CT) [6,19].

The clinical manifestation of Vibrio infections commences with the drinking of contaminated water or the eating of mishandled marine products. After passing through the acidic wall of the stomach, it attaches itself to the thin tissue lining the small intestine with the aid of toxin-coregulated pili (TCP) and with establishment factors like accessory colonization factor, diverse haemagglutinins, and core-encoded pilus. Human pathogenic species are known to produce several extracellular factors including haemolysin, cytotoxin, siderophore, phospholipase, collagenase, enterotoxin, and haemagglutinin [20].

all of the virulence properties, haemolysin, enterotoxin, and cytosine have a direct link to the clinical manifestation; conversely, siderophore and haemagglutinin are involved in the establishment of Vibrio pathogen disease conditions. One of the important means by which pathogens establish their pathogenicity is through the production of bacterial enzymes. Essential proteolytic enzymes that breakdown the amide bond in proteins and other short amino acids are vital for regulating homeostasis in prokaryotes and eukaryotes. Occasionally, the enzymes produced by virulent Vibrio species are found to be toxic to the infected human host.

Relevant Vibrio pathogens associated with human infections produce and form proteolytic enzymes; some of these enzymes are broadly classified as toxic factors processing other protein toxins [14,20].

Because V. cholerae O139 was recorded as the only non-O1 V. cholerae capable of causing epidemic outbreaks, immediately after the emergence of the O139 serogroup comparative analyses of V. cholerae O1 and O139 strains were carried out to investigate the origin of this new serogroup. The early studies indicated that O139 strains were closely related to O1 El Tor biotype strains, which is responsible for the seventh pandemic of cholera, and the initial O139 strain may have emerged from serotype-specific genetic changes in an ancestral El Tor strain. More detailed molecular epidemiological analyses, such as zymovar analysis, ribotyping, and pulsed-field gel electrophoresis showed that V. cholerae O139 Bengal strains are closely related to O1 El Tor strains. Furthermore, V. cholerae O139 strains had all of the virulence factors normally found in O1 El Tor strains, and both V. cholerae O1 and O139 Bengal cause cholera of comparable clinical severity. However, in contrast to O1 strains, O139 strains are encapsulated and the O139 serogroup antigen includes an O-antigen capsule and bacterial lipopolysaccharid. The LPS of serogroup O139 does not contain any long O-antigen side chains, whereas O1 strains have a core substituted with an average of 17 repeat units of 4-NH2-4,6-dideoxymannose, each substituted with 3-deoxy-l-glycero-tetronic acid . The O139 LPS appears to be an efficiently substituted core polysaccharide, even though it possesses only a few additional sugar moieties). Interestingly, these changes have rendered the O139 vibrios immunologically distinct from the O1 El Tor strains [13].

Analysis of the genetic regions associated with O-antigen biosynthesis in O1 and O139 strains suggested that the conversion of the ancestral El Tor strain involved insertion of a large foreign genomic region encoding the O139-specific genes and simultaneous deletion of most of the O1-antigen-specific gene cluster. Later, more detailed analysis and sequencing of the wbf genes responsible for the biosynthesis of O-antigen and genes downstream of the wbf gene cluster of V. cholerae O139 have been reported. These studies have now characterized the major genetic differences accounting for the phenotypically distinct surface polysaccharide of O1 El Tor and O139 Bengal. In brief, the genes responsible for the synthesis of O-antigen are present in a cluster designated as the wb region. A large portion of DNA corresponding to the wbe region of O1 strains was found to be missing in O139 strains, and O139 strains were found to have acquired a unique DNA region. It was also shown that the serogroup O139 resulted from a precise 22-kb deletion of the wbe (rfb) region of O1, with replacement by a 35-kb wbf region (wbfA through wbfX) encoding the O139 O-antigen [13,12].

The O139 antigen is quite different structurally from the O1 antigen. O139 contains a rare sugar, colitose, which is polymerized in long chains to form a capsule. Several lines of evidence suggest that O139 strains are generally closely related genetically to El Tor O1 strains. However, O139 lacks many of the genes required to make the O1 antigen. In the place of the O1 genes is a 36 000 bp insert predicted to encode genes involved in the biosynthesis of sugars and synthesis of polysaccharides. The G+C content of this region varies among the different open reading frames, but some are very dissimilar from the rest of the V. cholerae chromosome, suggesting that the insert may be derived from an organism other than Vibrio . Once again, we do not know how the transfer of this O139-specific DNA occurred or, indeed, how even the O1 antigen was first acquired. It seems

likely that the current epidemic O139 strains arose from an endemic O1 strain after acquisition of the new O139 DNA by homologous recombination, leading to replacement of O1 [13,20].

Virulence Factors

Studies have taught that one typically needs to ingest a large number of organisms at least a million viable cells to reproducibly cause disease. This may not be the case in natural settings where its association with organic material or aquatic organisms, like algae or plankton, may protect it from the acidic barrier that represents the most important defense mechanism against V. cholerae and other enteric pathogens. When the organism does make it to the small intestine in viable form, it then uses a variety of properties to efficiently colonize this site. For example, motility and chemotaxis direct the swimming of the organism toward the intestinal epithelium, where it then adheres using filamentous structures called 'pili' or 'fimbria'. It then multiplies and elaborates various toxins [22,20]

Cholera Toxin

The most important of the virulence factor is, of course, cholera toxin, a protein exotoxin that is responsible for most of the diarrhea seen in the disease. Cholera toxin causes secretion of chloride ion into the lumen of the intestine while also inhibiting sodium uptake. The result is an osmotic, watery diarrhea that takes on the appearance of rice-water — cloudy liquid with flecks of mucus. ingestion of only a few micrograms of cholera toxin is enough to cause 22 liters of diarrhea. Certainly all this diarrhea could aid in the dissemination of the organism. Typical diarrheal fluid from a cholera victim contains one hundred million organisms per milliliter [20].

Cholera toxin and the pilus also increase the fitness of V. cholerae within the human host. You can show this by specific mutations in these genes. Mutations inactivating cholera toxin decrease the extent of colonization, as measured, for example, by the number of bacteria shed per gram of stool in the volunteer. Mutations affecting the production of a special pilus called toxin co-regulated pilus (TCP) completely wipe out the ability of the organisms to colonize the intestine. Thus, the presence of these factors enhances the short-term replication of the organism in the human gut, and this enhanced replication must also contribute to the evolutionary fitness of strains carrying the genes for these virulence factors [13].

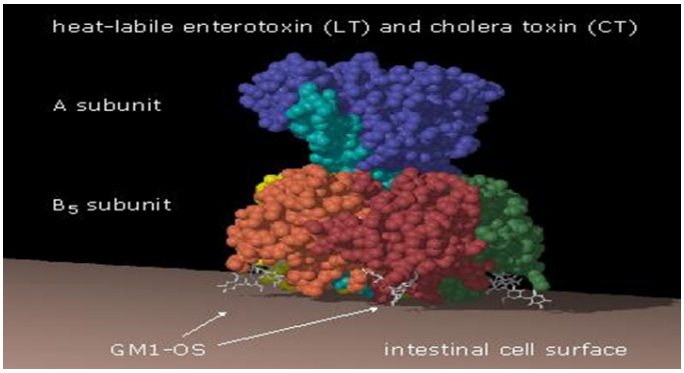

Figure 1: cholera toxin structure

CT produced by V. cholerae, is the main virulence factor in the development of cholera. The molecular characteristics of CT and its toxic effects in humans have been well characterized. CT is an 84 kD protein made up of two major subunits, CTA and CTB . The CTA subunit is responsible for the disease phenotype while CTB provides a vehicle to deliver CTA to target cells. CTA is a 28 kD subunit consisting of two primary domains, CTA1 and CTA2, with the toxin activity residing in the former and the latter acting as an anchor into the CTB subunit. The CTB subunit consists of a homopentameric structure that is approximately 55 kD (11.6 kD monomers) and binds to the GM1-ganglioside; found in lipid rafts, on the surface of intestinal epithelial cells [13].

The exact mechanism of delivering CTA1 into the intracellular space is still not fully resolved; however, the current understanding is that CT is endocytosed and travels through a retrograde transport pathway from the Golgi apparatus to the endoplasmic reticulum (ER). Recently, it has been shown that CT can also move from the apical to basolateral surface of epithelial cells via transcytosis, enabling transport of whole CT through the intestinal barrier. CTA is dissociated from CTB after the toxin reaches the ER and translocated to the cytosol via the ER-associated degradation pathway . Intoxication occurs when CTA1 enters the cell cytosol and catalyzes the ADP ribosylation of adenylate cyclase, which leads to increased intracellular cAMP. This increase in intracellular cAMP results in impaired sodium uptake and increased chloride outflow, causing water secretion and diarrhea [21].

Cholera toxin activates the adenylate cyclase enzyme in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H20, Na+, K+, Cl-, and HCO3- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin. The toxin has been characterized and contains 5 binding (B) subunits of 11,500 daltons, an active (A1) subunit of 23,500 daltons, and a bridging piece (A2) of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells. Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP. The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated. This situation can be illustrated Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl- into the intestinal contents. H2O, Na+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl-.

The lost H2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera [5,22].

Colonization Factors

A broad definition of V. cholerae colonization factors includes all bacterial gene products that specifically facilitate either the survival or the multiplication of the bacterium in the small intestine. This definition includes many bacterial factors and properties which include, but are not restricted to, those which mediate the attachment of vibrios to the small intestinal epithelium. By far the most important colonization factor of V. cholerae is TCP. This pilus was discovered by our lab as a structure that was coordinately regulated with cholera toxin. That is, under laboratory conditions when strains of V. cholerae express large amounts of cholera toxin, they also express large amounts of TCP [22,23].

Some years ago, laboratory studied the molecular basis for the coordinate regulation of cholera toxin and TCP. Curiously, the vast majority of V. cholerae strains will not make either cholera toxin or TCP under laboratory conditions. However, all clinical isolates make these virulence factors in the intestine. Thus, the expression of virulence by V. cholerae is a regulated property. We eventually cloned many of the genes involved in this regulation, but one of the most important is a gene called toxR. The expression of both cholera toxin and TCP is totally dependent on the toxR regulatory gene. ToxR, the gene product, is a membrane protein that perhaps senses environmental signals in the intestine and then activates the expression of cholera toxin and TCP genes [17,18].

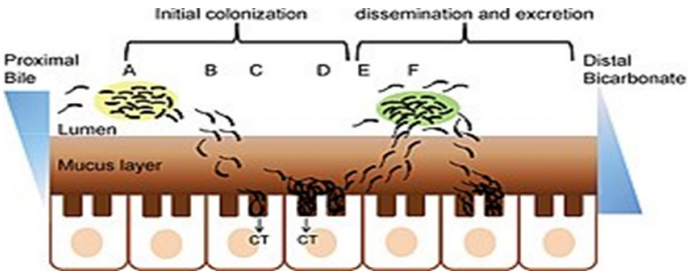

Figure 2: The role of biofilm in the intestinal colonization of Vibrio cholera

V. cholerae mutant strains which do not elaborate TCP are more than 1000-fold reduced in their capacity to colonize the infant mouse and essentially do not colonize the intestine of human volunteers at all . The mechanism by which TCP mediates colonization of the small intestine by V. cholerae has not yet been elucidated. Although it seems likely that some part of the pilus functions as an adhesin for enterocytes, to date no such binding has been clearly established. TCP are known to aggregate into 'bundles' and the expression of TCP causes auto-agglutination of V. cholerae cultures. This clumping behavior might reflect a similar role for TCP in the establishment of microcolonies on the intestinal epithelium [22,14].

There are several characteristics of pathogenic V. cholerae that are important determinants of the colonization process. These include adhesins, neuraminidase, motility, chemotaxis and toxin production. If the bacteria are able to survive the gastric secretions and low pH of the stomach, they are well adapted to survival in the small intestine. V. cholerae is resistant to bile salts and can penetrate the mucus layer of the small intestine, possibly aided by secretion of neuraminidase and proteases (mucinases). They withstand propulsive gut motility by their own swimming ability and chemotaxis directed against the gut mucosa. Specific adherence of V. cholerae to the intestinal mucosa is probably mediated by long filamentous fimbriae that form bundles at the poles of the cells. These fimbriae have been termed Tcp pili (for toxin coregulated pili), because expression of these pili genes is coregulated with expression of the cholera toxin genes. Not much is known about the interaction of Tcp pili with host cells, and the host cell receptor for these fimbriae has not been identified. Tcp pili share amino acid sequence similarity with N-methylphenylalanine pili of Pseudomonas and Neisseria.Two other possible adhesins in V. cholerae are a surface protein that agglutinates red blood cells (hemagglutinin) and a group of outer membrane proteins which are products of the acf (accessory colonization factor) genes. acf mutants have been shown to have reduced ability to colonize the intestinal tract. It has been suggested that V. cholerae might use these nonfimbrial adhesins to mediate a tighter binding to host cells than is attainable with fimbriae alone.V. cholerae produces a protease originally called mucinase that degrades different types of protein including fibronectin, lactoferrin and cholera toxin itself. Its role in virulence is not known but it probably is not involved in colonization since mutations in the mucinase gene (designated hap for hemagglutinin protease) do not exhibit reduced virulence. It has been suggested that the mucinase might contribute to detachment rather than attachment. Possibly the vibrios would need to detach from cells that are being sloughed off of the mucosa in order to reattach to newly formed mucosal cells [14,24].

4.1.3 TRANSMISSION OF VIRULENCE GENES

All epidemic strains of V. cholerae produce cholera toxin, consisting of five copies of the B subunit bound to a single copy of the A subunit, encoded by the genes ctxA and ctxB [23]. As the ctxAB genes were used in hybridization analysis of different V. cholerae strains, we and other investigators in the field started making curious observations. Not all strains of V. cholerae carry the cholera toxin genes. In fact only epidemic strains of V. cholerae had toxin genes, while environmental strains generally did not. Another characteristic of most, but not all, environmental strains of V. cholerae was the fact that they lacked the traditional antigenic surface coat of epidemic strains, the O1 antigen or serogroup. Thus, one could group V. cholerae strains into various subgroups, but only the O1 toxigenic strains were associated with epidemics before 1993. This suggested that there must exist a mechanism for these strains either to acquire the cholera toxin genes or to lose them. This observation prompted us to do a detailed analysis of the genetic structure of the DNA in the vicinity of the ctxAB genes [13,21].

Eventually we found that the ctxAB genes lie within a region on the V. cholerae chromosome which is referred to as the CTX element [19]. This segment of DNA was often duplicated in toxi-

genic strains and was about 7000 bp in size. Eventually several other labs, notably Jim Kaper's in Maryland, became interested in the CTX element and reported that two genes adjacent to ctx-AB appeared to encode proteins with enterotoxic activity. These were called ace and zot. Additionally, the CTX element encodes a peptide, the product of the cep gene which resembles the gene product of another pilus produced by the bacterium Aeromonas. Another gene, orfU, was also identified that encodes an unknown function. This group of genes is contained within a part of the CTX element known as 'core', because it is usually flanked on both sides by a different sequence called 'RS', for repetitive sequence. The RS element was known to encode a site-specific recombination system that catalyzes the integration of plasmids carrying this element into the V. cholerae genome [19]. As a whole, the ctx element was thought to be a site-specific transposon, but we did not have a clue how this transposon-like element moved into V. cholerae and where it came from originally. However, its chemical characteristics suggested it had an origin other than V. cholerae [10,12].

Antimicrobial Resistance

The upward trend of antibiotics resistance by microbial pathogens portends to weaken the idealistichope of public health gains made since the widespread use of antibiotics was adopted. The emergence of antibiotics resistance among various species of Vibrio pathogens is a well-established phenomenon and with the ongoing challenges of producing potent and effective new antibiotics, the management of communicable diseases has become a dire need in less industrialized countries where poor sanitation and malnutrition are prevalent. The indiscriminate use of antibiotics and chemotherapeutic agents as feed additives or immersion baths to establish preventive measures in farming and aquaculture environments has also been implicated in the emergence of multidrug resistance in aquatic microorganisms such as the Vibrio species [9,25].

Resistance to tetracycline and other antimicrobial agents among V. cholerae has been demonstrated in both endemic and epidemic cholera settings. Resistance can be acquired through the accumulation of selected mutations over time, or the acquisition of genetic elements such as plasmids, introns, or conjugative elements, which confer rapid spread of resistance. A likely risk factor for antimicrobial resistance is widespread use of antibiotics, including mass distribution for prophylaxis in asymptomatic individuals. Antibiotic resistance emerged in previous epidemics in the context of antibiotic prophylaxis for household contacts of cholera patients [3].

Analysis of O139 strains isolated during the last 9 years revealed interesting patterns of antibiotic resistance to various common antibiotics. Although the strains remained largely susceptible to ciprofloxacin, tetracycline, and gentamicin, resistance to ampicillin and susceptibility to cotrimoxazole (sulfamethoxazole, trimethoprim), chloramphenicol, and streptomycin varied during this period. The O139 serogroup of V. cholerae that emerged during 1992 and 1993 was sensitive to tetracycline and resistant to trimethoprim-sulfamethoxazole (SXT) and streptomycin. Waldor et al. reported the presence of a 62-kb self-transmissible transposon-like element (SXT element) encoding resistance to sulfamethoxazole, trimethoprim, and streptomycin in V. cholerae O139 strains isolated from this epidemic. The SXT element could be conjugally transferred from V. cholerae O139 to V. cholerae O1 and E. coli strains, where it integrated into recipient chromosomes in a site-specific recA-independent manner. Strains isolated from an O139 outbreak in Bangladesh in 1997 were found to be mostly sensitive to SXT and streptomycin . In keeping with the observation in Bangladesh, comparison of the antibiotic resistance patterns between the O139 strains isolated during 1992 and 1993 and those isolated in 1996 and 1997 in India also showed that the later strains were susceptible to SXT [9]. Analysis of genetic changes associated with the observed SXT sensitivity showed that sensitivity to SXT and streptomycin was associated with a deletion of an ≈3.6-kb region of the SXT element in strains that were sensitive to SXT and streptomycin [10]. Since 1997, the O139 strains isolated in India also showed an increased trend of resistance to ampicillin and neomycin and susceptibility to chloramphenicol and streptomycin [13,15].

This pattern of rapid shift in antimicrobial resistance is consistent with previous reports indicating substantial mobility of genetic elements, which confers resistance to antimicrobials, a phenomenon which has also been observed in V. cholerae O1 strains. A multiple antibiotic-resistance plasmid belonging to incompatibility group C has also been associated with drug resistance of V. cholerae O139 [7,13].

The O139 serogroup ofV. cholerae which emerged during 1992 to 1993 was sensitive to tetracycline . Although the new serogroup showed a trend of increased resistance to trimethoprim-sulfamethoxazole, it was more susceptible to ampicillin and tetracycline than the O1 serogroup). Waldor et al. reported the presence of a 62-kb self-transmissible transposon-like element (SXT element) encoding resistance to sulfamethoxazole, trimethoprim, and streptomycin in V. cholerae O139. The SXT element could be conjugally transferred from V. choleraeO139 to V. cholerae O1 and E. coli strains, where it integrated into recipient chromosomes in a site-specificrecA-independent manner [13,9].

Comparison of the antibiotic resistance patterns in the O139 strains isolated during 1992 and 1993 and those isolated in 1996 and 1997 in India showed that the latter strains were susceptible to co-trimoxazole, unlike the former. Recent studies have shown that O139 strains are becoming increasingly resistant to ampicillin and neomycin but increasingly susceptible to chloramphenicol and streptomycin). Considering the rapidly changing pattern of antibiotic resistance observed among V. cholerae strains, it appears that there is substantial mobility in genetic elements encoding antibiotic resistance in V.cholera [13,21].

The presence of this bacterium in the aquatic environment increases human fright on food safety owing to the latter possibly causing disease epidemics depending on the environmental conditions. The advent of antibiotics resistance is a challenging process repeatedly linking human, environmental, and pathogen-related features. In general, the antibiotic routine in humans and animals conveys an intrinsic threat of opting for antimicrobial resistance genes (ARGs). The predominance of resistance genes

in the environment is the outcome of an intricate combination of dynamics, which reveals an active balance of fitness costs and aids: costs of transporting the ARGs in the framework of the host genome and environment, relative to the sternness and recurrence of risk, pertinent to some physical environmental features, such as temperature and microbial ecology among others [12,16].

Several antibiotic resistance mechanisms in bacteria are usually enabled by exporting drugs through efflux pumps, chromosomal mutations, or developing genetic resistance via the exchange of conjugative plasmids, conjugative transposons, integrons, or self-transmissible chromosomally integrating SXT elements . Vibrio species are known to employ multi-drug efflux pumps to establish resistance against antimicrobial agents and other toxic compounds by a mechanism that prevents the accumulation of drugs inside the bacterial cells. V. cholerae has shown its ability in using multidrug efflux pumps to export a wide range of antibiotics, detergents, and dyes that are chemically and structurally unrelated [26]. Collectively, multi-drug efflux pumps are not employed only for drug resistance, but have also been implicated in the expression of important virulence genes in Vibrio pathogens. The spread of antibiotic-resistant pathogens in V. cholerae is known to be facilitated by horizontal gene transfer through self-transmissible mobile genetic elements, including SXT elements—mobile DNA elements belonging to the class of integrative conjugating elements (ICEs). The SXT genetic mobile element ICE conferring resistance to sulfamethoxazole-trimethoprim was first documented in V. cholerae O139 or a closely related ICE in Madras, India, owing to its ability to harbor resistance to trimethoprim, sulfamethoxazole, and streptomycin. The relationship between self-transmissible elements and multidrug resistance has been well documented in Vibrio species [26].

A recent study in Cameroun revealed that Vibrio cholerae O1 of environmental origin harbours heterogeneous multidrug resistance towards Amoxicillin (AML), Ampicillin (AMP), Tetracycline (TE), Chloramphenicol (C), Doxycycline (DXT), and Cotrimoxazole (SXT). The frequent usage of antibiotics as part of the Vibrio infection treatment regimen has resulted in the development of multidrug resistance in V. cholerae and seafood pathogens such as pathogenic Vibrio species [25].

As an environmental organism, V. cholerae has the means to acquire resistance genes fromintimate contact with indigenous resistant environmental bacteria through mobilizable genetic elements. The persistent discharge of antibiotics into WWTPs is associated with the release of resistance genes. These resistance genes in wastewater primarily originate from the gastrointestinal tracts of humans. However, most of the genetic determinants that confer resistance to antibiotics are located on plasmids. Acquired antibiotic resistance in bacteria is generally mediated by extrachromosomal plasmids and is transferable to other bacteria within the environment [14].

The co-location of antibiotics and ARGs in WWTPs can select for novel combinations of AMR that can be shared between microorganisms by horizontal gene transfer (HGT) on mobile genetic elements (MGEs), such as plasmids, thereby increasing the prevalence and combination of multiple drug resistance in the microbial community. Plasmid-mediated multidrug resistance is one of the most pressing problems in the treatment of infectious diseases. In the last decade, the emergence of antibiotic resistant genes in Vibrio species has been on the increase compared to previous years, and these genes include penicillin resistant genes penA, blaTEM-1 and Beta-lactam, chloramphenicol resistant genes , and tetracycline resistant genes. There has been little or no regulation of the choice of antibiotics administered to animals, with overlaps in the classes of antibiotics used for farming and human therapy in most of the sub-Saharan countries. The risk of multiple drug resistance found in environmental microorganisms being transferred to other pathogens is of significant public health concern that calls for concerted efforts in tackling the threat posed to disease control and management. These animals, animal products, farm workers, and the farming environment itself are potential reservoirs for resistance determinants. Antimicrobial resistance has been detected in farms; however, the extent of resistance and spill over in the country remains largely unknown. Hence, the transmission of resistance between animal feed and humans is important and requires investigation, as this has been linked to increasing clinical resistance in human medicine [6,21].

Mechanism by Vibro Cholera Disrupt Cell Function

When consumed, most bacteria do not survive the acidic conditions of the human stomach. The few surviving bacteria conserve their energy and stored nutrients during the passage through the stomach by shutting down protein production. When the surviving bacteria exit the stomach and reach the small intestine, they must propel themselves through the thick mucus that lines the small intestine to reach the intestinal walls where they can attach and thrive [24].

Once the cholera bacteria reach the intestinal wall, they no longer need the flagella to move. The bacteria stop producing the protein flagellin to conserve energy and nutrients by changing the mix of proteins which they express in response to the changed chemical surroundings. On reaching the intestinal wall, V. cholerae start producing the toxic proteins that give the infected person a watery diarrhea. This carries the multiplying new generations of V. cholerae bacteria out into the drinking water of the next host if proper sanitation measures are not in place [13,18].

The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A), and five copies of the B subunit (part B), connected by a disulfide bond. The five B subunits form a five-membered ring that binds to GM1 gangliosides on the surface of the intestinal epithelium cells. The A1 portion of the A subunit is an enzyme that ADP-ribosylates G proteins, while the A2 chain fits into the central pore of the B subunit ring. Upon binding, the complex is taken into the cell via receptor-mediated endocytosis. Once inside the cell, the disulfide bond is reduced, and the A1 subunit is freed to bind with a human partner protein called ADP-ribosylation factor 6 (Arf6).Binding exposes its active site, allowing it to permanently ribosylate the Gs alpha subunit of the heterotrimeric G protein. This results in constitutive cAMP produc-

tion, which in turn leads to the secretion of water, sodium, potassium, and bicarbonate into the lumen of the small intestine and rapid dehydration. The gene encoding the cholera toxin was introduced into V. cholerae by horizontal gene transfer. Virulent strains of V. cholerae carry a variant of a temperate bacteriophage called CTXφ [21,9].

Microbiologists have studied the genetic mechanisms by which the V. cholerae bacteria turn off the production of some proteins and turn on the production of other proteins as they respond to the series of chemical environments they encounter, passing through the stomach, through the mucous layer of the small intestine, and on to the intestinal wall.[Of particular interest have been the genetic mechanisms by which cholera bacteria turn on the protein production of the toxins that interact with host cell mechanisms to pump chloride ions into the small intestine, creating an ionic pressure which prevents sodium ions from entering the cell. The chloride and sodium ions create a salt-water environment in the small intestines, which through osmosis can pull up to six liters of water per day through the intestinal cells, creating the massive amounts of diarrhea. The host can become rapidly dehydrated unless an appropriate mixture of dilute salt water and sugar is taken to replace the blood's water and salts lost in the diarrhea [13,21].

By inserting separate, successive sections of V. cholerae DNA into the DNA of other bacteria, such as E. coli that would not naturally produce the protein toxins, researchers have investigated the mechanisms by which V. cholerae responds to the changing chemical environments of the stomach, mucous layers, and intestinal wall. Researchers have discovered a complex cascade of regulatory proteins controls expression of V. cholerae virulence determinants.[medical citation needed] In responding to the chemical environment at the intestinal wall, the V. cholerae bacteria produce the TcpP/TcpH proteins, which, together with the ToxR/ToxS proteins, activate the expression of the ToxT regulatory protein. ToxT then directly activates expression of virulence genes that produce the toxins, causing diarrhea in the infected person and allowing the bacteria to colonize the intestine [13,21].

Diagnosis

The laboratory diagnosis of cholera is made by isolating V. cholerae O139 in a proper medium. The TCBS medium is one the most commonly used enrichment medium is advised for handling environmental samples. Characteristic tiny yellow colonies are observed on the surface of the TCBS medium after within 48 hours of incubation. Specific tests for detecting the serotype and biotype of O1 V. cholerae are recommended. Detection of V. cholerae in a single tube reaction by using a quadruplex PCR that identifies simultaneously the serotype, biotype, toxigenic potential and virulence genes may allow early diagnosis). More sensitive methods than available PCR tests have been designed more recently. One of them, loop-mediated isothermal amplification detects cholera toxin producing V. cholerae in 35 minutes using culture samples or 70 minutes using direct stool samples from patients . Rapid diagnostic tests that detect the lipopolysaccharide of both O1 and O139 by the use of monoclonal antibodies have less than optimal sensitivity and specificity, but may be useful in the setting of outbreaks [6,13].

A rapid dipstick test is available to determine the presence of V. cholerae. In those samples that test positive, further testing should be done to determine antibiotic resistance. In epidemic situations, a clinical diagnosis may be made by taking a patient history and doing a brief examination. Treatment is usually started without or before confirmation by laboratory analysis [6].

Stool and swab samples collected in the acute stage of the disease, before antibiotics have been administered, are the most useful specimens for laboratory diagnosis. If an epidemic of cholera is suspected, the most common causative agent is V. cholerae O1. If V. cholerae serogroup O1 is not isolated, the laboratory should test for V. cholerae O139. However, if neither of these organisms is isolated, it is necessary to send stool specimens to a reference laboratory.Infection with V. cholerae O139 should be reported and handled in the same manner as that caused by V. cholerae O1. The associated diarrheal illness should be referred to as cholera and must be reported in the United States [21].

Cholera in Bioterrorism

Cholera as bioterrorism is terrorism by intentional releases or dissemination of cholera biological agents. Cholera as biological weapon is attractive from terrorists point of view because of low production costs, major range and easiness of transmission Cholera is classified as a category B bioterror disease. This high priority agent is considered to have bioterrorism potential because they.at the beginning of the 20th century a new form of bioterrorism occurred, which put humanity in the face a terrifying threat. Cholera is a deadly diseases that caused a worldwide phenomenon through history. Its imperative weapon, the vibrio cholerae bacterium, has allowed cholera to seize control and wipe out a huge percentage of human population. V.cholara's toxins are the primary cause of cholera lethal symptoms. The cholerae that helps it accomplish its job of invading the human system and defeating the body's powerful immune system [26].

Cholera as bioweapon agents classified as category B or second highest priority agents and they are:
- are moderately easy to disseminate;
- result in moderate morbidity rates and low mortality rates; and
- require specific enhancements of diagnostic capacity and enhanced disease surveillance.

During World War I, allegations were made that the Germans attempted to spread cholera in Italy. In 1936, Japan created Unit 731, a bio-warfare unit masquerading as a water-purification facility. In 1936, Japan created Unit 731, a bio-warfare unit masquerading as a water-purification facility. This 150-building complex near Harbin, Manchuria eventually orchestrated the deaths of over 9,000 victims.Unit 100, Another biological warfare site was also developed near Changchun for experimentation on Chinese civilians and soldiers.Tens of thousands died as a result of plague, cholera, anthrax and other diseases [25,8].

Treatment

Cholera is an easily treatable disease. The majority of people can be treated successfully through prompt administration of oral rehydration solution (ORS). The WHO/UNICEF ORS standard

sachet is dissolved in 1 litre (L) of clean water. Adult patients may require up to 6 L of ORS to treat moderate dehydration on the first day [16,19].

The treatment of the cholera disease condition is centered on the physiological ideology of replacing water and electrolytes and maintaining the intravascular volume. The main goal is to replenish potassium and bicarbonate, which were discharged along with choleric stool.

Severely dehydrated patients are at risk of shock and require the rapid administration of intravenous fluids. These patients are also given appropriate antibiotics to diminish the duration of diarrhoea, reduce the volume of rehydration fluids needed, and shorten the amount and duration of V. cholerae excretion in their stool [3,21]

For severely ill patients, the Centre for Disease and Control (CDC) recommends the use of antibiotics along with fluid replacement. Antimicrobial agents are useful in aiding the rehydration treatment of cholera, because their use reduces the duration of diarrhoea (which in turn reduces the spread of the disease), and treats acute illnesses (by reducing the volume of diarrhoea). CDC recommends that the class of antibiotics used for treating any infection should be based on indigenous antibiotic susceptibility patterns. The first line of treatment for adults in most countries as recommended is doxycycline; however, azithromycin is recommended as the primary treatment for pregnant women and children. For the period of an epidemic, an antibiogram must be observed by carrying out regular tests on all sample isolates from different geographic region [22,23].

Mass administration of antibiotics is not recommended, as it has no proven effect on the spread of cholera and contributes to increasing antimicrobial resistance. Rapid access to treatment is essential during a cholera outbreak. Oral rehydration should be available in communities, in addition to larger treatment centers that can provide intravenous fluids and 24 hour care. With early and proper treatment, the case fatality rate should remain below 1%. Zinc is an important adjunctive therapy for children under 5, which also reduces the duration of diarrhoea and may prevent future episodes of other causes on acute watery diarrhoea. Breastfeeding should also be promoted [16].

Prevention and Control

Since the infection primarily occurs via faeco-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits. Contaminated food and water are the main vehicles of transmission of V cholera and much can be done to keep transmission rates to a minimum. The measures include ensuring a safe water supply, (especially for municipal water systems), improving sanitation, making food safe for consumption by thorough cooking of high-risk foods (especially seafood), and health education through mass media. Some important messages for the media during outbreaks include the importance of purifying water and seafood, washing hands after defecation and before food preparation, recognition of the signs of cholera, and locations where treatment can be obtained to avoid delays in case of illness [4,7].

Figure 3: Dumping of sewage or fecal sludge from a UN camp into a lake in the surroundings of Port-au-Prince is thought to have contributed to the spread of cholera after the Haiti earthquake in 2010, killing thousands

The long-term prevention of cholera will require improved water and sanitation facilities, but these improvements are not happening rapidly in most regions where cholera is prevalent. A killed injectable vaccine was developed shortly after V cholerae was discovered in the 1880s, and it was widely used throughout the world. Vaccination was even a requirement for international travellers in the mistaken belief that it might prevent international spread of cholera. This vaccine was probably appropriate for those who could afford it during the early part of the 20th century when treatment was ineffective and sanitation standards were low. However, it was not cost-effective as a publichealth intervention because protection was short lived (6 months), it was associated with painful local inflammatory reactions, and it did not prevent the spread of disease.138 Vaccination was not practicable and was too expensive for people might benefit from it. Those who could afford it no longer needed it, and they did not like the side-effects. Thus, the whole-cell injectable vaccine is no longer recommended for any purpose, though it is still licensed. Dukoral was effective in field trials in less developed countries and it is now recommended for use in refugee settings at risk of cholera.Its cost-effectiveness in endemic areas is still not known. Orochol is highly protective in volunteer studies though its use in endemic areas is uncertain. Other live and killed oral vaccines are also being developed that may become useful in the future.A major problem in the development of these new oral vaccines will be to make them sufficiently inexpensive and to develop a formulation that can be readily distributed to huge populations at risk. Booster doses will probably be needed for each of the new oral vaccines, and the formulations will need to be sufficiently simple that the vaccine might even be self-administered at times of risk. The new oral vaccines will not prevent all cases of cholera because local intestinal immunity can be overcome with a high inoculum, but they should lower the risk by as much as 80% if used regularly. Also, a vaccine programme could work synergistically with sanitation programmes; the inoculum needed to cause disease would be raised and the numbers of pathogenic organism sentering the environment would be decreased. Thus vaccines and sanitation programmes should not be viewed as alternative preventive strategies but as complementary, perhaps even synergistic, ones [22,15].

The oral vaccines are made from a live attenuated strains of V. cholerae.The ideal properties of such a "vaccine strain" of the bacterium would be to possess all the pathogenicity factors required for colonization of the small intestine (e.g. motility, fimbriae, neuraminidase, etc.) but not to produce a complete toxin molecule. Ideally it should produce only the B subunit of the toxin which would stimulate formation of antibodies that could neutralize the binding of the native toxin molecule to epithelial cells. A new vaccine has been developed to combat the Vibrio cholerae Bengal strain that has started spreading in epidemic fashion in the Indian subcontinent and Southeast Asia. The Bengal strain differs from previously isolated epidemic strains in that it is serogroup 0139 rather than 01, and it expresses a distinct polysaccharide capsule. Since previous exposure to 01 Vibrio cholerae does not provide protective immunity against 0139, there is no residual immunity in the indigenous population to the Bengal form of cholera. The noncellular vaccine is relatively nontoxic and contains little or no LPS and other impurities. The vaccine will be used for active immunization against Vibrio cholerae O139 and other bacterial species expressing similar surface polysaccharides. In addition, human or other antibodies induced by this vaccine could be used to identify Vibrio cholerae Bengal for the diagnosis of the infection and for environmental monitoring of the bacterium [13].

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