Plant Archives Volume 20 No. 1, 2020 pp. 2737-2741 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

VIBRIO CHOLERAE: A REVIEW ON THE GENETICS OF PATHOGENESIS AND EPIDEMIOLOGY

Hadaf Mahdi Kadhim

Department of Biology, College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.

Abstract

Vibrio cholerae plays a global role in health of human, it causes cholera that is a severe diarrheal illness. The developing parts of the world and application of suitable water treatment measures leads to control and restricted this disease. *V. cholerae* can be transmitted and adhered to normal flora by raw shellfish that considered as a vehicle. Cholera can be diagnosed by the presence of the comma shaped of *V. cholerae* in the stools of patients who are suffering from severe diarrhoea. There is a relationship between the environment and *V. cholerae*. However, non-toxigenic strains of this bacterium have been more commonly isolated from the environment than toxigenic O139 and O1 strains. This review focused on the virulence factors, pathogenesis and epidemiologic patterns of *Vibrio cholerae*.

Key words: *Vibrio cholerae*, virulence factors, pathogenicity, epidemiology.

Introduction

In human history, Vibrios have a role in a global outbreak for example *V. cholerae* is a human pathogen that causes cholera. It is gram negative, polarly flagellated rods. It has been demonstrated that the climate is associated with outbreak of disease. Interestingly, zooplankton in systems of riverine and estuarine has an important role in the cholera cycle, likewise a vector in tularemia and malaria (Huq *et al*., 1983). Furthermore, adhesive ability in the human gut and in the environment is a significant feature of this bacterium that secrete a powerful chitinase to help its growth on mucinase and chitin surface in order to penetrate mucus barriers on plankton and that covers the epithelial of gastrointestinal tract (Islam, *et al*., 1999; Zhu and Mekalanose, 2003).

Environmental factors and bacterial prosperities were concluded to make *V. cholerae* highly adhesive in the gastro intestine; for example, serum of mussel hemolymph has a role to assist attachment in the gastrointestinal epithelium when this bacterium is ingested with seafood (Pruzzo *et al*., 2003). In Bangladesh, cholera outbreaks related to environmental variables including water temperature, salinity, rainfall and water depth (Alam *et al*., 2006; Hug *et al*., 2005). In 1999, cholera outbreak spreaded in Southeast Asia, this outbreak caused by serotype O139 (Ramamurthy *et al*., 1993).

***Author for correspondence :** E-mail: hadaf.mahdi@qu.edu.iq

Isolation and Bacteriological culture

V. cholerae is easy to isolate. It requires about 2.0-2.5% (w/v) concentrations of NaCl. It also requires bile salts to inhibit the gram positive growth. Both biochemical and molecular identification of *V. cholerae* are required. Alkaline peptone water medium is used for enrichment of *V. cholerae*. A non-selective medium is marine agar which is used to grow all Vibrios. Thiosulfate citrate bile salt sucrose agar (TCBS) is used to isolate *V. cholerae* from different sources. The best selective medium to isolate this bacterium was TCBS, however, some strains of *Aeromonas*, *Staphylococcus*, *Shewanella*, *Flavobecterium*, *Streptococcus* and *Pseudoalteromonas* may be slight growth present on this medium but these colonies looked extremely small and poorly developed (Nicholls *et al*., 1976). Furthermore, Taurocholate tellurite gelatin agar is used as a selective media for isolation and purification of *V. cholera* (O'Brien and Colwell, 1985). This bacterium can be isolated from clinical samples by plate stool specimens or rectal swabs directly on to TCBS agar. Moreover, this organism is an important species to monitor in food products and it may be fatal (Donovan and van Netten, 1995).

Virulence factors

• Cholera toxin (CT):

CT is considered as an important virulence factor that responsible for cholera symptoms. It is an ADP-ribosylating, this toxin composes from two subunits, A and B, subunit B forms binds to GM_1 gangliosides, thus tethers subunit A to the surface of cell (Gill and King, 1975; Gill, 1976; Madigan *et al*., 2003). The toxin is correlated with sphingo lipid and cholesterol in the membrane receptor on the cell surface (London and Brown, 2000). The lysogenic filamentous bacteriophage encodes *ctxA* and *ctxB* genes encoding CT (Waldor and Mekalanos, 1996). However, Boyd, *et al*., (2000) have demonstrated that phage can be infected *V. cholerae* nontoxigenic strains and converted to toxigenic strains. Moreover, *V. cholerae* produces other toxins including haemolysin (HlyA) and HA protease (HAP) that cause the inflammatory reaction during disease (Fullner *et al*., 2002).

• Toxin Coregulated Pilus (TCP)

Taylor *et al*., (1987) have reported that when *V. cholerae* colonizes the intestinal tract, it expressed a type IV bundle-forming pilus. The *tcp* operon encodes for pilin biosynthesis and it is encoding a regulatory protein ToxT and the pilus subunit TcpA which is responsible for *V. cholerae* pathogenicity island (VPI) (Karaolis *et al*., 1998; Heidelberg *et al*., 2000). This pilus has a crucial role for colonization of humans that is required for cholera symptoms. The pilus is formed a homopolymer of repeating TcpA subunit (Taylor *et al*., 1987; Taylor *et al*., 1988).

Lipopolysaccharide

It has been found to serve as a barrier for protecting the organisms from external stresses (Nikaido, 1988). On the basis of *V. cholerae* specific LPS O side chain can be classified strains of this bacterium. The serogroups O1 and O139 of *V. cholerae* are responsible for large scale epidemics. Strains of O1 are genetically very similar to strains of O139 (Dziejman *et al*., 2002). Knirel *et al*., (1997) have studied that the O139 antigen is a polysaccharide that contained from different carbohydrates. However, Kenne *et al*., (1982) have demonstrated that the O1 antigen is a homopolymer that contained from dideoxy-phoshpomannose substituted with tetronate.

Other virulence factors

V. cholerae has a single polar flagellum causing motility of this bacterium that identified as a virulence factor.

Chemotaxis is also considered as a virulence factor. It has a role not only to penetrate the mucous lining but also to colonize the intestinal epithelial cells (Freter *et al*., 1981). Microarray analysis of this bacterium in human patients stool established a repression of chemotaxis related genes that related to a hyper infectious for colonization of intestine (Merrell *et al*., 2002). Lee *et al*., (2001) have shown that chemotaxis has a role for the induction of CT.

Furthermore, *V. cholerae* produced RTX toxin, the gene cluster of RTX is formed from 4 genes including *rtxA, B, C* and *D* that encodes the toxin, secretory protein and the toxin activator, respectively (Lin *et al*., 1999).

Furthermore, the serogroups O1 and O139 *V.cholerae* have *hapA* gene that encodes hemagglutinin/ protease. It contains a mucinase activity and can perturb the barrier function of cell lines epithelium in vitro (Hase and Finkelstein, 1991).

HlyA hemolysin that encoded by *hlyA* gene. HlyA has a role in pathogenesis of *V.cholerae* but it remains unclear. However, Fullner *et al*., (2002) have studied that it can be contributed to the reactogenicity related to *ctx*- strains.

Moreover, different virulence-associated proteins have secreted by type II secretion system (EPS) in *V. cholerae* and required fourteen genes (*epsA-N*) and *vcpD*. These genes are coded the proteins for forming a secretion apparatus and secreting cholera toxin, chitinase, lipase, HA/protein and neuraminidase (Sandkvist *et al*., 1997; Connell *et al*., 1998; Marsh and Taylor, 1998). The crystal structures and interactions of the different components of this system have been suggested by Robien *et al*., (2003) and Abendroth *et al*., (2004). Furthermore, the *toxR* gene is coded a transmembrane protein. This protein controls production of cholera toxin and other virulence factors including outer membrane proteins and pili that required for colonization of this bacterium in the small intestine (Madigan *et al*., 2003). The expression of CT and TCP that considered as a major virulence factors that stimulates by ToxT and binds to the promoter regions that encoded CT and TCP and activated transcription (Carroll *et al*., 1997). The transcription of *toxT* initially occurs precisely during the intestine or under inducing conditions of defined laboratory and controlling by the regulatory cascade (Murley *et al*., 2000). Interestingly, the motility and chemotaxis of this bacterium can be stimulated by bile that is also stimulate ToxR and ToxT transcription of the promoter ctxA (Gupta and Chowdhury, 1997; Hung and Mekalanos, 2005).

There are various mobile genetic elements that evaluated the potential pathogenic *V.cholerae*; for example the genes encoding CT may carried by the bacteriophage. However, a lysogenic bacteriophage has been reported to correlate with VP1 (Karaolis *et al*., 1999). However, the *ctxA* and *ctxB* genes that encoded

cholera toxin and have also encoded within the genomic phage but not required for replication and integration of phage (Waldor and Mekalanos, 1996).

Pathogenicity

The ingestion of 10^8-10^9 *V. cholerae* is required to cause disease. This bacterium grows and release enterotoxin when this organism attaches small intestine epithelial cells. This toxin causes diarrhoea led to dehydration. The mortality rate is about 60% from untreated cholera.

The symptoms of cholera can be characterised by milky or rice watery diarrhoea and dehydration resulting from the massive fluid loss that can be cause circulatory failure and eventually death (Sack *et al*., 2004). Generally, cholera victims die resulting from extreme dehydration that caused by losing a massive net fluid. This disease can be treated by ion replacement and therapy of dehydration. However, the treatment by antibiotics cause limiting growth of *V. cholerae*, nevertheless it has no effect on toxin. However, antibiotics are of little benefit without electrolyte replacement and simultaneous fluid (Madigan *et al*., 2003).

The pathogenesis of *V. cholerae* can be summarised as shown in fig. 1.

Epidemiology

V. cholerae is a natural inhabitant of the coastal water, estuarine and brackish riverine (Almagro-Moreno, 2013). Cholera can be spread within environmental reservoir, potentially flies and food. It has been suggested that this disease is related to seasonal climatic patterns and tidal seawater intrusions (Sack *et al*., 2004). Cholera illness is considered a public health problem around the world, in Asia, several parts of Bangladesh and India are endemic for this disease, as well as, in Haiti, cholera has become endemic (Deen *et al*., 2020). In 2011, cholera vaccine has been created to cholera outbreak (Martin *et al*., 2014). Cholera outbreaks have been studied in many countries including Italy, Africa and south and Central America (Baine *et al*., 1974; Goodgame and Greenough, 1975; Weil and Berche, 1992). Furthermore, this bacterium is transmitted almost by contaminated water. It is also related to food consumption. In the Americas, cholera associated with consumption of raw shellfish and vegetables that contaminated by untreated sewage, as well as, washed in contaminated water. It has been reported that 250 thousand deaths out of 5 million cases of cholera in 1961. Moreover, 400 thousand cases and 9 thousand deaths in 1999 worldwide. In the United States, cholera outbreaks have been demonstrated in 250 total cases (Madigan *et al*., 2003).

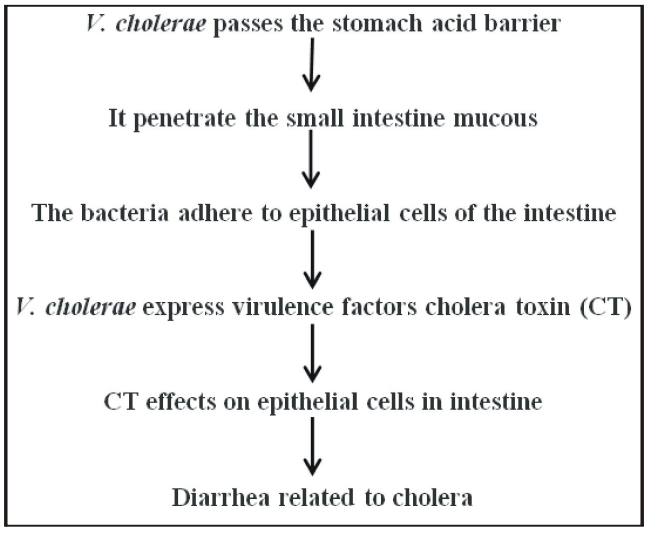

Fig. 1: The mechanisms pathway involved in *V. cholerae* (Prouty and Klose, 2006).

In Asia including China, Nepal and Pakistan, cholera outbreaks caused by O1 and O139 strains after that outbreak between 1992-1993; O139 strain became less regularly recorded (Mukhopadhyay *et al*., 2014). Furthermore, Griffith *et al*., (2006) have suggested that the risk of cholera can be increased depending on many factors including lack of development, immune status, poverty, high population density and use of unsafe water. However, in Bangladesh, the children under five have been suffered from cholera (Colombara *et al*., 2014). In Southeast Asia, cholera outbreaks have been associates with seasonal conditions and environmental factors including water sources that contaminated by frequent and widespread flooding and dislocate populations that can be affected the water supplies. Interestingly, Ahmed *et al*., (2018) have shown that since 2012 has no cases of this illness in Vietnam. Furthermore, in 1970 the pandemic of seventh cholera has been spread in Africa; isolates of 1070 *V. cholerae* O1 from 45 countries Africans have been collected from 1966 to 2014 (Weill, 2017). Increasing the incidence of cholera especially in poverty and poor housing, as well as, high population density has been reported in Africa.

In the Americans, the first cholera outbreak started in Chile and Peru in 1832 and lasted in 1851, however, the second outbreak occurred between 1853 and 1859 (Hays, 2005). The origin of epidemic cholera in Haiti was from Nepal, it has been investigated by epidemiology and genetic characterisation of *V. cholerae* isolates that isolated from the Haitain cholera epidemic (Frerichs *et al*., 2012).

Conclusion

V. cholerae is a pathogen; it causes cholera resulting

in severe dehydration. Based on adequate sanitation measures can be controlled of cholera. This bacterium is eliminated from wastewater within purification of water and treatment of sewage. *V. cholerae* is associated with marine animals gut flora, surface for nutrition and growth. However, based on epidemiological data can be decided making a vaccine against cholera at the regional and national levels. Cholera is a disease that continues to be a scientific issue and significant cause of mortality and morbidity worldwide.

References

Abendroth, J., A.E. Rice, K. McLuskey, M. Bagdasarian and W.G. Hol (2004). The crystal structure of the periplasmic domain of the type II secretion system protein EpsM from *Vibrio cholerae*: the simplest version of the ferredoxin fold. *J. Mol. Biol.*, **338:** 585-596.

Ahmed, M.U., M. Baquilod and C. Deola *et al*., (2018). Cholera prevention and control in Asian countries. *BMC Proc.*, **12:** 13-62.

Alam, M., M. Sultana, G.B. Nair, R.B. Sack, D.A. Sack, A.K. Siddique, A. Huq and R.R. Colwell (2006). Toxigenic *Vibrio cholerae* in natural environmental setting of Bangladesh. *Appl. Environ. Microbiol.*, **72:** 2849-2855.

Almagro-Moreno, S. and R.K. Cholera Taylor (2013). Environmental reservoirs and impact on disease transmission. *Microbiol. Spectrum.*, **1(2)**.

Baine, W.B., M. Mazzotri and D. Greco *et al*., (1974). Epidemiology of cholera in Italy in 1973. *Lancet.*, **2:** 1370-1374.

Boyd, E.F., A.J. Heilpern and M.K. Waldor (2000). Molecular analysis of a putative CTXphi precursor and evidence for independent acquisition of distinct CTX(phi)s by toxigenic *Vibrio cholerae*. *J. Bacteriol.*, **182:** 5530-5538.

Carroll, P.A., K.T. Tashima, M.B. Rogers, V.J. Di Rita and S.B. Calderwood (1997). Phase variation in *tcpH* modulates expression of the ToxR regulation in *Vibrio cholerae*. *Mol. Microbiol.*, **25:** 1099-1111.

Colombara, D.V., A.S. Faruque, K.D. Cowgill and J.D. Mayer (2014). Risk factor for diarrhea hospitalisation in Bangladesh. 2000-2008: a case-case study of cholera and shigellosis. *BMC Infect. Dis.*, **14:** 440.

Connell, T.D., D.J. Metzger, J. Lynch and J.P. Folster (1998). Endochitinase is transported to the extracellular milieu by the epsencoded general secretory pathway of *Vibrio cholerae*. *J. Bacteriol.*, **180:** 5591-5600.

Deen, J., M.A. Mengel and J.D. Clemens (2020). Epidemiology of cholera. *Vaccine.*, **38:** A31-A40.

Donovan, T.J. and P. Van Netten (1995). Culture media for the isolation and enumeration of pathogenic Vibrio species in food and environmental samples. *Int. J. Food. Microbiol.*, **26:** 77-91.

Dziejman, M., E. Balon, D. Boyd, C.M. Fraser, J.F. Heidelberg and J.J. Mekalanos (2002). Comparative genomic analysis of *Vibrio cholerae*: gene that correlate with cholera endemic and pandemic disease. *Proc. Natl. Acad. Sci. USA.*, **99:** 1556-1561.

Frerichs, R.R., P.S. Keim, R. Barrais and R. Piarroux (2012). Napalese origin of cholera epidemic in Haiti. *Clin. Microbiol. Infect: Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.*, **18:** E158-163.

Freter, R., P.C. O'Brien and M.S. Macsai (1981). Role of chemotaxis in the association of motile bacteria with intestinal mucosa: *in vivo* studies. *Infect. Immun.*, **34:** 234-240.

Fullner, K.J., J.C. Boucher, M.A. Hanes, G.K. Haines, B.M. Meehan, C. Walchle, P.J. Sansonetti and Mekalanos (2002). The contribution of accessory toxins of *Vibrio cholerae* O1 El Tor to the proinflammatory response in a murine pulmonary cholera model. *J. Exp. Med.*, **195:**1455-1462.

Gill, D.M. (1976). The arrangement of subunits in cholera toxin. *Biochemistry.*, **15:** 1242-1248.

Gill, D.M. and C.A. King (1975). The mechanism of action of cholera toxin in pigeon erythrocyte lysates. *J. Biol. Chem.*, **250:** 6424 - 6432.

Goodgame, R.W. and E.B. Greenough (1975). Cholera in Africa: a message for the west. *Ann Inter. Med.*, **82:**101-106.

Griffith, D.C., L.A. Kelly-Hope and M.A. Miller (2006). Review of reported cholera outbreaks worldwide 1995-2005. *An. J. Trop. Med. Hygiene.*, **75:** 973-977.

Gupta, S. and R. Chowdhury (1997). Bile affects production of virulence factors and motility of *Vibrio cholerae*. *Infect. Immun.*, **65:** 1131-1134.

Hase, C.C. and R.A. Finkelstein (1991). Cloning and nucleotide sequence of the *Vibrio cholerae* hemagglutinin/protease (HA/protease) gene and construction of an HA/protease-negative strain. *J. Bcteriol.*, **173:** 3311-3317.

Hays, J.N. (2005). Epidemics and pandemics: their impacts on human history. *Santa Barbara* (CA, USA): ABC-CLIO.

Heidelberg, J.F., J.A. Eisen, W.C. Nelson, R.A. Clayton, M.L. Gwinn, R.J. Dodson, D.H. Haft, E.K. Hickey, J.D. Peterson, L. Umayam, S.R. Gill, K.E. Nlson, T.D. Read and H. Tetteline *et al*., (2000). DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature.*, **406:** 477-483.

Hung, D.T. and J.J. Mekalanose (2005). Bile acids induce cholera toxin expression in *Vibrio cholerae* in a ToxT-independent manner. *Proc. Natl. Acad. Sci. USA.*, **102:** 3028-3033.

Huq, A., R.B. Sack, A. Nizam, I.M. Longini, G.B. Nair, A. Ali, J.G. Morris, M.N.H. Khan, A.K. Siddique, M. Yunus, M.J. Albert, D.A. Sack and R.R. Colwell (2005). Critical factors influencing the occurrence of *Vibrio cholerae* in the environmental of Bangladesh. *Appl. Environ. Microbiol.*, **71:** 4645-4654.

Huq, A., E.B. Small, P.A. West, M.I. Huq, R. Rahman and R.R. Colwell (1983). Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Appl. Environ. Microbiol.*, **45:** 275-283.

Islam, M.S., Z. Rahim, M.J. Alam, S. Begum, S.M. Moniruzzaman, A. Umeda, K. Amako, M.J. Albert, R.B. Sack, A. Huq and R.R. Colwell (1999). Association of *Vibrio cholerae* O1 with the *cyanobacterium*, *Anabaena* sp., elucidated by polymerase chain reaction and transmission electron microscopy, *Trans. R. Soc. Trop. Med. Hyg*., **93:** 36-40.

Karaolis, D.K., J.A. Johnson, C.C. Bailey, E.C. Boedeker, J.B. Kaper and P.R. Reeves (1998). A *Vibrio cholerae* pathogenicity island associated with epidemic and pandemic strains. *Proc. Natl. Acad. Sci. USA.*, **95:** 3134-3139.

Karaolis, D.K., S. Somara, D.R. Maneval, J.A. Jonhnson and J.B. Kaper (1999). A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature*., **399:** 375-379.

Kenne, L., B. Lindberg, P. Unger, B. Gustafsson and T. Holme (1982). Structural studies of the *Vibrio cholerae* O-antigen. *Carbohydr. Res*., **100:** 341-349.

Knirel, Y.A., S.N. Widmalm Senchenkova, P.E. Jansson and A. Weintraub (1997). Structural studies on the short-chain lipopolysaccharide of *Vibrio cholerae* O139 Bengal. *Eur. J. Biochem*., **247:** 402-410.

Lee, S.H., S.M. Butler and A. Camilli (2001). Selection for *in vivo* regulators of bacterial virulence. *Proc. Natl. Acad. Sci. USA*., **98:** 6889-6894.

Lin, W.K., J. Fullner, R. Clayton, J.A. Sexton, M.B. Rogers, K.E. Calia, S.B. Calderwood, C. Fraser and Mekalanos (1999). Identification of a *Vibrio cholerae* RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. *Proc. Natl. Acad. Sci. USA*., **96:** 1071-1076.

London, E. and D.A. Brown (2000). Insolubility of lipids in triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domain (rafts). *Biochim. Biophys. Acta*., **1508:** 182-195.

Madigan, M.T., J.M. Martinko and J. Parker (2003). Brock Biology of Microorganisms. Pearson Education, Inc. Prentice Hall.O'Brien M. and Colwell R. (1985). Modified taurocholate-tellurite-gelatin agar for improved differentiation of *Vibrio* species. *J. Cli. Microbiol*., **22:** 1011-1013.

Marsh, J.W. and R.K. Taylor (1998). Identification of the *Vibrio cholerae* type 4 prepilin peptidase required for cholera toxin secretion and pilus formation. *Mol. Microbiol*., **29:** 1481-1492.

Martin, S., A.L. Lopez and A. Bellos *et al*., (2014). Post-licensure deployment of oral cholera vaccines: a systematic review. *Bull World Health Org*., **92:** 881-893.

Merrell, D.S., S.M. Butler, F. Qadri, N.A. Dolganov, A. Alam, M.B. Cohen, S.B. Calderwood, G.K. Schoolnik and Camilli (2002). Host-induced epidemic spread of the cholera bacterium. *Nature*., **417:** 642-645.

Mukhopadhyay, A.K., Y. Takeda and G. Balakrish Nair (2014). Cholera outbreaks in the El Tor biotype era and the impact of the new El Tor variants. *Curr. Top. Microbiol. Immunol*., **379:** 17-47.

Murley, Y.M., J. Behri, R. Griffin and S.B. Calderwood (2000). Classical and El Tor biotypes of *Vibrio cholerae* differ in timing of transcription of tcpPH during growth in including conditions. *Infect. Immun*., **68:** 3010-3014.

Nicholls, K.M., V.J. Lee and T. Donovan (1976). An evaluation of commercial thiosulphate citrate bile salt sucrose agar (TCBS). *J. Appl. Bacteriol*., **41:** 265-269.

Nikaido, H. (1988). Structure and functions of the cell envelope of gram-negative bacteria. *Rev. Infect. Dis. 10 (Suppl.),* **2:** S279-S281.

O'Brien, M. and R. Colwell (1985). Modified taurocholate-tellurite-gelatin agar for improved differentiation of *Vibrio* species. *J. Clin. Microbiol*., **22:** 1011-1013.

Prouty, M.G. and K.E. Klose (2006). *Vibrio cholerae*: the genetics of Pathogenesis and Environmental Persistence. In: Thompson FL., Austin B. and Swings J. *The Biology of Vibrios*. Washington, DC., ASM Press.

Pruzzo, C., R. Tarsi, M. Mdel Mar Lleo, C. Signoretto, M. Zampini, L. Pane, R.R. Colwell and P. Canepari (2003). Persistence of adhesive properties in *Vibrio cholerae* after long-term exposure to sea water. *Environ. Microbiol*., **5:** 850-858.

Ramamurthy, T., S. Garg, R. Sharma, S.K. Bhattacharya, G.B. Nair, T. Shimada and T. Takeda *et al*., (1993). Emergence of novel strain of *Vibrio cholerae* epidemic potential in southern and eastern India. *Lancet*., **341:** 703-704.

Robien, M.A., B.E. Krumm, M. Sandkvist and W.G. Hol (2003). Crystal structure of the extracellular protein secretion NTPase EpsE of *Vibrio cholerae. J. Mol. Biol*., **333:** 657-674.

Sack, D.A., R.B. Sack, G.B. Nair and A.K. Siddique (2004). Cholera. *Lancet*., **363:** 223-233.

Sandkvist, M., L.O. Michel, L.P. Hough, V.M. Morales, M. Bangdasarian, M. Koomey and V.J. DiRita (1997). General secretion pathway (eps) genes required for toxin secretion and outer membrane biogenesis in *Vibrio cholerae*. *J. Bacteriol*., **179:** 6994-7003.

Taylor, R., C. Show, K. Peterson, P. Spears and J. Mekalanos (1988). Safe, live *Vibrio cholerae* vaccines? *Vaccine*., **6:** 151-154.

Taylor, R.K., V.L. Miller, D.B. Furlong and Mekalanos (1987). Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. *Proc. Natl. Acad. Sci. USA*., **84:** 2833-2837.

Waldor, M.K. and J.J. Mekalanos (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science*., **272:** 1910-1914.

Weil, O. and P. Berche (1992). The cholera epidemic in Ecuador: towards an endemic in Latin America. *Rev. Epidemiol. Sante. Publique*., **40:**145-155.

Weill, F.X., D. Domman and E. Njamkepo *et al*., (2017). Genomic history of the seventh pandemic of cholera in Africa. *Science*., **358:**785-789.

Zhu, J. and J.J. Mekalanos (2003). Quorum sensing-dependent biofilms enhance colonization in *Vibrio cholerae*. *Dev. Cell*., **5:** 647-656.