Open Access
Check for updates
International Journal of Medical Science and Dental Health (ISSN: 2454-4191)
Volume 11, Issue 10, October 2025
Doi: https://doi.org/10.55640/ijmsdh-11-10-09

Genetic Basis of Virulence Factors in Vibrio Cholerae: A Review

Russell Issam AL-Daher
Department of Biology, College of Science for women, University of Babylon, Iraq

Received: 17 September 2025, **accepted:** 29 September 2025, **Published Date:** 16 October 2025

Abstract

Cholera is a major epidemic disease worldwide, contributing significantly to global morbidity and mortality. The hallmark of the disease, severe watery diarrhea, results from a complex process in which multiple bacterial components enable *Vibrio cholerae* to reach the small intestinal epithelium, establish colonies, and produce toxins. The AraC/XylS-family transcriptional regulator, ToxT, acts as the primary activator of virulence genes in *V. cholerae*, the Gram-negative bacterium responsible for cholera. Unsaturated fatty acids (UFAs) present in bile can inhibit ToxT activity, thereby modulating virulence expression. Recent studies indicate that virulence in *V. cholerae* is not solely determined by the presence of key genes such as *ctxAB* and *tcp*. Accessory virulence-associated genes, mobile genetic elements (MGEs), regulatory networks, secretion systems, and small RNAs (sRNAs) collectively contribute to the pathogen's ability to infect, persist, and spread. The adhesion of *Vibrio cholerae* to the intestinal epithelium, as well as its capacity to obtain nutrients and manipulate host immune mechanisms, is facilitated by several molecular determinants. Among these are mucin-interacting proteins such as GbpA, hemagglutinin/protease (HapA), and neuraminidase (NanH), together with various regulatory networks. The bacterium's collective behavior and virulence expression are predominantly governed by the quorum-sensing (QS) system, which functions as a density-dependent communication network. When bacterial populations are at a low cell density (LCD), the regulatory molecules—including LuxO, HapR, and the signaling autoinducers AI-2 and CAI-1—coordinate gene expression patterns that enhance colonization capacity, stimulate biofilm development, and concurrently inhibit HapR-mediated repression. This review study highlights the genetic and molecular foundations underlying the virulence determinants that shape the pathogenic potential of *V. cholerae*.

Keywords: Genetic Basis, Virulence Factors, Vibrio cholerae, ToxT,

Introduction

Cholera is a severe, rapidly developing infectious disease that primarily manifests through profuse diarrhea. It results from ingesting food or drinking water contaminated with the bacterium *Vibrio cholerae*. This illness remains a major global public health concern, reflecting persistent inequalities and deficiencies in social and economic infrastructure. Ensuring universal access to clean drinking water, adequate sanitation facilities, and proper hygiene practices is fundamental for preventing cholera and similar waterborne infections (WHO, 2022). The causative organism, *Vibrio cholerae*, is a Gram-negative bacterium that naturally inhabits aquatic ecosystems (Huq et al., 2018). However, the actual magnitude of cholera's impact is frequently underestimated. One key reason is the diagnostic challenge—cholera symptoms often resemble those of other acute diarrheal conditions, making it difficult to

identify solely through clinical examination (Jubyda et al., 2023).

A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production. However, in 1992, a novel epidemic strain of *V. cholerae* representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule (Crisan et al., 2019 However, in 1992, a novel epidemic strain of *V. cholerae* representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule. (van Kessel, 2024).

A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor (Ghandour & Papenfort, 2023).

Thereofore, researchers have increasingly examined and utilized relationships between satellite-derived environmental factors—such as Phytoplankton abundance, chlorophyll concentration, sea surface height, salinity, and temperature —to enhance our understanding of bacterial pathogen ecology and to evaluate the risk of waterborne diseases across various spatial and temporal scales. Specifically for *Vibrio* species, remote-sensing data have been employed to describe the environmental conditions supporting the pathogens and their hosts, particularly through ocean-color remote sensing, which provides insights into biological variables linked to phytoplankton dynamics (Racault et al., 2019).

Cholera is a severe, rapidly developing infectious disease that primarily manifests through profuse diarrhea. It results from ingesting food or drinking water contaminated with the bacterium *Vibrio cholerae*. This illness remains a major global public health concern, reflecting persistent inequalities and deficiencies in social and economic infrastructure. Ensuring universal access to clean drinking water, adequate sanitation facilities, and proper hygiene practices is fundamental for preventing cholera and similar waterborne infections (WHO, 2022). The causative organism, *Vibrio cholerae*, is a Gram-negative bacterium that naturally inhabits aquatic ecosystems (Huq et al., 2018). However, the actual magnitude of cholera's impact is frequently underestimated. One key reason is the diagnostic challenge—cholera symptoms often resemble those of other acute diarrheal conditions, making it difficult to identify solely through clinical examination (Jubyda et al., 2023).

A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production (Rasmussen et al., 2007).

Lateral gene transfer (LGT) and the acquisition of foreign DNA including mobile genetic elements such as plasmids, bacteriophages, transposons, integrative and conjugative elements, and genomic islands (GIs) assist bacteria to increase their fitness under different environmental conditions. GIs encode for metabolic islands, degradation islands, resistance islands, symbiosis islands, and pathogenicity islands (PAI). It is proposed that during evolution, the V.

cholerae progenitor received several genomic islands (GIs) such as VPI-1, VPI-2 and GI-1 to GI-10, TLC and became V cholera O1 which then received VSP-1, VSP-2 and GI-11 by lateral gene transfer, as well as the acquisition of a CTX $^{\phi}$, RS1$^{\phi}$ to give rise to the contemporary V. cholerae O1 El Tor and O139 strains (Banerjee et al., 2014). The emergence of new pathogenic clones such as V. cholerae O1 El Tor, V. cholerae O139 and V. cholerae Atypical O1 El Tor clones are represented by the predicted extensive genetic recombination via lateral gene transfer mainly characterized by different assortments of laterally transferred genomic islands (Bhandari et al., 2021).

Architecture of virulence genes

Although Vibrio mimicus and Vibrio cholerae share considerable similarities, they differ notably at both the genetic and phenotypic levels. V. mimicus is capable of producing diarrheal illness that mirrors the clinical manifestations associated with V. cholerae. In the present investigation, attention was directed toward detecting CTXΦ—the lysogenic filamentous bacteriophage known to carry the cholera toxin genes in epidemic strains of V. cholerae—within clinical isolates of V. mimicus. Fully intact CTXΦ genomes were found in four isolates. Through Southern blot hybridization, it was determined that the V. mimicus strain PT5 contained two CTX prophage insertions at distinct chromosomal sites, whereas strains PT48, 523-80, and 9583 exhibited tandem integrations of CTXΦ. All four isolates were also shown to contain the replicative plasmid form of the phage, pCTX. Furthermore, infectious CTXΦ particles were successfully produced by the CTX prophage in strain PT5. Sequence comparison indicated that the orfU and zot genes of CTXΦ in V. mimicus PT5 were completely identical to those of V. cholerae N16961, providing evidence for a recent lateral gene transfer event between the two species. A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production. However, in 1992, a novel epidemic strain of *V. cholerae* representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule (Ramamurthy, 2020; Kumar, 2020).

The evolutionary origins and mechanisms underlying the diversification of AB-type toxins remain only partially understood. Some research indicates that bacteriophages are instrumental in the emergence of toxigenic bacterial strains by transferring genetic material, including toxin-encoding genes. A well-documented example is the *Vibrio cholerae* cholera toxin, an AB_5-type toxin whose genes are carried by the filamentous bacteriophage CTXϕ. (Sinha-Ray et al., 2019). Complementing CT is the TCP, encoded within Vibrio pathogenicity island-1 (VPI-1). TCP is a filamentous pilus required for efficient infection of the gastrointestinal tract and also serves as the receptor for CTXΦ, linking colonization and toxin acquisition mechanistically (Kumar, 2020). VPI-1 is a horizontally needed genomic island that contains *tcp* genes as well as accessory factors that promote colonization and fitness (Takahashi et al., 2021).

Although Vibrio mimicus and Vibrio cholerae share considerable similarities, they differ notably at both the genetic and phenotypic levels. V. mimicus is capable of producing diarrheal illness that mirrors the clinical manifestations associated with V. cholerae. In the present investigation, attention was directed toward detecting CTXΦ—the lysogenic filamentous bacteriophage known to carry the cholera toxin genes in epidemic strains of V. cholerae—within clinical isolates of V. mimicus. Fully intact CTXΦ genomes were found in four isolates. Through Southern blot hybridization, it was determined that the V. mimicus strain PT5 contained two CTX prophage insertions at distinct chromosomal sites, whereas strains PT48, 523-80, and 9583 exhibited tandem integrations of CTXΦ. All four isolates were also shown to contain the replicative plasmid form of the phage, pCTX. Furthermore, infectious CTXΦ particles were successfully produced by the CTX prophage in

strain PT5. Sequence comparison indicated that the orfU and zot genes of CTXΦ in V. mimicus PT5 were completely identical to those of V. cholerae N16961, providing evidence for a recent lateral gene transfer event between the two species. A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production. However, in 1992, a novel epidemic strain of *V. cholerae* representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule. (Crisan et al., 2019; Vizzarro et al., 2024 ; Maciel-Guerra et al., 2024).

Vibrio cholerae, the bacterium responsible for cholera, possesses a genome split into two chromosomes—an uncommon characteristic among bacteria. These chromosomes differ in size and are independently regulated by distinct initiator molecules during replication. Through advanced techniques such as marker frequency analysis and flow cytometry data analysis, it has been demonstrated that the smaller chromosome II begins replication later in the C phase of the cell cycle, after most of chromosome I has already replicated. This delayed initiation allows both chromosomes to complete replication nearly simultaneously, resulting in a increased frequency of replication origins for every cell for chromosome I compared to chromosome II (Ochi et al., A wide range of *Vibrio* species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic *Vibrio cholerae* O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production. However, in 1992, a novel epidemic strain of *V. cholerae* representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule (De et al., 2021).

Regulation of virulence genes

A wide range of Vibrio species exist freely in aquatic environments, among which several possess pathogenic potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic Vibrio cholerae O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup. Identification of these strains relied on their agglutination with O1-specific antisera that target the lipopolysaccharide component of the bacterial cell wall, in addition to confirmation of their enterotoxin production. However, in 1992, a novel epidemic strain of V. cholerae representing serogroup O139 (also referred to as "Bengal," being the 139th recognized serogroup) emerged in India and Bangladesh. This serovar can be distinguished by several characteristic features: it does not agglutinate with O1-specific antiserum, shows positive agglutination with O139-specific antiserum, and possesses a polysaccharide capsule (Dominguez & Blokesch,2024).

Small regulatory RNAs (sRNAs) make a secondary layer of fine-tuning gene expression, post-transcriptional control, involved in quorum sensing (QS), stress adaptation, and biofilm formation. Recent research has uncovered an RNA "sponge," QrrX, which interacts with Qrr1-4 sRNAs—key regulators of the QS network—thereby neutralizing their function and modulating the shift from individual cell activity to coordinated community behaviors (Gubensäk et al. 2023)

A wide range of Vibrio species exist freely in aquatic environments, among which several possess pathogenic

potential. Prior to 1992, All reported cholera cases were linked solely to two serotypes of toxigenic Vibrio cholerae O1—Inaba (AC) and Ogawa (AB)—and were associated with the two recognized biotypes, classical and El Tor. This indicates that the disease in these instances was confined to specific antigenic and phenotypic variants within the O1 serogroup (Lee et al., 2023).

Bacteria employ a communication mechanism known as quorum sensing (QS) to coordinate their behavior and regulate gene expression at the population level. This process depends on the collective detection of signaling compounds termed autoinducers. In the present study, QS was found to initiate a previously unrecognized form of multicellular organization in Vibrio cholerae, referred to as aggregation. Unlike the traditional surface-associated biofilm formation pathway—which is suppressed by QS—the aggregation process is triggered by autoinducers, develops rapidly within suspended cultures, and proceeds independently of cell division. These characteristics sharply contrast with those typically observed during *V. cholerae* biofilm development. While extracellular DNA was observed to restrict the size of aggregates, it alone was insufficient to initiate the process. A genetic mutagenesis analysis identified several genes necessary for aggregate formation, including those encoding proteins associated with intestinal colonization, stress adaptation, and a distinct protein marker that differentiates the current pandemic *V. cholerae* lineage from earlier epidemic strains. (Prentice et al., 2022).

Small regulatory RNAs (sRNAs) make a secondary layer of fine-tuning gene expression, post-transcriptional control, involved in quorum sensing (QS), stress adaptation, and biofilm formation. Recent research has uncovered an RNA “sponge,” QrrX, which interacts with Qrr1-4 sRNAs—key regulators of the QS network—thereby neutralizing their function and modulating the shift from individual cell activity to coordinated community behaviors. (Chin et al., 2020).

Environmental pressures play a critical role in driving variation in virulence genes. In aquatic and estuarine habitats, which serve as natural reservoirs for *Vibrio cholerae*, abiotic factors—such as fluctuations in temperature, salinity, pH, nutrient availability, and exposure to bile from animal or external sources—exert strong selective forces. During persistence in these environments, bacterial strains often downregulate or lose certain virulence traits, or their regulatory capacity, in favor of characteristics that enhance survival outside the host. Upon entering a host, however, these virulence networks and genes can be reactivated or restored, either through the action of mobile genetic elements (MGEs) or via allelic reversion, enabling the bacterium to regain its pathogenic potential (Vezzulli et al., 2020).

Clinical applications od virulence genes

Diagnostic Applications

Traditional approaches for isolating *Vibrio cholerae* from environmental water samples typically involve an initial enrichment in liquid broth, followed by plating on selective media. Identification is then confirmed through a combination of biochemical assays, PCR, and serological testing to determine the strain’s serotype. In situations where sufficient PCR reagents are available, biochemical confirmation can be omitted, and PCR alone may be used for verification. Because most water and plankton samples contain low bacterial densities, some form of sample concentration is usually necessary. Conducting pilot runs at the intended study site can help determine the optimal sample volumes needed to reliably achieve the study objectives. (Chaguza et al., 2024).

References

1. Abana, D., Gyamfi, E., Dogbe, M., et al. (2019). Investigating the virulence genes and antibiotic susceptibility patterns of *Vibrio cholerae* O1 in environmental and clinical isolates in Accra, Ghana. *BMC Infectious Diseases, 19*, 76. https://doi.org/10.1186/s12879-019-3714-z
2. Bhandari, M., Jennison, A. V., Rathnayake, I. U., & Huygens, F. (2021). Evolution, distribution and genetics of atypical *Vibrio cholerae*—A review. *Infection, Genetics and Evolution, 89*, 104726. https://doi.org/10.1016/j.meegid.2021.104726
3. Canals, A., Pieretti, S., Muriel-Masanes, M., et al. (2023). ToxR activates the *Vibrio cholerae* virulence genes by tethering DNA to the membrane through versatile binding to multiple sites. *Proceedings of the National Academy of Sciences U.S.A., 120*(29), e2304378120. https://doi.org/10.1073/pnas.2304378120
4. Chaguza, C., Ouso, D. O., Kabwama, S. N., Malama, K., Ng'oma, M., Musonda, K. G., Misinzo, G., &

Thomson, N. R. (2024). Genomic insights into the 2022–2023 *Vibrio cholerae* outbreak in Malawi. *Nature Communications, 15*(1), 45860. https://doi.org/10.1038/s41467-024-45860-6

5. Chin, C. S., Gutiérrez, R. A., Sorenson, J., DeLong, K., Tullman-Ercek, D., Korlach, J., & Waldor, M. K. (2020). Complete genome-wide reconstruction of mobile genetic elements reveals their contribution to *Vibrio cholerae* evolution and virulence. *Proceedings of the National Academy of Sciences, 117*(45), 28152–28162. https://doi.org/10.1073/pnas.2019637117

6. Crisan, C. V., Chande, A. T., Williams, K., et al. (2019). Analysis of *Vibrio cholerae* genomes identifies new type VI secretion system gene clusters. *Genome Biology, 20*, 163. https://doi.org/10.1186/s13059-019-1765-5

7. De, R. (2021). Mobile genetic elements of *Vibrio cholerae* and the evolution of epidemic traits. *Frontiers in Tropical Diseases, 1*, 691604. https://doi.org/10.3389/fitd.2021.691604

8. Dominguez, S. R., & Blokesch, M. (2024). The intersection between host–pathogen interactions and environmental signals in *Vibrio cholerae* pathogenesis. *Trends in Microbiology, 32*(5), 380–392. https://doi.org/10.1016/j.tim.2024.02.004

9. Sakib, S. N., Reddi, G., & Almagro-Moreno, S. (2018). Environmental role of pathogenic traits in *Vibrio cholerae*. *Journal of Bacteriology, 200*(15), e00795-17. https://doi.org/10.1128/JB.00795-17

10. Prentice, J. A., Bridges, A. A., & Bassler, B. L. (2022). Synergy between c-di-GMP and quorum-sensing signaling in *Vibrio cholerae* biofilm morphogenesis. *Journal of Bacteriology, 204*(10), e00249-22. https://doi.org/10.1128/jb.00249-22

11. Ghandour, R., & Papenfort, K. (2023). Small regulatory RNAs in *Vibrio cholerae*. *microLife, 4*. https://doi.org/10.1093/femsml/uqad030/7199165

12. Gubensäk, N., Sagmeister, T., Buhlheller, C., Geronimo, B. D., Wagner, G. E., Petrowitsch, L., Gräwert, M. A., Rotzinger, M., Berger, T. M. I., Schäfer, J., Usón, I., Reidl, J., Sánchez-Murcia, P. A., Zangger, K., Pavkov-Keller, T. (2023). *Vibrio cholerae's ToxRS bile sensing system*. *eLife, 12*, e88721. https://doi.org/10.7554/eLife.88721

13. Huber, M., Papenfort, K., & Bassler, B. L. (2022). An RNA sponge controls quorum sensing dynamics and biofilm formation in *Vibrio cholerae*. *Nature Communications, 13*(1), 3526. https://doi.org/10.1038/s41467-022-35261-x

14. Jubyda, F. T., Nahar, K. S., Barman, I., et al. (2023). *Vibrio cholerae* O1 associated with recent endemic cholera shows temporal changes in serotype, genotype, and drug-resistance patterns in Bangladesh. *Gut Pathogens, 15*, 17. https://doi.org/10.1186/s13099-023-00537-0

15. Kumar, A. (2020). Vibrio pathogenicity island-1: the master determinant of cholera pathogenesis. *Frontiers in Microbiology, 11*, 578. https://doi.org/10.3389/fmicb.2020.00757

16. Lee, D., Choi, H., Son, S., Bae, J., Joo, J., Kim, D. W., & Kim, E. J. (2023). Expression of Cholera Toxin (CT) and the Toxin Co-Regulated Pilus (TCP) by Variants of ToxT in *Vibrio cholerae* Strains. *Toxins, 15*(8), 507. https://doi.org/10.3390/toxins15080507

17. Lembke, M., Pennetzdorfer, N., Tutz, S., Koller, M., Vorkapic, D., Zhu, J., & Blokesch, M. (2018). Proteolysis of ToxR is controlled by cysteine-thiol redox state and bile salts in *Vibrio cholerae*. *Molecular Microbiology, 110*(5), 796–810. https://doi.org/10.1111/mmi.14125

18. Li, X. (2024). Diversity and complexity of CTXΦ and pre-CTXΦ families. *Frontiers in Microbiology*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11509585/

19. Maciel-Guerra, A., et al. (2024). Core and accessory genomic traits of *Vibrio cholerae* O1 linked to transmission and disease severity. *Nature Communications, 15*, 52238-0.

20. Mageto, L. M., Aboge, G. O., Mekuria, Z. H., Gathura, P., Juma, J., Mugo, M., Kebenei, C. K., Imoli, D., Ongadi, B. A., Kering, K., Mbae, C. K., & Kariuki, S. (2025). Genomic characterization of *Vibrio cholerae* isolated from clinical and environmental sources during the 2022-2023 cholera outbreak in Kenya. *Frontiers in microbiology*, *16*, 1603736. https://doi.org/10.3389/fmicb.2025.1603736.

21. McDonald, N. D., Regmi, A., Morreale, D. P., et al. (2019). CRISPR-Cas systems are present predominantly on mobile genetic elements in Vibrio species. *BMC Genomics, 20*, 105. https://doi.org/10.1186/s12864-019-5439-1

22. Pant, A., Bag, S., Saha, B., Verma, J., Kumar, P., Banerjee, S., … Das, B. (2020). Molecular insights into the genome dynamics and interactions between core and acquired genomes of *Vibrio cholerae*.

Proceedings of the National Academy of Sciences of the United States of America, 117(38), 23762–23773. https://doi.org/10.1073/pnas.2006283117

23. Montero, D. A., & Rodríguez, L. A. (2023). Vibrio cholerae: Classification, pathogenesis, immune response, and clinical management. Frontiers in Medicine, 10, 1155751. https://doi.org/10.3389/fmed.2023.1155751

24. Ochi, K., Mizuno, T., Samanta, P., Mukhopadhyay, A. K., Miyoshi, S. I., & Imamura, D. (2021). Recent Vibrio cholerae O1 Epidemic Strains Are Unable To Replicate CTXΦ Prophage Genome. mSphere, 6(3), e0033721. https://doi.org/10.1128/mSphere.00337-21

25. Ramamurthy, T., Nandy, R. K., Mukhopadhyay, A. K., Dutta, S., Mutreja, A., Okamoto, K., Miyoshi, S. I., Nair, G. B., & Ghosh, A. (2020). Virulence Regulation and Innate Host Response in the Pathogenicity of *Vibrio cholerae*. *Frontiers in cellular and infection microbiology*, *10*, 572096. https://doi.org/10.3389/fcimb.2020.572096

26. Rasmussen, T., Jensen, R. B., & Skovgaard, O. (2007). The two chromosomes of *Vibrio cholerae* are initiated at different time points in the cell cycle. *The EMBO Journal, 26*(13), 3124–3131. https://doi.org/10.1038/sj.emboj.7601747

27. Racault, M.-F., Abdulaziz, A., George, G., Menon, N., C, J., Punathil, M., McConville, K., Loveday, B., Platt, T., Sathyendranath, S., & Vijayan, V. (2019). Environmental Reservoirs of *Vibrio cholerae*: Challenges and Opportunities for Ocean-Color Remote Sensing. *Remote Sensing*, *11*(23), 2763. https://doi.org/10.3390/rs11232763

28. Saha, D., Aggarwal, S., & Singh, A. (2023). Attenuation of quorum sensing system and virulence in *Vibrio cholerae* by phytomolecules. *Frontiers in Microbiology, 14*, 1205387. https://doi.org/10.3389/fmicb.2023.1205387

29. Sajeevana, A., Ramamurthy, T., & Solomon, A. P. (2024). *Vibrio cholerae virulence and its suppression through the quorum-sensing system*. Critical Reviews in Microbiology. Advance online publication. https://doi.org/10.1080/1040841X.2024.2320823

30. Takahashi, E., Ochi, S., Mizuno, T., Morita, D., Morita, M., Ohnishi, M., Koley, H., Dutta, M., Chowdhury, G., Mukhopadhyay, A. K., Dutta, S., Miyoshi, S. I., & Okamoto, K. (2021). Virulence of Cholera Toxin Gene-Positive *Vibrio cholerae* Non-O1/non-O139 Strains Isolated From Environmental Water in Kolkata, India. *Frontiers in microbiology*, *12*, 726273. https://doi.org/10.3389/fmicb.2021.726273

31. van Kessel, J. C. (2024). *Vibrio cholerae: A fundamental model system for bacterial genetics*. *Journal of Bacteriology, 206*(11), e00248-24. https://doi.org/10.1128/jb.00248-24

32. Vezzulli, L., Baker-Austin, C., Kirschner, A., Pruzzo, C., & Martinez-Urtaza, J. (2020). Global emergence of *Vibrio cholerae* and other pathogenic vibrios in natural aquatic environments. *Nature Reviews Microbiology, 18*(11), 697–713. https://doi.org/10.1038/s41579-020-0402-3

33. Walton, M. G., Cubillejo, I., Nag, D., & Withey, J. H. (2023). Advances in cholera research: from molecular biology to public health initiatives. *Frontiers in microbiology*, *14*, 1178538. https://doi.org/10.3389/fmicb.2023.1178538.

34. Zhang, Q., Alter, T., & Fleischmann, S. (2024). Non-O1/Non-O139 *Vibrio cholerae* — an underestimated foodborne pathogen? An overview of its virulence genes and regulatory systems involved in pathogenesis. *Microorganisms, 12*(4), 818. https://doi.org/10.3390/microorganisms12040818