Unveiling the Toxin Genes of *Vibrio cholerae* in Seafood: A Collaboration of Phenotypic and Genotypic Approaches

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Abstract.
Vibrio cholerae is a pathogenic bacterium that causes cholera, a severe diarrheal disease transmitted through contaminated food or water, particularly seafood such as fish, shrimp, and shellfish. This review aims to provide a comprehensive overview of studies that have identified and characterized toxin genes of V. cholerae in seafood using phenotypic and genotypic approaches. The literature review was conducted using online databases including PubMed, ScienceDirect, Scopus, Web of Science, and Google Scholar with keywords "Vibrio cholerae", "virulence gene", "cholera toxin", "phenotypic-genotypic detection", "cholera infection". Selected articles were published between 2015 and 2025 in English or Indonesian and discussed the identification of V. cholerae toxin genes from marine products. The findings revealed that V. cholerae isolates from seafood commonly harbor virulence genes such as ctxA, hlyA, ompW, tcpA, zot, ace, and rtx, which play crucial roles in colonization, toxin production, and pathogenicity. Phenotypic detection using TCBS agar and hemolysis tests serves as an initial screening, whereas genotypic identification through PCR and sequencing offers higher sensitivity and specificity. Combining both methods enhances detection accuracy and risk assessment. Therefore, monitoring virulence genes of V. cholerae in seafood is essential for strengthening food safety surveillance and preventing cholera outbreaks in coastal and tourism areas.
Keywords: *Vibrio cholerae, virulence gene, cholera toxin, phenotypic-genotypic detection and cholera infection.*

I. INTRODUCTION

Cholera is an acute diarrheal infection that can be life-threatening if not properly treated. It is estimated that there are 1.3 to 4.0 million cases of cholera worldwide each year, resulting in 21,000 to 143,000 deaths. The primary cause of cholera infection is the consumption of food or water contaminated with the bacterium *Vibrio cholerae* (WHO, 2024). *Vibrio cholerae* is a Gram-negative bacterium belonging to the family *Vibrionaceae*, with a curved-rod shape and a length of approximately 2–4 µm (Fernandez *et al.*, 2020)(Yoon and Waters, 2020)(Jawetz and Adelberg's, 2019). Cholera is closely associated with poor sanitation, as well as direct contact with or ingestion of contaminated water and/or food (such as water used for drinking, cooking, bathing, and crop irrigation) (Jawetz and Adelberg's, 2019) . Seafood, including fish, shellfish, and shrimp, has been identified as one of the major sources of food-borne disease transmission, including cholera (Mumpuni and Hasibuan, 2020)(Brauge *et al.*, 2024).

There are more than 200 serogroups of *Vibrio cholerae* based on the O antigen, but only serogroups O1 and O139 are responsible for epidemic and pandemic cholera (Jawetz and Adelberg's, 2019). The virulence of *Vibrio cholerae* is closely related to its ability to produce *cholera toxin* (CT), the principal factor responsible for the disease's pathogenesis (Herrera and J.F.Satchell, 2020). The major toxin produced by *V. cholerae* is *cholera toxin (ctx)*, while additional accessory toxins include *toxin-coregulated pilus (TCP)*, *zonula occludens toxin (zot)*, *accessory cholera enterotoxin (ace)*, *hemolysin A* (*hlyA*), and several other supporting virulence genes (Montero *et al.*, 2023).

Several studies have demonstrated the presence of *V. cholerae* toxin genes in marine-derived samples, such as the detection of the ctxA gene in *Tenualosa ilisha* fish sold in traditional markets in Bangladesh (Hossain *et al.*, 2018). Another study conducted at Kedonganan Fish Market, Bali, reported the detection of *V. cholerae* carrying the hlyA gene in shrimp and shellfish samples (Sukrama *et al.*, 2017). Moreover, the *ompW* gene, which serves as a molecular marker for *V. cholerae* identification, was also detected in shrimp and shellfish from the same market (Praja *et al.*, 2019). Detection and characterization of *V. cholerae* and its toxin genes in seafood samples are generally performed using a phenotypic approach with Thiosulfate-

Citrate-Bile Salts-Sucrose (TCBS) agar to observe colony morphology, and a genotypic approach through molecular assays such as Polymerase Chain Reaction (PCR) (Jawetz and Adelberg's, 2019).

Given the high seafood consumption in Indonesia, early detection and characterization of virulence genes in various marine products are essential, considering the potential risk of cholera transmission through contaminated seafood, the limited local data on pathogenic *V. cholerae* strains, and the need to strengthen food safety and disease prevention efforts in coastal and tourism areas.

Based on these considerations, this article aims to provide a comprehensive, evidence-based review of existing studies on the toxin genes of *Vibrio cholerae* detected in commonly consumed seafood.

II. METHODS

This review employed a literature-based approach focusing on the toxin genes detected in seafood through both phenotypic and genotypic methods. Data were collected from scientific journals discussing the discovery of toxin genes from *Vibrio cholerae* in various marine products such as fish, squid, shrimp, shellfish, and others. Scientific articles were collected from major electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. The literature search was performed using combinations of the following keywords like *Vibrio cholerae*, virulence gene, cholera toxin, phenotypic-genotypic detection, cholera infection. Selected articles were published between 2015 and 2025 in English or Indonesian and discussed the identification of *V. cholerae* toxin genes from marine products.

This review not only highlights the development of phenotypic detection methods—such as the use of selective TCBS agar and hemolysis tests—but also evaluates the effectiveness of genotypic approaches including PCR and sequencing in identifying major toxin genes such as *ctxA*, *hlyA*, and *ompW*. The analysis was carried out by examining previous studies that provided data on pathogenesis, types of virulence genes, and detection techniques applied in *Vibrio cholerae* research. This methodological approach ensures that all presented findings are based on documented scientific evidence and reflect the most recent advances in molecular detection of *Vibrio cholerae*

II. RESULTS AND DISCUSSIONS

Pathogenesis

Transmission of *Vibrio cholerae* primarily occurs through the fecal–oral route, either via contaminated water or food, or through mechanical vectors such as houseflies (*Musca domestica*), which can carry the bacteria on their legs and mouthparts (Sebrina *et al.*, 2022). Once inside the host, *V. cholerae* must overcome the acidic barrier of the stomach before reaching the small intestine. Pathogenic strains possess acid tolerance mechanisms that allow them to survive and remain infectious under low-pH conditions (Guli, 2016). In the intestinal lumen, the bacteria encounter multiple layers of host defenses, including the commensal microbiota, epithelial integrity, rapid epithelial turnover, and protective mucus layer (Guntina, Agung and Kusuma, 2016). However, *V. cholerae* is able to overcome these barriers through adhesion mechanisms mediated by *toxin-coregulated pilus (TCP)*, fimbriae, adhesin proteins, and outer membrane proteins such as *ompW*, which bind to GM1 ganglioside glycolipid receptors on the intestinal mucosal membrane (Sebrina *et al.*, 2022).

Following colonization, *V. cholerae* produces its major virulence factor, *cholera toxin (CT),* encoded by the *ctxAB* gene. The toxin consists of two subunits: subunit B, which binds to GM1 receptors, and subunit A, which activates adenylate cyclase, leading to an increase in intracellular cyclic AMP (cAMP) levels (Guli, 2016)(Siregar *et al.*, 2025). Elevated cAMP inhibits sodium–chloride absorption and enhances chloride and bicarbonate secretion, resulting in massive fluid loss into the intestinal lumen. Additionally, increased prostaglandin levels further contribute to excessive fluid secretion (Sebrina *et al.*, 2022). This condition causes profuse watery diarrhea, which may exceed one liter per hour, often accompanied by vomiting, rapid dehydration, and circulatory shock**.**

Virulence Genes

In its pathogenesis, *Vibrio cholerae* employs a series of mechanisms that enable it to survive, colonize, and infect the human gastrointestinal tract. These processes are complex and are largely regulated by the expression of virulence genes that control colonization, toxin production, and immune evasion.
The major virulence genes of *V. cholerae* and their functions are summarized below.

Table 1. Virulence Genes of *Vibrio cholerae*

Virulence Factor	Function/Role	Refrence
Major Virulence Factor		
Cholera toxin (CTX)	The principal toxin responsible for severe, life-threatening diarrhea (cholera). It is encoded within the filamentous phage genome known as CTXΦ, which carries the ctxA and ctxB genes. The ctxA gene increases intracellular cAMP levels in intestinal epithelial cells, resulting in ion and water secretion into the intestinal lumen and leading to profuse watery diarrhea. The ctxB gene encodes the B subunit that binds to GM1 ganglioside receptors, facilitating entry of subunit A into the host cell.	(Pant, Das and Bhadra, 2019) (Hsiao and Zhu, 2020)
Virulence Accessory Factors		
Toxin-coregulated pilus (TCP)	Facilitates bacterial aggregation and microcolony formation through pilus–pilus interactions in the human intestine. TCP expression is usually coupled with CTX production and also acts as a receptor for CTXΦ, contributing to the evolution of virulence in non-toxigenic *V. cholerae* strains through sequential acquisition of VPI followed by CTXΦ.	(Montero *et al.*, 2023)
Zonula Occludens Toxin (Zot)	Disrupts tight junctions between intestinal epithelial cells, increasing mucosal permeability and inducing diarrhea. Zot also indicates pathogenic potential since it can be found in both CTXΦ and its precursor pre-CTXΦ, thus playing a role in genetic diversification and horizontal transfer of virulence factors among *V. cholerae* strains.	(Lopez-joven *et al.*, 2020)
Accessory Cholera Enterotoxin (Ace)	Alters ion transport in the ileum, leading to mild diarrhea. Ace can trigger early intestinal secretion before CTX acts by stimulating Ca^{2+}-dependent Cl^{-}/HCO_3^{-} symporters, resulting in extracellular Ca^{2+} influx.	(Montero *et al.*, 2023)
Hemolysin (HlyA)	Encodes the El Tor hemolysin, a pore-forming toxin with hemolytic, cytotoxic, and enterotoxic activities. It contributes to mucosal damage and intestinal fluid accumulation. Expression is regulated by complex factors such as HlyU (activator), HapR, and Fur (repressors), and is influenced by environmental conditions including oxidative stress.	(Diez *et al.*, 2023)
Repeat in Toxin (RTX toxin)	Exerts cytotoxic effects on host cells through actin depolymerization and cross-linking, causing cell morphological changes, tissue necrosis, and intestinal inflammation (21).	(Chow, Ng and Yuen, 2001)

Detection Methods

Phenotypic Tests

Phenotypic detection of *Vibrio cholerae* is performed through isolation and identification based on colony morphology, biochemical characteristics, and growth on selective media such as Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. Colonies of *V. cholerae* typically appear yellow on TCBS agar due to their ability to ferment sucrose (Guntina, Agung and Kusuma, 2016). Prior to isolation, environmental samples are

commonly subjected to an enrichment step using alkaline peptone water (APW) at pH 8.6, which selectively promotes the growth of *Vibrio* species due to their tolerance to alkaline conditions and ability to proliferate rapidly under microaerophilic conditions. Following enrichment, presumptive *V. cholerae* colonies may also be isolated on additional selective and differential media such as Tellurite Taurocholate Gelatin Agar (TTGA), where colonies appear translucent with a characteristic dark center and surrounding halo resulting from gelatin hydrolysis, as well as on CHROMagar™ Vibrio, which facilitates differentiation based on species-specific chromogenic reactions. Presumptive isolates are subsequently purified on non-selective media and further characterized using basic biochemical tests, including oxidase activity, which is typically positive for *V. cholerae*. Serogroup determination for epidemiologically significant strains is conducted using slide agglutination assays with O1 and O139 antisera, allowing phenotypic differentiation of pandemic-associated strains from non-O1/non-O139 environmental isolates (Huq *et al.*, 2013).

Genotypic Tests

Gene-based genotypic detection has become the primary method for identifying *V. cholerae* due to its high sensitivity and specificity. Several molecular approaches are commonly used:

1. Conventional PCR

Conventional PCR remains the mainstay method for the initial detection of *V. cholerae* because it is cost-effective, has simple procedures, and can be performed in many laboratories. Common species-specific markers include *ompW* and *toxR*, both known for their high sensitivity and specificity in detecting clinical and environmental strains. To distinguish epidemic serogroups, the rfb O1 and rfb O139 markers are often combined with virulence genes such as *ctxA* or *tcpA*. This multiplex approach allows for simultaneous identification of species, serogroup, and toxigenic status within a single amplification. Such dual-target strategies are particularly valuable in environmental surveillance, minimizing false positives caused by non-cholera *Vibrio* species frequently found in aquatic samples (Kachienga *et al.*, 2024).

2. Real-time PCR (qPCR) and Digital Droplet PCR (ddPCR)

Recent advances in genetic detection technologies have introduced digital droplet PCR (ddPCR) as a complementary or comparative method to real-time quantitative PCR (qPCR). One example of an advanced qPCR application is a quadruplex assay targeting *hlyA*, *rfb O1*, *rfb O139*, and *ctxA* genes simultaneously to determine species identity, serogroup, and toxigenic potential of *V. cholerae*. This assay demonstrates a limit of detection of approximately two colonies per reaction and 100% specificity for *V. cholerae* compared to non-*Vibrio* species (Yang *et al.*, 2023).

3. Sequencing

In addition to whole genome sequencing (WGS), amplicon sequencing—using either the Sanger method or targeted next-generation sequencing (tNGS)—remains an essential strategy for specific genetic characterization of *Vibrio cholerae* (Chaguza *et al.*, 2024). The targeted sequencing approach serves as a bridge between PCR-based molecular screening and comprehensive WGS analysis. During outbreak investigations, a stepwise sequencing strategy can be implemented. Initially, a PCR panel is used for rapid detection of key genetic markers, followed by amplicon sequencing of the *ctxB* and *wbeT* genes for preliminary strain stratification—such as identifying *ctxB7* alleles or determining Ogawa and Inaba serotypes. Subsequently, a subset of representative isolates is selected for WGS. This final stage enables detailed analyses, including determination of single nucleotide polymorphism (SNP) thresholds, mapping of antimicrobial resistance genes such as *SXT*, *R391*, *gyrA*, and *parC*, and phylogenetic tracing across geographical regions. Such a tiered framework ensures rapid response capabilities without compromising the resolution and precision required for public health decision-making (Chaguza *et al.*, 2024)(Lassalle *et al.*, 2023).

A Study On The Detection Of *Vibrio Cholerae* In Seafood

Various studies have reported the presence of *Vibrio cholerae* in seafood products using diverse phenotypic and genotypic approaches. A study on the transmission and toxigenic potential of *V. cholerae* in Hilsha fish (*Tenualosa ilisha*) in Bangladesh demonstrated that the majority of samples were contaminated with *V. cholerae*, with 81% of samples testing positive for the *ompW* gene and 20% harboring the *ctxA* gene.

A total of 158 isolates were identified, comprising serogroups O1 and non-O1/non-O139, highlighting the role of fish as a potential reservoir and transmission vehicle for toxigenic *V. cholerae* (Hossain *et al.*, 2018). Meanwhile, a study conducted in Shanghai involving 12 species of aquatic products revealed that although major virulence genes such as *ctxAB*, *tcpA*, *ace*, and *zot* were not detected, other virulence-associated genes, including *hapA*, *hlyA*, and *rtxCABD*, were frequently identified. This study also reported a high level of antibiotic resistance, with more than half of the isolates exhibiting multidrug resistance patterns (Lassalle *et al.*, 2023). Another study carried out at the Kedonganan Fish Market in Bali highlighted the pheno-genotypic profiles of *V. cholerae* isolated from shrimp and shellfish, where several isolates displayed hemolytic activity and carried the *hlyA* gene; however, some isolates were genotypically positive but did not phenotypically express hemolysis. These findings were further supported by subsequent research at the same location, which detected the *ompW* gene in a high proportion of shrimp samples and at a lower frequency in shellfish, with no detection of the *ctxA* gene. This indicates that *V. cholerae* contamination in local seafood products is predominantly associated with non-toxigenic strains. Overall, these findings underscore the variability in the distribution of *V. cholerae*, virulence profiles, and potential public health risks, which are influenced by the type of seafood commodity, geographical location, and detection methods employed(Praja *et al.*, 2019)(Sukrama *et al.*, 2017)**.**

IV. CONCLUSION

Vibrio cholerae is a pathogenic bacterium responsible for human cholera, typically transmitted through contaminated seafood such as fish and squid, especially when consumed raw or undercooked. Detection of virulence genes such as *ctxA*, *hlyA*, *ompW*, *tcpA*, *zot*, *ace*, and *rtx* is crucial for understanding its colonization, toxicity, and pathogenicity. Identification of *V. cholerae* can be initiated through phenotypic screening using TCBS agar, but confirmation via PCR-based genotypic methods is essential due to their superior sensitivity and specificity. The combination of phenotypic and genotypic approaches provides a more accurate assessment of infection risk. Therefore, fish and squid may serve as important reservoirs of *V. cholerae*, including virulent strains, making molecular detection of toxin genes vital for diagnosis, food safety surveillance, and prevention of seafood-associated cholera outbreaks.

V. ACKNOWLEDGMENT

The author declares that there is no acknowledgment.

VII. COMPETING INTERESTS

The author declares that there is no competing interest.

REFERENCES

[1]. Brauge, T. *et al.* (2024) ‘Sources and Contamination Routes of Seafood with Human Pathogenic *Vibrio spp* .: A Farm-to-Fork approach’, (July 2023), pp. 1–25. doi: 10.1111/1541-4337.13283.

[2]. Chaguza, C. *et al.* (2024) ‘Genomic insights into the 2022 – 2023 *Vibrio cholerae* outbreak in Malawi’, *Nature Communications*, (June 2023), pp. 1–15. doi: 10.1038/s41467-024-50484-w.

[3]. Chow, K. H., Ng, T. K. and Yuen, K. Y. (2001) ‘Detection of RTX Toxin Gene in *Vibrio cholerae* by PCR’, ***Journal of Clinical Microbiology***, 39(7), pp. 2594–2597. doi: 10.1128/JCM.39.7.2594.

[4]. Diez, C. M. P. *et al.* (2023) ‘Increased intracellular persul fi de levels attenuate *HlyU*-mediated hemolysin transcriptional activation in *Vibrio cholerae*’, ***Journal of Biological Chemistry***, 299(9), p. 105147. doi: 10.1016/j.jbc.2023.105147.

[5]. Fernandez, N. L. *et al.* (2020) ‘*Vibrio Cholerae* Adapts to Sessile and Motile Lifestyles by Cyclic di-GMP Regulation of Cell Shape’, *PNAS*, 117. doi: 10.1073/pnas.2010199117.

[6]. Guli, M. M. (2016) ‘Patogenesis Penyakit Kolera Pada Manusia’, 10(2).

[7]. Guntina, R. K., Agung, S. and Kusuma, F. (2016) ‘Deteksi *Vibrio cholerae*’, *Farmaka*, 15, pp. 92–104.

[8]. Herrera, A. and J.F.Satchell, K. (2020) ‘Cross-Kingdom Activation of *Vibrio Toxins* by ADP-Ribosylation Factor Family GTPases’, *American Society for Mikrobiology*, 202(November), pp. 1–15. doi: https://doi.org/10.1128/JB .00278-20.

[9]. Hossain, Z. Z. *et al.* (2018) 'Transmission and Toxigenic Potential of *Vibrio cholerae* in Hilsha Fish (*Tenualosa ilisha*) for Human Consumption in Bangladesh', *Frontier in Microbiology*, 9(February), pp. 1–13. doi: 10.3389/fmicb.2018.00222.

[10]. Hsiao, A. and Zhu, J. (2020) 'Pathogenicity and virulence regulation of *Vibrio cholerae* at the interface of host-gut microbiome interactions Abstract', 11(1), pp. 1582–1599. doi: https://doi.org/10.1080/21505594.2020.1845039 REVIEW.

[11]. Huq, A. *et al.* (2013) 'Detection, Isolation, and Identification of *Vibrio cholerae* from the Environment', *NIH Public Access*, pp. 1–58. doi: 10.1002/9780471729259.mc06a05s26.Detection.

[12]. Jawetz and Adelberg's, M. and (2019) *Medical Microbiology*. 28th Editi.

[13]. Kachienga, L. *et al.* (2024) 'Surveillance of *Vibrio cholerae* serogroups (O1 and O139) from surface and ground water sources in the Vhembe district , Limpopo province , South Africa', *Frontier in Enviromental Science*, (December), pp. 1–11. doi: 10.3389/fenvs.2024.1498893.

[14]. Lassalle, F. *et al.* (2023) 'Genomic Epidemiology Reveals Multidrug Resistant Plasmid Spread Between *Vibrio cholerae* lineages in Yemen', *Nature Microbiology*, 8(October). doi: 10.1038/s41564-023-01472-1.

[15]. Lopez-joven, C. *et al.* (2020) 'Analysis of the *Zonula occludens Toxin* Found in the Genome of the Chilean Non-toxigenic *Vibrio parahaemolyticus* Strain', *Frontier in Cellular and Infection Microbiology*, 10(September), pp. 1–13. doi: 10.3389/fcimb.2020.00482.

[16]. Montero, D. A. *et al.* (2023) 'Vibrio cholerae, Classification, Pathogenesis, Immune Response, and Trends in Vaccine Development', *Frontier in Medicine*, (May). doi: 10.3389/fmed.2023.1155751.

[17]. Mumpuni, F. S. and Hasibuan, S. (2020) 'Prevalensi Mikroba Pada Produk Pindang Tongkol Skala Ukm Di Pelabuhan Ratu, Sukabumi', 21(January 2019). Available at: https://www.researchgate.net/profile/Sawarni-Hasibuan/publication/330243901_Prevalensi_Mikroba_Pada_Produk_Pindang_Tongkol_Skala_Ukm_Di_Pelabuhan_Ratu_Sukabumi/Links/5fd83ccea6fdccdcb8c9c4e1/Prevalensi-Mikroba-Pada-Produk-Pindang-Tongkol-Skala-Ukm-Di-Pelabuhan-Ratu-Sukabumi.pdf?origin=publication_detail&_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InB1YmxpY2F0aW9uRG93bmxvYWQiLCJwcmV2aW91c1BhZ2UiOiJwdWJsaWNhdGlvbiJ9fQ&__cf_chl_tk=gJZG2gkdzokmu85mHZdQjZr3FcW2aBLEOv1KuzyfXaA-1752582061-1.0.1.1-6ICLbOptKHvsPiqjV82rSh3G1kMdtHUPR4Q7HuJaKbo.

[18]. Pant, A., Das, B. and Bhadra, R. K. (2019) 'CTX phage of *Vibrio cholerae*: Genomics and applications', *Elsevier*. doi: https://doi.org/10.1016/j.vaccine.2019.06.034.

[19]. Praja, R. K. *et al.* (2019) 'Detection of Genes Encoding *ompW* and *ctxA* of *Vibrio cholerae* Isolated from Shrimp and Shellfish at Kedonganan Fish Market , Bali-Indonesia', *Ocean Biomedicina Journal*, 2(1), pp. 1–14.

[20]. Sebrina, N. Z. *et al.* (2022) 'Analysis of *Vibrio cholerae* Bacteria in House Flies (*Musca domestica*) Causes Cholera. Analisis Bakteri *Vibrio cholerae* pada Lalat Rumah (*Musca domestica*) Penyebab Kolera', pp. 319–327.

[21]. Siregar, A. M. T. *et al.* (2025) 'Uji Efektifitas Antibakteri Ekstrak Etanol Rimpang Kunyit (*Curcuma Domestic Val*) Terhadap Pertumbuhan Vibrio Cholera Secara In Vitro Antibacterial Effectiveness Of Turmeric Rhizome Ethanol Extract (*Curcuma Domestic Val*) Agus Maruli Tua Siregar Pendahuluan Penyakiti', ***Jurnal Kedokteran STM (Sains dan Teknologi Medik)***, VIII(I), pp. 61–71.

[22]. Sukrama, D. M. *et al.* (2017) 'Pheno-genotypic profile of *Vibrio cholerae hemolysin* (hlyA) isolated from shrimp and shellfish at the Kedonganan fish market , Bali-Indonesia', ***Bali Medical Journal***, (June 2023). doi: 10.15562/bmj.v5i2.231.

[23]. WHO (2024) 'Cholera', *WHO*. Available at: https://www.who.int/news-room/fact-sheets/detail/cholera.

[24]. Yang, J. *et al.* (2023) 'Absolute quantification of viable *Vibrio cholerae* in seawater samples using multiplex droplet digital PCR combined with propidium monoazide', (June), pp. 1–11. doi: 10.3389/fmicb.2023.1149981.

[25]. Yoon, S. H. and Waters, C. M. (2020) '*Vibrio cholerae*', *HSS Public Access*, 27(9), pp. 806–807. doi: 10.1016/j.tim.2019.03.005.Vibrio.