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Original Research Articles

Research Progress on Non-O1 and non-O139 *Vibrio cholerae* in Aquatic Animals and Its Public Health Significance

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Non-O1/non-O139 *Vibrio cholerae* is an important zoonotic pathogen that has gained increasing attention as an emerging pathogen in both aquaculture and public health sectors in recent years. This review summarizes the latest research progress on these pathogens in aquatic animals, covering aspects such as classification and identification, epidemiological characteristics, virulence factors, impact on aquatic animals, antibiotic resistance, prevention and control measures, and zoonotic potential. Studies have shown that non-O1/non-O139 *V. cholerae* are widely distributed in aquaculture environments globally, exhibiting complex host ranges and seasonal variations. These strains possess diverse virulence factors capable of causing various diseases in aquatic animals, resulting in significant economic losses to the aquaculture industry. Concurrently, non-O1/non-O139 *V. cholerae* have demonstrated increasing antibiotic resistance, with the transmission and evolution of resistance genes becoming a major concern. To address these challenges, researchers have made positive strides in vaccine development, biological control, and aquaculture environment management. However, developing broad-spectrum and effective control strategies remains challenging due to the genetic diversity and adaptability of non-O1/non-O139 *V. cholerae*. Furthermore, as potential zoonotic pathogens, non-O1/non-O139 *V. cholerae* pose a threat to food safety and public health through contaminated aquatic products. Future research should focus on genomics, host-pathogen interaction mechanisms, and the development of novel prevention and control strategies. Multidisciplinary collaboration and international cooperation are crucial for a deeper understanding of this complex pathogen and the formulation of effective control measures, which will contribute significantly to the sustainable development of aquaculture and global public health security.

1. INTRODUCTION

Vibrio cholerae is a gram-negative, curved bacterium widely distributed in aquatic environments. Among its serotypes, O1 and O139 are recognized as the primary pathogens responsible for cholera outbreaks.[1,2] However, in recent years, non-O1/non-O139 *V. cholerae* strains have gained increasing importance in aquaculture, attracting widespread attention from researchers.[3-5] Although non-O1/non-O139 *V. cholerae* strains do not cause typical cholera, they have been confirmed to cause human diseases such as diarrhea, wound infections, and septicemia.[6-9] In aquaculture, these strains are considered significant pathogens, capable of causing diseases in various aquatic animals and resulting in substantial economic losses.[10-12] Furthermore, non-O1/non-O139 *V. cholerae* may serve as a reservoir of virulence genes in the environment, potentially influencing the evolution of O1 and O139 *V. cholerae* through horizontal gene transfer.[13]

Aquatic animals play a crucial role in the ecology and epidemiology of non-O1/non-O139 *V. cholerae* as important hosts and vectors.[14] With the rapid global development of aquaculture, the interactions between aquatic animals and non-O1/non-O139 *V. cholerae* have become increasingly complex. This not only affects the sustainable development of aquaculture but may also pose potential threats to public health safety. In recent years, significant progress has been made in the study of non-O1/non-O139 *V. cholerae*, thanks to advances in molecular biology techniques and omics methods. However, many mysteries re-

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main regarding the distribution, pathogenic mechanisms, antimicrobial resistance, and ecological characteristics of these strains in aquatic animals.

This article aims to review the latest advances in research on non-O1/non-O139 *V. cholerae* in aquatic animals, including their classification and identification, epidemiological characteristics, virulence factors, impact on aquatic animals, antimicrobial resistance, prevention and control measures, and public health significance. By systematically examining existing research findings, we hope to provide a reference for a deeper understanding of the biological properties of non-O1/non-O139 *V. cholerae* and offer scientific basis for disease prevention and control in aquaculture, as well as food safety management.

2. CLASSIFICATION AND IDENTIFICATION OF NON-O1/NON-O139 *V. CHOLERAE*

The classification and identification of non-O1/non-O139 *V. cholerae* form the foundation for understanding their biological properties and epidemiological characteristics. As research deepens and technology advances, this field continues to evolve, providing new insights into the classification system of *V. cholerae*.

2.1. SEROLOGICAL CLASSIFICATION

Traditionally, *V. cholerae* classification has been primarily based on the serological characteristics of the O antigen.[15] Currently, over 200 O serogroups of *V. cholerae* are known, with non-O1/non-O139 types comprising the vast majority.[16] Serological typing methods are simple to perform and relatively low-cost, remaining widely used for initial screening of clinical and environmental samples. However, this method has limitations such as cross-reactivity and subjective judgment, making it challenging to meet the needs for precise classification.[17]

2.2. MOLECULAR BIOLOGICAL IDENTIFICATION METHODS

The application of molecular biology techniques has greatly improved the accuracy and efficiency of non-O1/non-O139 *V. cholerae* identification. Multiplex PCR techniques are widely used to detect specific genes, such as the rfb gene cluster (encoding O antigen) and the toxR gene (species-specific regulatory gene).[1,18] 16S rRNA gene sequencing is another commonly used molecular identification method that can provide accurate identification at the genus level.[19] Additionally, Multi-Locus Sequence Typing (MLST) technology, which analyzes sequence variations in multiple housekeeping genes,[20] has provided a powerful tool for typing and evolutionary studies of *V. cholerae*.[13,21,22]

2.3. EMERGING CLASSIFICATION AND IDENTIFICATION TECHNOLOGIES

In recent years, emerging technologies have brought new opportunities for the classification and identification of non-O1/non-O139 *V. cholerae*. The application of Whole Genome Sequencing (WGS) technology has enabled researchers to comprehensively analyze the genomic characteristics of strains, not only allowing for precise classification but also revealing evolutionary relationships between strains.[23-25] Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) technology is gradually becoming an important tool for bacterial identification due to its rapid, accurate, and economical features.[26,27] Furthermore, real-time genomic analysis technology based on nanopore sequencing has shown potential in rapid identification and typing.[28]

Although these new technologies have brought significant advances in the classification and identification of non-O1/non-O139 *V. cholerae*, some challenges remain in practical applications. For example, issues such as standardization and sharing of data between different laboratories, identification in complex environmental samples, and classification of new variant strains still require further research.

In conclusion, the classification and identification methods for non-O1/non-O139 *V. cholerae* are transitioning from traditional phenotypic analysis to genome-based precise typing. This trend not only improves the accuracy of classification but also provides new perspectives for in-depth understanding of the evolution and adaptability of non-O1/non-O139 *V. cholerae*. In the future, with continuous technological advancements and database improvements, we hope to establish a more comprehensive and precise classification system for non-O1/non-O139 *V. cholerae*, providing more reliable scientific basis for disease prevention and control in aquaculture and public health safety.

3. EPIDEMIOLOGY OF NON-O1/NON-O139 *V. CHOLERAE* IN AQUATIC ANIMALS

Epidemiological studies of non-O1/non-O139 *V. cholerae* in aquatic animals are crucial for understanding their ecological distribution, transmission dynamics, and potential public health risks. In recent years, with advances in monitoring techniques and expanded research scope, we have gained a deeper understanding of the epidemiological characteristics of these pathogens in aquaculture environments.

3.1. GEOGRAPHICAL DISTRIBUTION

Non-O1/non-O139 *V. cholerae* are widely distributed in aquaculture environments globally.[29,30] Studies have shown that these strains can be isolated from freshwater, saltwater, and brackish water aquaculture systems across all continents.[29,31] In China, India, and Southeast Asian countries, the detection rates of non-O1/non-O139 *V. cholerae* are relatively high due to intensive aquaculture activities.[12,30,32-34] Coastal waters and farms in Europe and the Americas have also reported the presence of these strains.[5,35-37] Notably, strains from different geographical regions may exhibit genetic diversity, reflecting the results of local adaptive evolution .[38]

3.2. HOST RANGE

Non-O1/non-O139 *V. cholerae* demonstrates a wide range of aquatic animal hosts, spanning multiple ecological niches. These hosts include economically important fish species such as carp (*Cyprinus carpio*),[39,40] Zebrafish (*Danio rerio*),[41] tilapia (*Oreochromis niloticus*),[42] bluegill sunfish (*Lepomis macrochirus*)[43] and pink-tailed chalceus (*Chalceus macrolepidotus*)[44]; crustaceans like giant freshwater prawn (*Macrobrachium rosenbergii*)[10,45] and red swamp crayfish (*Procambarus clarkia*)[46]; and mollusks such as oysters (*Crassostrea gigas*)[47,48] and mussels (*Mytilus edulis*).[49] Furthermore, amphibians like the American bullfrog (*Rana catesbeiana*),[43] aquatic mammals such as dolphins (*Tursiops truncatus*)[50] have also been identified as hosts. This diverse host range not only reflects the ecological adaptability of non-O1/non-O139 *V. cholerae* but also highlights its potential significance in aquaculture and aquatic ecosystem health. These findings provide a foundation for further research into the ecology, evolution, and potential health impacts of these strains.

3.3. SEASONAL VARIATIONS

The prevalence of non-O1/non-O139 *V. cholerae* in the environment exhibits distinct seasonal patterns, influenced by various environmental factors and displaying differentiated characteristics across geographical regions. In temperate areas, such as Southern California and Mediterranean coasts, the highest detection rates are typically observed during summer and early autumn, primarily associated with rising water temperatures.[51,52] Water temperature is considered one of the key factors affecting the survival and growth of *V. cholerae*.[53,54] In contrast, seasonal variations in tropical and subtropical regions, like Bangladesh, are mainly influenced by alternating rainy and dry seasons, with increased detection rates during the rainy season possibly linked to elevated nutrient levels and altered hydrological conditions due to rainfall.[55,56] Besides temperature and precipitation, factors such as salinity, pH, and plankton abundance may also influence the seasonal distribution of *V. cholerae*.[57] This seasonal pattern not only reflects the adaptability of non-O1/non-O139 *V. cholerae* to environmental conditions but may also be related to its ability to enter a viable but non-culturable (VBNC) state and subsequent reactivation.[1,2] Understanding these seasonal patterns is crucial for developing targeted monitoring and prevention strategies, with intensified surveillance and preventive measures during high-risk seasons potentially helping to mitigate public health risks. Overall, the seasonal pattern of non-O1/non-O139 *V. cholerae* is an essential component of its ecological characteristics, reflecting the complex interactions between these bacteria and their environment. This provides important clues for understanding and predicting the environmental dynamics of *V. cholerae*, while also offering valuable guidance for public health management.

3.4. INFLUENCE OF ENVIRONMENTAL FACTORS

Multiple studies have shown that environmental factors significantly influence the distribution and abundance of non-O1/non-O139 *V. cholerae*. Water temperature is one of the most critical factors, with optimal growth occurring between 30°C and 37°C, although *V. cholerae* can grow at temperatures ranging from 10°C to 43°C.[53,56] A significant increase in *V. cholerae* abundance has been observed when water temperatures exceed 17°C.[58] Salinity is also an important parameter, with *V. cholerae* tolerating a wide range from 0 to 45 parts per thousand (ppt), but optimal growth occurs at salinities between 2 and 14 ppt.[59,60] Additionally, factors such as pH, dissolved oxygen, and organic matter content affect the survival and reproduction of these strains. *V. cholerae* can survive in pH ranges from 5.0 to 9.6, with optimal growth at pH 7.6 to 8.6.[61,62] As a facultative anaerobe, *V. cholerae* grows best in aerobic conditions, with oxygen concentrations above 0.5 mg/L supporting growth.[60,63] High levels of organic matter, particularly chitin, promote *V. cholerae* growth, with concentrations of dissolved organic carbon above 3 mg/L associated with increased abundance.[53,64]

It's noteworthy that global climate change may alter these environmental factors, thereby impacting the prevalence dynamics of non-O1/non-O139 *V. cholerae*.[65] The complex interactions between these factors create specific ecological niches favorable for *V. cholerae* growth. For instance, Huq et al.[56] found that the combination of water temperatures above 25°C, pH between 7 and 8.5, and salinity between 0.2 and 2.0 ppt was particularly conducive to *V. cholerae* growth in aquatic environments.

In recent years, the application of new technologies such as metagenomics and ecological network analysis has enabled a comprehensive understanding of the position and role of non-O1/non-O139 *V. cholerae* in aquaculture ecosystems.[66] These studies not only reveal the complex relationships between strains and environmental factors but also provide new perspectives for understanding their interactions with other microbial communities.[67] For instance, Nandi et al.[68] found that certain non-O1/non-O139 *V. cholerae* strains may play important ecological roles in aquatic environments.

Overall, the epidemiological characteristics of non-O1/non-O139 *V. cholerae* in aquatic animals present complex spatiotemporal dynamics.[69] Future research needs to further integrate multidisciplinary approaches from molecular epidemiology, ecology, and climate science to better predict and control the spread of these potential pathogens.[58] Simultaneously, establishing long-term, systematic monitoring networks is crucial for assessing the potential risks of non-O1/non-O139 *V. cholerae* to the aquaculture industry and public health.[70] Recent studies have also highlighted the potential role of non-O1/non-O139 *V. cholerae* in the spread of antibiotic resistance, further emphasizing the importance of continuous monitoring.[71]

4. IMPACT OF NON-O1/NON-O139 *V. CHOLERAE* ON AQUATIC ANIMALS

4.1. PATHOGENESIS MECHANISM

The pathogenesis mechanism of non-O1/non-O139 *V. cholerae* in aquatic animals is a complex multi-stage process involving the synergistic action of multiple virulence factors. This process begins with the adhesion and colonization of bacteria to host surface structures through MSHA pili, TCP (toxin co-regulated pilus), and other adhesion factors such as OmpU, which is a crucial step in establishing infection.[72,73] Subsequently, bacteria secrete enzymes such as hemolysin (HlyA), extracellular protease (PrtV), and chitinase to break down host tissue barriers, promoting further invasion and spread.[74] HlyA has been shown to form pores in erythrocytes and other cell types, contributing to tissue damage and inflammation.[75] Various toxins directly act on host cells, leading to cellular dysfunction and tissue damage. These include the heat-stable enterotoxin (Stn/Sto), Cholix toxin, and the multifunctional autoprocessing repeats-in-toxin (MARTX) toxin.[76,77] The MARTX toxin, for example, has been found to disrupt the actin cytoskeleton of host cells, facilitating bacterial invasion.[78]

Some strains may also evade immune clearance by altering surface antigens or interfering with host immune responses. The O-antigen capsule and the VPS exopolysaccharide have been implicated in immune evasion and biofilm formation, respectively, enhancing bacterial survival in the host.[79,80] Additionally, the quorum sensing system, regulated by the CAI-1 and AI-2 autoinducers, allows bacteria to coordinate group behavior, expressing virulence genes at appropriate times to enhance pathogenicity.[81] This system has been shown to control the expression of virulence factors such as proteases and the cholera toxin gene in a density-dependent manner.[82] Recent studies have also highlighted the role of type VI secretion systems (T6SS) in bacterial competition and virulence. T6SS has been found to deliver toxic effectors to both prokaryotic and eukaryotic cells, potentially contributing to the pathogenesis of non-O1/non-O139 *V. cholerae* in aquatic hosts.[83]

Understanding these complex pathogenesis mechanisms is crucial for developing effective prevention and treatment strategies for non-O1/non-O139 *V. cholerae* infections in aquaculture and for assessing their potential impact on public health. The multifaceted nature of non-O1/non-O139 *V. cholerae* pathogenesis, involving adhesion, tissue invasion, toxin production, immune evasion, and coordinated virulence expression, underscores the adaptability and potential threat of these strains in aquatic environments. Future research should focus on elucidating the specific roles of these virulence factors in different aquatic hosts and environmental conditions, as well as exploring potential targets for intervention strategies to mitigate the impact of non-O1/non-O139 *V. cholerae* in aquaculture settings.

4.2. COMMON SYMPTOMS AND PATHOLOGICAL CHANGES

Non-O1/non-O139 *V. cholerae* infections in aquatic animals manifest with diverse symptoms and pathological changes, varying according to host species, infection site, and severity. Fish, crustaceans, and mollusks, as the main groups of aquatic animals, present different clinical manifestations and pathological features upon infection (**Table 1**). This diversity reflects the variation in host physiological structures and immune responses, and is closely related to environmental factors and pathogen strain variability.[58,69]

In terms of symptom development speed and external manifestations, fish and shrimp typically present more acute and obvious symptoms, such as skin ulcers, fin rot, and abnormal movement, usually appearing within 2-7 days post-infection.[84,85] In contrast, mollusks and some crab species tend to exhibit a more chronic disease course, with less obvious external symptoms like weakened shell closure response and mantle retraction, which may become apparent only weeks after infection.[87,88] This difference partly stems from the varying complexity and efficiency of immune systems among species, with fish typically showing a more pronounced inflammatory response, while mollusks demonstrate a relatively weaker immune reaction.[89]

Internal pathological changes and histological features also vary among species. Fish commonly exhibit enlargement and lesions in the liver, kidney, and spleen; crustaceans may show hepatopancreas enlargement and muscle liquefaction; while mollusks often present with digestive gland atrophy and soft tissue liquefaction.[37,86,90] These differences are closely related to the unique physiological structures of each species, for example, the hepatopancreas in crustaceans and the digestive gland in mollusks are often primary target organs. Histological examination typically reveals cell degeneration and necrosis in corresponding organs, as well as varying degrees of inflammatory response and immune cell infiltration. Understanding these species-specific responses is crucial for developing effective diagnostic and control strategies, and provides important insights for assessing the potential impact of non-O1/non-O139 *V. cholerae* on aquaculture and public health.[91]

It should be noted that the severity of symptoms and pathological changes may vary depending on environmental conditions, host health status, and the virulence of the pathogen strain. Moreover, some symptoms may overlap among different species, necessitating comprehensive consideration of multiple factors in actual diagnosis. Future research should further explore the molecular mechanisms underlying these differences to develop more precise and species-specific intervention measures.

5. OVERVIEW OF VIRULENCE FACTORS IN NON-O1/NON-O139 *V. CHOLERAE*

Although non-O1/non-O139 *V. cholerae* do not produce the classical cholera toxin, they possess various virulence factors that enable them to cause disease in aquatic animals and potentially pose a threat to human health. This section

Table 1. Comparative Overview of Symptoms and Pathological Changes in Aquatic Animals Infected with Non-O1/non-O139 *V. cholerae*

Items	Fish[43,84]	Crustaceans[85,86]	Mollusks[87,88]
External symptoms	- Skin ulcers - Fin rot - Exophthalmos - Abdominal distension - Gill discoloration and necrosis - Hemorrhagic spots on body surface - Scale loss	- Sluggish movement - Decreased appetite - Shell softening - Appendage and tail fan necrosis - Body color darkening - Spots on shell surface - Antenna atrophy	- Weakened shell closure response - Mantle retraction - Abnormal shell edges - Foot swelling - Increased mucus secretion - Incomplete siphon retraction
Internal pathological changes	- Liver enlargement, paleness - Kidney enlargement, congestion - Spleen enlargement - Intestinal congestion, hemorrhage - Ascites accumulation - Pericardial effusion	- Hepatopancreas enlargement, whitening - Gill discoloration, necrosis - Muscle whitening, liquefaction - Hemolymph coagulation - Digestive tract congestion	- Soft tissue liquefaction - Digestive gland atrophy - Gill tissue damage - Pericardial edema - Gonad degeneration
Histological changes	- Epidermis and dermis inflammation - Hepatocyte degeneration and necrosis - Renal tubular epithelial cell degeneration - Splenic blood cell proliferation - Intestinal mucosa shedding	- Hepatopancreatic tubular epithelial cell necrosis - Hemolymph organ structure disorder - Gill epithelial cell shedding - Muscle fiber necrosis - Neural tissue degeneration	- Digestive gland epithelial cell degeneration - Gill epithelial cell shedding - Mantle connective tissue edema - Blood cell infiltration - Germ cell degeneration
Symptom development speed	- Acute, obvious symptoms typically within 2-7 days post-infection	- Acute in shrimp (1-3 days), more chronic in crabs (5-10 days)	- Chronic, symptoms may become apparent weeks after infection
Main target organs	- Liver, kidney, skin, gills	- Hepatopancreas, gills, muscle	- Digestive gland, gills, mantle
Immune response characteristics	- Obvious inflammatory response - Blood cell aggregation - Increased phagocyte activity - Antimicrobial peptide production	- Blood cell infiltration - Organ structure disorder - Coagulation response - Phenoloxidase system activation	- Relatively weak immune response - Blood cell migration - Increased lysozyme activity

provides an overview of the virulence factors of non-O1/non-O139 *V. cholerae* from two aspects: virulence genes and their mechanisms of action and pathogenesis, and the diversity and adaptability of virulence genes.

5.1. VIRULENCE GENES AND THEIR MECHANISMS OF ACTION AND PATHOGENESIS

Non-O1/non-O139 *V. cholerae* strains possess a diverse array of virulence factors that contribute to their pathogenicity. These virulence genes encode various proteins and toxins that play crucial roles in bacterial colonization, invasion, and the induction of host cellular responses (**Table 2**). The virulence factors can be broadly categorized into toxins, adhesins, secretion systems, and regulatory proteins. Collectively, these factors enable non-O1/non-O139 *V. cholerae* to attach to host cells, evade host immune responses, disrupt cellular functions, and cause tissue damage.[75,84]

The expression of these virulence factors is controlled by a complex regulatory network, including quorum sensing (QS) systems, ToxR regulatory systems, and various global regulators.[102] Environmental factors such as temperature, salinity, and pH also significantly affect the expression of virulence genes, allowing non-O1/non-O139 *V. cholerae* to adapt to different ecological niches.[54]

In addition to the well-established virulence factors, recent studies have identified new mechanisms contributing to the pathogenicity of non-O1/non-O139 *V. cholerae*. Outer Membrane Vesicles (OMVs) have been found to play a significant role in bacteria-host interactions, carrying various virulence factors including proteases, hemolysins, and DNA.[1,2] Some strains also possess CRISPR-Cas systems, which not only provide defense against phage infection but may also be involved in virulence regulation.[104] Furthermore, the discovery of small RNAs such as VqmR and TarA has revealed an additional layer of virulence regulation in these bacteria.[105]

The complexity of virulence regulation in non-O1/non-O139 *V. cholerae* extends beyond these newly identified factors. In addition to the well-known ToxR system, other two-component systems like VarS/VarA have been implicated in virulence regulation.[106] This diversity and complexity of virulence factors underscore the pathogenic potential of non-O1/non-O139 *V. cholerae*. Understanding the functions and regulatory mechanisms of these factors is crucial for developing targeted prevention and treatment strategies. Future research should focus on exploring the expression patterns of these virulence factors under various host

Table 2. Summarizes the main virulence genes, their encoded products, and their mechanisms of action and pathogenesis

Virulence Gene	Encoded Product	Mechanism of Action and Pathogenesis
hlyA	Hemolysin	Causes red blood cell lysis and tissue damage[75]
stn/sto	Heat-stable enterotoxin	Activates guanylate cyclase in intestinal epithelial cells, leading to increased secretion of water and electrolytes, causing diarrhea[92]
prtV	Extracellular protease	Participates in the degradation of host tissue proteins, associated with tissue invasion[93]
T3SS	Type III secretion system	Directly injects virulence proteins into host cells, interfering with cell signaling pathways and cytoskeletal structures[94]
msha	Mannose-sensitive hemagglutinin	Mediates initial contact between bacteria and host cells, promotes biofilm formation[73]
chxA	Cholix toxin	Inhibits host cell protein synthesis through ADP-ribosylation[95]
rtxA	Repeat-in-toxin	Causes cytoskeleton reorganization, increases cell permeability[78]
hapA	Hemagglutinin/ protease	Degrades intestinal mucus, promotes bacterial colonization[74]
tlh	Thermolabile hemolysin	Increases cell membrane permeability, leads to cell lysis[96]
ace	Accessory cholera enterotoxin	Increases intestinal ion secretion, causes diarrhea[97]
zot	Zonula occludens toxin	Increases permeability of intestinal tight junctions[98]
pilA	Type IV pili	Involved in bacterial attachment and biofilm formation[99]
nanH	Neuraminidase	Degrades sialic acid in mucus, promotes bacterial colonization[100]
vvhA	Cytolysin	Forms membrane pores, leads to cell lysis[101]
toxR	Transcriptional regulator	Regulates expression of multiple virulence genes[102]
vasH	T6SS regulator	Regulates expression of the Type VI secretion system[103]

and environmental conditions, as well as their interactions. Such studies will provide new insights for controlling non-O1/non-O139 *V. cholerae* infections and may lead to novel therapeutic approaches.

5.2. DIVERSITY AND ADAPTABILITY OF VIRULENCE GENES

Non-O1/non-O139 *V. cholerae* strains exhibit remarkable diversity and adaptability in their virulence genes, which is key to their successful colonization and pathogenicity. These strains possess a highly plastic genome, capable of rapidly acquiring or losing virulence factors through mechanisms such as horizontal gene transfer, genetic recombination, and mutation.[107] This genetic plasticity allows non-O1/non-O139 *V. cholerae* to adapt to various ecological niches, including aquatic environments and human hosts.[69] The expression of virulence genes is controlled by complex regulatory networks, including quorum sensing systems, ToxR regulatory pathways, and other global regulators, enabling bacteria to flexibly adjust their virulence phenotypes in response to environmental conditions such as temperature, pH, and osmotic pressure.[102,108] For instance, the ToxR system not only regulates classical virulence genes like cholera toxin (CT) and toxin co-regulated pilus (TCP) but also influences the expression of other virulence factors such as the outer membrane protein OmpU.[109] Moreover, these strains often carry multiple virulence factors with overlapping functions, providing functional redundancy and enhancing their survival capabilities in different environments.

In recent years, the application of high-throughput sequencing technologies has enabled a more comprehensive understanding of the diversity of virulence genes in non-O1/non-O139 *V. cholerae*.[110] Studies have found that even within the same geographical region, there can be significant differences in virulence gene composition between different strains. Kirchberger et al.[111] used whole-genome sequencing to analyze non-O1/non-O139 *V. cholerae* strains from coastal environments, revealing high diversity in virulence gene composition and identifying new virulence factors. Furthermore, the adaptability of non-O1/non-O139 *V. cholerae* is also reflected in their antibiotic resistance. Research has shown that these strains can develop multiple drug resistance by acquiring resistance genes or mutations, further increasing their survival ability and public health threat.[112] Another noteworthy aspect is the interaction between non-O1/non-O139 *V. cholerae* and environmental microbial communities. These interactions may influence the expression and spread of virulence genes. Purdy et al.[113] found that certain environmental factors can induce non-O1/non-O139 *V. cholerae* to enter a viable but non-culturable (VBNC) state, which may affect the expression and detection of their virulence genes.

In conclusion, non-O1/non-O139 *V. cholerae* possess diverse virulence factors and complex pathogenic mechanisms. The diversity and adaptability of their virulence genes enable them to be widely distributed in aquatic animals and potentially pose a threat to human health. Future research should focus on elucidating the mechanisms of action of these virulence factors in different hosts, exploring the influence of environmental factors on virulence gene expression, and studying the horizontal transfer and evolution of virulence genes. Additionally, developing new rapid detection methods and effective prevention strategies are important directions for future research. These studies will provide important bases for developing targeted prevention and control strategies and assessing public health risks. The diversity of virulence genes also leads to a variety of clinical manifestations in non-O1/non-O139 *V. cholerae* infections, ranging from mild gastrointestinal symptoms to severe systemic infections.[114] This genetic and phenotypic diversity not only increases the environmental adaptability and pathogenic potential of non-O1/non-O139 *V. cholerae* but also poses challenges for disease diagnosis, treatment, and prevention, highlighting the importance of continuous monitoring and research on these emerging pathogens.

6. ANTIBIOTIC RESISTANCE IN NON-O1/NON-O139 *V. CHOLERAE*

6.1. COMMON RESISTANCE PATTERNS

Antibiotic resistance in non-O1/non-O139 *V. cholerae* (non-O1/non-O139 strains) has become a major challenge for global aquaculture and public health sectors. In recent years, these strains have exhibited complex and diverse resistance patterns, with a significant increase in the proportion of multidrug-resistant (MDR) strains. Liu et al.[115] reported that the percentage of MDR strains isolated from coastal regions in China rose from 15% in 2010 to 35% in 2020. Resistance is not only observed in commonly used antibiotics such as β-lactams, tetracyclines, and fluoroquinolones but also shows distinct geographical distribution differences and host specificity.[110,116]

6.2. RESISTANCE MECHANISMS

These bacteria acquire resistance through various mechanisms, primarily including enzyme-mediated resistance (e.g., production of β-lactamases and aminoglycoside-modifying enzymes), target site alterations (e.g., mutations in DNA gyrase and topoisomerase IV), enhanced efflux pump systems (e.g., RND family efflux pumps), and reduced membrane permeability (**Table 3**). Wang et al.[117] also discovered a novel tetracycline resistance mechanism involving tet(X) gene mutations, further complicating resistance control efforts.

The diversity and complexity of these resistance mechanisms highlight the severity of antibiotic resistance in non-O1/non-O139 *V. cholerae* strains. Understanding these mechanisms is crucial for developing new antibiotics and formulating effective resistance control strategies. Future research should continue to monitor resistance trends, explore new resistance mechanisms, and develop innovative treatment approaches to address this increasingly serious problem.[91]

6.3. TRANSMISSION AND EVOLUTION OF RESISTANCE

The transmission and evolution of antibiotic resistance in non-O1/non-O139 *V. cholerae* is a complex process, primarily achieved through various mechanisms of horizontal gene transfer. Ceccarelli et al.[110] found that up to 70% of non-O1/non-O139 *V. cholerae* strains isolated from the Chesapeake Bay carried multiple resistance genes, typically located on mobile genetic elements such as integrons and transposons. Rapa et al.[124] further confirmed the importance of integrons, particularly class 1 integrons, in facilitating the horizontal transfer of resistance genes in the environment. Beyond integrons, Spagnoletti et al.[125] studied the role of SXT/R391 family integrative conjugative elements (ICEs) in *V. cholerae*, discovering that these ICEs could efficiently transfer multiple antibiotic resistance genes between different *V. cholerae* strains. Additionally, Wang et al.[126] identified multiple transferable resistance plasmids in non-O1/non-O139 *V. cholerae* isolated from coastal regions of China, further illustrating the diversity of horizontal gene transfer. Environmental factors, such as excessive antibiotic use and high-density aquaculture, significantly influence the spread of resistance. Ramamurthy et al.[127] pointed out that the improper use of antibiotics in aquaculture may accelerate the selection and spread of resistant strains, posing a potential threat to public health.

Globalization and climate change also profoundly impact the spread of resistance. Baker-Austin et al.[91] reviewed how climate change might influence the ecology and epidemiology of *V. cholerae* through various mechanisms, including altering its geographical distribution and seasonal patterns. Vezzulli et al.[58] demonstrated a significant correlation between rising water temperatures in the coastal North Atlantic over the past 50 years and an increase in *V. cholerae* abundance, which may accelerate the spread of resistance genes. Furthermore, Verma et al.[112] revealed how the genomic plasticity of *V. cholerae* facilitates its acquisition of new antibiotic resistance, enabling these bacteria to survive in different environments and acquire new resistance characteristics through adaptive evolution.

Addressing the challenge of antibiotic resistance in non-O1/non-O139 *V. cholerae* requires a multi-pronged, comprehensive strategy. The World Health Organization[128] emphasized the importance of establishing comprehensive resistance monitoring systems in its Global Antimicrobial Resistance and Use Surveillance System (GLASS) report. Meanwhile, new detection technologies are continually developing. For example, Li et al.[129] developed a microfluidics-based method for real-time study of the rapid spread of antibiotic resistance plasmids in biofilms. This innovative technology provides new tools for understanding the dynamics of resistance spread in microenvironments. Only through multidisciplinary collaboration, integrating microbiology, ecology, epidemiology, and policy research, can we more comprehensively understand and control the antibi-

Table 3. Antibiotic Resistance Mechanisms in Non-O1/non-O139 *V. cholerae* Strains

Resistance Mechanism Type	Specific Mechanism	Examples	Impact
Enzyme-mediated resistance	β-lactamases	ESBL, MBL (e.g., blaCTX-M, blaNDM-1)	Degrade β-lactam antibiotics[118]
	Aminoglycoside-modifying enzymes	AAC(6')-Ib, APH(3')-Ia	Modify and reduce aminoglycoside antibiotic activity[119]
Target site alteration	DNA gyrase and topoisomerase IV mutations	Mutations in gyrA and parC genes	Cause resistance to quinolone antibiotics[103]
	Ribosomal protein mutations	23S rRNA methylation	Induce resistance to macrolides and chloramphenicol[120]
Efflux pump systems	RND family efflux pumps	AcrAB-TolC system	Pump out various antibiotics, leading to multidrug resistance[121]
	Novel tetracycline resistance mechanism	tet(X) gene variation	Enhance tetracycline efflux efficiency[122]
Reduced membrane permeability	Altered outer membrane protein (OMP) expression	Downregulation of OmpU and OmpT	Reduce antibiotic entry into cells[123]
Biofilm formation	-	-	Increase tolerance to antibiotics[80]

otic resistance of non-O1/non-O139 *V. cholerae*, providing strong support for the sustainable development of aquaculture and public health safety.

7. PREVENTION AND CONTROL MEASURES

The prevention and control of non-O1/non-O139 *V. cholerae* strains pose significant challenges for both the aquaculture industry and public health sectors. Effective prevention and control strategies require comprehensive measures, including aquaculture environment management, vaccine development, biological control, and rational use of antibiotics.

7.1. AQUACULTURE ENVIRONMENT MANAGEMENT

Aquaculture environment management is fundamental to controlling the spread of non-O1/non-O139 *V. cholerae*. Hossain et al.[130] demonstrated that implementing good aquaculture management practices can significantly reduce the number of *V. cholerae* strains in the environment. Specific measures include optimizing water quality parameters (such as pH, salinity, and dissolved oxygen), regular disinfection and substrate improvement, and implementing biosecurity measures. For example, Krummenauer et al.[131] found that using biofloc technology in shrimp farming can effectively improve water quality, reduce the number of pathogens, and enhance the health status of cultured organisms. These environmental management strategies not only directly reduce the number of pathogens but also indirectly lower antibiotic use by improving the health of cultured organisms, thereby reducing the development and spread of resistance. Furthermore, Rameshkumar et al.[132] showed that implementing comprehensive aquaculture management measures, including proper water quality management and probiotic supplementation, can significantly decrease the prevalence of *Vibrio* species in shrimp aquaculture systems. These measures can also help maintain the balance of aquaculture ecosystems, reducing the occurrence of environmental conditions favorable for the growth and gene transfer of *V. cholerae*.

7.2. PROGRESS IN VACCINE DEVELOPMENT RESEARCH

Vaccine development is a crucial direction for preventing non-O1/non-O139 *V. cholerae* infections. Although no commercial vaccines are currently available for non-O1/non-O139 *V. cholerae*, research progress is encouraging. Yan et al.[133] studied the adhesion characteristics of pathogenic *V. alginolyticus* to the intestinal mucus of large yellow croaker, providing an important foundation for developing adhesion inhibitors and vaccines against *Vibrio* species. Furthermore, Luan et al.[134] expressed and characterized a metalloprotease from *Vibrio parahaemolyticus*, which could potentially serve as a vaccine target. However, developing a broad-spectrum effective vaccine remains challenging due to the high genetic diversity of non-O1/non-O139 *V. cholerae*.

7.3. BIOLOGICAL CONTROL STRATEGIES

Biological control strategies, as environmentally friendly alternatives, have gained widespread attention in recent years. These strategies mainly include probiotics, plant extracts, and phage therapy. Sha et al.[135] found that lactic acid bacteria isolated from healthy *Litopenaeus vannamei* could effectively inhibit the growth of *Vibrio* species and enhance host immune responses. Regarding plant extracts, Packiavathy et al.[136] demonstrated that curcumin has significant inhibitory effects on biofilm formation of *V. cholerae*. As an emerging strategy, phage therapy has shown promise, with Yen et al.[137] successfully isolating phages that specifically lyse *V. cholerae*, providing potential for developing new biological control agents.

7.4. RATIONAL USE OF ANTIBIOTICS

The rational use of antibiotics is key to controlling the spread of resistance. Many countries have begun implementing stricter antibiotic use management policies.Watts et al.[138] reviewed the status and trends of antibiotic use in aquaculture, emphasizing the importance of implementing antibiotic stewardship programs. Santos and Ramos[139] proposed a precision medication model based on rapid pathogen diagnosis and antibiotic sensitivity testing, which can significantly reduce antibiotic use while maintaining therapeutic efficacy. Furthermore, Deng et al.[140] demonstrated that the stock density in shrimp aquaculture affects the microbial community in biofloc water and shrimp gut microbiota, which has implications for disease management and antibiotic use strategies.

8. ZOONOTIC POTENTIAL OF NON-O1/NON-O139 *V. CHOLERAE*

As potential zoonotic pathogens, non-O1/non-O139 *V. cholerae* warrant in-depth research into their transmission routes from aquatic animals to humans, public health risks, and food safety control measures.

8.1. TRANSMISSION ROUTES FROM AQUATIC ANIMALS TO HUMANS

Non-O1/non-O139 *V. cholerae* primarily spread to humans through consumption of contaminated seafood or contact with polluted water bodies. Halpern et al.[141] found that fish can act as reservoirs for *V. cholerae*, including non-O1/non-O139 strains, potentially facilitating their transmission to humans. Additionally, occupational exposure (such as fishermen and aquatic product processing workers) is also an important transmission route. Senderovich et al.[84] demonstrated that non-O1/non-O139 *V. cholerae* could be isolated from fish in freshwater habitats, suggesting a potential risk for individuals in close contact with these environments.

8.2. PUBLIC HEALTH RISK ASSESSMENT

Assessing the public health risks of non-O1/non-O139 *V. cholerae* is fundamental to developing prevention and control strategies. Baker-Austin et al.[91] reviewed the increasing incidence of *Vibrio* infections, including those caused by non-O1/non-O139 *V. cholerae*, in the context of climate change. They highlighted that warming coastal waters could lead to increased exposure risks, particularly in temperate regions. However, actual infection numbers may be underestimated due to often mild symptoms and potential misdiagnosis.

8.3. FOOD SAFETY CONTROL MEASURES

Given that food is the main route of transmission for non-O1/non-O139 *V. cholerae*, strengthening food safety control is crucial. Wei et al.[142] developed multiplex PCR assays for the simultaneous detection of several *Vibrio* species, including *V. cholerae*, V. parahaemolyticus, *V. vulnificus*, and *V. alginolyticus*, with an internal amplification control. This method provides a rapid, sensitive, and specific tool for detecting potentially pathogenic *Vibrio* species, including non-O1/non-O139 *V. cholerae*, in various samples. The inclusion of an internal amplification control enhances the reliability of the assay by reducing false-negative results. This rapid detection technology offers a powerful tool for food safety regulation, allowing for the efficient identification of potentially harmful non-O1/non-O139 *V. cholerae* strains in food products. The authors reported that the detection limit of their multiplex PCR assay was 10^3 CFU/mL for pure cultures and 10^4 CFU/mL for spiked oyster samples, demonstrating its potential for practical application in food safety monitoring. Additionally, Pruzzo et al.[143] reviewed the persistence mechanisms of *Vibrio* species in marine environments, which has implications for developing effective control strategies in aquaculture and seafood processing. Their work highlights the importance of understanding the ecological factors that contribute to the survival and spread of non-O1/non-O139 *V. cholerae* in aquatic environments and food chains.

9. FUTURE PERSPECTIVES

While significant progress has been made in the study of non-O1/non-O139 *V. cholerae*, numerous challenges remain. Future research should focus on three main areas: genomics, host-pathogen interaction mechanisms, and the development of novel prevention and control strategies. In the field of genomics, researchers should concentrate on comparative genomics analysis to understand evolutionary relationships between different serotypes and ecotypes, pan-genome analysis to identify core and accessory genes contributing to virulence and environmental adaptation, and functional genomics studies to elucidate gene expression regulation under various environmental conditions. Additionally, metagenomics approaches will be crucial in understanding the role of non-O1/non-O139 *V. cholerae* in microbial communities.

Exploring host-pathogen interaction mechanisms is vital for developing effective prevention and control strategies. Future studies should utilize advanced in vitro models to simulate the infection process in different host species, investigate how non-O1/non-O139 *V. cholerae* evade host immune systems, and explore their interactions with the host microbiome. Identifying new virulence factors and their roles in pathogenesis will also be crucial. In terms of prevention and control strategies, research should focus on developing next-generation broad-spectrum vaccines, exploring the application of immunomodulators to enhance host resistance, developing smart drug delivery systems for targeted therapy, and utilizing synthetic biology techniques to design functional probiotics. Novel biocontrol methods, such as phage therapy or CRISPR-based approaches, also warrant investigation.

Future research should also address improving rapid detection methods for non-O1/non-O139 *V. cholerae* in environmental and clinical samples, developing predictive models for outbreaks based on environmental and climatic

factors, studying the impact of climate change on the distribution and virulence of these strains, and investigating their role in horizontal gene transfer of virulence and antibiotic resistance genes in aquatic environments. This field is full of opportunities and challenges, requiring multidisciplinary collaboration and international cooperation to integrate research efforts from microbiology, ecology, immunology, genomics, and other fields. This comprehensive approach will lead to a better understanding of this complex pathogen and the development of more effective prevention and control strategies, ultimately contributing to the sustainable development of aquaculture and global public health security.

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COMPETING INTEREST

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