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Prevalence, Virulence, and Antibiotic Resistance of *Vibrio parahaemolyticus* from Seafood and its Environment: An Updated Review

Deniz Ürünleri ve Çevresinde *Vibrio parahaemolyticus*'un Yaygınlık, Virülans ve Antibiyotik Direnç Profilleri: Güncel Bir Derleme

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Abstract

Vibrio parahaemolyticus is a curved, rod-shaped, Gram-negative, halophilic bacterium that is widely disseminated in coastal, marine, and estuarine environments and causes acute gastroenteritis due to raw or undercooked seafood consumption, wound infection, and septicemia in humans. A wide variety of virulence factors, such as its toxins, type 3 secretion system, type 6 secretion system, adhesins, urea hydrolysis, and flagellar motility, are responsible for initiating infection and causing illness to the host. The pandemic clone emergence that causes global outbreaks is a major concern. Additionally, *V. parahaemolyticus* has emerged as a shrimp pathogen that causes acute hepatopancreatic necrosis disease or early mortality syndrome, which threatens the viability of the shrimp aquaculture industry. Moreover, the emergence of multidrug-resistant *V. parahaemolyticus* strains in seafood and environmental samples in recent years raises a serious concern of human health on seafood safety. This review highlights the prevalence of *V. parahaemolyticus* in various countries and newly emerging inland saline aquaculture areas, pathogen-associated seafood-borne outbreaks, and various virulence factors. Additionally, it provides updated literature on antibiotic resistance profiles of *V. parahaemolyticus* from seafood and environmental samples in recent years.

Keywords: Antibiotic resistance, prevalence, seafood, virulence, *Vibrio parahaemolyticus*

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Öz

Vibrio parahaemolyticus, kıyı, deniz ve nehir ağzı ortamlarında yaygın olarak görülen kavisli, çubuk şeklinde, Gram-olumsuz, halofilik bir bakteridir. İnsanlarda çiğ veya az pişmiş deniz ürünleri tüketimi, yara enfeksiyonu ve septisemi ile ilişkili akut gastroenterite neden olur. Toksinleri, tip 3 salgılama sistemi, tip 6 salgılama sistemi, adezinler, üre hidrolizi ve flagellar motilite gibi çok çeşitli virülans faktörleri, enfeksiyonu başlatmaktan ve konakçıda hastalığa neden olmaktan sorumludur. Küresel salgınlara neden olan pandemik klonların ortaya çıkması büyük bir endişe kaynağıdır. Ayrıca, *V. parahaemolyticus*, karides yetiştiriciliği endüstrisinin canlılığını tehdit eden akut hepatopankreatik nekroz hastalığı veya erken ölüm sendromuna neden olan bir karides patojeni olarak ortaya çıkmıştır. Ayrıca, son yıllarda deniz ürünleri ve çevresel örneklerde çoklu ilaca dirençli *V. parahaemolyticus* suşlarının ortaya çıkması, deniz ürünleri güvenliği konusunda insan sağlığı açısından ciddi bir endişe yaratmaktadır. Bu derleme, *V. parahaemolyticus*'un çeşitli ülkelerde ve yeni ortaya çıkan iç su balıklandırma alanlarındaki prevalansını, bu patojenle ilişkili deniz ürünleri kaynaklı salgınları ve enfeksiyonu başlatmaktan ve konakçıda hastalığa neden olmaktan sorumlu çeşitli virülans faktörlerini vurgulamaktadır. Ayrıca son yıllardaki, deniz ürünleri ve çevre örneklerinde *V. parahaemolyticus*'un antibiyotik direnç profilleri hakkında güncel literatür sunmaktadır.
Anahtar Kelimeler: Antibiyotik direnci, yaygınlık, deniz ürünleri, virülans, *Vibrio parahaemolyticus*

Introduction

Over the decades, a significant contribution from aquaculture-based fish-food supply has taken place, whereas supply from capture fisheries is leveled out. In both cases, fish products are often associated with certain food safety issues, as the risk of chemical and biological agent contamination is greater in freshwater and coastal ecosystems than in the open seas. Largely, the associated food safety issues differ between regions and habitat of collection/harvest, apart from the management practices and environmental conditions. Therefore, proper assessment and regulation of any food safety concerns are becoming increasingly essential and indispensable. These days, the presence of pathogenic bacteria in marine habitats raises a big concern on food safety worldwide due to the inherent potentials of these microbial groups to cause disease outbreaks. Among the potential disease-causing hazards, *Vibrio parahaemolyticus*, a Gram-negative, halophilic bacterium, which is widely disseminated in estuarine, marine, and coastal surroundings, emerges as a very obvious human threat example[1]. It is curved or rod-shaped and can grow at sodium chloride concentrations of 3-8% with an optimum salt concentration of 2-4%. *V. parahaemolyticus* is susceptible to the vibriostatic agent O/129, a fermentative bacteria, motile, with a single polar flagellum. It is typically found in a free-swimming state or attached to inert and animate surfaces, including zooplankton, fish, shellfish, or any suspended matter underwater[2]. On thiosulfate citrate bile salt sucrose (TCBS) agar, *V. parahaemolyticus* is distinguished from other *Vibrio* species, such as *Vibrio cholerae* and *Vibrio alginolyticus,* by its sucrose non-fermenting characteristics and appear as green colored colonies[3]. *Vibrio vulnificus* also appears as green-colored colonies on TCBS agar and differs from *V. parahaemolyticus* and other *Vibrio* species by its ability to ferment lactose[4]. *V. parahaemolyticus* is classified based on the antigenic properties of the somatic (O) and capsular (K) antigen produced in various environmental conditions[5,6].

V. parahaemolyticus causes acute gastroenteritis in humans due to the consumption of contaminated raw or undercooked seafood with virulent strains[7,8]. It also causes infections through open wounds that are exposed to seawater and cause septicemia, wound infection, or ear infection that may be life-threatening to individuals with pre-existing medical conditions[9,10]. Apart from being a human pathogen, it is now considered an aquatic zoonotic pathogen that can cause vibriosis in many fish and shellfish species and is one of the pathogenic agents that threaten the viability of the aquaculture industry, especially shrimp[10,11]. *V. parahaemolyticus* that carry *pirA* and *pirB* toxin genes is the cause of acute hepatopancreatic necrosis disease (AHPND) or early mortality syndrome (EMS) in shrimp, which causes heavy losses in the shrimp industry[12]. AHPND was first reported in China in 2009, followed by Malaysia in 2010, Vietnam in 2011, Thailand in 2012, and Mexico in 2013[11]. The mortality rate of shrimps due to AHPND is very high, reaching up to 100%[13]. Species of shrimps that are susceptible to *V. parahaemolyticus* are *Litopenaeus vannamei*, *Penaeus monodon,* and *Penaeus chinensis*[14].

V. parahaemolyticus is a member of the indigenous flora of marine and brackish water environments and is detected in a wide variety of marine species, including eels, octopus, squid, sardines, tuna, mackerel, perch, flounder, rockfish, red snapper, pompano, etc.[15]. Additionally, it is most commonly found in bivalve mollusk and shellfish[16]. Lesmana et al.[17] reported that warm summer months are considered peak periods for the isolation of this bacterium. Environmental factors for the prevalence and distribution of *V. parahaemolyticus* include water temperature, salinity, oxygen concentrations, plankton density, presence of sediment, organic matter in suspension, and marine organisms[18]. Earlier, few reviews on *V. parahaemolyticus* have described its virulence and outbreaks globally. However, these studies lack focus on the antibiotic resistance of the pathogen. Given the importance of its associated pathogenicity and food safety concerns, the recent works were updated with

its prevalence in many countries and newly emerging inland saline aquaculture areas, recent reports on pathogen-related foodborne outbreaks, various virulence factors for host infection and illness, and antibiotic resistance profiles of this bacterium that is isolated from seafood and its culturing environments.

Prevalence

V. parahaemolyticus was first reported in Japan in 1950 in an outbreak of food poisoning case that caused 272 illnesses and 20 deaths[19]. The bacterium was isolated from victims of the epidemic in Japan and was found to be associated with the consumption of shirasu, a Japanese boiled and semi-dried sardine dish[20]. Since then, *V. parahaemolyticus* has been commonly found to be prevalent in seafood samples in South East Asian countries[21-23]. *V. parahaemolyticus* has accounted for several gastrointestinal disorder cases in Japan[20,24,25], Taiwan[26,27], China since the early 90's[28-30], Laos[31], Bangladesh[32], Hong Kong, and Indonesia[31,33].

Yano et al.[34] reported the prevalence of pathogenic *V. parahaemolyticus* in Thailand, which is one of the major producers and exporters of cultured shrimp worldwide. Pathogenic and antimicrobial-resistant *V. parahaemolyticus* was isolated from shrimps and cockles in Malaysia[35]. In India, *V. parahaemolyticus* was first isolated from fecal samples of patients with acute diarrhea admitted to the Johns Hopkins Unit of the Infectious Diseases Hospital, Calcutta[36]. A recent study isolated *V. parahaemolyticus* strains from patients with acute diarrhea who are admitted to Infectious Diseases Hospital, Kolkata, from 2001 to 2012[37]. Reyhanath and Kutty[38] have reported the prevalence of multidrug-resistant strains of *V. parahaemolyticus* from the fish landing center in Ponnani, South India. Narayanan et al.[39] isolated pathogenic *V. parahaemolyticus* with high genetic diversity and carbapenam resistance from shrimp aquaculture farms in central Kerala, India. Yu et al.[26] reported the prevalence of *V. parahaemolyticus* in oysters and clam culturing environments in Thailand. The isolated strains exhibited hemolytic or urease activities and the presence of gene markers for *tdh*, *trh*, type 3 secretion system T3SS1 (*vcrD1*), or T3SS2α (*vcrD2*). Guin et al.[40] reported a high prevalence of pathogenic *V. parahaemolyticus* in fish and water samples in and around Kolkata, India. The study also revealed the emergence of several new serovars of pandemic *V. parahaemolyticus* and was closely related to O3:K6 serovar (60-85%) by pulsed-field gel electrophoresis analysis.

In Europe, *V. parahaemolyticus* has been isolated from the North Sea, the Mediterranean Sea, the Baltic Sea[41], and the Black Sea[42]. *V. parahaemolyticus* was found prevalent in 53 of 100 water samples in the coastal waters of Guadeloupe[43]. Outbreaks associated with *V. parahaemolyticus* infections are rarely reported in European countries compared to Asian countries. In 1999, a total of 64 illnesses were reported in three episodes due to the consumption of raw oysters from a typical outdoor street market in Galicia, Northwest Spain[44]. Robert-Pillot et al.[45] reported the prevalence of pathogenic *V. parahaemolyticus* from environmental samples of French coastal areas and seafood products that were imported into France. In 2016, New Delhi metallo-beta-lactamase 1 producing *V. parahaemolyticus* strain was isolated from seafood samples imported from Vietnam to France[46]. In 2004, a *V. parahaemolyticus* outbreak of 80 illnesses occurred in A Coruña, Spain[47]. All patients had attended a wedding ceremony in the same restaurant. The epidemiologic investigation revealed that the outbreak was caused by the consumption of boiled crabs, which were prepared under unsanitary conditions. *V. parahaemolyticus* infections are usually rare and intermittent across all of Europe except Galicia in northwestern Spain. This region is considered a hotspot for *V. parahaemolyticus* infections with recurring cases of foodborne vibriosis and outbreaks since the late 1990s[48]. Additionally, Rodriguez-Castro et al.[49] reported the prevalence of pathogenic *V. parahaemolyticus* in coastal waters of Galicia, Spain. In Italy, pandemic *V. parahaemolyticus* O3:K6 strain was first isolated from a stool sample of a patient with diarrhea who was hospitalized in central Italy in the summer of 2007[50]. Lamon et al.[51] reported the occurrence of potentially pathogenic *V. parahaemolyticus* from shellfish samples from two harvesting areas of Sardinia, Italy.

In the United States, *V. parahaemolyticus* was first identified as an etiological agent in 1971 after the three food-related epidemics of gastroenteritis in Maryland, which was associated with crab food product consumption[52]. Since then, recurrent *V. parahaemolyticus* outbreaks have been reported throughout the US coastal regions due to the consumption of raw or uncooked seafood. Between 1973 and 1998, the Centers for Disease Control and Prevention (CDC) have reported approximately 40 outbreaks of *V. parahaemolyticus* infection[53]. Among them, four major epidemics occurred in the Gulf Coast, Pacific Northwest, and Atlantic Northeast regions between 1997 and 1998, which involved >700 cases of illness that are associated with the consumption of raw oysters. In 1997, a massive outbreak was reported in North America, which included 209 people (including one death) of *V. parahaemolyticus* infections associated with consumption of raw oysters harvested from Oregon, Washington, and California in the US, and British Columbia (BC) in Canada[54]. Oyster-associated outbreaks of 43 cases in Washington and 416 cases in Texas in 1998 were caused by *V. parahaemolyticus* in the US[55]. In the summer of 2004, 22 passengers onboard a cruise ship developed gastroenteritis symptoms after ingesting raw oysters from the Alaskan waters[56]. In the summer of 2006, an outbreak of *V. parahaemolyticus* infection occurred, involving

177 cases due to raw oyster ingestion that were harvested in Washington and BC[57]. DePaola et al.[58] reported a prevalence of O4:K12, a serovariant of pandemic *V. parahaemolyticus* O3:K6 in the US. In the summer of 2010, another outbreak due to *V. parahaemolyticus* infection occurred in Maryland, which was linked to the consumption of oysters[59]. Furthermore, *V. parahaemolyticus* cases have increased in the Northeast USA, with outbreaks in New York in 2012 and New York, Connecticut, and Massachusetts in 2013[60]. Almuhaideb et al.[61] reported the prevalence of pathogenic *V. parahaemolyticus* in oyster (*Crassostrea virginica*) and water samples from Delaware Bay from June to October of 2016. Pathogenic *V. parahaemolyticus* is also reported from water, oyster, and sediment samples from the Chesapeake Bay, Maryland[62].

In recent years, inland saline water has emerged as a potential farming aquaculture resource for rearing fish/shellfish species[34]. Some studies revealed the prevalence of *V. parahaemolyticus* in these sources[34,63,64]. Inland saline aquaculture refers to the culture of fish/shellfish or plants using inland sources of saline groundwater. Currently, inland saline aquaculture is practiced in many countries, including the USA, Israel, India, and Australia, to produce seafood[65]. Singh et al.[64] reported the prevalence of *V. parahaemolyticus* in inland saline farms of Punjab. Sanathkumar et al.[63] reported a high incidence of *V. parahaemolyticus* from shrimps in low saline (1-6 ppt) inland saline shrimp farms in the southeastern coast of India. Yano et al.[34] reported the prevalence of *V. parahaemolyticus* (38%) in shrimp samples from low saline (1-5 ppt) inland saline areas of Thailand.

Virulence Factors

The major virulence factors associated with *V. parahaemolyticus* are its toxins [thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH)], type 3 secretion systems (T3SS1 and T3SS2), type 6 secretion systems, such as T6SS1 and T6SS2[66-68], and other virulence factors like adhesins, lipase, gelatinase activity, and urea hydrolysis[69]. Additionally, *V. parahaemolyticus* has two different types of flagellar systems, which help in swimming and swarming. These features are likely to assist in the strains' survival in the environment and the colonization of a human host[70]. Herein, we describe some of the virulence factors associated with *V. parahaemolyticus,* including studies on quorum sensing (QS), adhesins, toxins, type 3 secretion systems, type 6 secretion systems, and some other related factors to virulence, such as polar and lateral flagella, etc. (Figure 1).

Quorum Sensing

The expression of virulence factors in *V. parahaemolyticus* is modulated by the phenomenon known as QS. Bacterial QS is the regulation of gene expression in response to fluctuation in cell-population density. Quorum sensing bacteria produce and release signaling molecules (known as auto-inducers) that increases in concentration as a cell density function. It leads to gene expression alteration, which results in cell-to-cell communication when a minimum threshold stimulatory concentration of an auto-inducer is detected[9]. These signaling molecules bind to receptor proteins on the bacterial surface and trigger a phosphorylation/dephosphorylation signal transduction cascade[71,72]. Bacteria use QS communication circuits to regulate a diverse array of physiological activities, such as virulence factor secretion, where bacterial cells function in harmony to coordinate alter their gene expression and control their synchrony-requiring activities[73]. At high cell densities, *V. parahaemolyticus* produces transcriptional regulator OpaR as a result of a response to QS system stimulation by auto-inducers, including auto-inducer 2 (AI-2)[71]. OpaR is the primary QS regulator that controls virulence factor gene expression, such as swarming motility, type 3 secretion, and type 6 secretion systems in *V. parahaemolyticus*[74]. Additionally, OpaR also controls the colony and cellular morphology that are associated with growth on a surface and biofilm formation[73]. Kernell Burke et al.[73] suggested that the 11 transcription factors downstream of OpaR presumably play an essential role in the regulatory network that controls phenotypic output that is critical to the survival and

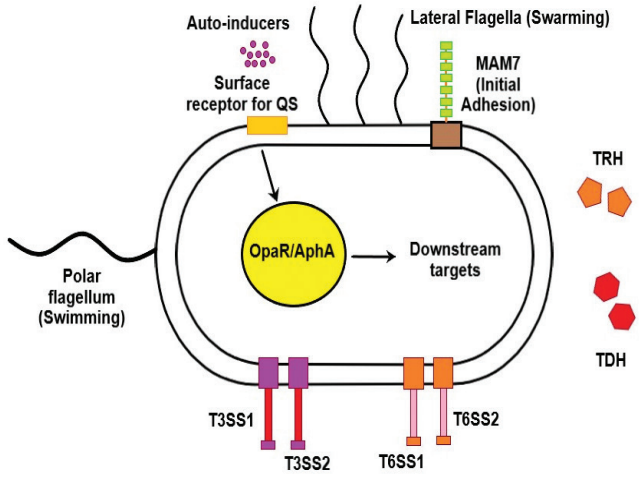

Figure 1. Virulence factors of *Vibrio parahaemolyticus.* The surface receptor for Quorum sensing (QS) reacts to stimulation and response by secreted auto-inducers (signaling molecule). OpaR/AphA are the transcriptional regulators that respond to auto-inducer stimulation and affect downstream targets. MAM7 is a multivalent adhesion protein that is responsible for its initial host cell attachment. A single polar flagellum is required for swimming motility in a moist environment, whereas lateral flagella aids in swarming motility and biofilm formation. T3SS (T3SS1 and T3SS2) to inject virulence proteins into the host cells. T6SS (T6SS1 and T6SS2) aids in adhesion to host cells. Toxins (TDH and TRH) are the major virulence factors

virulence of the organism. OpaR production is ceased and AphA is expressed at low cell densities, which is another transcriptional regulator and functions opposite to OpaR[71,75]. Expression of AphA represses the transcription of T3SS1 genes allowing *V. parahaemolyticus* to utilize this system for survival[76]. The cytotoxicity caused by *V. parahaemolyticus* infection on tissue culture cells was significantly reduced with the deletion of AphA, supporting the role of QS in *V. parahaemolyticus* virulence[77].

Adhesion to Host Cells

The initial binding of bacteria to host cells is essential for the activation and delivery of virulence factors and thus is a vital determinant of the pathogen's success[78]. Multivalent adhesion molecule is an adhesin that is present in a wide range of Gram-negative pathogens, which enables them to establish a high-affinity binding to host cells during the early stages of infection[78]. Krachler et al.[78] reported MAM7 as the outer membrane protein mediating host cell attachment in *V. parahaemolyticus.* MAM7 contains a transmembrane motif at the N-terminus and seven mammalian cell entry (mce) domains that are also found in *Mycobacterium* spp. and some Gram-positive bacteria species[78]. MAM7 has two host receptors: extracellular matrix protein fibronectin and plasma membrane phospholipid phosphatidic acid (Table 1)[68]. MAM7 facilitates the attachment of bacteria to host cells by interacting with these two receptors, likely resulting in a tripartite complex on the bacterial and eukaryotic cell surface[78,79]. Furthermore, MAM7-mediated attachment augments T3SS-mediated cell death in some cell types. MAM7 discovery and characterization have led to new research insights as a novel therapeutic or prophylactic agent in combating not only *V. parahaemolyticus* but many other Gram-negative bacterial infections[78].

Toxins

The TDH and TRH are the two virulence-associated factors with *V. parahaemolyticus,* which causes hemolysis and cytotoxicity in the host cell (Table 1)[70]. *V. parahaemolyticus* is extensively present in marine and estuarine environments, but not all strains are considered pathogenic[80]. Pathogenic strains are usually absent in environmental samples and lack the genes *tdh* and *trh,* which cause diseases to humans and marine animals[22,81]. However, studies from Europe, Asia, and the US have reported approximately 0-6% of the environmental samples as positive for the presence of *V. parahaemolyticus* strains with *tdh* and *trh* genes[55,82-84]. The isolated pathogenic strains from humans with gastroenteritis are differentiated from the environmental strains based on their ability to produce TDH. *V. parahaemolyticus* strains, which are TDH-positive, exhibits β-hemolytic properties on a special high-salt mannitol medium, Wagatsuma agar; this event is known as Kanagawa phenomenon (KP)[85,86]. The KP test is commonly used to identify pathogenic *V. parahaemolyticus* in seafood as well as patient samples. Kanagawa phenomenon test reproducibility is dependent on pH, media salinity, and erythrocyte type. Thus, the identification of pathogenic serovars by this method is not always accurate. Only 1-2% of samples from the environment are reported as KP-positive and the rest are considered KP-negative strains[86]. Molecular epidemiological studies indicate that *V. parahaemolyticus* KP-negative strains did not harbor the *tdh* gene but had a *trh* gene. Qadri et al.[87] reported the isolation of a KP-negative *V. parahaemolyticus* strain that carries the *trh* gene from a gastroenteritis outbreak in the Republic of Maldives in 1985. The *trh* gene is very similar to the *tdh* as it plays a similar role in *V. parahaemolyticus* pathogenesis and is therefore regarded to be a *V. parahaemolyticus* virulence factor[88].

TDH is a pore-forming toxin that consists of 165 amino acids[89,90]. During infection, a fairly large size of the pore causes the ion flux alteration in the intestine, which in turn causes diarrhea and other gastrointestinal disorders[91]. Studies explained its hemolytic, cytotoxic, enterotoxic, and cardiotoxic activities[90-92]. Approximately, 90% of TDH pathogenicity is contributed by the *tdh2* gene compared to *tdh1*, which produces nearly 10% of the total TDH[93]. This gene has been identified in some strains of *V. mimicus*, *V. cholerae* non-O1/non-O139, and *V. hollisae*[94].

TRH is a heat-labile toxin of 23 kDa in size and can be destroyed by heat treatment at 60 °C for 10 min. Takahashi et al.[95] demonstrated the TRH-induced chloride secretion and intracellular calcium elevation in cultured human colonic epithelial cells. TRH-bearing strains are also capable of producing urease enzymes[93]. Studies revealed that *trh* gene bearing *V. parahaemolyticus* are more frequently distributed in tropical seafood than *tdh* gene bearing *V. parahaemolyticus*[22,96,97]. The *trh* sequences consist of *trh1* and *trh2* genes and are approximately 70% identical to the *tdh* sequence[98].

Thermolabile hemolysin (TLH) is expressed by all clinical and environmental *V. parahaemolyticus* strains and can cause red blood cell lysis and shows lecithin-dependent phospholipase activity[99]. Studies revealed that TLH protein displays a sign of severe cytotoxicity on HeLa, Changliver, and RAW264.7 cells[100]. This suggests that TLH may have similar biological functions to TDH and TRH toxins, thereby playing a pivotal role in *V. parahaemolyticus* infection.

Secretion Systems

Type 3 Secretion Systems

Type 3 secretion systems, such as T3SS1 and T3SS2, and type 6 secretion systems, such as T6SS1 and T6SS2, have also been reported as virulence factors in *V. parahaemolyticus* like many other Gram-negative bacteria[66-68]. Type 3 secretion systems

Table 1. List of known *Vibrio parahaemolyticus* virulence factors

Effectors	Gene	Domain	Activity	Effects on host cells	References
Toxins and adhesins					
TDH	*tdh*	Thermostable direct hemolysin	Forms tetrameric pore complexes in the host cell membrane	Causes cytotoxicity and enterotoxicity	Yanagihara et al.[105]; Matsuda et al.[90]; Ohnishi et al.[106]
TRH	*trh*	TDH-related hemolysin	Forms tetrameric pore complexes in the host cell membrane	Causes cytotoxicity and enterotoxicity	Shinoda[94]; Ohnishi et al.[106]; Shimohata and Takahashi[107]
MAM7	*vp1611*	mce domain	Binds to fibronectin and phospholipid phosphatidic acid	Initial attachment of the bacterium to a host cell	Krachler et al.[78]; Krachler and Orth[79]
T3SS1 effectors					
VopQ	*vp1680*	Non-conserved	Forms pores and binds to V-ATPase	Induces autophagy	Burdette et al.[108]; Ono et al.[109]; Matsuda et al.[110]
VopR	*vp1683*	Unknown	Binds PIP2 in membrane	Promotes refolding of T3SS effectors	Wang et al.[67]
VopS	*vp1686*	Fic domain	AMPylates Rho-family GTPases	Disrupts actin cytoskeleton	Yarbrough et al.[111]; Luong et al.[112]
VPA0450	*vpa0450*	Inositol polyphosphate 5-phosphatase	Hydrolyzes $PI(4,5)P_2$ to PI4P	Disrupts plasma membrane integrity	Broberg et al.[113]
T3SS2 effectors					
VopC	*vpa1321*	Cytotoxic necrotizing factor-1 homolog	Deamidates Rac and CDC42 at their switch-2 region	Disregulation of actin network, Promotes invasion to host cell	Zhang L et al.[114]
VopT	*vpa1327*	ADP-ribosyltransferase	ADP-ribosylation of Ras	Induces cytotoxicity, inhibits growth of yeast	Kodama et al.[115]
VopA/P	*vpa1346*	Acetyltransferase	Inhibits MAPK signaling by acetylation of MAPK kinases (MKKs)	Suppress immune response and cytokine production	Trosky et al.[116]; Kodama et al.[115]
VopL	*vpa1370*	Wiskott-Aldrich homology 2 (WH2) and proline rich region	Nucleation of actin polymerization	Induces actin stress fiber and filoform formations, remodels host cell adherents and tight junction, promotes intestinal colonization, inhibits host reactive oxygen species (ROS)	Liverman et al.[117]; Miller et al.[118]; Zahm et al.[119]
VopZ	*vpa1336*	Unknown	Inhibits TAK1 activation, Prevents NF-kB, and MAPK signaling	Enterotoxicity and promotes colonization	Zhou et al.[120]; de Souza Santos et al.[121]
VopV	*vpa1357*	F-actin binding domains (LR and C-Terminal domain)	Actin binding and bundling	Causes cytotoxicity and enterotoxicity, remodels actin cytoskeleton and intestinal brush border, promotes colonization and fluid accumulation	Hiyoshi et al.[122]; Zhou et al.[123]; Chaand et al.[124]
VPA1380	*vpa1380*	Unknown	Cysteine catalysis dependent on inositol hexakisphosphate (IP6)	Inhibits growth of yeast	Calder et al.[125]

Adapted from Wang et al.[67]

(T3SS) or an injectisome is a nanomachine or needle-like bacterial machinery used to inject bacterial protein effectors across eukaryotic cell membranes without encountering the extracellular environment[101]. The primary role of type 3 secretion systems in *V. parahaemolyticus* is the host environment survival by releasing the crucial nutrients from the host cells through infected host cell lysis[102]. The T3SS1 is present in all environmental and clinical *V. parahaemolyticus* strains and is located on chromosome 1[102]. The T3SS2 is more commonly associated with pathogenic strains that carry the *tdh* gene but

not *trh* and is encoded on a pathogenicity island (Vp-PA1) on chromosome 2[102]. Another T3SS2 (T3SS2β) of a different lineage has been identified in a *tdh*-negative, *trh*$^+$ *V. parahaemolyticus* strain[103]. TTSS1 is related to cytotoxic activity, whereas TTSS2 for enterotoxic activity[104]. T3SS1 initiates a series of events that involve autophagy, membrane blebbing, cell rounding, and lastly, cell lysis during tissue cell infection. The effectors associated with TTSS1 are VopQ (VP1680), VPA0450, VopR (VP1638), and VopS (VP1686) (Table 1). The effectors of T3SS2 include VopC (VPA1321), VopT (VPA1327), VopA/P (VPA1346), VopL (VPA1370), VopZ, VopV, and VPA1380 (Table 1)[67].

Type 6 Secretion Systems

The type 6 secretion systems, T6SS1 (VP1386-VP1420) and T6SS2 (VPA1030-VPA1043) are located on chromosomes 1 and 2, respectively, on *V. parahaemolyticus* RIMD 2210633[126,127]. A study suggested that the T6SS systems in *V. parahaemolyticus* are functional for host cell adhesion and are not involved in cytotoxicity, as is the case with other bacterial T6SS[128]. T6SS1 and T3SS2 systems co-exist, thus both systems are suggested to cooperate during host infection. T6SS1 plays its role in adhesion, the first step of infection, and the T3SS2 export effectors that induce enterocytotoxicity[104,128]. T6SS gene is reported to be used as a virulence marker to distinguish pandemic and non-pandemic strains. Ceccarelli et al.[129] reported the presence of T6SS gene in all pandemic strains during his study, whereas the non-pandemic strains had a partial set of T6SS genes. Additionally, researchers have reported that T6SS1 and T6SS2 require different temperature and salinity conditions to be active. T6SS1, which is predominantly found in clinical isolates, is most active under warm marine-like conditions, whereas T6SS2 is only active under low salt conditions and that surface sensing and QS differentially regulate both systems[130].

Other Virulence Factors

Flagella

Apart from above- mentioned virulence factors, different types of flagella help in the strains' survival and colonization on a human host[70]. *V. parahaemolyticus* have two different types of flagellar systems, namely polar and lateral flagella, in which the polar flagellum is responsible for swimming and the lateral flagella for the swarmer cell type transformation and biofilm formation (Figure 1). *V. parahaemolyticus* is capable of swimming at speeds up to 60 µm/s with the aid of polar flagellum. The energy to rotate this flagellum is provided by a sodium motive force, which is advantageous in saltwater with an average pH of 8.0[131]. A decreased polar flagellum speed due to increased growth environment viscosity or growth under iron-limiting conditions induces the lateral flagella (swarmer cell type). These flagella are powered by proton motive force[131].

Others

Other virulence factors include adhesiveness, lipase, gelatinase activity, and urea hydrolysis[69]. *Ure* gene is responsible for urease production in *V. parahaemolyticus,* and *trh* and *ure* gene are genetically linked[132]. Studies revealed that urease produced by *V. parahaemolyticus* causes intestinal fluid accumulation and shows a positive result in the suckling mouse test, thereby suggesting that the urease from *V. parahaemolyticus* may be an essential virulence factor in *trh*$^+$ *V. parahaemolyticus* strains[133,134]. The *Uh* gene encodes urease production, and the toxic effects of urease on intestinal mucosa permeability are thought to be due to ammonium ions accumulation during the infection process[135].

Pandemic Strains

Gastroenteritis due to *V. parahaemolyticus* occurs as sporadic cases and is caused by *V. parahaemolyticus* of different serotypes. However, since 1996, incidences of gastroenteritis due to *V. parahaemolyticus* serotype O3:K6 have increased in many countries[47,136-138].

This serotype was first recognized during the active surveillance of *V. parahaemolyticus* infection among hospitalized patients in Calcutta, India, between January 1994 and August 1996[139]. The study identified a sudden increase in this serotype since 1996 and accounted for 50-80% of the *V. parahaemolyticus* strains isolated during the study period. This highly virulent strain was subsequently isolated from travelers who arrive in Japan from various Southeast Asian countries and was recovered at a high rate in other Southeast Asian countries[136,137,139]. *V. parahaemolyticus* O3:K6 serotype was first identified in the US in 1998 and caused the largest outbreak (416 people) due to the consumption of oysters from Galveston Bay[140]. The same serotype was later isolated from another outbreak of *V. parahaemolyticus* infection associated with eating raw oysters and clams among residents in Connecticut, New Jersey, and New York in July-September 1998[141]. In 2004, *V. parahaemolyticus* O3:K6 strain was isolated from victims of outbreaks that occurred in Chile[138] and Spain[47].

Currently, >20 serotypes of *V. parahemolyticus* are identified, including O3:K6, O4:K68, O1:K25, and O1:KUT[5]. Molecular analysis of the worldwide clinical isolates of *V. parahaemolyticus* demonstrated that a 24 kb region named *V. parahaemolyticus* island-1 (VPaI-1) encompassing ORFs VP0380 to VP0403 is present only in new O3:K6 and related strains recovered after 1995. Further investigation showed the presence of 3 additional regions, VPaI-4 (VP2131 to VP2144), VPaI-5 (VP2900 to VP2910), and VPaI-6 (VPA1254 to VPA1270) in the pandemic strains[142]. Nishioka et al.[143] suggested VPAI-1 as one of the pandemicity markers due to the presence of

a virulence gene. In China, *V. parahaemolyticus* strains were isolated and screened for pandemic O3:K6 clone strains, the isolates in the pandemic group carried the *tdh* but not the *trh* gene, and *orf8* gene. Pandemic clonal serovars included O3:K6, O1:KUT, O1:K25, O1:K26, and O4:K68 and the newly emerging serovars O1:K36, O3:K25, and O3:K68[144]. Matsumoto et al.[31] reported a novel *toxRS*-targeted polymerase chain reaction method that detected pandemic clones and suggested that the technique will be useful in differentiating between pandemic and non-pandemic *V. parahaemolyticus* strains. The differences among and between O3:K6 strains led to the definition of non-pandemic O3:K6 strains isolated in 1980–1990 in South Asian countries, including Taiwan, India, Thailand, Japan, and Bangladesh[129].

In Chile, pandemic *V. parahaemolyticus* serotype O3:K6 strain caused one of the world's worst diarrhea outbreaks that are related to seafood consumption, with >10,000 clinical cases[145]. In 2005, epidemics peaked in the Region de Los Lagos, Chile, where most seafood is produced. However, cases gradually decreased and disappeared a few years later[146]. In recent years, pandemic strains from environmental samples are growing, which constitute a new threat to seafood safety and human health. Meparambu Prabhakaran et al.[147] isolated new serovars of pandemic *V. parahaemolyticus* strains from water, plankton, and seafood samples collected from the Indian coast. Caburlotto et al.[148] reported pandemic strains of *V. parahaemolyticus* from environmental water samples in the Northern Adriatic, Italy. Recently, a new type of *V. parahaemolyticus* serotype named 'O4:KUT-recAin' was isolated from patients with acute diarrhea in coastal hospitals of China[149]. Hu et al.[150] also reported the prevalence of O3:K6 *V. parahaemolyticus* serotype from aquatic products in the Southern Fujian coast, China.

Antibiotic Resistance Profiles

In addition to routine human and animal therapy applications, antibiotics were often used at sub-therapeutic levels in livestock, poultry production, and aquaculture to promote growth and prevent infection[151]. Antibiotic resistance has emerged and evolved in many bacterial genera, including *Vibrio* sp., over the past few decades due to excessive use of antibiotics in human, agricultural, and aquaculture systems[152,153]. Antibiotics from both urban and agricultural sources enter and persist in the aquatic environment, which results in resistant bacteria selection and survival. This selection pressure has promoted the evolution and spread of hundreds of antibiotic resistance genes that confer resistance to various bacteria, regardless of their origins[154]. *Vibrio* spp. are usually susceptible to most antibiotics of veterinary and human significance[155]. However, many studies reported that *V. parahaemolyticus* are gaining resistance to multiple antibiotics due to antibiotic misuse to control aquaculture infections (Table 2). Most frequently observed antibiotic resistance profiles involve ampicillin, penicillin, and tetracycline regardless of the countries[156]. The presence of multiple-antibiotic-resistant *V. parahaemolyticus* in aquatic environments and seafood is a major concern in fish and shellfish farming and human health. Most of these studies have been conducted in South Asian countries like India, China, Malaysia, Thailand, and South Korea (Table 2). Studies from other countries, like Brazil, Nigeria, Egypt, and Saudi Arabia, have also reported the prevalence of antibiotic resistance in *V. parahaemolyticus* from seafood and environmental samples in recent years (Table 2).

The increased bacterial resistance toward many clinical antibiotics affects many countries' healthcare and food production sectors. The CDC recommends antibiotics, such as fluoroquinolones (levofloxacin), cephalosporin (cefotaxime and ceftazidime), aminoglycosides (amikacin and gentamicin), and folate pathway inhibitors (trimethoprim-sulfamethoxazole) for *Vibrio* spp. Infection treatment[171]. However, various antibiotic resistance patterns among *V. parahaemolyticus* isolated from seafood and its environment in different countries have been observed (Table 2). A recent study on the antibiotic resistance of AHPND-causing *V. parahaemolyticus* strains isolated from shrimps (*P. vannamei*)[172] revealed that most isolates were resistant to colistin, ampicillin, and streptomycin but susceptible to other antibiotics. Another study revealed that *V. parahaemolyticus* isolates from oysters in coastal parts of West Bengal, India, exhibited resistance to cefpodoxime (100%) followed by ampicillin and cefotaxime (90%), ceftizoxime (60%), tetracycline (50%), ceftriaxone (40%), ciprofloxacin, and nalidixic acid (10% each)[173]. Mok et al.[174] reported that *V. parahaemolytics* strains from water samples and aquatic animals (fish and shrimps) from aquaculture farms along the Korean coast exhibited resistance to two antibiotics (colistin and ampicillin). According to Ali et al.[175], *V. parahaemolyticus* strains from marine fishes in Bangladesh were resistant to ampicillin (100%) and streptomycin (78.9%). The study of da Silva et al.[176], revealed that *V. parahaemolyticus* from water and blue crab (*Callinectes sapidus*) samples from the Maryland Coastal Bays, United States, were resistant to cephalothin (61%), cefoxitin (31%), and ceftazidime (29%). The reported high multiple antibiotic resistance of *V. parahaemolyticus* from seafood and its environment is of public health concern. Therefore, frequent investigation on the antimicrobial resistance of *V. parahaemolyticus* for epidemiological purposes and healthcare treatment guidance is necessary.

Table 2. Antibiotic resistance profiles of *Vibrio parahaemolyticus* in some countries

Country	Sampling site	Sample type	Resistant (%)	Intermediate (%)	Susceptible (%)	References
India	Shrimp farms in Andhra Pradesh and Tamil Nadu	Water and shrimp	Tetracycline (100); amoxyclav (40); cefotaxime (9); ticarcillin (5); ofloxacin and ampicilin/sulbactam (3); levofloxacin, minocycline, chloramphenicol and ciprofloxacin (2)	Cephalothin (39); cefotaxime (31); amikacin (2)	Nalidixic acid, meropenem, norfloxacin and gentamicin (100); levofloxacin, minocycline, chloramphenicol, ciprofloxacin and amikacin (98); ampicilin/ sulbactam and ofloxacin (97)	Navaneeth et al.[157]
	Retail markets in Cochin, Kerala	Fish and shellfish	Ampicillin (79.3); cefotaxime (41.4); cefepime (10.3); cefoxitin and ceftazidime (3.4)	Cefepime (86.2); cefoxitin (58.6); ceftazidime (37.9); cefotaxime (34.5); ampicillin (6.9); ciprofloxacin (3.4)	Amoxicillin/clavulanic acid, chloramphenicol, gentamicin, meropenem, tetracycline and trimethoprim/sulfamethoxazole (100); ciprofloxacin (96.6); ceftazidime (58.6); cefoxitin (37.9); cefotaxime (24.1); ampicillin (13.8)	Narayanan et al.[39]
	Cochin, Kerala	Shellfish from retail market	Ampicillin, streptomycin and cephalothin (100); amoxycillin (90); carbencillin (95); ceftazidime (96); colistin (95); gentamicin (10); trimethoprim (10)		Chloramphenicol and tetracycline (100)	Sudha et al.[158]
	East coast of India	Water, sediment and shrimp (*Panaeus monodon*)	Ampicillin (100); furazolidone and neomycin B (80); ceftriaxone and ciprofloxacin (60); chlortetracycline chloramphenicol and kanamycin (40); nalidixic acid, oxytetracycline and streptomycin (20)	Oxytetracycline and streptomycin (80); chloramphenicol, erythromycin and nalidixic acid (60); chlortetracycline, ceftriaxone, ciprofloxacin and kanamycin (40); furazolidone, gentamicin and neomycin B (20)	Gentamicin (80); erythromycin (40); chlortetracycline, kanamycin and nalidixic acid (20)	Vaseeharan et al.[159]
South Korea	Restaurants in Seoul	Water (restaurant fish tank)	Ampicillin (51.4); amikacin and tetracycline (11.4); ceftazidime (8.6); cefotaxime and ciprofloxacin (5.7); ampicillin/sulbactam and cefepime (2.9)		Piperacillin, imipenem, gentamicin and trimethoprim/ sulfamethoxazole (100); ampicillin/sulbactam and cefepime (97.1); cefotaxime and ciprofloxacin (94.3); ceftazidime (91.4); amikacin and tetracycline (88.6); ampicillin (48.6)	Jeong et al.[160]
	Fishery auction markets, fish markets, and online markets	Fishery samples and environmental samples	Ampicillin (100)		Ciprofloxacin, amoxycillin/ clavulanic acid, ampicillin-sulbactam, chloramphenicol, tetracyclines, and gentamicin (100)	Lee et al.[161]
Italy	Italian coastal waters (Adriatic sea and Tyrrhenian sea)	Shellfish and clinical samples (feces)	Ampicillin and amoxicillin (100); colistin sulfate, polymyxin B, erythromycin, kanamycin and neomycin (<20)	Tetracycline (11.2); oxytetracycline (8.4) and trimethoprim/ sulfamethoxazole (3.7)	Chloramphenicol and doxycycline (100); oxolinic acid, nalidixic acid, nitrofurantoin, trimethoprim/sulfamethoxazole and oxytetracycline (>90); tetracycline and ciprofloxacin (>80)	Ottaviani et al.[162]
	Fish farm (Adriatic sea)	Water, sediment, and biofilm samples	Tetracycline (17); trimethoprim- sulfadiazine (7); trimethoprim (2)			Labella et al.[163]
Thailand	Thap Put district, Phang Nga Province	Oyster (*Crassostrea lugubris* and *C. belcheri*) and estuarine water	Erythromycin (54.2); sulfamethoxazole (34.7); trimethoprim (27.9); ampicillin (10.2); streptomycin (0.8); tetracycline (0.5)		Chloramphenicol and ciprofloxacin (100)	Jeamsripong et al.[164]

Table 2. Contiuned

Country	Sampling site	Sample type	Resistant (%)	Intermediate (%)	Susceptible (%)	References
China	Hebei province	Seafoods (fish, mussel, shrimp, crab, sea-irchin, scallop, clam, and oyster)	Ampicillin (100); sulfisoxazole (47.36); nitrofurantoin (34.21); tobramycin (31.57); sulfamethoxazole-trimethoprim (26.31); ceftriaxone (5.26); gentamicin, cefoperazone and cephalothin (2.63)	Nitrofurantoin (42.1); cefoperazone (39.47); tobramycin (36.84); gentamicin (31.57); ciprofloxacin and cephalothin (26.31); ceftriazone (21.05); ceftriaxone (15.78); sulfamethoxazole-trimethoprim and ofloxacin (5.26); norfloxacin (2.63)	Chloramphenicol (100); norfloxacin (97.36); ofloxacin (94.73); ceftriaxone (78.94); ciprofloxacin (73.68); cephalothin (71.05); trimethoprim (68.42); gentamicin (65.78); cefoperazone (57.89); sulfamethoxazole- Sulfisoxazole (52.63); tobramycin (31.57); nitrofurantoin (23.68)	Liu et al.[165]
Brazil	Retail markets in Natal (Rio Grande do Norte, Brazil)	Shrimp (*Litopenaeus vannamei)*	Ampicillin (90); amikacin (60)	Nitrofurantoin (30); tetracycline (40); amikacin (20); ciprofloxacin (90); sulfamethoxazole-trimethoprim (10)	Chloramphenicol (100); nitrofurantoin (70); tetracycline (60); amikacin (20); ciprofloxacin (10); sulfamethoxazole-trimethoprim (90)	De Melo et al.[166]
Malaysia	Wet markets in Selangor	Seafood sample (blood clam, shrimp, surf clam, and squid)	Penicillin G (100); ampicillin and cefazolin (84.17); cephalothin (54.17); cefuroxime sodium (51.67) ; amikacin (37.5); gentamicin (6.67); ceftazidime and cefotaxime (5); ofloxacin (2.5); amoxicillin-clavulanic acid (0.83);	Cefotaxime (60); cefuroxime sodium (37.5); cephalothin (35.83); amikacin (33.33); gentamicin (29.17); ofloxacin (27.5); ceftazidime (24.17); amoxicillin-clavulanic acid (23.33); cefazolin (14.17); ampicillin (10.83); tetracycline (5.83); trimethoprim-sulfamethoxazole (5)	Chloramphenicol (100); trimethoprim-sulfamethoxazole (95); tetracycline (94.17); amoxicillin-clavulanic acid (75.83); ceftazidime (70.83); ofloxacin (70); gentamicin (64.17); cefotaxime (35); amikacin (29.17); cefuroxime sodium (10.83); cephalothin (10); ampicillin (5); cefazolin (1.67)	Tan et al.[167]
Nigeria	Open markets in Edo and Delta states	Ready-to-eat shrimp samples	Amoxicillin (82.6); penicillin (86.9); doxycycline and trimethoprim (41.3); oxytetracycline (36.9); sulfamethoxazole (32.6); cefotaxime (30.4); tetracycline (28.3); amoxicillin/clavulanate and ampicillin/sulbactam (23.9)	Chloramphenicol (78.3); erythromycin (63); ciprofloxacin (52.2); streptomycin (50); doxycycline (39.1); cefotaxime (26.1); oxytetracycline (21.7)	Gentamycin and colistin (100)	Beshiru et al.[168]
Egypt	Fish markets in Sharkia Governorate	Shrimp (*Penaeus semisulcatus*) and crabs (*Portunus pelagicus*)	Ampicillin, ampicillin-sulbactam and tetracycline (100); ceftazidime (97.2); cefotaxime and ciprofloxacin (91.7); trimethoprim-sulfamethoxazole (75); kanamycin (72.2); nalidixic acid (69.4); chloramphenicol (61.1); gentamicin (50); amikacin (30.6)	Chloramphenicol (22.2); kanamycin (11.1); cefotaxime and nalidixic acid (8.3); ciprofloxacin (2.8)	Amikacin (69.4); gentamicin (50); trimethoprim-sulfamethoxazole (25); nalidixic acid (22.2); kanamycin and chloramphenicol (16.7); ciprofloxacin (5.6); ceftazidime (2.8)	Ahmed et al.[169]
Saudi Arabia	Coastline of the Arabian Gulf	Seawater	Carbenicillin (98); ampicillin (88); cephalothin (76); cefaclor (61); ticarcillin (44); streptomycin (29); aztreonam (27); amikacin (12); cefoxitin and kanamycin (5); amoxy/clavulanic (2)	Kanamycin (71); streptomycin (66); ticarcillin (54); cefoxitin (51); aztreonam (49); cefotaxime (41); amoxy/clavulanic (39); amikacin and ceftriaxone (29); ciprofloxacin (20); ampicillin and nitrofurantoin (12)	Pipera/tazobactam, ceftazidime, chloramphenicol, imipenem, meropenem, nalidixic acid, levofloxacin, and sulf./trimethoprim (100); tetracycline (98); cefepime (95); nitrofurantoin (85); ciprofloxacin and piperacillin (80); ceftizoxime (76); ceftriaxone (71)	Ghenem and Elhadi[170]

Conclusion

V. parahaemolyticus is a halophilic bacterium that naturally occurs in estuarine, marine, and coastal environments worldwide. It causes foodborne gastroenteritis, wound infection, and septicemia in humans and is an emerging threat to the shrimp aquaculture industry, which causes AHPND or EMS in shrimps. This review highlighted the prevalence of *V. parahaemolyticus* in various countries. The emergence of the pandemic clone and its ability to cause large outbreaks is of global concern. Routine monitoring and surveillance of seafood, environmental samples, and aquaculture areas, especially newly emerged inland saline areas, are of prime importance. Many virulence factors are associated with this pathogen, such as toxins, T3SS, T6SS, adhesins, urea hydrolysis, and flagellar motility, which alters the homeostasis and integrity of human cells. Most studies determine the virulence factors done *in vitro* with tissue culture cells, thus further studies are needed *in vivo* models. Additionally, the detailed mechanism of the combined effects of the virulence factors, which have evolved to work together, and the distinct functions of the individual effectors in causing pathogenicity are yet to be investigated. *V. parahaemolyticus* are usually susceptible to the majority of antibiotics of veterinary and human significance. However, many studies have reported multiple-antibiotic resistant *V. parahaemolyticus* from seafood and environmental samples in recent years. A high percentage of ampicillin and penicillin resistance suggests excluding these antibiotics as a treatment for infections due to this microorganism. Further research is needed to test the effectiveness of various antibiotics against *V. parahaemolyticus.* Effective control measures that combine novel drugs and other strategies such as probiotics and phage therapy to control infection in aquaculture are urgently required to avoid public health threats due to massive antibiotic misuse.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.N., D.B., R.H., Design: S.M., S.K.S., R.H., S.V., C.N., M.S.D., Data Collection or Processing: S.N., S.M., D.B., S.V., D.W., A.S.S., Analysis or Interpretation: S.N., S.K.S., D.B., C.N., D.W., M.S.D., Literature Search: S.N., S.M., S.K.S., S.V., M.S.D., A.S.S., Writing: S.N., R.H., C.N., D.W., A.S.S.

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