Control and Prevention Strategies of *Vibrios* in Asia

Subjects: Fisheries

Contributor: Kangping Xu, Yushu Wang, Wangxiaohan Yang, Hongyan Cai, Youyu Zhang, Lixing Huang

It is estimated that vibriosis account for about half of the economic losses in Asian fish culture. Consequently, the prevention and control of vibriosis is one of the priority research topics in the field of Asian fish culture disease. Relevant measures have been proposed to control some *Vibrios* that pose a threat to Asian fish culture, there are currently only a few effective vaccines available to combat these *Vibrios*.

Keywords: Asian ; fish culture ; vibriosis ; prevention

1. Introduction

Twenty years ago, aquatic products played a secondary role in people's food choices. However, now aquatic products have become one of the mainstream food categories. Looking back on the development of global aquaculture from 1997 to 2017, aquaculture has made a substantial contribution to food production throughout the world, especially in Asia. According to the current consumption, aquaculture production needs to increase from 82,087 kilotons in 2018 to 129,000 kilotons in 2050 to meet global needs [1][2]. By 2050, aquaculture will dominate the global seafood supply [3].

Vibrio is one of the important pathogenic microorganisms of humans and marine animals. It widely exists in marine and freshwater ecosystems. Because of its high abundance and biomass, *Vibrio* plays a crucial role in the aquatic environment. More than 80 species of *Vibrio* have been reported, some of which are pathogenic to animals, especially aquatic animals, some to humans, and some to both animals and humans [4]. The outbreak of vibriosis will not only seriously affect marine biomass but also lead to serious economic losses in Asian fish culture.

With the rapid development of Asian fish culture in recent decades, the cases of *Vibrio* infection through aquatic products at home and abroad, causing human disease or huge economic losses, are also increasing year by year. At the same time, the prevention and control measures for *Vibrio* are also developing. At present, the use of antibiotics is the most important treatment for vibriosis in Asian fish culture [5]. At the same time, the overuse of broad-spectrum antibiotics has resulted in an increase in the number of drug-resistant bacteria. The resistance genes of these bacteria can be transferred to other bacteria that have never been exposed to the antibiotic [6]. Therefore, it is necessary to develop some antibiotic-free methods. For example, using vaccines, probiotics, bacteriophages and other technologies.

Before considering the prevention and control of *Vibrio*, it is essential first to identify the exact pathogen. At present, the mainly used identification methods are still conventional physiological, biochemical analyses, 16S rDNA sequencing and drug sensitivity test. In addition to these widely used assays, some convenient, fast and highly sensitive detection methods have been developed in recent years, for example, the identification of biomarkers based on host genes [7], exosomic miRNAs [8] and so on.

Vaccination in Asian fish culture can prevent or mitigate the spread of disease and is effective against many related pathogens [9]. Vaccination is usually a secure and economic precaution. For this reason, illness prevention based on stimulating the immune system of aquatic animals has proved to be the basis of the development of modern Asian fish culture. Nevertheless, there are only a few *Vibrios* with vaccine control technology.

The control and prevention strategies of seven *Vibrio* species that are seriously harmful to Asian fish culture, including *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, *Vibrio mimicus*, *Vibrio anguillarum*, *Vibrio alginolyticus* and *Vibrio cholerae*. For each *Vibrio*, researchers describe their prevention and treatment methods (**Figure 1**), especially vaccine prevention methods, in order to provide views for better prevention and control of vibriosis in Asian fish culture in the future.

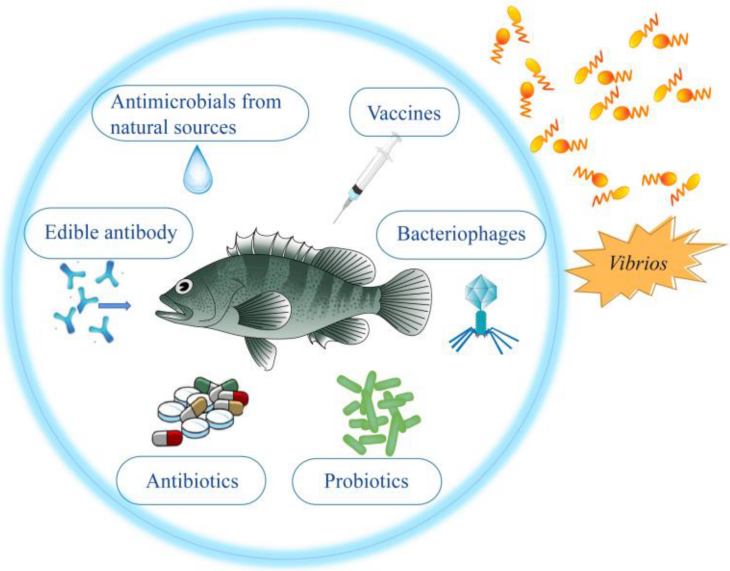

Figure 1. Strategies for prevention and control of vibriosis in Asian fish culture.

2. Control and Prevention Strategies of *Vibrios*

2.1. V. harveyi

V. harveyi is a luminous marine bacterium and is also a well-recognized and acute pathogen of marine fish [10]. The research on the control and prevention measures of *V. harveyi* started early, and now there are a variety of control technologies (**Table 1**).

2.1.1. Antibiotics

Antibiotic methods are generally used in the initial stage of prevention and treatment of vibriosis or emergency treatment. In the case of skin ulcer disease of young hybrid groupers, researchers confirm that the pathogen of this sickness is *V. harveyi* ML01 strain, which is sensitive to minocycline, doxycycline and ceftriaxone. In other words, these three antibiotics can be used for emergency treatment of *V. harveyi* infection [11]. In 2017, the drug sensitivity test of *V. harveyi* extracted from the diseased cultured hippocampus was carried out, and the results showed that *V. harveyi* is highly sensitive to doxycycline and tetracycline. This provides a reference for the prevention strategy of vibriosis in seahorse culture in eastern China [12]. Although antibiotics are widely used, the rapid increase in antibiotic resistance is really puzzling.

2.1.2. Bacteriophages

As people gradually realize the risk of using antibiotics in Asian fish culture, probiotics, bacteriophage, antimicrobials from natural sources and so on are gradually replacing antibiotics. In bacteriophage therapy, phages such as lytic *Vibrio* phage VhKM4 [13] can resist *V. harveyi* efficiently due to its strong lytic activity. Although several research studies have proven these methods effective, there have not been enough similar studies of each method to prove that they should be promoted in practical application. At the same time, whether these biological control methods have potential threats still needs further study in future research.

2.1.3. Vaccines

One of the research hot spots of *Vibrio* prevention is vaccine development. Most research on *V. harveyi* vaccine is targeted at fish. Many excellent achievements have been made in the research of *V. harveyi* vaccine. Vaccine exploration started from the traditional whole-cell inactivation method, followed by the study on the method of purifying subcellular components, making vaccine technology enter the era of modern vaccines represented by DNA vaccine [10].

Whole-cell vaccines can be categorized into two types, attenuated live vaccine and inactivated vaccine. The production cost of these vaccines is not high [14]. This traditional vaccine is the most widely used in the prevention of aquatic animal diseases.

- Inactivated vaccines

FKVh, a vaccine mainly composed of formalin-inactivated *V. harveyi* Vh1 strain, may be an effective vaccine, and the survival rate of hybrid tilapia increased from 20% to 87% after vaccination [15]. There is also a combined vaccine (VICV)

against *V. vulnificus*, *V. alginolyticus*, *V. harveyi* and infectious spleen and kidney necrosis virus (ISKNV). Huang et al. have proved its immunization effectiveness by immunizing orange-spotted grouper *Epinephelus coioides* with the VICV vaccine and attacking the above four pathogens [16]. Compared with the monovalent vaccine, this kind of vaccine can more conveniently protect fish from a variety of pathogens.

Although the production cost of inactivated vaccines is relatively lower compared with other kinds of vaccines, their performance still needs to be continuously improved. This can be achieved by combining adjuvants, liposome embedding and other methods. Research has proposed a greatly effective vaccine that can prevent *V. harveyi*. The vaccine consists of inactivated *V. harveyi* cells and ISA763 AVG adjuvant. The experiment observed that the RPS of grouper inoculated with this vaccine was 100% in the sixth week and 91.7% in the twelfth week after being attacked by *V. harveyi* [17]. The formalin-inactivated cell of *V. harveyi* adjuvanted with Montanide TMISA 763 AVG induced efficient immune protection in turbot [18]. Similarly, the application of liposomes-entrapped *V. harveyi* WCV or *V. harveyi* WC can actively strengthen the immune system and provide protection for *V. harveyi* infection in *Epinephelus bruneus* [19]. The expression standard of various immune substances in the grouper‘s spleen is significantly up-regulated after inoculation in the laboratory, using a vaccine made of inactivated *V. harveyi* ZJ0603 combined with β-glucan [20].

- Attenuated live vaccines

An attenuated live vaccine has been developed to highly protect Japanese flounder (*Paralichthys olivaceus*) infected with *V. harveyi* in the experiment. The vaccine is made of live *Escherichia coli*, which can express and secrete Vhp1 with impaired cytotoxicity [21]. Moreover, a study shows that *V. harveyi* WC13DH51 strain can be made into a live attenuated vaccine and has a significant protective effect on groupers [22]. Furthermore, an attenuated live vaccine was developed by constructing recombinant Et15VhD. The infection experiment shows that this vaccine can effectively prevent the infection of *V. harveyi* and *E. tarda* [23]. Similarly, the attenuated mutant strain T4DM of *V. harveyi* can also be used as a live attenuated vaccine. On the medium containing rifampicin with increased concentration, T4DM was obtained by selecting T4D mutants repeatedly with a relatively narrow antibiotic resistance profile and no detectable plasmid. T4DM is also a cross-protection vaccine, which can effectively protect Japanese flounder from the infection of *V. alginolyticusvia* and *V. harveyi*, especially through immersion (10^8 CFU/mL) and intraperitoneal injection (10^8 CFU/mL) [24].

- Subunit vaccines

A subunit vaccine made of purified recombinant Vhp1 can effectively render Japanese flounder *V. harveyi*-resistant [21]. There is also a *V. harveyi* subunit vaccine encoding TssJ antigen that was found to emerge a moderate protective role against *V. harveyi* in fish. The full-length sequence of TssJ was obtained from the *V. harveyi* strain QT520 and was predicted as a new candidate antigen, whose relative percentage survival was 52.39% [25]. Moreover, based on VirB11, a recombinant protein vaccine was developed and became a candidate vaccine to prevent *V. harveyi* infection [26]. In addition, recombinant cell vaccines expressing the DnaJ and OmpK have strong cross-protection against *V. alginolyticus, V. parahaemolyticus* and *V. harveyi* [27].

- Anti-idiotypic vaccines

A great deal of studies have shown that antibodies may have a regulatory effect on the immune system. Consequently, they have the conditions for making vaccines. The vaccine developed according to this principle is called an anti-idiotypic vaccine. As an anti-Id vaccine, anti-Id IgG is a vaccine that can provide protection by imitating the antigen epitope of *V. harveyi*. It may have a good application prospect in Asian fish culture against *V. harveyi* [28].

- DNA vaccines

A series of experimental results suggest that DNA vaccines represented by pDV are positive vaccines against *V. harveyi* [29]. DNA vaccine can also be obtained by cloning the *ompU* gene into pEGFP-N1 plasmid. After the infection test of the turbot, the RPS was 51.4% [30]. Moreover, a *V. harveyi* DNA vaccine encoding TssJ antigen could produce a moderate protective role against *V. harveyi* in fish, and the relative percentage survival was 69.11% [25]. However, the exact route of protection in fish for these vaccines is still unclear at present [14].

- mRNA vaccines

In 1990, the successful use of in vitro transcription (IVT) mRNA in animals was first reported, and related research has developed extremely rapidly since then. The production cost of the mRNA vaccine is low. The application safety is high, and the development turnaround time is short and pretty efficient. Therefore, the mRNA vaccine may have a better

prospect compared with the traditional vaccine [31]. mRNA vaccines applied to aquatic animals are rare. Researchers found a study on the mRNA vaccine against *V. harveyi* infection in fish. The researchers first used computational techniques to find potential T-and B-cell epitopes in *V. harveyi* hemolysin proteins and then sutured these epitopes into multi epitope mRNA vaccines. However, more experiments are needed to further prove the effectiveness of this vaccine [32].

Table 1. Control and prevention strategies of *V. harveyi.* NR: Not Relevant, None: the method has not been tested in vivo or relevant data has not been found.

Pathogen	Prevention and Control Technology	Concrete Measure/ Vaccine Type	Host	Vaccine Antigen Components	Route of Infection	Ref.
V. harveyi	Antibiotics	Ceftriaxone, Doxycycline, Minocycline	Juvenile hybrid groupers	NR	Bath, Injection (IP)	[11]
		Doxycycline, Tetracycline	Hippocampus	NR	Injection (IP)	[12]
	Bacteriophages	Phage VhKM4	Finfish	NR		[13]
	Vaccines	Inactivated	Marine Red Hybrid Tilapia	*V. harveyi* strain Vh1 (Formalin-Inactivated)	Injection (IP)	[15]
			E. coioides	VICV	Injection (IP)	[16]
			Orange-spotted grouper	*V. Harveyi* (formalin-killed, Adjuvant: ISA763 AVG)	Injection (IP)	[17]
			Turbot	*V. Harveyi* (formalin-killed, Adjuvant: TMISA763 AVG)	Injection (IP)	[18]
			Pearl gentian grouper	*V. harveyi* ZJ0603 (Formalin-killed, combine with β-glucanhas)	Injection (IP)	[20]
		Attenuated	Grouper	Non-toxic *V. harveyi*	Bath, Injection (IP)	[22]
			Japanese flounder	Attenuated mutant *V. Harveyi* T4DM	Bath, Injection (IP)	[24]
		Subunit	Japanese flounder	Recombinant Vhp1	Injection (IP)	[21]
			Golden pompano	Antigen encoding TssJ	Injection (IP)	[25]
			Orange-spotted grouper	VirB11	Injection (IP)	[26]
			Juvenile sea bass	Expressed r-OmpK of *Vibrio*	Injection (IP)	[27]
		Anti-idiotypic	Grouper	Anti-Id IgG (Fab)	Injection (IP)	[28]
		DNA	Japanese flounder	Plasmid pDV	Injection (IP, IM)	[29]
			Turbot	Plasmid with OmpU	Injection (IP, IM)	[30]
		mRNA	Fish	and B-cell epitopes in hemolysin protein	None	[32]

2.2. V. vulnificus

V. vulnificus is a gram-negative bacterium that can cause wound infection and septicemia. Unlike other *Vibrios*, it is able to ferment lactose. According to genetic, biochemical and serological tests and host infection, *V. vulnificus* is currently classified into three biotypes. Biotype 1 strains are the source of most human infections, and biotype 2 strains mainly infect eels. The recently discovered biotype 3 has the biochemical characteristics of biotype 2 and 1 and can result in human wound infection [33].

In this era of environmental protection and sustainable development, the biological control strategy of *Vibrio* is gradually emerging, but there are few examples in fish farming.

Vaccines

- Inactivated vaccines

Since *V. vulnificus* is one of the most harmful *Vibrios* to Asian fish culture, its vaccine control technology has been continuously developed (**Table 2**). The early vaccines against *V. vulnificus* are generally inactivated vaccines. For example, a formalin-inactivated *V. vulnificus* vaccine was effective at exciting a humoral antibody response in sex-reversed hybrid tilapia [34]. The combined vaccine (VICV) also can be effective against *V. harveyi* [16].

Table 2. Control and prevention strategies of *V. vulnificus.* NR: Not Relevant.

Pathogen	Prevention and Control Technology	Concrete Measure/ Vaccine Type	Host	Vaccine Antigen Components	Route of Infection	Ref.
V. vulnificus	**Vaccines**	**Inactivated**	**Tilapia (Sex reversed hybrid)**	**Atypical *V. vulnificus* (Formalin killed cells)**	**Injection (IP)**	[34]
			E. coioides	**Vh + Vv + Va inactive vaccine and ISKNV whole cell inactive vaccine**	**Injection (IP)**	[16]
		Subunit	**Japanese eel**	**Expressed OmpU of *V. vulnificus***	**Injection (IP)**	[35]
		Multivalent	**Japanese eel**	**Recombinant Omp containing both OmpA and OmpU**	**Injection (IP)**	[36]
			European eel	**Trivalent outer membrane protein (OmpⅡ-U-A)**	**Injection (IP)**	[37]

- Subunit vaccines

There are also a few subunit vaccines against *V. vulnificus* under development. Some scientists have found that the expressed OmpU of *V. vulnificus* was capable of resisting the infection of *V. vulnificus* in Japanese eels and evidently raised the immune ability of eel. Therefore, the OmpU is proposed as a potential subunit vaccine against *V. vulnificus* [35].

- Multivalent vaccines

Multivalent vaccines may be more practical in aquaculture due to their multiple protective effects. There is a bivalent protein that can be used against *V. vulnificus* and *Edwardsiella anguillarum* in Japanese eel as a vaccine. This fresh recombinant Omp vaccine with OmpA and OmpU shows its strong immunogenicity by significantly increasing the RPS rate of eels when infected with *E. anguillarum* and *V. vulnificus* [36].

In addition to these bivalent vaccines, a trivalent outer membrane protein, OmpII-U-A, containing part sequences of OmpU from *V. vulnificus*, OmpA from *E. anguillarum,* and OmpII from *A. hydrophila*, can also be made into a vaccine. According to the study of He et al. [37], the OmpII-U-A is able to prevent eel from being infected by *V. vulnificus* and *A. hydrophila*. This is the first time the expression and immunogenicity of a trivalent Omp are being reported, and the outcomes of this research will supply valuable guidelines for the exploration of multiplex vaccines in fish.

V. vulnificus has many bivalent and trivalent vaccines that can protect aquatic animals from *A. hydrophila*, *E. anguillarum* and other pathogens. These vaccines can provide ideas for the advancement of aquatic animal multiplex vaccines.

2.3. V. parahaemolyticus

V. parahaemolyticus is a Gram-negative, slightly halophilic bacterium that inhabits brackish aquatic environments such as coastal and estuarine waters. Apart from being pathogenic to aquatic organisms, *V. parahaemolyticus* is also known as a global food-borne pathogen and one of the most common causes of gastroenteritis in East Asia due to the local dietary habit of eating raw fish and shellfish [38][39].

V. parahaemolyticus is antibiotic-resistant, so it cannot be treated with antibiotics which is currently the most commonly used measure in Asian fish culture [40]. Consequently, there is a pressing need to exploit fresh, effective alternatives for antibiotics against *V. parahaemolyticus* (**Table 3**), while the vaccine is the most promising approach due to its economy, efficacy and safety in public awareness [40][41][42].

Table 3. Control and prevention strategies of *V. parahaemolyticus.* NR: Not Relevant.

Pathogen	Prevention and Control Technology	Concrete Measure/ Vaccine Type	Host	Vaccine Antigen Components	Route of Infection	Ref.
V. parahaemolyticus	**Vaccines**	**Inactivated**	**Tiger grouper**	**Vaksin polivalen *Vibrio* (formalin killed cells)**	**Injection, Bath**	[43]
		Recombinant	**Juvenile sea bass**	**Expressed r-OmpK of *Vibrio***	**Injection (IP)**	[27]

Vaccines

- Inactivated vaccines

A polyvalent *Vibrio* vaccine had already been commercially used in Indonesian fish farming recently for tiger grouper (*Mycteroperca tigris*) and had shown effective protection against *V. parahaemolyticus* and two other *Vibrio* pathogens [43].

- Recombinant vaccines

A study on cross-protection also found a recombinant cell vaccine had successfully induced an immune response to *V. parahaemolyticus* in juvenile sea bass by expressing the OmpK of *Vibrio* [27]. At the same time, another study has pointed out the limitation of recombinant OmpK in preparing diagnostic antibodies [44]. For this reason, using modern methods for understanding and developing new *V. parahaemolyticus* immunogenic proteins and antibodies are necessary [45][46].

2.4. V. cholerae

V. cholerae is a Gram-negative motile bacterium that can cause fatal pandemic diseases. There are millions of cholera cases worldwide every year, and the mortality rate is extremely high [47]. Consuming contaminated seafood by mistake is one of the reasons why people are infected with *V. cholerae*. As an important food-borne pathogen, *V. cholerae* is widely distributed in fish, which brings serious safety hazards to human and aquatic animal health [48].

2.4.1. Antibiotics

V. cholerae is one of the important pathogens related to fish vibriosis. In the bluegill sunfish that died in the farms of Guangdong around 2018, the pathogen identified was non-O1/non-O139 *V. cholerae.* The antibiotic sensitivity displayed that the isolated strain was sensitive to azithromycin, chloramphenicol, neomycin, norfloxacin, doxycycline, etc. The possible method to prevent infection of bluegill sunfish is to give neomycin or doxycycline for seven days [49].

2.4.2. Vaccines

After consulting a large number of data, researchers found that the current prevention and control of *V. cholerae* in Asian fish culture is still based on antibiotics, and no *V. cholerae* vaccine for aquatic animals has been found yet. Nevertheless, in recent decades, the misuse of antibiotics has resulted in the emergence and spread of drug-resistant bacteria in the environment, which is likely to pose a threat to public health [50]. Therefore, people are also constantly exploring new methods for the prevention and control of *V. cholerae* (**Table 4**).

Table 4. Control and prevention strategies of *V. cholerae.* NR: Not Relevant, None: the method has not been tested in vivo or relevant data has not been found.

Pathogen	Prevention and Control Technology	Concrete Measure/ Vaccine Type	Host	Route of Infection	Ref.
V. cholerae	Antibiotics	Neomycin, Doxycycline	Bluegill sunfish	Injection (IP)	[49]
	Edible antibody	Anti-non-O1 *V. cholerae* egg yolk powder	Carp	Injection	[51]
	Vaccines	None	None	None	None

2.4.3. Edible Antibodies

In 2015, a dominant non-O1 *V. cholerae* L1 strain was isolated from diseased carp in a breeding farm in Jiangsu, China. The researchers used egg yolk powder (IgY) against non-O1 vibrio cholerae to prove its effective effect on diseased carp [51]. This is one of the new methods to control *V. cholerae*.

2.4.4. Bacteriophages

In addition to the above emerging strategies, a phage prevention and control method has also been proposed [52], which has become a highly potential prevention and control measure in the future. Nevertheless, researchers have not found any information about the bacteriophage therapy of *V. cholerae.* This may be due to the difficulty in developing efficient phage administration mechanisms, different types of aquaculture systems, and the lack of a specific regulatory frame [53].

As a kind of pathogenic bacteria that is very harmful to Asian fish culture, the lack of *V. cholerae* vaccine prevention technology is indeed a big gap in the prevention of aquatic animal diseases.

References

1. Naylor, R.L.; Hardy, R.W.; Buschmann, A.H.; Bush, S.R.; Cao, L.; Klinger, D.H.; Little, D.C.; Lubchenco, J.; Shumway, S.E.; Troell, M. A 20-year retrospective review of global aquaculture. Nature 2021, 591, 551–563.
2. Boyd, C.E.; McNevin, A.A.; Davis, R.P. The contribution of fisheries and aquaculture to the global protein supply. Food Secur. 2022, 14, 805–827.
3. Stentiford, G.D.; Holt, C.C. Global adoption of aquaculture to supply seafood. Environ. Res. Lett. 2022, 17, 041003.
4. Thompson, F.L.; Iida, T.; Swings, J. Biodiversity of Vibrios. Microbiol. Mol. Biol. Rev. 2004, 68, 403–431.
5. Schar, D.; Klein, E.Y.; Laxminarayan, R.; Gilbert, M.; Van Boeckel, T.P. Global trends in antimicrobial use in aquaculture. Sci. Rep. 2020, 10, 1–9.
6. Verschuere, L.; Rombaut, G.; Sorgeloos, P.; Verstraete, W. Probiotic Bacteria as Biological Control Agents in Aquaculture. Microbiol. Mol. Biol. Rev. 2000, 64, 655–671.
7. Zhou, Q.; Zhu, X.; Li, Y.; Yang, P.; Wang, S.; Ning, K.; Chen, S. Intestinal microbiome-mediated resistance against vibriosis for Cynoglossus semilaevis. Microbiome 2022, 10, 153.
8. Zhao, N.; Zhang, B.; Xu, Z.; Jia, L.; Li, M.; He, X.; Bao, B. Detecting Cynoglossus semilaevis infected with Vibrio harveyi using micro RNAs from mucous exosomes. Mol. Immunol. 2020, 128, 268–276.
9. Miccoli, A.; Manni, M.; Picchietti, S.; Scapigliati, G. State-of-the-Art Vaccine Research for Aquaculture Use: The Case of Three Economically Relevant Fish Species. Vaccines 2021, 9, 140.
10. Zhang, X.-H.; He, X.; Austin, B. Vibrio harveyi: A serious pathogen of fish and invertebrates in mariculture. Mar. Life Sci. Technol. 2020, 2, 231–245.
11. Shen, G.M.; Shi, C.Y.; Fan, C.; Jia, D.; Wang, S.Q.; Xie, G.S.; Li, G.Y.; Mo, Z.L.; Huang, J. Isolation, identification and pathogenicity of Vibrio harveyi, the causal agent of skin ulcer disease in juvenile hybrid groupers Epinephelus fuscoguttatus × Epinephelus lanceolatus. J. Fish Dis. 2017, 40, 1351–1362.
12. Xie, J.; Bu, L.; Jin, S.; Wang, X.; Zhao, Q.; Zhou, S.; Xu, Y. Outbreak of vibriosis caused by Vibrio harveyi and Vibrio alginolyticus in farmed seahorse Hippocampus kuda in China. Aquaculture 2020, 523, 735168.
13. Lal, T.M.; Sano, M.; Ransangan, J. Isolation and Characterization of Large Marine Bacteriophage (Myoviridae), VhKM4 Infecting Vibrio harveyi. J. Aquat. Anim. Health 2017, 29, 26–30.
14. Mondal, H.; Thomas, J. A review on the recent advances and application of vaccines against fish pathogens in aquaculture. Aquac. Int. 2022, 30, 1971–2000.

15. Abu Nor, N.; Zamri-Saad, M.; Md Yasin, I.S.; Salleh, A.; Mustaffa-Kamal, F.; Matori, M.F.; Azmai, M.N.A. Efficacy of Whole Cell Inactivated Vibrio harveyi Vaccine against Vibriosis in a Marine Red Hybrid Tilapia (Oreochromis niloticus × O. mossambicus) Model. Vaccines 2020, 8, 734.
16. Huang, Z.; Tang, J.; Li, M.; Fu, Y.; Dong, C.; Zhong, J.F.; He, J. Immunological evaluation of Vibrio alginolyticus, Vibrio harveyi, Vibrio vulnificus and infectious spleen and kidney necrosis virus (ISKNV) combined-vaccine efficacy in Epinephelus coioides. Vet. Immunol. Immunopathol. 2012, 150, 61–68.
17. Nguyen, H.T.; Nguyen, T.T.T.; Tsai, M.-A.; Ya-Zhen, E.; Wang, P.-C.; Chen, S.-C. A formalin-inactivated vaccine provides good protection against Vibrio harveyi infection in orange-spotted grouper (Epinephelus coioides). Fish Shellfish Immunol. 2017, 65, 118–126.
18. Xu, W.; Jiao, C.; Bao, P.; Liu, Q.; Wang, P.; Zhang, R.; Liu, X.; Zhang, Y. Efficacy of Montanide™ ISA 763 A VG as aquatic adjuvant administrated with an inactivated Vibrio harveyi vaccine in turbot (Scophthalmus maximus L.). Fish Shellfish Immunol. 2018, 84, 56–61.
19. Harikrishnan, R.; Kim, J.-S.; Balasundaram, C.; Heo, M.-S. Vaccination effect of liposomes entrapped whole cell bacterial vaccine on immune response and disease protection in Epinephelus bruneus against Vibrio harveyi. Aquaculture 2012, 342–343, 69–74.
20. Wei, G.; Tan, H.; Ma, S.; Sun, G.; Zhang, Y.; Wu, Y.; Cai, S.; Huang, Y.; Jian, J. Protective effects of beta-glucan as adjuvant combined inactivated Vibrio harveyi vaccine in pearl gentian grouper. Fish Shellfish Immunol. 2020, 106, 1025–1030.
21. Cheng, S.; Zhang, W.-W.; Zhang, M.; Sun, L. Evaluation of the vaccine potential of a cytotoxic protease and a protective immunogen from a pathogenic Vibrio harveyi strain. Vaccine 2010, 28, 1041–1047.
22. Bai, J.-Y.; Long, H.; Cui, J.; Zhang, X.; Cai, X.-N.; Xie, Z.-Y. Characterization of a pathogenic Vibrio harveyi strain from diseased Epinephelus coioides and evaluation of different methods to control its infection. Aquaculture 2020, 526, 735371.
23. Hu, Y.-H.; Cheng, S.; Zhang, M.; Sun, L. Construction and evaluation of a live vaccine against Edwardsiella tarda and Vibrio harveyi: Laboratory vs. mock field trial. Vaccine 2011, 29, 4081–4085.
24. Hu, Y.-H.; Deng, T.; Sun, B.-G.; Sun, L. Development and efficacy of an attenuated Vibrio harveyi vaccine candidate with cross protectivity against Vibrio alginolyticus. Fish Shellfish Immunol. 2012, 32, 1155–1161.
25. Sun, Y.; Ding, S.; He, M.; Liu, A.; Long, H.; Guo, W.; Cao, Z.; Xie, Z.; Zhou, Y. Construction and analysis of the immune effect of Vibrio harveyi subunit vaccine and DNA vaccine encoding TssJ antigen. Fish Shellfish Immunol. 2019, 98, 45–51.
26. Atujona, D.; Huang, Y.; Wang, Z.; Jian, J.; Cai, S. Vibrio harveyi (VirB11) recombinant vaccine development against vibriosis in orange-spotted grouper (Epinephelus coioides). Aquac. Res. 2019, 50, 2628–2634.
27. Silvaraj, S.; Md Yasin, I.S.; MMAK; Saad, M.Z. Elucidating the Efficacy of Vaccination against Vibriosis in Lates calcarifer Using Two Recombinant Protein Vaccines Containing the Outer Membrane Protein K (r-OmpK) of Vibrio alginolyticus and the DNA Chaperone J (r-DnaJ) of Vibrio harveyi. Vaccines 2020, 8, 660.
28. Huang, W.-L.; Chuang, S.-C.; Yang, C.-D. Anti-Idiotype Vaccine Provides Protective Immunity Against Vibrio Harveyi in Grouper (Epinephelus coioides). Vaccines 2019, 7, 210.
29. Hu, Y.-H.; Sun, L. A bivalent Vibrio harveyi DNA vaccine induces strong protection in Japanese flounder (Paralichthys olivaceus). Vaccine 2011, 29, 4328–4333.
30. Wang, Q.; Chen, J.; Liu, R.; Jia, J. Identification and evaluation of an outer membrane protein OmpU from a pathogenic Vibrio harveyi isolate as vaccine candidate in turbot (Scophthalmus maximus). Lett. Appl. Microbiol. 2011, 53, 22–29.
31. Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. mRNA vaccines—A new era in vaccinology. Nat. Rev. Drug Discov. 2018, 17, 261–279.
32. Islam, S.I.; Mou, M.J.; Sanjida, S.; Tariq, M.; Nasir, S.; Mahfuj, S. Designing a novel mRNA vaccine against Vibrio harveyi infection in fish: An immunoinformatics approach. Genom. Inform. 2022, 20, e11.
33. Bisharat, N.; Agmon, V.; Finkelstein, R.; Raz, R.; Ben-Dror, G.; Lerner, L.; Soboh, S.; Colodner, R.; Cameron, D.N.; Wykstra, D.L.; et al. Clinical, epidemiological, and microbiological features of Vibrio vulnificus biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. Lancet 1999, 354, 1421–1424.
34. Shoemaker, C.A.; Lafrentz, B.R.; Klesius, P.H. Vaccination of sex reversed hybrid tilapia (Oreochromis niloticus × O. aureus) with an inactivated Vibrio vulnificus vaccine. Biologicals 2011, 39, 424–429.
35. Le, H.; Lihua, D.; Jianjun, F.; Peng, L.; Songlin, G. Immunogenicity study of an expressed outer membrane protein U of Vibrio vulnificus in Japanese eel (Anguilla japonica). J. Appl. Microbiol. 2018. Online ahead of print.

36. Guo, S.; Hu, L.; Feng, J.; Lin, P.; He, L.; Yan, Q. Immunogenicity of a bivalent protein as a vaccine against Edwardsiella anguillarum and Vibrio vulnificus in Japanese eel (Anguilla japonica). Microbiologyopen 2018, 8, e00766.
37. He, L.; Wu, L.; Lin, P.; Zhai, S.; Guo, S.; Xiao, Y.; Wan, Q. First expression and immunogenicity study of a novel trivalent outer membrane protein (OmpII-U-A) from Aeromonas hydrophila, Vibrio vulnificus and Edwardsiella anguillarum. Aquaculture 2020, 519, 734932.
38. Jun, J.W.; Kim, J.H.; Choresca, C.H., Jr.; Shin, S.P.; Han, J.E.; Han, S.Y.; Chai, J.Y.; Park, S.C. Isolation, molecular characterization, and antibiotic susceptibility of Vibrio parahaemolyticus in Korean seafood. Foodborne Pathog. Dis. 2012, 9, 224–231.
39. Lee, J.-K.; Jung, D.-W.; Eom, S.-Y.; Oh, S.-W.; Kim, Y.; Kwak, H.-S.; Kim, Y.-H. Occurrence of Vibrio parahaemolyticus in oysters from Korean retail outlets. Food Control. 2008, 19, 990–994.
40. Hu, Q.; Chen, L. Virulence and Antibiotic and Heavy Metal Resistance of Vibrio parahaemolyticus Isolated from Crustaceans and Shellfish in Shanghai, China. J. Food Prot. 2016, 79, 1371–1377.
41. Kang, C.-H.; Shin, Y.; Jang, S.; Yu, H.; Kim, S.; An, S.; Park, K.; So, J.-S. Characterization of Vibrio parahaemolyticus isolated from oysters in Korea: Resistance to various antibiotics and prevalence of virulence genes. Mar. Pollut. Bull. 2017, 118, 261–266.
42. Friedman, M. Antibiotic-Resistant Bacteria: Prevalence in Food and Inactivation by Food-Compatible Compounds and Plant Extracts. J. Agric. Food Chem. 2015, 63, 3805–3822.
43. Istiqomah, I.; Sukardi; Murwantoko; Isnansetyo, A. Review Vibriosis Management in Indonesian Marine Fish Farming. E3S Web Conf. 2020, 147, 01001.
44. Wang, W.; Guo, S.; Gao, Y.; Liang, X.; Liu, L.; Pan, S. Comparative immunogenicity of outer membrane protein K and whole-cell antigens of Vibrio parahaemolyticus for diagnosis. Lett. Appl. Microbiol. 2021, 73, 460–470.
45. Chukwu-Osazuwa, J.; Cao, T.; Vasquez, I.; Gnanagobal, H.; Hossain, A.; Machimbirike, V.I.; Santander, J. Comparative Reverse Vaccinology of Piscirickettsia salmonis, Aeromonas salmonicida, Yersinia ruckeri, Vibrio anguillarum and Moritella viscosa, Frequent Pathogens of Atlantic Salmon and Lumpfish Aquaculture. Vaccines 2022, 10, 473.
46. Liu, X.; Yang, M.-J.; Wang, S.-N.; Xu, D.; Li, H.; Peng, X.-X. Differential Antibody Responses to Outer Membrane Proteins Contribute to Differential Immune Protections between Live and Inactivated Vibrio parahemolyticus. J. Proteome Res. 2018, 17, 2987–2994.
47. Peterson, K.M.; Gellings, P.S. Multiple intraintestinal signals coordinate the regulation of Vibrio cholerae virulence determinants. Pathog. Dis. 2018, 76, ftx126.
48. Ahmed, H.A.; El Bayomi, R.M.; Hussein, M.A.; Khedr, M.H.; Remela, E.M.A.; El-Ashram, A.M. Molecular characterization, antibiotic resistance pattern and biofilm formation of Vibrio parahaemolyticus and V. cholerae isolated from crustaceans and humans. Int. J. Food Microbiol. 2018, 274, 31–37.
49. Yang, Y.; Zhang, H.; Liu, Y.; Dong, J.; Xu, N.; Yang, Q.; Zhou, S.; Ai, X. Identification of Vibrio cholerae as a bacterial pathogen of bluegill sunfish. Aquac. Rep. 2022, 23, 101092.
50. Elmahdi, S.; DaSilva, L.V.; Parveen, S. Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: A review. Food Microbiol. 2016, 57, 128–134.
51. Gao, X.; Chen, N.; Zhang, Y.; Zhang, X.; Bing, X. Non-O1 Vibrio cholerae pathogen from Cyprinus carpio and control with anti-non-O1 V. cholerae egg yolk powder (IgY). Aquaculture 2017, 479, 69–74.
52. Wittebole, X.; De Roock, S.; Opal, S.M. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence 2013, 5, 226–235.
53. Plaza, N.; Castillo, D.; Pérez-Reytor, D.; Higuera, G.; García, K.; Bastías, R. Bacteriophages in the control of pathogenic vibrios. Electron. J. Biotechnol. 2018, 31, 24–33.