The Pharma Innovation Journal 2022; SP-11(10): 1956-1961

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(10): 1956-1961

www.thepharmajournal.com
Received: 03-08-2022
Accepted: 05-09-2022

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A comprehensive review of the new emerging bacterial fish pathogen *Aeromonas veronii*

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Abstract
The current expansion of aquaculture production correlates with an increase in disease outbreaks, which has a detrimental impact on the productivity, economic success, and long-term viability of the worldwide aquaculture sector. Aeromonosis is one of the most prevalent infections in Asia that causes a high rate of mortality in cultured shrimp, fish, and shellfish. In this regard, vaccines have proven to be a successful pathogen-prevention measure to increase fish production. It has proven particularly effective to prevent infection by immunizing fish and stimulating their immune systems in aquaculture. For instance, monovalent and multivalent vaccinations have been created to protect fish against a number of bacterial infections. However, it would be challenging to create such preemptive techniques as rapidly as necessary when pathogens and infections occasionally appear. The aim of this review is to gather knowledge about bacterial pathogenesis, virulence factors, and diagnosis and to have a better understanding of various management strategies that have been employed in order to come up with a successful commercial vaccine for use in aquaculture.

Keywords: *Aeromonas veronii*, virulence factor, diagnosis, PCR, inactivated vaccines

1. Introduction
Globally fish production was observed to be 179 million tonnes in 2020 with a total first sale value estimated at US$ 401 billion. Asia is a major producer in the world, with 89% of the global total in volume terms in the last 20 years. China is the world's largest producer of tilapia with an annual production of 1.8 million tonnes (FAO, 2020). Aquaculture is one of the most rapidly growing food animal-producing sectors that utilize advanced various farming systems. Successful aquaculture practices are depending on the physical and chemical factors such as water, biological factors, and health factors of the farmed fish (FAO, 2019, Prabu *et al*. 2019) [45]. Intensification and diversification in aquaculture lead to high production in fish farming, but rapid movement of aquatic animals and animal products around the world, generating significant disease outbreaks due to unregulated transboundary movements. In the Intensive culture system fish were growing under high stocking density which will lead the stressful condition to fish. Abiotic and biotic factors include poor water quality parameters and environmental temperature, and bacteria, viruses, parasites, and fungi are responsible for stress.

2. Health management and the immune system
The growing worldwide population, rising demand, and limited resources available in capture fisheries will surely lead to the continued global growth of aquaculture, with the inherent risk of aquatic animal infections and their hosts spreading with them. (Bondad-Reantaso and Subasinghe, 2008) [12]. Fish are aquatic organisms loaded with a fascinating number of infectious agents of various types, sizes, and characteristics, all of which they have developed an incredibly effective defense mechanism against, which is remarkably similar around the vertebrate kingdom. They have humoral and cell-mediated immune responses to bacterial infections, as well as non-specific and specific immunological responses. It is only through these defense systems that infection immunity can be developed (Ellis, 1999) [20]. The innate defense mechanism is a collection of nonspecific antimicrobial systems known as innate defense mechanisms, which are constitutional and do not improve with repeated exposure to the same pathogenic agent.

3. Mode and Source of infection
In the intensive culture system fish were growing under high stocking density which will lead the stressful condition to fish.

Abiotic and biotic factors include poor water quality parameters and environmental temperature, and bacteria, viruses, parasites, and fungi are responsible for stress. Stress is a risk factor for disease because opportunistic pathogens are normally present in the water making it a favorable environment for disease-causing. The stressful condition will lead to an imbalance between the host, pathogen, and environment (Snieszko, 1974) [54]. Contaminated water serves as the reservoir of pathogenic microorganisms and variations in water quality parameters such as dissolved oxygen, pH, ammonia, nitrite, hydrogen sulfide, etc. are also responsible for favorable conditions for microorganism multiplication (Boyd and Tucker, 2019) [13]. Disease or infection depends on fish species, age, nutritional status of fish, health management practices, biosecurity, level of infection, host immune response, and environmental condition in the aquaculture system.

4. Genus *Aeromonas* and classification

Members of the genus *Aeromonas* are gram-negative cells, straight, coccobacillary to bacillary with rounded ends. Cells are 0.3 - 1 µm diameter and 1- 3.5 µm in length. It can occur as single, pair, or even as short chains (Altwegg, 1999) [4]. Most of the members are motile by a single polar flagellum of 1.7µm wavelength. They exhibit many fine structures like the S layer, flagella, piled, and capsule outside the cell membrane and cell wall (Austin *et al.*, 1998) [13]. They are facultatively anaerobic and chemoorganotrophic organisms. They have the ability to perform oxidative and fermentative metabolism of D-glucose, and acid and often produce acid with gas from many carbohydrates. In addition to glucose, Aeromonads also utilize a number of other carbohydrates (Popoff, 1984; Renaud *et al*. 1988; Altwegg *et al*. 1999; Abbott *et al*. 1992) [49, 53, 4, 32]. Most isolates utilize ammonium salt as the sole source of nitrogen. They are positive for both oxidase and catalase. They are enzymatically very active and reduce nitrates. They are reported to produce amylase, DNAase, chitinase, elastase, esterases, peptidases, arylamidases, and other hydrolytic enzymes (Joseph *et al*. 1988) [31]. The optimum growth temperature varies between 22 °C and 37°C. Growth temperature can range between 0 to 45 °C. They are generally resistant to 150 µg of vibriostatic agent 2, 4 diamino 6, 7 disisopropyl pteridine (O/129) (Abbott *et al*. 1992) [7]. *Aeromonas* sp. can cause "Aeromonosis" or "Motile *Aeromonas* Septicemia" or "Ulcer disease" Red-Sore disease" or "Red Past", "Tail, and Fin rot" disease in fish, which causes ulcers and hemorrhages in the skin and organs (Roberts, 2001) [54]. The genus *Aeromonas* consists of 31 species (Chen *et al*. 2016) [22]. Motile *Aeromonas* septicemia is worldwide distributed and it can affect freshwater as well as marine water fish. Initially based on phenotypic expression, the genus *Aeromonas* was allocated to the family Vibrionacea. Molecular genetic studies using 16S rRNA cataloging, 5S rRNA gene sequence comparisons, and rRNA DNA hybridization reveal that *Aeromonas* is sufficiently different from the vibrionacea family (Colwell *et al*. 1986; Ruimy *et al*. 1994) [18, 50]. Finally, *Aeromonas* has transferred from the family Vibrionaceae to a new family, Aeromonadaceae (Colwell *et al*. 1986) [18].

Super kingdom : Bacteria
Phylum: proteobacteria
Class: Gammoproteobacteria
Order: Aeromonadales
Family: Aeromonadacea.

The interest in the taxonomy of the genus *Aeromonas* has increased markedly in recent years and it classification, with 31 species currently recognized, remains challenging. Novel species are continuously being described, strains and species described so far are being rearranged, and DNA-DNA hybridization studies have observed discrepancies (Janda and Abbott, 2010) [29].

4.1 *Aeromonas veronii*

A. veronii is ubiquitous in fresh water and is related to a variety of vertebrates and invertebrates showing both beneficial and pathogenic outcomes (Janda and Abbott, 1998; Janda and Abbott, 2010) [28, 30]. This species was first reported by Hickmen-Brenner *et al*. (1987) [26]. They are gram-negative, facultatively anaerobic rod-shaped bacteria and are oxidase and catalase positive. *A. veronii* taxon comprised two biovars namely biovar veronii (HG 10) and biovar sobria (HG 8), with the latter being considered more virulent (Janda and Abbott, 2010) [29]. Both biovars are genotypically indistinguishable but can be readily separated on the basis of a few biochemical key tests (Altwegg *et al*. 1999, Joseph *et al*. 1991) [4, 31]. The first biovar was originally proposed for ornithine decarboxylase-positive species *A.veronii* (HG 10), whereas the second, *A.sobria* is negative for ornithine decarboxylase and positive for both arginine and lysine decarboxylase (Hickman Brenner *et al*. 1987) [26].

4.2 *A. veronii bv. veronii*

A. veronii bv. veronii grow well in the absence of added NaCl and are negative for the string test. They are resistant to 0/129, ampicillin (10 µg), and carbenicillin (30 µg) but are susceptible to cephalothin (30 µg) and colistin (10 µg). They show positive for indole and Voges prousker and are β hemolytic on sheep blood agar (5%). They produce gas from glucose, and acid from D-mannitol and sucrose but fail to produce acid from L-arabinose and are pyrazinamidase negative. They are characteristically ornithine decarboxylase positive, arginine dehydroxylase negative, and esculin and arbutin hydrolysis positive (Bergey's manual).

4.3 *A. veronii* bv. sobria

A. veronii bv. sobria grows in the absence of added NaCl and is negative for the string test. They are resistant to O/129, ampicillin (10 µg) and carbenicillin (30 µg) whereas sensitive to cephalothin and colistin (10µg). They show positive for indole and Voges prousker and are β hemolytic on sheep blood agar (5%). They produce gas from glucose, acid from D-mannitol, and sucrose and are unable to produce acid from L-arabinose, and are also pyrazinamidase negative. They are characteristically ornithine decarboxylase negative, lysine decarboxylase and arginine dihydrolase positive, and esculin and arbutin hydrolysis negative (Bergey's manual). Among the *Aeromonas* species, A. *veronii* has the greatest host range in virulence and is pathogenic to both fish and humans (Janda and Abbott, 2010) [29]. Within the motile species, *A. hydrophila, A. caviae, and A. veronii* are commonly associated with wound infections, diarrhea, and life-threatening septicemic problems in humans (Janda and Abbott, 1998) [33]. Abbott *et al*. (1994) [3] reported the first bactericemia involving Av. *bv. veronii* in humans and also highlighted the difficulty in routine identification of this organism. In fish, they have been reported as pathogens and digestive tract symbionts of zebrafish (Janda and Abbott, 1998; Bates *et al*. 2006) [33, 9]. The wide range of infections caused by *A. veronii* strains indicated that this species is a

generalist. *A. veronii* is also the causative agent of the bacterial hemorrhagic septicemia (motile aeromonads septicemia) of cultured warm-water fish, and like *A. salmonicida and A. hydrophila*, it is also considered as a major economic problem for the aquaculture industry (Austin and Austin, 2007) [7].

5. Pathogenicity of *Aeromonas* spp. on fish

Aeromonads are major bacteria in freshwater, coastal water, and sewage. They have also been described in connection with fish and human diseases (Wu *et al*. 2007) [63]. *Aeromonas* infections are responsible for high mortalities in fish hatcheries and in natural fish populations (Muduli *et al*. 2021) [39]. It is well established that *Aeromonas* spp. infections produce septicemia, ulcerative and hemorrhagic disease in fish, leading to significant mortality in the aquaculture sector which can cause heavy loss. The bacterial adhesion and colonization are mainly confined to gills, skin, and gastrointestinal tract, lesions, or ulcers (Jutfelt *et al*. 2008; Figueras *et al*. 2011) [33, 22]. Haemorrhages, ulceration, exophthalmia, ascitic fluid, liver and kidney lesions, and superficial lesions on the skin are the main characteristic of the disease (Garcia *et al*. 2007) [23]. Qin *et al*. (2014) [46] reported the outbreak of *A.veronii biovar sobria* causing 60% mortality in *Misgurnus anguillicaudatus*, with symptoms of redness of the body, bleeding spots on the body surface and ulcers in foci in severe cases of the moribund fishes. There is a number of commercial important fish susceptible to aeromonad infection including Brown trout (*Salmo trutta*), Rainbow trout (*O. mykiss*), Ayu (*Plecoglossus altevelis*), Common carp (*Cyprinus carpio*), Japanese (*Anguilla anguilla*), Channel catfish (*Ictalurus punctatus*), Tilapia (*Oreochromis niloticus*), Clariid catfish (*Clarius batrachus*) and some ornamental fishes also infected with aeromonads such as guppy (*Poecilia reticulata*), platy (*Xiphophorus maculates*), tiger barb (*Barbus pentazona*), dwarf gourami (*Colisa lalia*), etc. (Musa *et al*. 2008) [40].

5.1 Virulence factors and toxins of *A. veronii*

Virulence factors contribute to the pathogenicity of any bacterium. Understanding the virulence factors helps to know more about the interaction between pathogenic bacteria and host at cellular and molecular levels. In the case of *Aeromonas* spp, structural components, extracellular products, iron acquisition system, quorum sensing, secretion systems, and associated toxins (with hemolytic, cytotoxic, enterotoxin activities) are reported (Wang *et al*. 2003; Janda and Abbott, 2010) [60, 29]. Type III secretion system delivers toxins directly to the cytosol of the eukaryotic host through a needle-like structure and can be used as a general indicator of virulence (Stuber *et al*. 2003) [57]. Some researchers reported that all motile *Aeromonas* species produce a single polar flagellum allowing the bacteria to swim through liquid environments (Canals *et al*. 2007; Wihelms *et al*. 2009) [14, 61]. Haemolytic activity is associated with the production of both hemolysin and aerolysin genes, thereby affecting the cells by pore formation (Wong *et al*. 1998) [67]. *Aeromonas* produces a wide range of proteases that cause tissue damage, aid invasiveness and establishment of infection by overcoming host defenses, and provide nutrients for cell proliferation (Shieh, 1987; Leung *et al*. 1988) [68, 69]. *A. veronii* produces more enterotoxins (Trower *et al*. 2000) [58]. However the role of enterotoxin in fish is not fully understood. Krzymińska *et al*. (2012) [34] reported that the production of the *act* gene in *A. veronii* will interact with epithelial cells and leads to the generation of reactive oxygen species and nitric oxide radical and thus leading to cytotoxicity. Cytotoxic enterotoxin *Act* is described in *Aeromonas*, which is type II secreted having a hemolytic activity (Sha *et al*. 2002) [51]. Extracellular proteases such as collagenase are reported as an important virulent factor in *A. veronii bv. veronii*. Biofilm development is regulated by quorum sensing in *Aeromonas* (Lynch *et al*. 2002) [36]. It has a major role in virulence and is a complex process involving both pili and flagella.

5.2 Difference between *A. veronii* and other major *Aeromonas* species

Hickmen-bernner *et al*. (1987) [26] named *A. veronii* to those isolates which are ornithine decarboxylase positive and resembled *Vibrio cholera* biochemically except that they produced gas during fermentation and were negative for string test. A positive reaction in esculin or salicin and the production of gas from glucose will identify the strain as *A. veronii*. *Aeromonas* can be separated from *Vibrios* in their ability to grow in nutrient broth without NaCl, their inability to grow on TCBS agar, and their resistance to vibriostatic agent 2, 4-diamino-6, 7-diisopropyl-pteridine (O/129). *V. cholera* O1 and *V. cholera* O139 are resistant to O/129, but their decarboxylase pattern is different from aeromonads except the case of *A. veronii* (Janda and Abbott, 2010) [30]. Joseph *et al*. (1991) [31] described the differentiating features between *A. veronii* and other major aeromonads. The unique differentiating factor of *A. veronii* from other major aeromonads is positive to ornithine decarboxylase reaction. Presently, in genus *Aeromonas*, ornithine decarboxylase positive strains could be either *Av. bv. veronii* (most common) or *A. allosachrophila* (only one strain) has been reported (Abbott *et al*. 2003) [1]. *A. veronii* differs from *A. caviae* by showing a positive result for lysine decarboxylase, gas from glucose and production of hydrogen sulfide (H_2S) from GCF (gelatin-cysteine-thiosulfate medium). *A. veronii* differs from *A. hydrophila* because of negative pyrazinamidase activity and susceptibility to cephalothin and cefazolin. It was different from *A. sobria* by virtue of salicin fermentation, esculin, and arbutin hydrolysis.

5.3 Diagnosis

Aeromonas veronii infection can be detected based on clinical signs, serological, and isolation of causative agents. Clinical signs and isolation techniques will be a different fish host and severity of infection. Mohanty and Sahoo (2007) [38] suggested that gross signs or symptoms are not much helping accurate in a disease diagnosis because many bacterial infections have similarities in the case of gross signs or symptoms. The gross pathological signs of *A. veronii* 10 indicate ulcers and hemorrhagic infiltrate on fins and skin and loss of muscular mass of infected organisms (Guzman-Murillo *et al*. 2000) [24]. Amal *et al*. (2018) [5] reported severe hemorrhages of the liver, spleen, and kidney with enlarged gall bladder in hybrid red tilapia. Raj *et al*. (2019) [47] studied that infected fishes have a loss of scales, enlarged liver and gall bladder, hemorrhages on the liver, and exophthalmia. Histological characteristics observed the in brain and liver showed severe blood congestion, but more severe signs were found in the liver and the infected liver exhibited tissue degeneration and accumulation of hemosiderin around the vessels and liver cells (Dong *et al*. 2017) [19].

Hematological measures have been found to be important

indicators of fish health (Fazio, 2019) [21]. The bacterial infection showed a decrease in the number of erythrocytes in the blood and the percentage of hematocrit (Benli and Yildiz 2004) [11]. *Aeromonas* veronii infection can be detected by PCR assay, amplification of the aerolysin (aerA), cytotoxic enterotoxin (act), cytotonic enterotoxins (ast, alt), lipase (lip), glycerophospholipid: cholesterol acyltransferase 11 (gcat), serine protease (ser), DNase (exu), elastase (ahyB) and the structural gene, flagellin (fla) were performed with the template DNA of the isolates (Shameena *et al*. 2020) [52]. Nawaz *et al*. (2010) [42] reported that oligonucleotide primers amplified a 231- bp region of the act gene from the template DNA of 97.0% of the isolates and primers specific for the amplification of the aerA gene amplified a 431-bp region of the aerA gene from the template DNA of 96.0% of the isolates. Shameena *et al*. (2020) [52] reported that *A. veronii* bv. sobria is more pathogenic than *A. veronii* bv. *veronii*.

Table 1: Various techniques used to develop a vaccine against *A. veronii* in different fish species

Fish	Antigen types	Conc./ Dose	Preparation method	Route of administration	RPS	Ref.
M. anguillicaudatus	Whole cell	1 × 107 CFU/mL	Live attenuated	Injection	65.66%	[59]
Cyprinus carpio	Extracellular products	391.6 µg/fish	FKC	Injection (IP)	50-75%	[60]
Crucian carp	WC	1 × 108 CFU/mL	Inactivated vaccine	Injection	78.37%	[61]
Paralabrax maculatofasciatus	outer membrane proteins (OMPs)	20 µg of omp38 and omp48 DNA vaccines	DNA vaccine	Injection	50-60%	[62]
Cyprinus carpio	OmpAI, OmpW	109 CFU/g	Recombinant	Oral	40- 50%	[63]
Pelteobagrus fulvidraco	Bivalent	1.0 × 108 CFU mL−1	formalin-inactivated	Injection (IP)	-	[64]
Zebrafish	Bivalent and Monovalent	5 × 108 cells/fish	formalin-inactivated	Injection (IP)	66-100%	[65]
Cyprinus carpio	Subunit	10 µg/mL	Recombinant	Oral	64.29%	[66]
Carassius auratus gibelio	OmpAII	0.2 mL rOmpAII	Recombinant	Injection (IP)	38-100%	[67]

6. Conclusion

Aeromonas veronii is considered a threatening pathogen to aquaculture as it has resulted in devastating outbreaks and economic losses in fish aquaculture. The aim of this review is to gather knowledge about bacterial pathogenesis, virulence factors, and diagnosis and to have a better understanding of various management strategies that have been employed in order to come up with a successful commercial vaccine for use in aquaculture.

7. Acknowledgments

The authors would like to thank Dr. Megha Bedekar, Principle Scientist, AEHM Division, ICAR-CIFE, Mumbai.

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