



Species Diversity, Existence of Virulence Gene Profile and *in-vivo* Pathogenicity Study of *Aeromonas* spp. Isolated from Diseased *Catla catla*

Chethurajupalli Lavanya^{1*}, Tambireddy Neeraja¹, Thatavarthi Venkata Ramana², Arumugam Balasubramanian³ and Ogirala Sudhakar²

¹Department of Aquatic Animal Health Management, College of Fishery Science, Andhra Pradesh Fisheries University, Muthukur, Andhra Pradesh, India

²Andhra Pradesh Fisheries University, Vijayawada, Andhra Pradesh, India

³Department of Fisheries Resource Management, College of Fishery Science, Andhra Pradesh Fisheries University, Muthukur, Andhra Pradesh, India

ABSTRACT

This study aimed to isolate *Aeromonas* species from hemorrhagic septicemia diseased *Catla catla* freshwater fish in Andhra Pradesh, India, in order to learn more about them, locate its virulence genes, and evaluate their pathogenicity. A total of nine *Aeromonas* spp., were isolated and identified by API 20E kit. Out of nine *Aeromonas* spp., *A. veronii* bv *veronii* (28%) was most prevalent species than other *Aeromonas* spp. The isolated species were tested for frequency of six virulence genes viz., aerolysin, enterotoxin, elastase, hemolysin, lipase and serine protease. We found heterogeneous distribution pattern of virulence genes among the *Aeromonas* spp. with dominance of elastase (87%). Furthermore, catla were challenged with nine *Aeromonas* spp. with various virulence gene profile by intraperitoneal injection at 10⁶cfu/ml and studied for 96 h to determine co-relation between the number of virulence genes and their pathogenicity. *In-vivo* pathogenicity test revealed that the mortality did not depend on number of genes rather it depended on the type of combination of aerolysin, elastase and protease genes. The study concluded that isolated *Aeromonas* spp. harbored various virulence genes indicating their pathogenicity for fishes.

Article Information

Received 25 May 2024

Revised 05 July 2024

Accepted 16 July 2024

Available online 5 December 2024

(early access)

Published 13 December 2025

Authors' Contribution

CL conducted the study and wrote the manuscript. TN planned the work, analysed the data and supervised the project. TVR, AB and OS edited the manuscript.

Key words

Aeromonas species, Biochemical characterization, Virulence genes, *In-vivo*, Pathogenicity, *Catla catla*

INTRODUCTION

The Gram-negative, facultative anaerobic bacteria known as aeromonads are seriously risky. They may be found in many natural aquatic habitats (Igbiosa *et al.*, 2012). Fresh water fishes especially carps are the important food sources and study models all around the world (Sanyal *et al.*, 2018). Most popular amongst carps is *Catla catla* which is a native fish of regions of riverine systems (Shahzad *et al.*, 2014). The genus *Aeromonas* has experienced a number of revisions to its classification, adding complexity to an already convoluted subject. *A. hydrophila*, *A. caviae*, *A. salmonicida*, and *A. sobria*

are the four *Aeromonas* spp. reported in 1980s (Hu *et al.*, 2012). Then, recently, the number of *Aeromonas* spp. has increased to 41 (Fernandez-Bravo and Figueras, 2020). These species are responsible for the diseases furunculosis, hemorrhagic septicemia, skin ulcers, fin/tail rot, dropsy, and skin ulcer-causing hemorrhagic septicemia (Reith *et al.*, 2008). In humans, some *Aeromonas* species, including *A. caviae*, *A. hydrophila*, *A. veronii*, *A. trota*, *A. schubertii*, and *A. jandaei*, cause gastroenteritis (Sinha *et al.*, 2004), septicemia, peritonitis, meningitis and eye infections (Kelly *et al.*, 1993).

Aeromonad's pathogenesis is very complex because of its wide range of virulence factors that interact with one another in a multifactorial manner. Bacterial pathogenesis has been linked to a variety of proteases, including aerolysin, hemolysin, enterotoxin, temperature-sensitive protease and serine protease (Albert *et al.*, 2000; Nawaz *et al.*, 2010; Hu *et al.*, 2012; Li *et al.*, 2020). Aeromonad isolates pathogenicity varies widely in terms of the amount, and presence of virulence genes within and between species. Perhaps there is a geographical explanation for the disparities that exist (Ghenghesh *et al.*, 2014). Therefore, the continuous monitor about these species in

* Corresponding author: kavyaaa029@gmail.com
0030-9923/2026/0001-0071 \$ 9.00/0



Copyright 2026 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

fish culture is necessary to improved disease management and preventative approaches, as it understanding that *Aeromonas* are highly virulent in nature. Therefore, the present study was carried out on *Aeromonas* species identification and characterization of virulence genes with main focus on their pathogenicity.

MATERIALS AND METHODS

Fish sampling

Thirty-two infected *Catla catla* fish were collected from farms in the West Godavari and SPSR Nellore districts of Andhra Pradesh, India. All of the collected samples were brought to the lab at the College of Fishery Science in Muthukur, Nellore district.

Isolation and identification of *Aeromonas* spp.

Infected catla fish showed symptoms of bacterial hemorrhagic septicemia such as hemorrhages on body surface and internal organs, dropsy, pale gills, fin and tail rot, and discoloration of internal organs. Based on severity of infections, inoculums were collected from kidney, liver and gill tissues and were streaked on a rimler shotts (RS) medium. After 18-24 h of streaking on RS plates, the most prominent colonies were sub cultured on trypticase soy agar (TSA). Initially, the standard technique of identifying bacterial isolates relied on biochemical assays through API 20E test kit (Hi-Media, India). Taxonomic keys proposed by Abbott *et al.* (2003), Martinez-Murcia *et al.* (2008) and Beaz-Hidalgo *et al.* (2010) were followed for identification of *Aeromonas* spp.

Virulence genes detection in *Aeromonas* spp.

The DNAs of all the isolated strains were extracted

by a DNA extraction kit (Bangalore Genei, Bangalore) according to the company's guidelines with minor adjustments. Briefly, each strain mixed in 110 µl of extraction solution, incubated for 20 min at 35°C then centrifuged for 10 min at 10,000 rpm. 100 µl supernatant collected into another tube having 100 µl 100% ethanol, then centrifuged for 5 min at 10,000 rpm. The DNA pellet was washed for two times at 5000 rpm for 5 min with 100 µl of 95% ethanol. Next allow to dry DNA pellet at room temperature and dissolved in 50 µl of nucleus free water. Aerolysin, cytotoxic enterotoxin, elastase, lipase, hemolysin and serine protease virulence genes were amplified by PCR. The virulence genes primers and their thermal cycling conditions are given in Table I. PCR amplification of virulence genes was performed in a reaction volume of 25 µl using a thermal cycler (BioRad, T100, Germany). The reaction mixture consists: 1 µl of template DNA, 2 µl of F and R primer, 10 µl of master mix (Taq DNA polymerase, 2.0 x master mix red, MgCl₂ 2.0 mM, Thermo Scientific) and 12 µl of molecular grade water. The amplified product was checked on a 1.2% agarose gel.

In-vivo pathogenicity study of *Aeromonas* spp.

To determine the correlation between the number of virulence genes and the pathogenicity mortality rates, In-vivo test was conducted. A total of 330 catla (average weight 25 ± 2 g) were used for this study. Before acclimatization, fishes were treated with 2 ppm potassium permanganate for 10 min (Barkoh *et al.*, 2010) and maintained for 15 days. During that period fishes were fed with 35% protein commercial pellet (CP, Chennai) @ 3% of body weight.

Table I. Primers used in PCR for virulence genes expression of *Aeromonas*.

Virulence gene	Primer sequence (5'-3')	Product size (bp)	Denaturation	Annealing	Extension	Reference
<i>Aerolysin</i>	CCTATGGCCTGAGCGAGAAG CCAGTTCCAGTCCCACCACT	431	94°C, 30 s	55.5°C, 30 s	72°C, 30s	Mansour <i>et al.</i> (2019)
<i>Enterotoxin</i>	TGACCCAGTCCTGGCACGGC GGTGATCGATCACCACCAGC	442	94°C, 30 s	63°C, 50 s	72°C, 30s	Nawaz <i>et al.</i> (2010)
<i>Haemolysin</i>	GGCCGGTGGCCGAAGATACGGG GGCGGCGCCGACGAGACGGGG	597	94°C, 30 s	62°C, 30 s	72°C, 2 min	Sreedharan <i>et al.</i> (2012)
<i>Lipase</i>	GACTCCCTCAAGGACAGCAG AGAGGCTTTCAGGGCATTG	594	94°C, 45 s	58°C, 30 s	72°C, 30s	U-taynapun <i>et al.</i> (2020)
<i>Elastase</i>	ACACGGTCAAGGAGATCAAC CGCTGGTGTGGCCAGCAGG	540	94°C, 30 s	60.6°C, 30s	72°C, 30s	Mansour <i>et al.</i> (2019)
<i>Serine protease</i>	ATTGGATCCCTGCCTATCGCTTCAGTTCA GCTAAGCTTGCATCCGTGCCGTATTCC	911	94°C, 30 s	55°C, 30s	72°C, 30s	Zheng <i>et al.</i> (2012)

For bacterial cell suspension preparation, we used the protocol described by [Sasmal et al. \(2014\)](#). Initially, a pure culture of *Aeromonas* spp. was obtained from trypticase soya agar (TSA) slants, then streaked onto the TSA plate. After 24 h of culture at $32 \pm 1^\circ\text{C}$ the young colonies were transferred into 10 ml of trypticase soya broth (TSB). The bacterial cell suspensions were then inoculated into a 250 ml conical flask containing 90 ml of TSB for mass culture and incubated on a shaker at $32 \pm 1^\circ\text{C}$ for 24 h. The cultures were centrifuged at 7500 rpm for 20 min. After three washes with saline, the resulting cell pellet was resuspended in 10 ml of the solution. We used the spread plate method ([Collins et al., 1989](#)) to determine the number of bacterial cells per ml of neat suspension on TSA after 24 h of incubation at 32°C .

For challenge test, healthy fish were divided into three categories viz., first category for *Aeromonas* spp. injection, second category for sterile normal saline injection as sham control and third category as control with no injection and maintained as triplicates. Prior to injection, fish were anaesthetized by tricaine methanesulfonate (MS222, Sigma, 150 mg/L) ([Das et al., 2019](#)). Each catla fish in the first group was injected I/P with 0.2 ml of a bacterial cell solution of *Aeromonas* spp. containing 10^8 cfu/ml. A 0.2 ml I/P injection of sterile normal saline was administered to the second group of fish. All of the fishes in the experimental category were monitored for a full 96 h after the challenge to analyze any changes in behavior, any clinical symptoms, and any deaths that happened. The mortality rate (total number of dead fishes in a time period/ total number of fishes stocked $\times 100$) was calculated based on [Sung et al. \(2000\)](#). To confirm Koch's postulates, the moribund/freshly dead fishes with obvious clinical indications were randomly chosen for re-isolation of challenged *Aeromonas* spp.

Statistical analysis

R studio software (3.6.1 version) was used for analysing pathogenicity studies difference by multivariate ANOVA at 5% level of significance.

RESULTS AND DISCUSSION

The motile Aeromonads are the opportunistic Gram negative bacteria responsible to cause various diseases in fish like septicaemia, fin rot, haemorrhages, and dropsy, commonly called motile aeromonas septicaemia ([Lewbart, 2001](#)) and in humans, they cause gastrointestinal diseases, peritonitis, meningitis, and eye infections ([Reith et al., 2008](#)). The infected samples of the present study also recorded various motile aeromonas septicaemia disease symptoms such as pinpoint haemorrhages on the kidney,

on body and fin bases, fluid discharge from a reddened vent, excess fluid in the gut and visceral cavity, pale gills, fin and tail rot, and discoloration of internal organs like the kidney, liver, and spleen.

Table II. Prevalence and distribution of *Aeromonas* spp. between two sampling locations.

S. No	<i>Aeromonas</i> species	Prevalence (N=56)	Location wise prevalence	
			W. Godavari (N=37)	SPSR Nellore (N=19)
1	<i>A. veronii</i> bv. <i>veronii</i>	28% (n=16)	n = 9	n = 7
2	<i>A. veronii</i> bv. <i>sobria</i>	17% (n=10)	n = 6	n = 4
3	<i>A. trota</i>	10% (n=6)	n = 3	n = 3
4	<i>A. tecta</i>	10% (n=6)	n = 4	n = 2
5	<i>A. schubertii</i>	7% (n=4)	n = 2	n = 2
6	<i>A. popoffii</i>	7% (n=4)	n = 3	n = 1
7	<i>A. media</i>	7% (n=4)	n = 4	0
8	<i>A. aquariorum</i>	7% (n=4)	n = 4	0
9	<i>A. allosaccharophila</i>	3% (n=2)	n = 2	0

Characterization of *Aeromonas* spp.

The biochemical characterization of isolated *Aeromonas* spp. are given in [Supplementary Table I](#). Based on the severity of disease condition, inoculums were taken from kidney, liver and gills. A total of 56 of nine *Aeromonas* species were identified from 32 diseased catla. [Table II](#) shows the prevalence and distribution of *Aeromonas* spp. among the two districts. Many researchers isolated *Aeromonas* species from fishes, including *A. hydrophila*, *A. veronii*, *A. sobria*, *A. caviae*, *A. salmonicida*, *A. allosaccharophila*, *A. media* and *A. jandaei* ([Nawaz et al., 2010](#); [Hu et al., 2012](#); [Sanayal et al., 2018](#)). Among them, *A. veronii* is a significant cause of infections in fish ([Silver et al., 2011](#); [Nawaz et al., 2010](#); [Li et al., 2020](#)). Our study reports also suggested that *A. veronii* bv. *veronii* and *A. veronii* bv. *sobria* are the major pathogenic bacteria causing disease in freshwater fishes. In the present study, we found that *A. veronii* bv. *veronii* (28%, 16/56) was more prevalent spp. when compared to others species. This is completely in accordance with several studies ([Hu et al., 2012](#); [Li et al., 2020](#); [U-taynapun et al., 2020](#); [Sayuti et al., 2021](#)). Moreover, we have seen variation in species distribution among the two sampling locations. From W. Godavari district, 37 isolates of nine *Aeromonas* spp. recovered, while 19 isolates of six *Aeromonas* spp. found in SPSR Nellore district. Which might be influenced by type of water intake, because in W. Godavari district

Table III. Mortality rate (Mean±SD) of catla fish challenged with *Aeromonas* species.

Aeromonas species	No. of virulence genes	Virulence gene profile	Bacterial injection (cfu/ml) (10 ¹ dilution)	Mortality (mean number of fish died)					Mortality (%)
				0-12 h	12- 24 h	24 - 48 h	48 - 72 h	72 - 96 h	
<i>A. schubertii</i>	3	<i>Aer, Ela, AhyB</i>	2.0 x10 ⁸	-	2.00 ^a ±0.57	3.00 ^a ±0.57	2.33 ^b ±0.57	2.67 ^b ±1.00	100.0 ^a ±0.00
<i>A. tecta</i>	4	<i>Aer, Ela, AhyB, Lip</i>	2.1 x10 ⁸	-	2.33 ^a ±0.57	2.67 ^a ±0.57	2.33 ^b ±0.57	2.67 ^b ±0.57	100.0 ^a ±0.00
<i>A. aquariorum</i>	4	<i>Aer, Ela, AhyB, Lip</i>	2.3 x10 ⁸	-	3.00 ^a ±0.57	3.33 ^b ±0.57	2.67 ^b ±0.57	1.00 ^a ±1.15	100.0 ^a ±0.00
<i>A. allosaccharophila</i>	5	<i>Aer, Ela, AhyB, Lip, Alt</i>	1.4 x10 ⁸	-	2.67 ^a ±0.57	3.67 ^b ±0.57	2.67 ^b ±0.57	1.00 ^a ±0.00	100.0 ^a ±0.00
<i>A. media</i>	5	<i>Aer, Ela, AhyB, Lip, Hly</i>	1.2 x10 ⁸	-	2.33 ^a ±0.57	3.33 ^b ±0.57	2.67 ^b ±0.00	1.67 ^a ±0.57	100.0 ^a ±0.00
<i>A. trola</i>	6	<i>Aer, Ela, AhyB, Lip, Alt, Hly</i>	1.4 x10 ⁸	-	5.00 ^b ±0.57	2.67 ^a ±0.57	1.33 ^a ±0.57	1.00 ^a ±1.00	100.0 ^a ±0.00
<i>A. popoffii</i>	6	<i>Aer, Ela, AhyB, Lip, Alt, Hly</i>	2.1 x10 ⁸	-	5.33 ^b ±0.57	3.67 ^b ±0.57	1.00 ^a ±1.00	-	100.0 ^a ±0.00
<i>A. veronii</i> bv. <i>Sobria</i>	6	<i>Aer, Ela, AhyB, Lip, Alt, Hly</i>	1.2 x10 ⁸	-	5.33 ^b ±0.57	3.33 ^b ±0.57	1.33 ^a ±0.57	-	100.0 ^a ±0.00
<i>A. veronii</i> bv. <i>veronii</i>	6	<i>Aer, Ela, AhyB, Lip, Alt, Hly</i>	2.4 x10 ⁸	-	5.67 ^b ±1.00	3.33 ^b ±0.57	1.00 ^a ±1.00	-	100.0 ^a ±0.00
Sham control group	-	-	-	-	-	-	-	-	00.00
Control group	-	-	-	-	-	-	-	-	00.00

Aer, Aerolysin; Ela, Elastase; AhyB, Serine protease; Lip, Lipase; Alt, Enterotoxin; Hly, Hemolysin.

*Figures having different super scripts are significantly different

culture is mainly using creek water as intake where as ground water is a major intake source for Nellore district. Our findings are supported by [Altwegg *et al.* \(1989\)](#) who suggested that the prevalence of *Aeromonas* is likely to vary with geographical locations.

Determination of virulence genes in *Aeromonas* spp.

There is a heterogeneous distribution of virulence genes pattern was observed among the nine *Aeromonas* species. It believed that virulence factors contributing to the severity of many diseases through the release of a wide range of toxins [Sen and Rodgers \(2004\)](#). However, all the *Aeromonas* species cannot produce all the toxins ([Chopra and Houston, 1999](#)). The *Aeromonas* pathogenesis process was rather complicated, since no one suspected virulence-associated factor could be definitively linked to any given set of symptoms or disease ([Albert *et al.*, 2000](#)). Major virulence factors in *Aeromonas* were aerolysin, cytotoxic enterotoxin and serine protease ([Chopra *et al.*, 1996](#); [Sha *et al.*, 2002](#); [Nawaz *et al.*, 2010](#); [Tomas, 2012](#)). The present study findings showed that, 87% of the isolates possessed at three or more virulence gene. Of the six virulence genes, elastase gene was found to be dominant with prevalence of 87% (49/56) followed by serine protease 73% (41/56), aerolysin 62% (35/56), cytotoxic enterotoxin 48% (27/56), lipase 46% (26/56) and haemolysin 39% (22/56).

Similar to our findings, [Shuang *et al.* \(2020\)](#) also found elastase in 100% of *Aeromonas*. Elastase is a zinc

metalloprotease enzyme that involved in pathogenesis ([Tomas, 2012](#)). Further, Serine proteases participate in β -hemolysin precursor activation, which includes the stimulation of aerolysin and other cellular enzymes, which may have an impact on the total virulence of *Aeromonas* ([Nawaz *et al.*, 2010](#)). In the present study, we found serine protease in 73% of the isolates and aerolysin in 62% of the isolates. The aerolysin gene participates in the secretion of adhesins, hemagglutinins, and several hydrolytic enzymes, all of which are important in pathogenesis ([Sreedharan *et al.*, 2012](#)). More or less similar frequencies of virulence genes are found by ([El-Gohary *et al.*, 2020](#); [Li *et al.*, 2020](#); [Nawaz *et al.*, 2010](#)). The present study isolates had enterotoxin in 48%, which is very similar to ([Gashgari and Selim, 2015](#)) who found 42% in *A. veronii* isolated from sea bream. These enterotoxins are implicated in tissue destruction and the release of fluid in infected fish intestines ([Sha *et al.*, 2002](#)). Further, lipase gene recorded in 46% of present study isolates, similarly ([U-taynapun *et al.*, 2020](#)) found 46% of lipase in *Aeromonas* spp. Lipase enzymes involving in the modification of the animal cell membrane, increasing the seriousness of the disease ([Tomas, 2012](#)). In our isolates haemolysin gene was found in 39% of the strains, which are involving in lysis of the erythrocytes ([Wang *et al.*, 2008](#)).

In-vivo pathogenicity of *Aeromonas* spp. to Catla catla

The mortality pattern of catla challenged with nine

Aeromonas spp. is shown in Table III. *In-vivo* pathogenicity studies showed that the bacteria were able to induce severe infections in catla fishes. We found 100% mortality in challenged fishes with *Aeromonas* spp. possessed three (elastase, serine protease and aerolysin) to six virulence genes (elastase, serine protease, aerolysin, enterotoxin, lipase and haemolysin). Indicating that, even presence of only three virulence genes such as elastase, serine protease and aerolysin could cause 100% mortality. The *In-vivo* pathogenicity results were supported by the prevalence of virulence genes in isolated *Aeromonas* spp. of the present study. The results completely agreed with those of Nawaz *et al.* (2010); Hu *et al.* (2012) and Li *et al.* (2011, 2020), who suggested that aerolysin, enterotoxin elastase, and protease in *Aeromonas* pose a high threat to the animals. Moreover, we have observed prominent pathological signs after 24 h of post injection, such as hemorrhages on various body parts, tail rot and internal fluid accumulation, which might be due to presence of aerolysin, enterotoxins and proteases. In addition, mortality was not found in sham control and control groups. Our findings suggested that a bacterial strain's pathogenicity was actually associated with the type of virulence genes possessed rather than its number.

CONCLUSION

Catla fish farms often have problems with *Aeromonas* spp., especially by *A. veronii* bv *veronii* and *A. veronii* bv *sobria*. In addition, the present study isolates found with elastase, enterotoxin, protease and aerolysin genes, indicating the pathogenic potential of our isolates. The *in-vivo* pathogenicity test also confirmed the virulence of *Aeromonas* spp. to catla. It was suggesting that pathogenicity was related to the type of virulence genes possessed by *Aeromonas* spp. but not on number of genes. Hence, our findings suggesting that, *Aeromonas* spp. pose more serious threat to the freshwater fishes.

DECLARATIONS

Funding

The study was funded by College of Fishery Science, Muthukur.

Ethical approval

The catla fishes were used in this study handled with very care as per Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) and study was approved by Sri Venkateswara Veterinary University committee, 2019.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Abbott, S.L., Cheung, W.K. and Janda, J.M., 2003. The genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J. clin. Microbiol.*, **41**: 2348–2357. <https://doi.org/10.1128/JCM.41.6.2348-2357.2003>
- Albert, M.J., Ansaruzzaman, M., Talukder, K.A., Chopra, A.K., Kuhn, I., Rahman, M., Faruque, A.S., Islam, M.S., Sack, R.B. and Mollby, R., 2000. Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. *J. clin. Microbiol.*, **38**: 3785–3790. <https://doi.org/10.1128/JCM.38.10.3785-3790.2000>
- Altwegg, M., Geiss, H.K. and Freij, B.J., 1989. *Aeromonas* as a human pathogen. *CRC Crit. Rev. Microbiol.*, **16**: 253–286. <https://doi.org/10.3109/10408418909105478>
- Barkoh, A., Smith, G.D. and Southard, M., 2010. *Prymnesium parvum* control treatments for fish hatcheries. *J. Am. Water Resour. Assoc.*, **46**: 161–169. <https://doi.org/10.1111/j.1752-1688.2009.00400.x>
- Beaz-Hidalgo, R., Alperi, A., Bujan, N., Romalde, J.L. and Figueras, M.J., 2010. Comparison of phenotypical and genetic identification of *Aeromonas* strains isolated from diseased fish. *Syst. appl. Microbiol.*, **33**: 149–153. <https://doi.org/10.1016/j.syapm.2010.02.002>
- Chopra, A.K. and Houston, W.C., 1999. Enterotoxins in *Aeromonas* associated gastroenteritis. *Microb. Infect.*, **1**: 1129–1137. [https://doi.org/10.1016/S1286-4579\(99\)00202-6](https://doi.org/10.1016/S1286-4579(99)00202-6)
- Chopra, A.K., Peterson, J.W., Xu, X.J., Coppenhaver, D.H. and Houston, C.W., 1996. Molecular and biochemical characterization of heat-stable cytotoxic enterotoxin from *Aeromonas hydrophila*. *Microb. Pathogen.*, **21**: 357–377. <https://doi.org/10.1006/mpat.1996.0068>
- Collins, C.H., Lyne, P.M. and Grange, G.M., 1989. *Collins and lyne microbiological methods*, 6th Ed. Butterworth, London.
- Das, S., Aswani, R., Jasim, B., Sebastian, K.S., Radhakrishnan, E.K. and Mathew, J., 2019. Distribution of multi-virulence factors among *Aeromonas* spp. isolated from diseased *Xiphophorus hellerii*. *Aquacult. Int.*, **28**: 235–248. <https://doi.org/10.1007/s10499-019-00456-5>
- El-Gohary, F.A., Zahran, E., El-Gawad, A., Eman, A.

- and El-Gohary, A.H., 2020. Investigation of the prevalence, virulence genes, and antibiogram of motile aeromonads isolated from Nile Tilapia fish farms in Egypt and assessment of their water quality. *Animals*, **1432**: 10. <https://doi.org/10.3390/ani10081432>
- Fernandez-Bravo and Figueras, 2020. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. *Microbiology*, **8**: 1-39. <https://doi.org/10.3390/microorganisms8010129>
- Gashgari, R.M. and Selim, S.A., 2015. Detection and characterization of antimicrobial resistance and putative virulence genes in *Aeromonas veronii* Biovar *Sobria* isolated from Gilthead sea bream (*Sparus aurata* L.). *Fd. Borne Pathog. Dis.*, **12**: 620, 806-811. <https://doi.org/10.1089/fpd.2015.1976>
- Ghenghesh, K., Ahmed, S.F., Cappuccinelli, P. and Klena, D.J., 2014. Genospecies and virulence factors of *Aeromonas* species in different sources in a North African country. *Libyan J. Med.*, **9**: 25497. <https://doi.org/10.3402/ljm.v9.25497>
- Hu, M., Wang, N., Pan, Z.H., Lu, C.P. and Liu, Y.J., 2012. Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls and water environment in China. *Lett. appl. Microbiol.*, **55**: 224-233. <https://doi.org/10.1111/j.1472-765X.2012.03281.x>
- Igbinsosa, I.H., Igumbor, E.U., Aghdasi, F., Tom, M. and Okoh, A.I., 2012. Emerging *Aeromonas* species infections and their significance in public health. *Sci. World J.*, pp. 1-13. <https://doi.org/10.1100/2012/625023>
- Kelly, K.A., Koehler, J.M. and Ashdown, L.R., 1993. Spectrum of extraintestinal disease due to *Aeromonas* species in tropical Queensland, Australia. *Clin. Infect. Dis.*, **16**: 574-579. <https://doi.org/10.1093/clind/16.4.574>
- Lewbart, G.A., 2001. Bacteria and ornamental fish. *Semin. Avian Exotic Pet Med.*, **10**: 48-56. <https://doi.org/10.1053/saep.2001.19543>
- Li, J., Ni, X.D., Liu Y.J. and Lu, C.P., 2011. Detection of three virulence genes *alt*, *ahp* and *aerA* in *Aeromonas hydrophila* and their relationship with actual virulence to zebraish. *J. appl. Microbiol.*, **110**: 823-830. <https://doi.org/10.1111/j.1365-2672.2011.04944.x>
- Li, T., Raza, S.Z., Bintong, Y., Yufeng, S., Wang, J., Sun, W., Qian, A., Wang, C., Yuanhuan, K. and Shan, K., 2020. *Aeromonas veronii* infection in commercial freshwater fish: A potential threat to public health. *Animals*, **10**: 608. <https://doi.org/10.3390/ani10040608>
- Mansour, A., Mahfouz, N.B., Husien, M. and El-Magd, M.A., 2019. Molecular identification of *Aeromonas hydrophila* strains recovered from Kafrel Sheikh fish farms. *Slov. Vet. Res.*, **56**: 201-208. <https://doi.org/10.26873/SVR-758-2019>
- Martinez-Murcia, A.J., Saavedra, M.J., Mota, V.R., Maier, T., Stackebrandt, E. and Cousin, S., 2008. *Aeromonas aquariorum* sp. nov., isolated from aquaria of ornamental fish. *Int. J. Syst. Evol. Microbiol.*, **58**: 1169-1175. <https://doi.org/10.1099/ijs.0.65352-0>
- Nawaz, M., Khan, S.A., Khan, A.A., Sung, K., Tran, Q., Kerdahi, K. and Steele, R., 2010. Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. *Fd. Microbiol.*, **27**: 327-331. <https://doi.org/10.1016/j.fm.2009.11.007>
- Reith, M.E., Singh, R.K., Curtis, B., Boyd, J.M., Bouevitch, A., Kimball, J., Munholl, J., Murphy C., Sarty, D., Williams, J., Nash, J.H., Johnson, S.C. and Brown L.L., 2008. The genome of *Aeromonas salmonicida* subsp. *salmonicida*, insights into the evolution of a fish pathogen. *BMC Genom.*, **9**: 427. <https://doi.org/10.1186/1471-2164-9-427>
- Sanyal, K.B., Mukherjee, D., Guchhait, A. and Dash, G., 2018. Phenotypic and molecular identification of bacterial species in Indian major carps and exotic carps from south 24 Parganas, West Bengal, India. *Int. J. Curr. Microbiol. appl. Sci.*, **7**: 534-547.
- Sasmal, D., Surendhra, B.C. and Abaham, T.J., 2014. Effect of garlic (*Alliumsativum*) extract on the growth and disease resistance of *Carassius auratus* (Linnaeus, 1758). *Indian J. Fish*, **52**: 207-214.
- Sayuti, M.A., Salwany, M.D., Saad, M., Yusof, Annas, S., Yunus, N., Liles, M., Monir, S., Zaidi, Z. and Amal, Z., 2021. The prevalence, putative virulence genes and antibiotic resistance profiles of *Aeromonas* spp. isolated from cultured freshwater fishes in peninsular Malaysia. *Aquaculture*, **540**: 736719-736728. <https://doi.org/10.1016/j.aquaculture.2021.736719>
- Sen, K. and Rodgers, M., 2004. Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: A PCR identification. *J. appl. Microbiol.*, **97**: 1077-1086. <https://doi.org/10.1111/j.1365-2672.2004.02398.x>
- Sha, J., Kozlova, E.V. and Chopra, A.K., 2002. Role of various enterotoxins in *Aeromonas hydrophila*-induced gastroenteritis: Generation of enterotoxin gene-deicient mutants and evaluation of their enterotoxic activity. *Infect. Immununol.*, **60**: 1924-1935. <https://doi.org/10.1128/IAI.70.4.1924->

1935.2002

- Shahzad, A., Iahtasham, K., Qurban, A., Safwan, U., Zahid, M. and Shamim, A., 2014. Isolation and characterization of *Aeromonas sobria* in *catla catla* affected with hemorrhagic septicemia. *Bull. Eur. Assoc. Fish Pathol.*, **34**: 35-44.
- Shuang, Y., Yong, Lu, W., Chen, C., Jing, Y., Min, Y., Ning, N., Dong, D., Rong, L., Gang, C. and Juan, L., 2020. Genetic diversity, antimicrobial resistance, and virulence genes of *Aeromonas* isolates from clinical patients. *Tap Water Syst. Fd. Biomed. Environ. Sci.*, **33**: 385-395.
- Silver, A.C., Williams, D., Faucher, J., Horneman, A.J. and Gogarten, J.P., 2011. Complex evolutionary history of the *Aeromonas veronii* group revealed by host interaction and DNA sequence data. *PLoS One*, **6**: 16751.
- Sinha, S., Shimada, T., Ramamurthy, T., Bhattacharya, S.K., Yamasaki, S., Takeda, Y. and Nair, G.B., 2004. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *J. med. Microbiol.*, **53**: 527-534. <https://doi.org/10.1099/jmm.0.05269-0>
- Sreedharan, K.R., Philip, S. and Singh, I.S.B., 2012. virulence potential and antibiotic susceptibility pattern of motile aeromonads associated with freshwater ornamental fish culture systems: A possible threat to public health. *Braz. J. Microbiol.*, **43**: 754-765. <https://doi.org/10.1590/S1517-83822012000200040>
- Sung, H.H., Hwang, S. and Tasi, F., 2000. Responses of giant freshwater prawn (*Macrobrachium rosenbergii*) to challenge by two strains of *Aeromonas* spp. *J. Invert. Pathol.*, **76**: 278-284. <https://doi.org/10.1006/jipa.2000.4981>
- Tomas, J.M., 2012. The main *Aeromonas* pathogenic factors. *Int. Sch. Res. Netw. Microbiol.*, pp. 1-22. <https://doi.org/10.5402/2012/256261>
- U-taynapun, K., Nganwisuthiphan, T. and Chirapongsatunkul, N., 2020. Species diversity and existence of virulence genes in clinical *Aeromonas* spp. causing motile *Aeromonas septicemia* (MAS) isolated from cultured Nile tilapia (*Oreochromis niloticus*). *Int. J. Agric. Technol.*, **16**: 749-760.
- Wang, C.L., Cao, X., Liu, X.D., Guo, L.P., Hu, T., Ni, X.D., Liu, Y.J. and Lu, C.P., 2008. Detection of biochemical characters and extracellular proteases in the different isolates of *Aeromonas hydrophila*. *Anim. Husband. Vet. Med.*, **40**: 16-19.
- Zheng, W., Cao, H. and Yang, X., 2012. Grass carp (*Ctenopharyngodonidellus*) infected with multiple strains of *Aeromonas hydrophila*. *Afr. J. Microbiol. Res.*, **6**: 4512-4520. <https://doi.org/10.5897/AJMR11.1405>