Article AR2483

DOI: HTTPS://DOI.ORG/10.23910/1.2021.2483

International Journal of Bio-resource and Stress Management

Crossref

October 2021

Print ISSN 0976-3988
Online ISSN 0976-4038

IJBSM 2021, 12(5):506-515

Research Article

Stress Management

Antimicrobial Resistance of *Aeromonas* Species Isolated from Cultured Indian Major Carp, *Labeo rohita*: Possible Public Health Concern

Chethurajupalli Lavanya[1], Tambireddy Neeraja[1*], P. Hari Babu[1], T. V. Ramana[1], A. Balasubramanian[1], Supradhnya Meshram[2] and P. Sruthi[1]

[1]Dept. of Aquatic Animal Health Management, College of Fishery Science, SVVU, Muthukur, Nellore, Andhra Pradesh (524 344), India

[2]Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai (400 061), India

Open Access

Corresponding Author

Tambireddy Neeraja

e-mail: tambinee@yahoo.com

Citation: Lavanya et al., 2021. Antimicrobial Resistance of *Aeromonas* Species Isolated from Cultured Indian Major Carp, *Labeo rohita*: Possible Public Health Concern. International Journal of Bio-resource and Stress Management 2021, 12(5), 506-515. HTTPS://DOI.ORG/10.23910/1.2021.2483.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

Conflict of interests: The authors have declared that no conflict of interest exists.

Abstract

In India, *Labeo rohita* is widely cultured and consumed freshwater fish. Aeromonads are etiological agents of major bacterial fish diseases like furunculosis, haemorrhagic septicaemia, skin ulcers, fin/tail rot and dropsy, causing significant economic losses in carp culture. *Aeromonas* species are widely distributed in aquatic environment which is considered as important vehicle of *Aeromonas* infections to fish and humans. Some of the *Aeromonas* spp. causes gastroenteritis, septicaemia, peritonitis, meningitis and eye infections in humans. In the present study *Aeromonas* species were isolated from diseased freshwater fish *Labeo rohita* collected from two districts *viz*., West Godavari and SPSR Nellore of Andhra Pradesh, India. A Total of 12 *Aeromonas* spp. were isolated and identified by biochemical tests. *A. veronii* bv. *veronii* (35%) was dominant when compared to other *Aeromonas* spp. Further, Antimicrobial resistance and multiple Antimicrobial resistance (MAR) of all *Aeromonas* spp. were tested against 17 antibiotics being frequently used for human diseases. The Antimicrobial resistance of all the 12 *Aeromonas* spp. have shown significantly high ($p<0.05$) resistance (100%) to ampicillin, amoxyclave and oxytetracycline except *A. cavernicola* when compared to other antibiotics. The MAR index of *Aeromonas* spp. ranged from 0.18-0.76, which indicates origination of isolated *Aeromonas* spp. from high risk sources of contamination. *A. hydrophila*, *A. veronii* bv. *sobria, A. veronii* bv. *veronii*, *A. schubertii* and *A. jandaei* isolated in this study were found to be pathogenic to humans also. The results revealed the pathogenic potential of *Aeromonas* infections in freshwater fish culture and emerging threats to public health.

Keywords: *Aeromonas* species, antimicrobial resistance, biochemical characterization, *Labeo rohita*

1. Introduction

Among the freshwater fishes, Indian major carps are the most widely cultivated freshwater fishes in India contributing to 80% of production (Anonymous, 2017). In India, the increase in aquaculture production particularly an expansion into semi-intensive and intensive methods of production led to an increase in fish diseases due to higher stocking densities and stress conditions that favour the occurrence and spread of infectious diseases (Das and Mishra, 2014). The occurrence of bacterial

Article History

RECEIVED on 02nd July 2021 RECEIVED in revised form on 16th September 2021 ACCEPTED in final form on 27th October 2021

diseases in freshwater fish was considered to be a serious problem in India (Mishra et al., 2017). The most common bacteria associated with disease outbreaks in fish culture systems are *Aeromonas* spp. (Tomas, 2012). *Aeromonas* spp. are etiological agents of fish diseases like furunculosis (Reith et al., 2008), haemorrhagic septicemia, skin ulcers (Figuras et al., 2009), fin/tail rot (Cizek et al., 2010) and dropsy (Sreedharan, 2008). *Aeromonas* spp. are pathogenic to fish and humans, their presence in fish culture systems is of concern (Bomo et al., 2003). The genus *Aeromonas* is widely distributed in the aquatic environment (Popoff, 1984; Daskalov, 2006) and can easily transmit to humans posing threat to public health (Austin and Adams, 1996; Borrell et al.,1998; Janda and Abbott, 1998). The diseases caused by aeromonads in humans are generally the result of ingestion of contaminated water and aquatic food (Alavandi and Ananthan, 2003). *Aeromonas* species are also frequently isolated from various meat products, milk, vegetables (Stratev et al., 2016) which are considered as an important vehicle of *Aeromonas* infections to humans (Altwegg et al., 1991; Kirov, 1993). Some of the*Aeromonas* species such as *A. caviae*, *A. hydrophila*, *A. veronii* bv. *sobria, A. veronii* bv. *veronii*, *A. trota, A. schubertii*, *A. jandaei* causing gastroenteritis in adults (Sinha et al., 2004); *A. hydrophila*, *A. veronii* bv. *sobria, A. caviae*, *A. media*, *A. trota* and *A. jandaei* causing gastroenteritis in children (Albert et al., 2000); *A. caviae* causing septicaemia (Dwivedi et al., 2008) and in rear occasions *A. caviae*, *A. hydrophila*, *A. sobria* causing peritonitis, meningitis and eye infections in humans especially in immune compromised patients (Kelly et al., 1993). Among the Aeromonads, *A. hydrophila, A. caviae* and *A. veronii* bv. *sobria* are responsible for 85% of gastrointestinal diseases (Kuijper et al., 1989; Altwegg et al.,1990; Havelaar et al., 1992; Khun et al., 1997; Borrell et al., 1998; Stratev et al., 2012).

Antibiotics are a major source to treat bacterial infections in aquaculture. However, overuse of antibiotics for the treatment of bacterial diseases or their incorporation in animal feeds as growth promoters in sub-therapeutic doses may lead to the development of antibiotic resistance (Vivekanadhan et al., 2002). The antibiotic resistance of *Aeromonas* species is considered a serious threat worldwide (El-ghareeb et al., 2019). The present study aimed to evaluate the prevalence of *Aeromonas* spp. isolated from majorly cultured and consumed freshwater fish, *Labeo rohita* from Andhra Pradesh, India and their antimicrobial resistance patterns against most commonly used antibiotics in treating human infections.

2. Materials and Methods

2.1. Fish Sampling

The diseased *Labeo rohita* fishes were collected from fish farms of two districts *viz.,*West Godavari and SPSR Nellore of Andhra Pradesh, India. All the samples were aseptically brought to the laboratory of the Department of Aquatic animal health management, College of fishery science, Muthukur, Nellore district for microbiological study.

2.2. *Isolation of Aeromonas spp.*

A total of 33 rohu fish showing clinical signs of bacterial hemorrhagic septicemia were collected and *Aeromonas spp.* were isolated from skin, liver, abdominal fluid and kidney. The inoculums from these organs were streaked on Rimlershotts (RS) medium and Glutamate Starch Phenol red Agar base (GSPA). On RSA plates, *Aeromonas* forms yellow coloured, convex and glossy colonies of 0.5-3.0 mm diameter, after 18-24 h of streaking. On GSPA medium, *Aeromonas* produce yellow, circular, convex and glossy colonies of 0.5-3.0 mm diameter after 48-72 h of streaking. Typical colonies were picked from the plates, sub-cultured in trypticase soy agar (TSA) slants for biochemical characterization and antibiotic resistance tests.

2.3. *Characterization of Aeromonas spp.*

Identification of bacterial isolates was carried out by biochemical tests in the conventional method. A series of biochemical reactions as described by Martin-Carnahan and Joseph (2005) were performed to identify bacteria at the genus and species level. Taxonomic keys proposed by Abbott et al. (2003), Martinez-Murcia et al. (2008) and Beaz-Hidalgo et al. (2009 and 2010) were followed for identification of *Aeromonas* spp.

2.4. Antimicrobial resistance assay

Antibiotic resistance of *Aeromonas* spp. was carried out against 17 potential antibiotics by disc diffusion method on Mullen Hinton agar.The antibiotic discs and sterile swabs were procured from HiMediaDCM™, Mumbai, Maharashtra, India. The antibiotics used, their concentrationand the interpretation of antimicrobial resistance of bacteria to various antibiotics are shown in Table 1. Young cultures of bacteria (20 h old) from TSA slants were inoculated individually into Tryptone Soya Broth (TSB) and incubated for 10-12 h at 30±2°C. Inoculum from TSB grown culture of each bacterium was collected using sterile cotton swabs and spread onto Mullen Hinton agar (MHA) plate. Antibiotic impregnated discs were placed aseptically onto the inoculated agar plates at equal distances and sufficiently separated from each other to avoid overlapping of the zone of inhibition and at least 15 mm away from the edge. The plates were then incubated for 24 h at 30±2°C and the diameter of the zone of inhibition was measured.

2.5. Multiple antibiotic resistance (MAR) and MAR index

The resistance profiles and resistance patterns of *Aeromonas* spp. for 17 potential antibiotics were determined using antibiogram data (Table 1). Multiple antibiotic resistance (MAR) of each *Aeromonas*sp. at least resistant to three antibiotics was recorded and MAR index was calculated as per Orozova et al. (2010).

MAR Index=(Number of antibiotics to which the bacterium is resistant/The total number of antibiotics tested)

2.6. *Statistical analysis*

Statistical analysis for antibiotic resistance was carried out

Table 1: List of antibiotics and antibiotic resistance interpretation chart (Himedia, 2009)

Antibiotics with code	Disc content (µg disc^{-1})	Diameter of zone of inhibition (mm)		
		R (<)	IM	S (>)
Amikacin (AK)	30	14	15-16	17
Amoxyclav (AMX)	30	13	14-17	18
Ampicillin (AMP)	10	13	14-16	17
Chloramphenicol (C)	30	12	13-17	18
Ciprofloxacin (CIP)	5	15	16-20	21
Co-trimoxazole (CoT)	25	10	11-15	16
Doxycycline hydrochloride (DO)	30	10	11-13	14
Enrofloxacin (EX)	10	15	16-20	21
Erythromycin (E)	15	13	14-22	23
Gentamicin (GEN)	10	12	13-14	15
Nalidixic acid (NA)	30	13	14-18	19
Neomycin (N)	30	12	13-16	17
Nitrofurantoin (NIT)	300	14	15-16	17
Novobiocin (NV)	30	12	13-16	17
Oxacillin (O)	5	17	-	18
Oxytetracycline (OX)	30	11	12-14	15
Trimethoprim (TR)	5	10	11-15	16

R: Resistant; IM: Intermediate; S: Sensitive

using R studio software (3.6.1 version) and graphs were obtained using Microsoft Excel –2007. Pearson chi square test was used to test the significant difference in the prevalence of isolated *Aeromonas* spp. and their significant difference towards antimicrobial resistance.

3. Results and Discussion

3.1. Gross pathological signs of diseased fish

The clinical symptoms observed in the infected fish were haemorrhages on the body surface and fin bases; reddish bulged vent with bloody fluid discharge; fluid accumulation in the intestine and visceral cavity; pale gills; fin and tail rot; discoloration of internal organs like kidney, liver, spleen and pinpoint haemorrhages on the kidney. The major gross pathological signs observed in diseased fish are presented in Plate 1.

3.2. Identification of Aeromonas spp.

The isolated *Aeromonas* spp. were characterized by conventional biochemical tests and the results are given in Table 2. Twelve *Aeromonas* species isolated from *Labeo rohita* were *A. veronii* bv. *veronii* (n=21), *A. tecta* (n=10), *A. veronii* bv. *sobria* (n=4), *A. aquariorum* (n=4), *A. popoffii* (n=4), *A. schubertii* (n=4), *A. jandaei* (n=3), *A. molluscorum* (n=3), *A. hydrophila* (n=3), *A. piscicola* (n=2), *A. enchelia* (n =1) and *A.*

a) Haemorrhagic fins, body surface with reddish vent

b) Tail rot and hemorrhages on body

c) abdominal dropsy and haemorrhagic eye

d) Bloody fluid accumilation in the intestine and discoloured liver

Plate 1: Gross pathological signs of diseased *Labeorohita* collected in fish farms of Andhra Pradesh

cavernicola (n=1). The letter ‘n’ represents number of each species of *Aeromonas* isolated. Among the 60 *Aeromonas* isolates of *L. rohita* identified*,* the highest prevalence (35%)

Table 2: Biochemical characterization of *Aeromonas* spp. isolated from *Labeo rohita* in conventional methods

Sl. No.	Aeromonas species	Grams reaction	Morphology	Motility	Oxidase	Catalase	O/F	MR	VP	Esculin	Indole test	Citrate test	Gas from glucose	Lysine decarboxylase	Ornithine decarboxylase	Arginine hydrolase	H2S Production
		Biochemical tests															
1.	*A. aquariorum*																
2.	*A. cavernicola*	-	R	+	+	+	+/+	+	+	+	-	-	-	+	+	+	-
3.	*A. enchelia*	-	R	+	+	+	+/+	-	-	+	+	-	+	-	-	v	v
4.	*A. hydrophila*	-	R	+	+	+	+/+	+	+	+	+	+	n	+	-	+	+
5.	*A. jandaei*	-	R	+	+	+	+/+	+	+	-	+	+	+	+	-	+	v
6.	*A. molluscorum*	-	R	+	+	+	+/+	-	-	+	-	v	-	-	-	v	-
7.	*A. popoffii*	-	R	+	+	+	+/+	+	+	-	v	+	+	-	-	+	+
8.	*A. piscicola*	-	R	+	+	+	+/+	+	+	+	+	v	+	+	-	v	v
9.	*A. schubertii*	-	R	+	+	+	+/+	+	+	-	-	v	-	+	-	+	-
10.	*A. tecta*	-	R	+	+	+	+/+	+	+	+	-	v	+	+	-	v	v
11.	*A. veronii* bv. *sobria*	-	R	+	+	+	+/+	+	+	-	+	v	+	+	-	+	v
12.	*A. veronii* bv. *veronii*	-	R	+	+	+	+/+	+	+	+	+	+	+	+	+	-	v

Table 2: Continue...

Sl. No.	Aeromonas species	Biochemical tests							
		Acid produced from			Fermentation from				
		Sucrose	Lactose	Maltose	Arabinoose	Salicin	Mannitol	Sorbitol	Cellobiose
1.	*A. aquariorum*	n	-	n	-	n	n	-	n
2.	*A. cavernicola*	-	-		+	-	-	-	_
3.	*A. enchelia*	n	n	n	-	n	n	n	n
4.	*A. hydrophila*	+	n	n	v	+	+	n	n
5.	*A. jandaei*	-	n	n	n	n	n	n	n
6.	*A. molluscorum*	n	n	n	n	-	n	n	n
7.	*A. popoffii*	n	n	n	n	n	n	n	-
8.	*A. piscicola*	n	n	n	n	n	n	+	n
9.	*A. schubertii*	n	n	+	-	n	-	n	n
10.	*A. tecta*	n	n	n	n	n	n	n	n
11.	*A. veronii* bv. *sobria*	+	n	n	-	n	n	n	n
12.	*A. veronii* bv. *veronii*	n	n	N	n	n	n	n	n

R: Rods; n: not applicable; v: variable (either +ve or –ve)

was recorded for *A. veronii*bv. *veronii*, followed by 16.7% of *A. tecta,* 6.67% each of *A. veronii* bv. *sobria, A. aquariorum, A. popoffii*, *A. schubertii* and 5% *A. jandaei*, *A. molluscorum, A. hydrophila* and 3.33% of *A. piscicola*, 1.67% each of *A. cavernicola* and *A. enchelia*. The prevalence of *Aeromonas* species in rohu was given in Figure 1.

3.3. Antimicrobial resistance assay

The 60 isolates of *Aeromonas* spp. were individually tested for antimicrobial resistance against 17 antibiotics. The percentage of antimicrobial resistance of *Aeromonas* spp. to the 17 antibiotics is given in Figure 2. The *Aeromonas* isolates displayed 91.67% resistance to ampicillin, amoxyclav, and

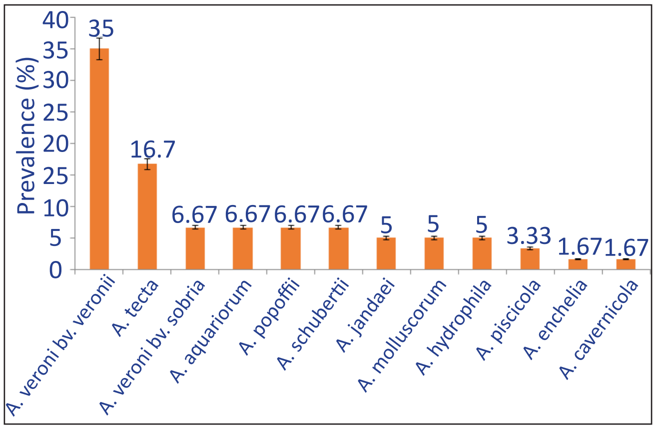

Figure 1: Prevalence (%) of *Aeromonas* spp. isolated from *Labeo rohita*

oxytetracycline except for *A. cavernicola*; 78.77% resistance to erythromycin; 65.02% resistance to chloramphenicol; 59.72% resistance to novobiocin; 57.14% resistance to doxycycline hydrochloride; 55.87% resistance to nitrofuratoin; 52.94% resistance to oxacillin; 51.68% resistance to amikacin; 47.62% resistance to ciprofloxacin; 42.95% resistance to neomycin; 42.66% resistance to trimethoprim; 31.78% resistance to co- trimoxazole; 27.42% resistance to nalidixic acid; 24.62% resistance to enrofloxacin and 22.62% resistance to gentamycin.

Statistical analysis by Pearson chi-square showed that the antibiotic resistance of *Aeromonas* isolates to ampicillin, amoxyclav and oxytetracycline was significantly high (p<0.05) in comparison to the other 14 antibiotics tested. The calculated chi-square value was 151.97 while the table value for chi-square at 16 degrees of freedom is 26.296. The results of the statistical analysis showed a significant difference in antibiotic resistance of *Aeromnas* isolates to various tested antibiotics.

3.4. Multiple antibiotic resistance (MAR) index and percentage

The Multiple antibiotic resistance patterns of 12 *Aeromonas* species were calculated and details of the MAR index are presented in Table 3. The minimum and maximum MAR index observed for each species of *Aeromonas* ranged between 0.18 and 0.76. The MAR index ranged between 0.29-0.58 in *A. aquariorum,* 0.47 in *A. cavernicola,* 0.76 in *A. enchelia,* 0.29-0.53 *in A. jandaei,* 0.29 to 0.58 in *A. hydrophila;* 0.59-0.71 in *A. molluscorum,* 0.18-0.41 in *A. piscicola,* 0.24-0.41 in *A. popoffii,* 0.18-0.70 in *A. schubertii,* 0.29-0.52 in *A. tecta,* 0.24-0.58 in *A. veronii* bv. *sobria* and 0.24-0.71 in *A. veronii* bv. *veronii.* All the isolates of 12 *Aeromonas* species were found to be multiple antibiotic resistant**.**

Table 3: Multiple antibiotic resistance (MAR) index and percentage of *Aeromonas* spp. in diseased rohu fish of Andhra Pradesh

Bacteria species	No. of isolates	No. of isolates with MAR	MAR index (range)
A. aquariorum	4	4	0.29-0.58
A. cavernicola	1	1	0.47
A. enchelia	1	1	076
A. hydrophila	3	3	0.29-0.58
A. jandaei	3	3	0.29-0.53
A. molluscorum	2	2	0.59-0.71
A. piscicola	2	2	0.18-0.41
A. popoffii	3	3	0.24-0.41
A. schubertii	4	4	0.18-0.70
A. tecta	10	10	0.29-0.52
A. veroniibvsobria	4	4	0.24-0.58
A. veroniibvveronii	21	21	0.24-0.71

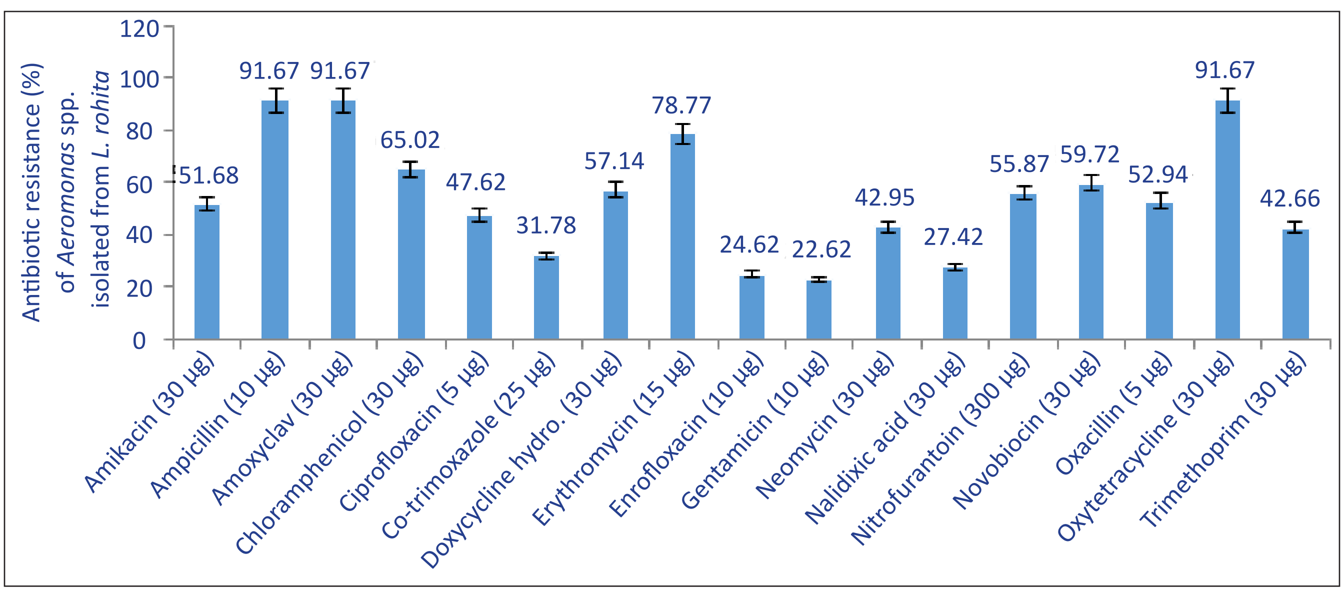

Figure 2: Antimicrobial resistance (%) of *Aeromonas* spp. isolated from *Labeo rohita*

Aeromonas species are widely distributed in aquatic environments and were isolated from drinking water (Havelaar et al., 1992), squid (Baldrias and Alvero, 1999), shrimp (Thayumanavan et al., 2003; Vivekanandhan et al., 2005), mussels (Ottaviani et al., 2006), ornamental fishes (Sreedharan et al., 2012, Mohan and Unni, 2012). *Aeromonas* contaminated water, food and their products caused health issues to the consumers (Joseph et al., 2013). *Aeromonas* spp. are opportunistic, which provokes clinical signs in stressed fish or fish affected by concurrent infections (van der Marel et al., 2008). These *Aeromonas* species are causing hemorrhagic septicaemia, tail and fin rot, ulcer disease, or red-sore disease in a variety of freshwater and marine fish of the world (Roberts et al.,1989) and causing significant economic losses in the aquaculture industry worldwide (Maniati et al., 2005). Motile *Aeromonads* like *A. hydrophila* (Dallal and Moez Ardalan, 2004), *A. sobria* (Taher et al., 2000) associated with gastroenteritis in humans. Janda and Abbott (1998) classified *A. veronii* bv. *veronii*, *A. jandaei* and *A. schubertii* as minor human pathogens. *A. aquariorum* was reported to cause bacteraemia and wound infections in humans (Figueras et al., 2009) and *A. punctata* caused gastroenteritis (Gilardi, 1967). In aquaculture and animal husbandry antibiotics are used for treating bacterial diseases and sub-therapeutic doses causing the emergence of several environmental and public health problems, such as the generation of multi-resistant bacteria (Moore et al., 2014).

In the present investigation, *Aeromonas* infected rohu exhibited clinical signs like haemorrhages on the body surface, fin bases, reddish vent, discharge of bloody fluid from the vent, pale gills, fin rot, tail rot, scale loss, reduced mucus on the body surface, distended abdomen, spleen enlargement, discoloration of internal organs like kidney and liver. More or less similar observations were also made by Mathur et al. (2005) in mrigal; Alsaphar and Al-Faragi (2012), Stratev et al. (2015) in common carp and Sruthi et al. (2021) in catla and rohu.

Hu et al. (2012) isolated *A. veronii* (60%) as the dominant species in fish followed by *A. hydrophila, A. sobria, A. media, A. caviae, A. jandaei, A. salmonicida, A. allosaccharophila, A. bivalvium.* Tong Li et al. (2020) also isolated 54% of *A. veronii* from several freshwater fish like *Carassius auratus*, *Cyprinus carpio*, *Ctenopharyngodon idella* and *Silurusa sotus*. Further, U-taynapun et al. (2020) also recorded *A. veronii* bv. *veronii* (78%) followed by *A. hydrophila* (12%), *A. veronii* bv. *sobria* (6%) and *A. jandaei* (4%) in motile *Aeromonas septicemia* tilapia. Among the 12 *Aeromonas* isolated in the present study, the prevalence of *A. veronii*biovar*veronii* in diseased rohu was significantly highest with 35% than *A. tecta*, *A.veronii* biovar*sobria*, *A. aquariorum*, *A. popoffii*, *A. schubertii*, *A. jandaei*, *A. molluscorum*, *A. hydrophila*, *A. piscicola*, *A. cavernicola* and *A. enchelia* and these results are agreeing with the previous findings. The prevalence of *Aeromonas* spp. are more in the freshwater culture system where *A. hydrophila, A. veronii* bv. *sobria, A. caviae* were reported to be the major human pathogens and *A. veronii* bv. *veronii, A . jandaei* and *A. schubertii* are the minor human pathogens (Janda and Abbott, 1998). The present investigation also revealed occurrence of zoonotic Aeromonads in rohu *viz., A. veronii* bv. *veronii* to greater extent and *A. veronii* bv. *sobria, A. schubertii* and *A. hydrophila* to lesser extent warranting the chances of public health concern, if the fish are handled and cooked improperly. Albert (2000), Alavandi and Ananthan (2003) cautioned that high prevalence of *Aeromonas* spp. in the environment is considered a threat to public health through ingestion of contaminated water and fish.

In recent years the number of antibiotic-resistant bacteria in aquaculture has increased dramatically in different parts of the world. In aquaculture and animal husbandry systems antibiotics are extensively applied for controlling bacterial diseases (Praveen et al., 2014). According to Redmayne (1989), the continued usage of medicated feeds and their application to the rapidly developing fish and shellfish farming can foster greater dissemination of virulent and resistant bacterial pathogens in the natural environment and thus potentially into the human food chain. In our study, the antimicrobial resistance pattern of 12 *Aeromonas* spp. isolated from cultured rohu fish from fish farms of Andhra Pradesh against 17 antibiotics revealed significantly high antibiotic resistance to three antibiotics *viz*., ampicillin, amoxyclav and oxytetracycline. Further, significantly low resistance ($p < 0.05$) was shown for the remaining 14 antibiotics. Similar results were recorded by Yucel et al. (2004) isolated *A. hydrophila, A. veronibv. sobria, A. caviae* from fish were 100% resistant to ampicillin. Hatha et al. (2005) found *Aeromonas* spp. were highly resistant to oxytetracycline, amoxycillin, ampicillin, novobiocin and polymixin-B. Further, Roy et al. (2013) reported that *A. veronii* isolated from Fresh Water Loach, *Lepidocephalycthys guntea* showed resistance erythromycin, kanamycin, ciprofloxacin and gentamycin. Further, Raj et al. (2019) reported that *A. veronii* isolated from tilapia showed resistant to bacitracin, nitrofurantoin, furazolidone, erythromycin, azithromycin, cefalexin, amoxycillin, cenrofloxacin, ampicillin. Further, Hassan et al. (2017) reported the resistance of *A. veronii* to ampicillin and other beta-lactam antibiotics such as amoxicillin. Wassif (2018) indicating that A. *veronii*bv*sobria* was resistant to ampicillin, amoxicillin, oxytetracycline. Zdanowicz et al. (2020) reported that isolates from the water of carp ponds were mostly resistant to amoxicillin, ampicillin, clindamycin and penicillin. The present study findings were completely in concurrence with the above studies. Further, in contrast to our findings Sanyal et al. (2018) reported high antibiotic sensitivity of *Aeromonas* to chloramphenicol. The *Aeromonas* spp. isolates from a number of clinical and environmental sources were 100% sensitive to amikacin, ciprofloxacin, chloramphenicol, gentamycin (Kampfer et al., 1999), in contrast to the resistance to ciprofloxacin and gentamycin found in the present study.

In contrast to the present study, Sreedharan et al. (2012) reported 100% sensitivity of *Aeromonas* spp. to trimethoprim isolated from freshwater ornamental fish culture systems. The variation in resistance/sensitivity of *Aeromonas* spp. to similar antibiotics in different studies might be due to the utilization of water in aquaculture systems from high/low risk sources.

The multiple antibiotic resistance (MAR) index is clinically very important due to its zoonotic importance (Le et al., 2018). A high MAR index of zoonotic bacterial pathogens is a potential risk for humans by direct contact with diseased fish (Lowry and Smith, 2007). MAR among *Aeromonas* spp. has been reported from many parts of the world (Koet al.,1996; Mirand and Zemelman, 2002; Vivekanandhan et al., 2002; Holmstrom et al., 2003; Sinha et al., 2004). According to Krumperman (1985), the MAR index value is more than 0.2 or more is said to have originated from high risk sources of contamination where antibiotics are often used. In the present study, all the *Aeromonas* spp. showed a MAR index of more than 0.2, indicating that the pathogenic *Aeromonas* spp. isolated from rohu could have been originated from high-risk antibiotic contamination water sources. Our study is supported by Abraham (2011), who reported the association of opportunistic human bacterial pathogens and their sensitivity to broad spectrum antibiotics in cultured freshwater fishes where the MAR index was high in the bacterial flora of carps grown in sewage-fed farms than the carps grown in non sewage fed aquaculture systems of Kolkata, India. Zhou et al. (2019) studied MAR of *Aeromonas* spp. from 115 human samples, among which 28.7% exhibited multiple-drug resistance (MDR) patterns to 15 antimicrobial agents. 80% strains of *A. hydrophila* and 81.2% strains of *A. dhakensis* presented with MDR, while fewer MDR isolates were *A. caviae* (39.6%) and A. *veronii* (16.7%) from hospital patient samples. Isolation of antimicrobial resistance and multiple antibiotic resistant *Aeromonas* spp. from freshwater aquaculture systems in many countries along with our findings has shown a growing concern in the treatment of *Aeromonas* infections in fish and humans.

4. Conclusion

Occurence of *Aeromonas* spp. in farm grown rohu and their resistance to antimicrobials with high multi-antibiotic resistant index indicates the origin of bacteria from high-risk sources and intern their threat to public health. Proper measures required in the selection of water source for aquaculture and the food fish need to be handled and cooked appropriately in order to prevent the introduction of antimicrobial resistant bacteria in fish and humans respectively.

5. Acknowledgement

The first author is thankful to the Vice Chancellor, Prof. V. Padmanabha Reddy and the former Dean of Fishery Science, Dr. T. VenkataRamana of Sri Venkateswara Veterinary University (SVVU), Tirupati for providing stipend and the facilities provided for carrying out research work during her Ph.D. (Aquatic Animal Health) study. The chemicals, consumables and fish samples of the project entitled 'National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) as NSPAAD sub-center- 12 being operated at the Department of Aquatic Animal Health Management, College of Fishery Science, SVVU, under the principal investigatorship of the corresponding author was provided to the first author. The authors duly acknowledge NFDB, Govt. of India, Hyderabad and NBFGR (ICAR), Lucknow, India for providing funds under the NSPAAD project.

6. References

Abbott, S.L., Cheung, W.K., Janda, J.M., 2003. The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. Journal of Clinical Microbiology 41(6), 2348–2357.

Abraham, T.J., 2011. Food safety hazards related to emerging antibiotic resistant bacteria in cultured freshwater fishes of Kolkata, India. Journal of Food Science and Technology 3(1), 69–72.

Albert, M.J., Ansaruzzaman, M., Talukder, K.A., Chopra, A.K., Kuhn, I., Rahman, M., Faruque, A.S., Islam, M.S., Sack R.B., Mollby, R., 2000. Prevalence of enterotoxin genes in *Aeromonas*spp. isolated from children with diarrhoea, healthy controls, and the environment. Journal of Clinical Microbiology 38, 3785–3790.

Alsaphar, S.A., Al-Faragi, J.K., 2012. Detection and study of the experimental infectionof Aeromonas strain in the common carp (*Cyprinus carpio* L.). The Iraqi Journal of Veterinary Medicine 36(2), 222–230.

Altwegg, M., Steigerwalt, A.G., Altwegg-Bissig, J., Luthy-Hottenstein, J., Brenner, D.J., 1990. Biochemical identification of *Aeromonas* geno species isolated from humans. Journal of Clinical Microbiology 28, 258–264.

Altwegg, M., MartinettiLucchini, G., Luthy-Hottenstein, J., Rohrbach, M., 1991. Aeromonas-associated gastroenteritis after consumption of contaminated shrimp. European Journal of Clinical and Microbiological Infections and Diseases 10, 44– 45.

Alavandi, S.V., Ananthan, S., 2003. Biochemical characteristics, serogroups and virulence factors of *Aeromonas* species isolated from cases of diarrhoea and domestic water samples in Chennai. Indian Journal of Medical Microbiology 21, 233–238.

Anonymous, 2017. Department of animal husbandry, dairying and fisheries. Ministry of Agriculture, Annual report 2016-17, Government of India, 162.

Austin, B., Adams, C., 1996. Fish pathogens. In: Austin, B., Altwegg, M., Gosling, P.J., Joseph, S. (Eds.), The Genus Aeromonas. Wiley, Chichester, 198–243.

Baldrias, L.R., Alvero, M.A.C., 1999. *Aeromonas* species from Raw Squid Balls: A preliminary study of a potential food-borne pathogen and health hazard. Journal of

Environmetal Science Management 2, 48–52.

Beaz-Hidalgo, R., Alperi, A., Bujan, N.,Romalde, J.L., Figueras, M.J., 2010. Comparison of phenotypical and genetic identification of *Aeromonas* strains isolatedfrom diseased fish. Systematic and Applied Microbiology 33(3), 149–153.

Beaz-Hidalgo, R., Alperi, A., Figueras, M.J., Romalde, J.L., 2009. *Aeromonas piscicola* sp. nov., isolated from diseased fish. Systematic and Applied Microbiology 32(7), 471–479.

Bomo, A.M., Husby, A., Stevik, T.K., Hanssen, J.F., 2003. Removal of fish pathogenic bacteria in biological sand filters. Water Resources Management 37(11), 2618–2626.

Borrell, N., Figueras, M.J., Guarro, J., 1998. Phenotypic identification of *Aeromonas* genomo species from clinical and environmental sources. Clinical Journal of Microbiology 40, 103–108.

Cizek, A., Dolejska, M., Sochorova, R., Strachotova, K., Piackova, V., Vesely, T., 2010. Antimicrobial resistance and its genetic determinants in aeromonads isolated in ornamental (koi) carp (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio*). Veterinary Microbiology 142(3-4), 435–439.

Das, B.K., Mishra, S.S., 2014. Diseases in Freshwater aquaculture, In: Training manual on model training course on preventive health management practices in freshwater aquaculture. ICAR-Central Institute of Freshwater aquaculture, Bhubaneswar, Odisha, India.

Dasklov, H., 2006. The importance of *Aeromonas hydrophila* in food safety. Food control 17(6), 474–483.

Dwivedi, M.A., Mishra, A., PrasadA., Azim, R.K., Singh, A.K., Baronia, K.N., Prasad, U.N., Dwivedi, 2008. *Aeromonas caviae* septicemia in immunocompetent gastrointestinal carriers. Brazilian Journal of Infectious Diseases 12(6).

El-ghareeb, H.M., Zahran, E., Elghany, M.A., 2019. Occurecne, molecular characterization and antimicrobial resistence of pathogenic Aeromonashydrophila from retail fish. Alexandria Journal of Veterinary Science 62(1), 172–181.

Figueras, M.J., Alperi, A., Saavedra, M.J., Ko, W., Gonzalo, N., Navarro, M., Martinez-Murcia, A.J., 2009. Clinical relevance of the recently described species *Aeromonas aquariorum*. Journal of Clinical Microbiology 47, 3742–3746.

Gilardi, G.L., 1967. Morphological and biochemical characteristics of *Aeromonas punctata (hydrophila, liquefaciens)* isolated from human sources. Journal of Applied Microbiology 15, 417–421.

Hassan, M.A., Noureldin, E.A., Mahmoud, M.A, Fita, N.A., 2017. Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. The Egyptian Journal of Aquatic Research 43(2), 161–167.

Hatha, M., Vivekanandhan, A.A., Joice, G.J., 2005. Antibiotic resistance pattern ofmotile aeromonads from farm raised fresh water fish. International Journal of Food Microbiology 98(2), 131–134.

Havelaar, A.H., Schets, F.M., van Silfhout, A., Jansen, W.H., Wieten, G., van der Kooj, D., 1992. Typing of Aeromonas strains from patients with diarrhoea and from drinking water. Journal of Applied Bacteriology 72, 435–444.

HiMedia, 2009. The HiMedia Manual. A Manual of Microbiology Laboratory Practice HiMedia Laboratories Pvt. Ltd., Mumbai, pp.1194.

Holmstrom, K., Graslund, S., Wahlstrom A., Poungshompoo, S., Bengtsson, B.E., Kautsky, N., 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food Science and Technology 38, 255–266.

Hu, M., Wang, N., Pan, Z.H., Lu, C.P., Liu, Y.J., 2012. Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls and water environment in China. Letters in Applied Microbiology 1–10.

Janda, J.M., Abbott S.L., 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clinical Infectious Diseases 27, 332–344.

Joseph, A.V., Sasidharan, R.S., Harisree, P., Sarita, G., 2013. Occurrence of potential pathogenic *Aeromonas* species in tropical seafood, aquafarms and mangroves off Cochin coast in South India.Veterinary world 300–306

Kampfer, P., Christamann, C., Swings, J., Huys, G., 1999. In vitro susceptibility of *Aeromonas* genomic species to 69 antimicrobial agents. Systematic and Applied Microbiology 22(4), 662–669.

Kirov, S.M., 1993. The public health significance of *Aeromonas* spp. in foods.International Journal of Food Microbiology 20, 179–198.

Krumperman, P.H., 1985. Multiple antibiotic indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Applied Environtal Microbiology 46, 165–170

Khun, I., Albert, M.J., Ansaruzzaman, M., Bhuiyan, N.A., Alabi, S.A., Islam, M.S., Neogi, P.K., Huys, G., Janssen, P., Kersters, K., Mollby, R., 1997. Characterization of *Aeromonas* spp. isolated from humans with diarrhoea, from healthy controls, and from surface water in Bangladesh. Journal of Clinical Microbiology 35, 369–373.

Ko, C.W., Yu, K.W., Liu, C.Y., Huang, C.T., Leu, S.H., Chuang, Y.C., 1996. Increasing antibiotic resistance in clinical isolates of Aeromonas strains in Taiwan. Antimicrobial Agents and Chemotherapy 40, 1260–1262.

Kuijper, E.J., Bol, P., Peeters, M.F., Steigerwalt, R., Zanen, H.C., Brenner, D.J., 1989. Clinical and epidemiologic aspects of members of Aeromonas DNA hybridization groups isolated from human feces. Journal of Clinical Microbiology 27, 1531–1537.

Kelly, K.A., Koehler, J.M., Ashdown, L.R., 1993. Spectrum of extraintestinal disease due to *Aeromonas* species in Tropical Queensland, Australia.Clinical Infectious Diseases 16, 574–579.

Le, T.S., Thi Hien, N., Hong Phuong, V., Van Cuong, D., Hong Loc, N., Minh Trung, T., Trong Tuan, T., Paul, C., Southgate, D.I., 2018. Protective effects of bacteriophages against *Aeromonas hydrophila* causing motile aeromonas septicemia (MAS) in striped catfish. Journal of Antibiotics 7, 1–16.

Lowry, T., Smith, S.A., 2007. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. Journal of Animal Veterinary Medical Association 231, 876–880.

Maniati, M., Petinaki, E., Maniatis, K.A.N., 2005. Antimicrobial susceptibility of Aeromonas sp., Vibrio sp. and Plesiomonasshigelloides isolated in the Philipines and Thailand. International Journal of Antimicrobial Agents 25, 345–353.

Martin-Carnahan, A., Joseph, S.W., 2005. Aeromonadaceae. In: The Proteobacteria, Part B, Bergey's Manual of Systematic Bacteriology (Eds. Brenner, D.J.,.Krieg, N.R., Staley, J.T. and Garrity, G.M). Second edition Springer Verlag, New York.

Martinez-Murcia, A.J., Saavedra, M.J., Mota, V.R., Maier, T., Stackebrandt, E., Cousin, S., 2008. *Aeromonas aquariorum*sp. nov., isolated from aquaria of ornamentalfish. International Journal of Systematic and Evolutionary Microbiology 58(5), 1169–1175.

Mathur, A.K., Kumar, P., Mehrotra, S.C., 2005. Abdominal dropsy disease in majorcarps of Meghalaya: isolation and characterization of *Aeromonas hydrophila*. Current Science 88(12), 1897–1900.

Moore, L.S.R., Rachel, F., Mark, J.G., Myriam, G., Eimear, T.B., Hugo, D., David, M.L., Alison, H.H., 2014. Homogeneity of antimicrobial policy, yet heterogeneity of antimicrobial resistance: antimicrobial non-susceptibility among clinical isolates from primary, secondary and tertiary care patients in London. Journal of Antimicrobial Chemotherapy 69(12), 3409–3422.

Mirand, C.D., Zemelman, R., 2002. Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. Science of the Total Environment 293, 207–218.

Mishra, S.S., Das, R., Das, B.K., Choudhary, P., Rathod, R., Giri, B.S., Debbarma, J., Sahoo, S.N., Barua, A., Sahu A., Patil, P.K., Swain, P., 2017. Status of aquamedicines, drugs and chemicals use in India: A survey report. Journal of Aquaculture and Fisheries 1(004).

Orozova, P., Chikova, V., Najdenski, H., 2010. Antibiotic resistance of pathogenic forfish isolates of *Aeromonas* spp. Bulgarian Journal of Agricultural Sciences 16(3), 376–386.

Ottaviani, D., Santarelli, S., Bacchiocchi, S., Masini, L., Ghittino, C., Bacchiocchi, I., 2006. Occurrence and characterization of *Aeromonas*spp. in mussels from the adriatic sea. Food Microbiology 23, 418–422.

Popoff, M., 1984. Genus III. Aeromonas. In Bergey's Manual of Systematic Bacteriology,.Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins, 545–548.

Praveen, P.K., Debnath, C., Pramanik, A.K., Shekhar, S., Dalai, N., Rai, R., 2014. Antibiotic sensitivity and virulence potential study of *Aeromonas*species isolated from retail fish and chicken in and around Kolkata. Journal of Cell and Tissue Research 14(3), 4613–4616.

Raj, S., Raja Swaminathan, T., Arathi Dharmaratnama, S., Arun Rajaa, D., Ramraj, K.K., 2019. *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. Aquaculture 512, 734278.

Redmayne, P.C., 1989. World aquacultural developments. Food Technology 43, 80–86.

Reith, M.E., Singh, R.K., Curtis, B., Boyd, J.M., Bouevitch, A., Kimball, J., Munholland, J., Murphy, C., Sarty, D., Williams, J., Nash, J.H.E., Johnson, S.C., Brown, L.L., 2008. The Genome of *Aeromonas salmonicida* subsp *salmonicida* A449: Insights in to the Evolution of a Fish Pathogen. BMC Genomics 9, 427.

Roberts, R.J., 1989. Fish Pathology, Bailliere Tindall, London, Second edition. 467.

Roy, R.P., Bahadur, M., Barat, S., 2013. Isolation and identification of multi-antibiotic resistant *Aeromonas veronii* by 16s rDNA gene sequencing from the gut of fresh water loach, *Lepidocephalycthys guntea* (Hamilton Buchanan). International Journal of Bio-resource and Stress Management 4(4), 496–499.

Sinha, S., Shimada, T., Ramamurthy, T., Bhattacharya, S.K., Yamasaki S., Takeda, Y., Nair, G.B., 2004. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic*Aeromonas*species isolated from hospitalized diarrhoeal cases in Kolkata, India. Journal of Medical Microbiology 53, 527–534.

Sreedharan, K., 2008. *Aeromonas* associated with freshwater ornamental fish culture systems: characterization, pathogenicity and management. Doctoral Thesis, Cochin University of Science and Technology, India.

Sreedharan, K., Philip, R., 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. Brazilian Journal of Microbiology 754–765.

Sruthi, P., Neeraja, T., Balasubramanian, A., Supradhnya Meshram, G., Prasad, S., Lavanya, C., Sai Ram, V., Singh, S.M., 2021. Phenotypic characterization and antimicrobial resistance of *Aeromonas* species isolated from *Catla catla* and *Labeo rohita* collected in two districts of Andhra Pradesh, India. Journal of Experimental Zoology, India 24(2), 1337–1346.

Stratev, H., Vashin, D., 2015. Characterisation and determination of antimicrobial resistance of b-haemolytic *Aeromonas* spp. isolated from common carp (*Cyprinus carpio*L.). Reviews Medical Veterinary 166, 1-2, 54–61.

Stratev, D., Olumide, A., Odeyemi, A., 2016. Antimicrobial resistence of *Aeromonas hydrophila* isolated from different food sources: Amini review. Journal of Infection and Public Health 9(5),527–534.

Thayumanavan, T., Vivekhanandhan, G., Savithakumari, K., Subashkumar, R., Lakshmanaperumalsamy, P., 2003. Incidence of haemolysin positive and drug resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of Coastal South India. FEMS Immunology and Medical Microbiology 36, 41–45.

Tong, L., Sayed, H.A.R., Bintong, Y., Yufeng, S., Guiqin, W., Wuwen, S., Aidong, Q., Chunfeng, W., Yuanhuan, K., Xiaofeng, S., 2020. .*Aeromonas veronii* infection in commercial freshwater fish: a potential threat to public health**.** Animals (Basel) 2, 10(4), 608.

Tomas, J.M., 2012. The main *Aeromonas* pathogenic factors. International Scholarty Research Network, 1–22.

U-taynapun, K., Nganwisuthiphan, T., Chirapongsatonkul, 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*). International Journal of Agricultural Technology 16(3), 749–760.

Van der Marel, M., Schroers, V., Neuhaus, H., Steinhagen, D., 2008. Chemotaxis towards, adhesion to, and growth in carp gut mucus of two*Aeromonas hydrophila* strains with different pathogenicity for common carp, *Cyprinus carpio*. Journal of Fish Diseases 31, 321–330.

Vivekanandhan, G., Savithamani, K., Hatha A.A.M., Lakshmanaperumalsamy, P., 2002. Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fishand prawn of South India. International Journal of Food Microbiology 76, 165–168.

Vivekanandhan, G., Hatha, A.A.M., Lakshmanaperu-malsamy, C.P., 2005. Prevalence of *Aeromonas hydrophila* in fish and prawns from the seafood market of Coimbatore, South India*.* Food Microbiology 22, 133–137.

Wassif, I.M., 2018. Biochemical and molecular characterization of *Aeromonas* species isolated from fish. Alexandria Journal for Veterinary Sciences 57(1).

Yucel, N., Aslim, B., Beyatli, Y., 2004. Prevalence and resistance to antibiotics for *Aeromonas* species isolated from retail fish in turkey. Journal of Food Quality 28, 313–324.

Zdanowicz, M., Zbigniew, J.M., Perlinsk, P., 2020. Abundance and antibiotic resistance of *Aeromonas* isolated from the water of three carp ponds. Veterinary Research Communications 44, 9–18.

Zhou, Y., Li, Y., Zheng, N., Pingping, Z., Biao, K., Donghui, Y., Jianrong, S., 2019. Taxonomy, virulence genes and antimicrobial resistance of *Aeromonas* isolated from extra-intestinal and intestinal infections. BMC Infectious Diseases 19, 1–9.