Original Article

TJVR 2019; 3(1): 33-36

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Determination of antibiotic resistance pattern in *Aeromonas hydrophila* isolated from Reared *Oncorhynchus mykiss* in Tabriz city, Iran

Abolfazl Jafari-Sales[1] Azizeh Shadi-Dizaji[2]

[1] *Department of Microbiology, Kazeroon branch, Islamic Azad University, Kazeroon, Iran,*
[2] *Department of Biotechnology, Ataturk University, Erzurum, Turkey*

Correspondence: *Abolfazl Jafari Sales (A.jafari_1392@yahoo.com)*

Received: 28.03.2019 Accepted: 21.04.2019

ABSTRACT

Objective: *Aeromonas hydrophila* is a Gram negative, positive oxidase, anaerobic, and opportunistic bacteria that, under certain conditions, become a pathogen (in humans and fish). This bacterium causes toxin and host infection in which different antibiotic resistance in isolated strains has been reported in different regions of the world. The aim of this study was to determine the prevalence of this bacterium and its susceptibility to common antibiotics in Tabriz city.

Materials and Methods: 50 samples from 5 Reared *Oncorhynchus mykiss* farms in Tabriz city (For each farm, 10 numbers) were randomly assigned to suspected fish to the disease.

Results: By using biochemical tests, 14 samples (28%) from 2 Fish farms (40%) were identified as *A. hydrophila*. Antibiogram for these specimens showed that the bacterium had the highest resistance to vancomycin (100 %) and clindamycin (92.8%) antibiotics, and has the most sensitivity to the antibiotics of sultrim, tetracycline and oxytetracycline with 71.4%.

Discussion: Considering the different antibiotic resistance pattern in this study and other similar studies, the necessity of examining the pattern of resistance in each region seems necessary.

Keywords: *Aeromonas hydrophila, Oncorhynchus mykiss, Antibiotic resistance*

INTRODUCTION

The *Aeromonas* spp. were originally located in the *Vibrionaceae* family, but after genetic studies, it was found that they are not closely related to the vibrio. Thus, the *Aeromonas* spp. are located in a new family called *Aeromonadaceae* (Janda and Abbott, 2010; Ceylan et al., 2009; Gülaydın et al., 2018). Pathogenicity of Aeromonas species is very common in drinking water samples and types of food samples, especially marine foods. Today, marine products for humans are one of the most important food products. These products can easily be contaminated with a variety of pathogens (Puthucheary et al., 2011; Villari et al., 2003; Yogananth et al., 2009). *Aeromonas hydrophila* is an opportunistic pathogen, which can host a wide range of hosts, including various species of fish such as *Cyprinus carpio*, catfish, Tilapia, Eels, Goldfish, Trout and humans (Xia et al., 2004; Zhou et al., 2010). Today, the presence of the bacterium *A. hydrophila* in the aquatic ecosystem is well documented. This bacterium is part of the aquatic intestinal flora, which can be pathogenic when caused by tension (Yogananth et al., 2009). *A. hydrophila* causes many complications and diseases in fish, including Hemorrhagic Septicemia, Dark skin, skin ulcers, Pop-*Eye* and asymptomatic septicemia. This bacterium often causes irreparable damages in farmed fish populations and infection control depends on eliminating the predisposing

factors of the infection (Khushiramani et al., 2007; Jayavignesh et al., 2011). Recent studies indicate that, based on the severity of infection and the age of breeding fish with *A. hydrophila*, different clinical symptoms and even mortality are expected (Esmaeli and Peighan, 1997; Vega-Sánchez et al., 2014). In the last few decades, the widespread use of antibiotics in the treatment of human, animal, agricultural and Fishery infections has increased the presence of these compounds in the environment and, consequently, the resistance of some bacteria (Yousr et al., 2007). Today, trout (*Oncorhynchus mykiss*) is known as one of the most important fish species in Most of the cold-water fish farms, in most parts of the world (Tafi et al., 2014). Considering that trout (*Oncorhynchus mykiss*) is a diet of human societies, the aim of this study was to determine the antibiotic resistance pattern in *A. hydrophila* isolated from trout in Tabriz city.

MATERIALS and METHODS

Sampling and biochemical identification of isolates:

In this experimental study, 50 trout samples with suspected symptoms were collected from 5 farms in Tabriz city (10 fish per farm) at a 20-day interval from June to September 2018 and in suitable conditions and in the transport box of fish, with appropriate oxygenation was transferred to the microbiological laboratory. The Symptoms in all suspected fish included: abdominal distension, Inflammation of the Anus, Pop-Eye and Dark skin. All field conditions such as water factors (temperature and oxygen), food (type and physical and chemical characteristics) and fish (apparent observations) were recorded before the experiment was performed. Prior to sampling, each piece of fish was killed by cutting the spinal cord and then weighed. The body surface of the fish was washed with 70 ° C ethanol and the liver and Kidney and all of them were isolated from the abdominal area under sterile conditions and were washed twice with sterile physiology serum. By inserting the Inoculation needle into the tissues, the specimens were transferred to a blood-agar culture medium and incubated for 24 hours at 22 ° C. Suspicious colonies (white to gray, convex and semi-transparent colonies) based on biochemical tests including mobility, Gram stain, oxidase and catalase tests, indole, methyl red, citrate, nitrate recovery, sulfate production, KOH Identification and examples with features Gram-negative, Catalase and Oxidase-positive properties were transferred to Shotts-Rimler medium (Shotts and Rimler, 1973) as a selective medium of *A.hydrophila*.

Antibiotic susceptibility testing:

To determine the antimicrobial resistance pattern, disk diffusion method was used in Muller Hinton Agar (Merck, Germany) culture media (Bauer et al., 1966). Antibiotic disks include Vancomycin (30 µg), Ciprofloxacin (25 µg), Amoxicillin (30 µg), Sultrim (10 µg), Tetracycline (30 µg), Streptomycin (20 µg), Gentamicin (15 µg), Cefotaxime (25 µg), Nalidixic Acid (30 µg), Oxytetracycline (20 µg), Chloramphenicol (20 µg) and Clindamycin (30 µg) from the company PADTAN TEB(Iran) were used. Interpretation of antibiogram results was performed according to the CSLI (CLSI, 2009) standard.

RESULTS

Out of 50 samples taken from 5 trout farms with suspected symptoms, 14 specimens (28%) from 2 farms (40%) were identified as positive *A. hydrophila* specimens. By analyzing the results of the antibiogram, 14 isolates of *A. hydrophila* and the standard strain of *A. hydrophila* showed that the highest resistance among the isolates was related to vancomycin antibiotics (100%) and clindamycin (92.8%) and the least resistance in The isolates were associated with gentamicin antibiotics (7.2%) and ciprofloxacin (14.2%), and the most susceptible to sultrim, tetracycline and oxytetracycline antibiotics was 71.4%.

Table 1. Results of study on determination of microbial susceptibility of isolated strains of *A. hydrophila* (%)

Standard antibiotics	R	I	S
Vancomycin	100	0	0
Ciprofloxacin	14.3	35.7	50
Amoxicillin	28.6	50	21.4
Sultrim*	21.4	7.2	71.4
Tetracycline	21.4	7.2	71.4
Streptomycin	71.4	0	28.6
Gentamicin	7.2	42.8	50
Cefotaxime	0	42.8	57.2
Nalidixic Acid	0	50	50
Oxytetracycline	21.4	7.2	71.4
Chloramphenicol	0	57.2	42.8
Clindamycin	92.8	0	7.2

*: Trimethoprim – Sulphadiazine; R: Resistant; I: Intermediate; S: Sensitive;

DISCUSSION and CONCLUSION

One of the causes of heavy losses in fish farms is bacterial infection, which causes severe economic damage to the aquaculture industry annually (Nielsen et al., 2001). Among these, the *A. hydrophilia* bacteria is one of the most important opportunistic bacteria in aquatic organisms, which causes Hemorrhagic Septicemia and high mortality in aquaculture system (Casiano et al., 2010). Although other factors such as viral infection, nutrition, environmental factors also contribute to this mortality. But some studies have shown the presence of Aeromonas in these high-profile casualties (Ahangarzadeh et al., 2017). In this study, 50 specimens from 5 trout farms with suspicious symptoms were identified as 14 specimens (28%) from 2 farms (40%) as the positive specimen of *A. hydrophila*. Fadaeifard, of the 60 samples taken from 10 farms, identified 9 samples (15%) of *A. hydrophila* Positive (Fadaeifard, 2014). Igbal et al. (1999) examined various species of Aeromonas, which showed that the high percentage of Aeromonas isolated to tetracycline is susceptible. Which was consistent with the findings of the present study. Arzani et al. (2016), by investigation the antibiotic susceptibility of *A. hydrophila* bacteria in Tonekabon fish farming pools showed that *A. hydrophila* isolates were 100% sensitive to antibiotics tetracycline, gentamicin, kanamycin, norfloxacin, ofloxacin, amikacin, nalidixic acid, azithromycin and ciprofloxacin, and 100% resistant to oxacillin, meticillin, erythromycin, ampicilin and amoxicillin. Bakhtiari et al. (2017) in Khuzestan province showed the highest resistance of *A. hydrophila* to vancomycin and clindamycin (90%) streptomycin (70%) and the highest susceptibility to Sultrim, tetracycline and oxytracycline (75%). Which are consistent with the findings of this study. Hosseinzadeh and Tukmechi (2016) with the study of *A. hydrophila* isolated from common carp, showed that the bacteria were most susceptible to ciprofloxacin (84.11%), oxytetracycline (59.44%), gentamicin (59.22%) and streptomycin (41.06%). Sarder et al. (2016) by Examined antibiotic susceptibility of A. hydrophila isolated from freshwater fish Showed that the bacteria had the highest susceptibility to gentamicin antibiotics (100%), ciprofloxacin (93.53%), chloramphenicol (81.25%) and the highest resistance to amoxicillin (93.75%). Bektas and Iscimen (2005) in Turkey, by studying *A. hydrophila*, showed that the bacterium is susceptible to chloramphenicol, tetracycline and oxytracycline antibiotics and resistant to amoxicillin antibiotics. The antibacterial function of tetracycline is by through the disruption of the translation of the Aerolysin gene and thus inhibit protein synthesis in the bacterial ribosome, thus, by stopping the production of proteins in *A. hydrophila*, these bacteria are condemned to death (Pakravan and Akbarzadeh, 2017). Revina et al. (2017) examined antibiotic resistance patterns in *A. hydrophila* isolates, which showed that all isolates were resistant to amoxicillin, ampicillin, cefalexin and erythromycin and had the lowest resistance to gentamicin (4.4%), Streptomycin (10%). Saavedra et al., (2004) determined that *A. hydrophilia* isolated from rainbow trout showed the highest resistance to antibiotic amoxicillin (88%) and the most sensitive to cefotaxime (88%). The findings of this study have differences with similar studies. In order to justify this difference, as many studies have shown, a bacteria of one species may have several subtypes. Also, the acuity of these bacteria in different sources of water may vary. Therefore, the lack of specific information to describe the actuality of organisms that have been used in laboratory evaluation of the antimicrobial properties of bacteria in fish breeding or water resources can be considered as one of the problems. This study provides basic information on resistance to common antibiotics in *A. hydrophilia* isolates from trout (Oncorhynchus mykiss) farms in Tabriz city. The findings of this study indicate that *A. hydrophilia* bacteria is responsible for causing septicemia in trout (Oncorhynchus mykiss). It can also be said that if the bacteria enter the human food chain through infected fish, there is a risk of human bloody diarrhea. On the other hand, it can be concluded that the probability of a horizontal gene transfer is one of the determinants of antibiotic resistance in these isolates and excessive consumption of antibiotics in aquaculture has led to the emergence of resistant strains in *A. hydrophilia*.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Ahangarzadeh M, Peyghan R, Ghorbanpour najafabadi M, Sharifrouhani M, Soltani M. Role of Aeromonas hydrophila in bacterial septicemia of cultured carps in khuzestan province. *Iranian Veterinary Journal*, 2015; 11(3):5-16.

Arzani, N., Darvishi, H., Sharifian, G., Rabie pur, A. Evaluation of antibiotic susceptibility of Aeromonas hydrophilia in fish pools of Tonekabon city. *IJMST*, 2016; 20(79): 65-72.

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*. 1966; 45(4):493-6.

Bektas S, Iscimen S. Antibiotic Resistance of Aeromonas hydrophila Strains Isolated from Karasu Stream (Sinop/Turkey). *Environment*. 2005; 1(1-2):10-21.

Casiano H, Choresca JR, Dennis K, Jee-Eun Han G, Sang-Phil S, Ji-Hyung K, Jin-Woo J, Se-Chang P. Molecular detection of *Aeromonas hydrophila* isolated from albino catfish, *Clarias* sp. reared in an indoor commercial aquarium. *Korean J Vete Res*. 2010; 50(4): 331-333.

Ceylan E, Berktas M, Ağaoğlu, Z. The occurrence and antibiotic resistance of motile Aeromonas in livestock. *Trop Anim Health Prod*. 2009; 41: 199-204.

Clinical and laboratory standards institute (CLSI), Document M39-A3. Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data; Approved Guideline. 3th Edition. CLSI, 2009; 940.

Esmaili F, Peighan R. Isolation of Aeromonas like bacteria from the grass carp (Ctenopharyngodon idella). *Iranian Sci. Fish. J.* 1997; 6(2):1-8.

Fadaeifard, F. Molecular detection of Aeromonas hydrophila in the aquarium gold fish and cultured rainbow trout in Chaharmahal va Bakhtiary province. , 2014; 8(1 (29): 401-409.

Gülaydın Ö, Öztürk C, Önalan Ş, Karapınar Z, Arabacı M, Ekin İH, Ilhan Z, Gürtürk K, Ilhan F. The Investigation of The Presence of Some Bacterial and Viral Agents In Pearl Mullet (Chalcalburnus tarichii, Pallas 1811) By Real-Time PCR and The Histopathological Examination, *Fresen Environ Bull*, 2018; 27: 8286-8296.

Hosseinzadeh, M., Tukmechi, A. Isolation and identification of enterotoxin producing Aeromonas hydrophila from common carp (Cyprinus carpio). *Animal Environment*, 2016; 7(4): 173-178.

Iqbal MM, Chowdhury MB, Islam MA, Baqui M, Karim SM, Tajima K, Ezura Y. Seasonal Fluctuations of Motile Aeromonads and Pseudomonads in a Cultured Pond of Mrigal, Cirrhinus mrigala, in Bangladesh. *Asian Fisheries Science*. 1999; 12:17-24.

Janda JM, Abbott SL. The genus Aeromonas: taxonomy, pathogenicity, and infection. *Clin Microbiol Rev*. 2010; 23(1):35-73.

Jayavignesh V, Sendesh Kannan K, Bhat AD. Biochemical characterization and cytotoxicity of the Aeromonas hydrophila isolated from Catfish. *Arch Appl Sci Res*. 2011; 3(3):85-93.

Khushiramani R, Girisha SK, Karunasagar I, Karunasagar I. Cloning and expression of an outer membrane protein ompTS of Aeromonas hydrophila and study of immunogenicity in fish. *Protein Expr Purif*. 2007; 51(2):303-7.

Moori Bakhtiari N, Peyghan R, Monzavi SF. Determination of isolated Aeromonas hydrophila antibiotic resistance profile from farmed common carp (Cyprinus carpio) in Khuzestan Province. *IJFS*, 2017; 25(5):41-50.

Nielsen ME, Høi L, Schmidt AS, Qian D, Shimada T, Shen JY, Larsen JL. Is Aeromonas hydrophila the dominant motile Aeromonas species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China?. *Diseases of aquatic organisms*. 2001; 46(1):23-9.

Pakravan S, Akbarzadeh A. The review of pathogenic mechanism of Aeromonas hydrophila and action of tetracycline against it in aquatic animals. *J. Aqu. Eco*. 2017; 6 (4):1-9.

Puthucheary SD, Puah SM, Chua KH. Molecular characterization of clinical isolates of Aeromonas species from Malaysia. *PloS one*. 2012; 7(2):e30205.

Revina O, Avsejenko J, Cīrule D, Valdovska A. Antimicrobial resistance of Aeromonas spp. isolated from the sea trout (Salmo trutta L.) in Latvia. *Research for Rural Development*. 2017; 1.

Saavedra MJ, Guedes-Novais S, Alves A, Rema P, Tacão M, Correia A, Martínez-Murcia A. Resistance to β-lactam antibiotics in Aeromonas hydrophila isolated from rainbow trout (Onchorhynchus mykiss). *International microbiology*. 2004; 7(3):207-11.

Sarder H, Khan T, Saha ML, Punom NJ, Mandal SC and Rahman MS. Prevalence and antibiotic susceptibility of *Aeromonas hydrophila* isolated from freshwater fishes. *Journal of Fisheries*, 2016; 4(3): 411-419.

Shotts, E.B, Rimler, R, Medium for the isolation of Aeromonas hydrophila. *Appl. Environ. Microbiol.*, 1973; *26*(4):550-553.

Tafi AA, Meshkini S, Tukmechi A. Effects of Chitosan on some immune response of rainbow trout (Oncorhynchus mykiss) and enhance resistance against a pathogenic Aeromonas hydrophila fallowing experimental infection. *Journal of Animal Research*, 2014; 26(4): 468-477.

Vega-Sánchez V, Latif-Eugenín F, Soriano-Vargas E, Beaz-Hidalgo R, Figueras MJ, Aguilera-Arreola MG, Castro-Escarpulli G. Re-identification of Aeromonas isolates from rainbow trout and incidence of class 1 integron and β-lactamase genes. *Vet Microbiol*. 2014; 172(3-4):528-33.

Villari P, Crispino M, Montuori P, Boccia S. Molecular typing of Aeromonas isolates in natural mineral waters. *Appl. Environ. Microbiol.*. 2003; 69(1):697-701.

Xia C, Ma ZH, Rahman MH, Wu ZG. PCR cloning and identification of the β-haemolysin gene of Aeromonas hydrophila from freshwater fishes in China. *Aquaculture*. 2004; 229(1-4):45-53.

Yogananth N, Bhakyaraj R, Chanthuru A, Anbalagan T, Nila KM. Detection of virulence gene in Aeromonas hydrophila isolated from fish samples using PCR technique. *Global J Biotech Biochem*. 2009; 4:51-3.

Yousr AH, Napis S, Rusul GR, Son R. Detection of aerolysin and hemolysin genes in Aeromonas spp. isolated from environmental and shellfish sources by polymerase chain reaction. *ASEAN Food Journal*. 2007; 14(2):115.

Zhou L, Wang X, Liu Q, Wang Q, Zhao Y, Zhang Y. A novel multivalent vaccine based on secretary antigen-delivery induces protective immunity against Vibrio anguillarum and Aeromonas hydrophila. *Journal of biotechnology*. 2010; 146(1-2):25-30.