

Pathology of *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss*) of Himachal Pradesh

Rajendra Damu Patil*, Ekta Bisht, Abhishek Verma, Rinku Sharma¹ and Rajesh Kumar Asrani

Department of Veterinary Pathology, Dr G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, ¹Disease Investigation Laboratory, ICAR-Indian Veterinary Research Institute, Regional Station, Palampur-176 061, Himachal Pradesh, India

Address for Correspondence

Rajendra Damu Patil, Associate Professor, Department of Veterinary Pathology, Dr G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, Himachal Pradesh, E-mail: rdpatil02@gmail.com

Received: 10.10.2024; Accepted: 22.1.2025

ABSTRACT

Trout fish farming is playing an important role in providing livelihood and nutritional security to the people of hilly state like Himachal Pradesh. The present investigation was conducted during a natural outbreak of *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss*) in a commercial trout fish farm located in the Mandi district of Himachal Pradesh, India. The overall mortality was 30%. A total of twenty-one moribund rainbow trout of around 5 months age were investigated. The clinical signs exhibited by the fish before death were lethargy, slow movement and gasping. The gross lesions at necropsy were distended abdomen (dropsy), cutaneous haemorrhages and/or ulcerative lesions at the base of fins and anal area, ascites and mild to severe degree of congestion and haemorrhage in the visceral organs. The samples collected at necropsy included swabs from the skin lesions, liver and kidney for bacteriological studies and representative tissues for histopathological evaluation. Bacteriological studies revealed the presence of *Aeromonas hydrophila* in the samples of moribund trout. Microscopically, gills, liver, kidneys and skeletal muscles revealed predominant vascular changes such as congestion, haemorrhage and oedema, vacuolar or Zenker's degeneration, necrosis and an inflammatory reaction. In addition, decreased haematopoiesis in the anterior kidney and melano-macrophage center infiltration in the liver and kidney were also observed. In conclusion, the outbreak was diagnosed as acute severe septicemic *Aeromonas hydrophila* infection associated with significant mortality in rainbow trout leading to economic losses.

Keywords: *Aeromonas hydrophila*, *Oncorhynchus mykiss*, pathology, trout fish

Aquaculture production is nowadays gaining popularity in meeting the nutritional requirements of humans due to increasing population¹. Rainbow trout (*Oncorhynchus mykiss*) is a cold-water fish belonging to *Salmonid* family and is highly valued for its nutritional potential. Himachal Pradesh is one of the leading states in rainbow trout farming and seed production in India with a total production of 849.7 tonnes in the recent past². The rich water resources of Himachal Pradesh offer a great potential for trout fish farming, providing livelihoods to the native population and ensuring food security.

Aeromonas hydrophila is a ubiquitous bacterial pathogen in marine and freshwater habitats but can emerge as a primary or opportunistic pathogen if it encounters favourable conditions, affecting fish farming on a large scale and causing significant economic losses³. *A. hydrophila* is facultatively anaerobic, motile, Gram-negative bacterium that optimally grows at 22-32°C, with a few strains surviving even at low temperature, affecting and causing diseases in all classes of vertebrates including fishes^{4,5}. It is a non-spore forming, rod-shaped bacterium belonging to *Aeromonadaceae* family of the class, *Gammaproteo bacteria*. The biochemical characteristics are positive for catalase, oxidase and indole tests⁶. Many studies have demonstrated freshwater bodies to be the reservoirs of *A. hydrophila* which is the etiological agent involved in causing disease conditions such as motile aeromonas septicemia (MAS), hemorrhagic and ulcerative diseases in fishes including rainbow trout, catfish, carp and salmon^{1,6,7}.

Fish farmers are greatly impacted by acute *A. hydrophila* outbreaks in which mortality rates can reach up to 100 percent⁸. Food, animals,

How to cite this article : Patil, R.D., Bisht, E., Verma, A., Sharma, R. and Asrani, R.K. 2025. Pathology of *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss*) of Himachal Pradesh. Indian J. Vet. Pathol., 49(1) : 85-89.

groundwater and wastewater are some of the other sources of this pathogen contributing to its zoonotic potential and hence raising public health concerns^{1,9}. Overcrowding, increased organic content, trauma, change in temperature, unhygienic handling, contaminated feed, pollution and deficit of dissolved oxygen are some of the stressors that may contribute in the development of the infection in fishes¹⁰. The natural infection of *A. hydrophila* in fish depends on the species affected, season and environmental conditions¹¹.

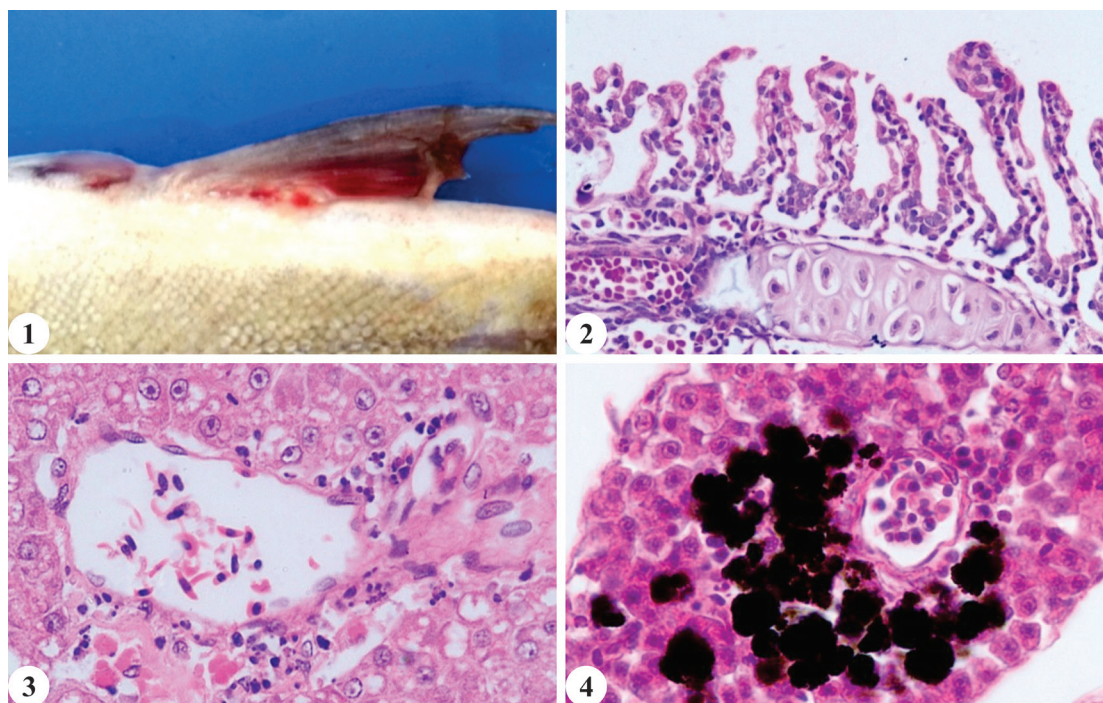


Fig. 1. Hemorrhages at the base of anal fin of rainbow trout (*Oncorhynchus mykiss*); **Fig. 2.** Gills: Engorged and dilated vasculature, short and thickened primary and secondary lamellae and mild lymphocytic infiltration in gill lamellae (H&E x200); **Fig. 3.** Liver: Congestion, vacuolar degeneration and necrosis of a few hepatocytes in the centrilobular region (H&E x400); **Fig. 4.** Liver: Melano-macrophage center (MMC) aggregation around the central vein (H&E x400).

The present investigation was conducted during a natural outbreak of *A. hydrophila* infection in rainbow trout (*O. mykiss*) in a commercial farm with a total stock of 4000 fish located in the Mandi district of Himachal Pradesh, India. The disease outbreak was recorded in the month of July, 2023. A total of twenty-one moribund rainbow trout aged 5 months and of either sex were investigated in the Department of Veterinary Pathology, Dr G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh. The body weights of the affected fish ranged from 110 to 150 g and the body lengths varied from 13 to 18 cm. A thorough post-mortem examination was performed and gross pathological lesions were recorded. Simultaneously, swabs were collected aseptically from the lesions on the skin and visceral organs including liver and kidney for microbiological analysis. For isolation and identification of bacteria, the swabs were streaked onto tryptic soy agar (TSA) plates and incubated at 22°C for 48 hrs. The subculturing was performed to obtain pure colonies and the biochemical characterization of the bacterial isolates was performed as per the standard procedure¹². Various biochemical tests including catalase, oxidase, indole, methyl red, citrate, Voges-Proskauer, gelatin liquefaction and motility tests were employed for species level identification of *Aeromonas* spp.

Representative tissue samples were collected at necropsy and fixed in 10% neutral buffered formalin.

Thereafter, the tissue samples were processed and embedded in paraffin blocks¹³. Haematoxylin and eosin-stained sections (3-4 µm) were then examined under the light microscope.

In the present investigation, overall 30% mortality was recorded in rainbow trout. Prior to death, the affected moribund fishes exhibited clinical signs including lethargy, slow movement, gasping and distended abdomen (dropsy). At necropsy, external examination revealed eroded scales along with focally extensive areas of well-demarcated, ulcerative lesions with or without haemorrhagic borders on the skin, base of dorsal, pectoral, caudal and anal fins and anal region (Fig. 1). Internal examination showed ascitic fluid accumulation and mild to severe degree of congestion and/or haemorrhages in almost all the internal organs suggestive of septicaemia.

Histopathological examination of the tissues revealed lesions in gills, liver, kidneys and skeletal muscles. Microscopically, gills revealed severe congestion, foci of haemorrhages, short, curled to thickened primary and secondary lamellae and infiltration of lymphocytes in both primary and secondary lamellae (Fig. 2). Liver sections revealed vascular and sinusoidal congestion, areas of haemorrhage, vacuolar degeneration and necrosis of hepatocytes and infiltration of mononuclear cells, predominantly, lymphocytes (Fig. 3). Hepatic

parenchyma also revealed the presence of melanomacrophage center (MMC) aggregation in a few foci (Fig. 4). Histologically, anterior kidney exhibited decreased haematopoiesis and presence of bacterial colonies (Fig. 5). However, posterior kidney exhibited congestion, areas of haemorrhages, degeneration and necrosis of tubular epithelium, epithelial cast formation and multifocal areas of MMC aggregation in the interstitium (Fig. 6). Histopathology of the skeletal muscles revealed necrotizing myositis characterized by extensive exudation between the muscle bundles, Zenker's degeneration and necrosis of muscle fibers with loss of striations and infiltration of mononuclear inflammatory cells mainly lymphocytes (Fig. 7).

The bacterial isolates obtained from the skin lesions, liver and kidney showed yellowish opaque, round, convex, smooth-edged colonies of *A. hydrophila* on TSA media (Fig. 8). The Gram staining of the isolates revealed Gram-negative, bacilli present singly, in pairs and a few in short chains. Confirmatory diagnosis of *A. hydrophila* was made on the basis of biochemical characterization of the isolates that included positive reactions for catalase, oxidase, indole, methyl red, citrate, Voges-Proskauer, gelatin liquefaction and motility tests.

Aeromonas hydrophila is an emerging aquatic pathogen, widely distributed in the environment. It can cause disease in fish, humans and in other animals. The clinical signs including lethargy, slow movement,

gasping and distended abdomen were more or less similar to those described earlier in *A. hydrophila* infected rainbow trout¹⁴.

Aeromonas infection in fish causes world economic problems because of the high number of fish mortalities. The mortality rate of *A. hydrophila* in fish can vary (up to 100%) depending on the strain of the bacteria and the type of fish infected⁸. In the present outbreak, overall 30% mortality was recorded in rainbow trout. The susceptibility of rainbow trout to *A. hydrophila*, may be related to the environment of trout fish, which lives on clear running water, that is less exposed and less resistant to this pathogenic bacterium. Besides this, *A. hydrophila* bacteria multiplies at faster rate, colonize and cause mortality quickly in comparison to other pathogenic bacteria in fish. Additionally, the bacteria can directly cause death and co-infect with viruses, fungi, and other bacteria and synergistically aggravate the disease severity or mortality in fish¹⁵. Interestingly, the present disease outbreak occurred in the rainy season i.e. in the month of July, when water temperature is relatively higher than the other months of the year.

The gross findings such as eroded scales, dermal haemorrhages, ulcerations, ascitic fluid accumulation, congestion and/or haemorrhages in the internal organs are more or less similar to those reported by Zepeda-Velázquez and co-workers during the natural infection of *Aeromonas* species in rainbow trout¹⁴. The gross and

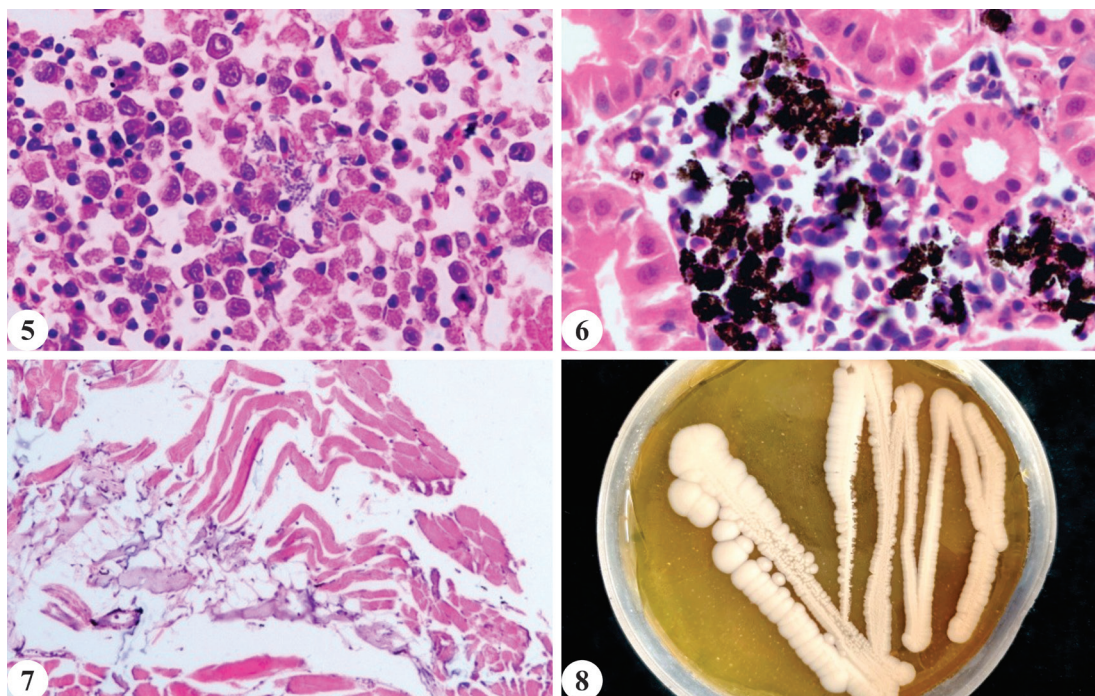


Fig. 5. Anterior kidney: Decreased hematopoietic activity and presence of bacilli (arrow) along with infiltration of lymphocytes (H&E x400); **Fig. 6. Posterior kidney:** Degeneration and necrosis of tubular epithelium and MMC aggregation in the interstitium (H&E x400); **Fig. 7. Skeletal muscles:** Severely disrupted muscle bundles showing extensive necrosis of muscle fibers and infiltration of lymphocytes (H&E x40); **Fig. 8.** Yellowish opaque, round, convex smooth-edged colonies of *A. hydrophila* on TSA media.

histopathological findings of this study confirmed that *A. hydrophila* is pathogenic to rainbow trout. The microscopic lesions such as vascular changes, degeneration and/or necrosis, inflammatory response, presence of bacterial colonies and/or MMC aggregates in the internal organs or tissues are more or less consistent with those reported during experimental studies in infected fishes^{14,16}. In fishes, kidney is one of the target organs of an acute septicemia caused by aeromonads and this organ is apparently attacked by bacterial toxins and loses its structural integrity as also observed in the present study. The MMC or macrophage aggregates, are the distinctive groupings of pigment-containing phagocytic cells within the tissues of fish and it plays an important role in the humoral adaptive immune response to foreign materials, including infectious agents¹⁷.

A. hydrophila has a natural habitat in water and can thrive at temperatures ranging from 0 to 45°C with an optimum temperature of 22 to 32°C. In addition, environmental and stress conditions such as overcrowding, low dissolved oxygen, poor water quality, higher organic content, physical injuries, temperature fluctuation, factory pollution, unhygienic handling, contaminated feed and poor nutrition may influence *A. hydrophila* infection in fish^{6,10}.

Isolation, identification and confirmation of fish pathogenic bacteria are important in the accurate diagnosis of suspected disease¹⁸. The conventional bacteriological and biochemical methods used for the present investigation have proven effective for identification of *A. hydrophila*¹⁰. The morphology of the Gram-negative motile bacterium, colony characteristics on TSA media and the biochemical properties of *A. hydrophila* isolates recovered in this study are consistent with those in other reports¹⁸⁻²⁰.

A. hydrophila is also considered the most important zoonotic pathogen of concern. Fish play a fundamental role in *Aeromonas* transmission to humans²¹. In humans, it may transmit through ingestion of contaminated fish flesh and may lead to various intestinal and extra-intestinal diseases. The diseases in humans include gastroenteritis, traveler's diarrhoea, septic arthritis, skin and wound infections, blood-borne infections, pneumonia, meningitis and fulminating septicaemia^{22,23}. Fever, abdominal pain and signs of respiratory distress are commonly exhibited by patients infected with *A. hydrophila*²³. *A. hydrophila* isolated from fish and humans showed several virulence factors and exhibit a wide range of antibiotic resistance which also considered as a public health hazard²². Moreover, the occurrence of *Aeromonas* septicemia is reported to be 32 to 45% in immunocompromised patients and the risk of mortality during the infection increases with the number of comorbidities in human patients²³. *A. hydrophila* is a

widespread, emerging food pathogen and frequently isolated from different environmental or clinical samples as well as from retail foods including fish, seafood, raw milk, poultry and red meats^{10,24}. Hence, investigation of *Aeromonas* outbreaks in table fish like rainbow trout must be carried out with due precaution due to its seemingly high zoonotic potential to consumers.

In conclusion, the study reports a natural outbreak of acute *A. hydrophila* infection associated with septicemia and dermal lesions in rainbow trout of Himachal Pradesh resulting in significant mortality among trout fish with a significant economic impact on the cold water fisheries of the state.

ACKNOWLEDGEMENTS

The authors are grateful to CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh for providing the facilities to conduct the research work.

REFERENCES

1. Abdella B, Abozahra NA, Shokrak NM, Mohamed RA and El-Helow ER. 2023. Whole spectrum of *Aeromonas hydrophila* virulence determinants and the identification of novel SNPs using comparative pathogenomics. *Sci Rep* **13**: 7712.
2. Zahoor S, Jan A and Husain N. 2024. Current status of rainbow trout (*Oncorhynchus mykiss*) production in India and global world. *Int Creat Res Thoughts* **12**: 2402232.
3. Pauzi NA, Mohamad N, Azzam-Sayuti M, Yasin ISM, Saad MZ, Nasruddin NS and Azmai MNA. 2020. Antibiotic susceptibility and pathogenicity of *Aeromonas hydrophila* isolated from red hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) in Malaysia. *Vet World* **13**: 2166-2171.
4. Rasmussen-Ivey CR, Figueras MJ, McGarey D and Liles MR. 2016. Virulence factors of *Aeromonas hydrophila*: In the wake of reclassification. *Front Microbiol* **7**: 217-548.
5. Daskalov H. 2006. The importance of *Aeromonas hydrophila* in food safety. *Food Control* **17**: 474-483.
6. Semwal A, Kumar A and Kumar N. 2023. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. *Heliyon* **9**: e14088.
7. Shahi N, Mallik SK, Sahoo M and Das P. 2013. Biological characteristics and pathogenicity of a virulent *Aeromonas hydrophila* associated with ulcerative syndrome in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum) in India. *Isr J Aquac* **65**: 926-936.
8. Kusdarwati R, Kurniawan H and Prayogi YT. 2017. Isolation and identification of *Aeromonas hydrophila* and *Saprolegnia* spp. on catfish (*Clarias gariepinus*) in floating cages in Bozem Moro Krembangan Surabaya. *IOP Conf Series: Earth & Env Sci* **55**: 012-038.
9. Awan F, Dong Y, Wang N, Liu J, Ma K and Liu Y. 2018. The fight for invincibility: Environmental stress response mechanisms and *Aeromonas hydrophila*. *Microb Pathog* **116**: 135-145.
10. Moya-Salazar J, Diaz CR, Cañari B, Badillo RX, Verano-Zelada M, Chicoma-Flores K and Contreras-Pulache H. 2022. Detection of pathogenic *Aeromonas hydrophila* from two rainbow trout (*Oncorhynchus mykiss*) farms in Peru. *Braz J Vet Med* **44**: e000922.
11. Yardimci B and Aydin Y. 2011. Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Ankara Univ Vet Fak Derg* **58**: 47-54.

12. Carter GR and Cole Jr JR. 1990. Diagnostic Procedure in Veterinary Bacteriology and Mycology. 5th ed. Academic Press, San Diego, USA.
13. Luna LG. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed., Mc-Graw-Hill Book, New York.
14. Zepeda-Velazquez AP, Vega-Sanchez V, Salgado-Miranda C and Soriano-Vargas E. 2015. Histopathological findings in farmed rainbow trout (*Oncorhynchus mykiss*) naturally infected with 3 different *Aeromonas* species. *Can J Vet Res* **79**: 250-254.
15. Nicholson P, Monon N, Jaemwimol P, Tattiyapong P and Surachetpong W. 2020. Coinfection of tilapia lake virus and *Aeromonas hydrophila* synergistically increased mortality and worsened the disease severity in tilapia (*Oreochromis* spp.). *Aquac* **520**: 734-746.
16. Marinho-Neto FA, Claudiano GS, Yunis-Aguinaga J, Cueva-Quiroz VA, Kobashigawa KK, Cruz NR, Moraes FR and Moraes JR. 2019. Morphological, microbiological and ultrastructural aspects of sepsis by *Aeromonas hydrophila* in *Piaractus mesopotamicus*. *PLoS One* **14**: e0222626.
17. Agius C and Roberts RJ. 2003. Melano-macrophage centres and their role in fish pathology. *J Fish Dis* **26**: 499-509.
18. Yazdanpanah-Goharrizi L, Rokhbakhsh-Zamin F, Zorrie-hzahra MJ, Kazemipour N and Kheirikhah B. 2020. Isolation, biochemical and molecular detection of *Aeromonas hydrophila* from cultured *Oncorhynchus mykiss*. *Iran J Fish Sci* **19**: 2422-2436.
19. Bakiyev S, Smekenov I, Zharkova I, Kobegenova S, Sergaliyev N, Absatirov G and Bissenbaev A. 2022. Isolation, identification and characterization of pathogenic *Aeromonas hydrophila* from critically endangered *Acipenser baerii*. *Aquac Rep* **26**: 101-293.
20. Das R, Sarma K, Hazarika G, Choudhury H and Sarma D. 2023. Identification and characterisation of emerging fish pathogens *Aeromonas veronii* and *Aeromonas hydrophila* isolated from naturally infected *Channa punctata*. *ALJMAO* **28**: 117.
21. Abd-El-Malek A. 2017. Incidence and virulence characteristics of *Aeromonas* spp. in fish. *Vet World* **10**: 34-37.
22. Ahmed HA, Mohamed ME, Rezk MM, Gharieb RM and Abdel-Maksoud SA. 2018. *Aeromonas hydrophila* in fish and humans, prevalence, virulotyping and antimicrobial resistance. *Slov Vet Res* **55**: 113-124.
23. Kaki R. 2023. A retrospective study of *Aeromonas hydrophila* infections at a university tertiary hospital in Saudi Arabia. *BMC Infect Dis* **23**: 671.
24. Tahoun AB, Ahmed HA, Abou Elez RM, El-Gedawy AA, Elsohaby I and Abd El-Ghaffar AE. 2016. Molecular characterisation, genotyping and survival of *Aeromonas hydrophila* isolated from milk, dairy products and humans in Egypt. *Int Dairy J* **63**: 52-58.