

Case Report

Outbreak of *Aeromonas Hydrophila* Affecting Fish Health in Babogaya Lake, Ethiopia

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Abstracts

On October 6, 2023, reports emerged of a fish mortality outbreak in Babogaya Lake, in Bishoftu, in Ethiopia's Oromia Region. The Oromia Regional Government formally referred the occurrence to the National Animal Health Institute (AHI) in order to ascertain its cause. Samples from infected fish, lake water, and sediment were gathered for laboratory study as part of field research. *Aeromonas hydrophila* was detected in 5 (15.6%) of the 32 fish tissue samples that were analyzed. Selective culture, hemolysis tests on 7% sheep blood agar (β -hemolytic activity was seen in 5/32 samples, or 15.6%), biochemical assays, and PCR targeting the *aerA* gene were used to confirm the isolates. Identification was further validated by phenotypic profiling utilizing the Biolog GEN III system, which showed significant metabolic plasticity. *Escherichia coli* O157 and other pathogens were also recovered from two samples, indicating a more widespread problem of microbial pollution in the lake ecosystem. The extensive distribution of *A. hydrophila* and fecal indicator bacteria in various environmental sources points to deteriorating water quality and environmental stress as key causes of the outbreak. These results highlight the necessity of regular water quality monitoring, efficient waste management, and improved aquatic health surveillance in order to safeguard fish populations and public health in the Babogaya Lake area.

Keywords

Aeromonas Hydrophila, Babogaya Lake, Biolog System, Outbreak

1. Introduction

One of the well-known crater lakes, Babogaya Lake, is situated in the central Ethiopian area of Bishoftu, some 45 kilometers southeast of Addis Ababa [10]. The lake is important both ecologically and economically, supporting a range of activities like residential water consumption, tourism, recreation, and small-scale aquaculture [12]. But growing human demands have started to jeopardize its ecological stability [19].

Aeromonas hydrophila is a Gram-negative, facultative anaerobic bacterium that is frequently found in freshwater

settings [22]. Even though it is a component of the natural microbial community, overcrowding, high organic loads, low dissolved oxygen levels, and warm water temperatures—all of which are frequently found in aquaculture settings—can make it pathogenic [18]. Motile *Aeromonas septicemia* (MAS), a disease characterized by hemorrhagic lesions, skin ulcerations, and systemic infections that can result in significant mortality, is known to be caused by this opportunistic pathogen in fish [5]. *Aeromonas hydrophila* serious concerns have been expressed in recent years regarding fish health,

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public safety, and the sustainability of aquaculture operations due to the occurrence and recurrence of bacterial diseases in aquatic environments [1]. The capacity of *A. hydrophila* to impact a variety of aquatic creatures and sporadically result in zoonotic infections in people makes it stand out among these pathogens [14].

Babogaya Lake, Bishoftu (Debre Zeit), Ethiopia, was the site of a major *A. hydrophila* outbreak on October 6, 2023. *A. hydrophila* high mortality rates among fish populations were linked to the outbreak, with the Nile tilapia (*Oreochromis niloticus*), a species of significant economic significance in the area, being disproportionately affected [2]. Concurrently, the scenario was accompanied by a rise in anthropogenic stressors that contribute to the lake's deteriorating water quality, including uncontrolled tourism, agricultural runoff, and urban encroachment [4]. Investigating the root causes of the fish mortality outbreak in Babogaya Lake is the goal of this project, which will focus on the identification, isolation, and characterization of *Aeromonas hydrophila* and other possible bacterial infections that impact fish and the aquatic environment [1].

2. Objectives

1. To collect and examine fish, water, and sediment samples from Babogaya Lake during the outbreak for evidence of bacterial infection.
2. To isolate and identify *Aeromonas hydrophila*** using cultural, biochemical, and phenotypic methods.
3. To confirm the presence of *A. hydrophila* through molecular techniques, including PCR.
4. To characterize the phenotypic properties of the isolates using the Biolog GEN III system.

3. Materials and Methods

Study Area

Babogaya Lake, is one of the crater lakes in Bishoftu (formerly Debre Zeit), in the Oromia Regional State of central Ethiopia, served as the study's site. Located roughly 45 kilometers southeast of Addis Ababa, the capital, Bishoftu is renowned for its collection of volcanic crater lakes that are essential socioeconomic and biological resources for the area [16]. In particular, aquaculture, tourism, and domestic water use at Babogaya Lake sustain local livelihoods.

Sample Collection and Transportation

In order to collect samples for laboratory diagnosis, a field research team was sent to Babogaya Lake after the epidemic was reported. From several locations throughout the lake, representative samples were gathered, paying particular attention to regions showing the highest recorded fish mortality rates. For bacteriological and physicochemical analysis, all samples were properly labeled and sent to the diagnostic lab in a cold chain.

Isolation and Identification of *Aeromonas hydrophila*

In order to isolate and identify *Aeromonas hydrophila* from fish and water samples, all culture media were produced in accordance with the manufacturer's instructions, and standard operating procedures were closely adhered to [8].

Biochemical Characterization

The isolates' morphological and biochemical traits were assessed using the identification standards described in practical manuals for the diagnosis of fish infections and Bergey's Manual of Systematic Bacteriology [11]. Gram staining, colony shape, cellular organization, and hemolytic activity on 7% sheep blood agar were the original criteria used for the presumed identification of *Aeromonas hydrophila* [17]. Voges-Proskauer (VP) test, deoxyribonuclease (DNase) activity assay, triple sugar iron (TSI) agar test, hemolysin production, motility test, oxidase test, citrate utilization, and indole production were among them.

Biolog GEN III Identification of *Aeromonas hydrophila*

Following the manufacturer's recommended procedure, presumed pure isolates of *Aeromonas hydrophila* were further verified using the Biolog GEN III microbial identification system (OmniLog ID System, Hayward, CA, USA) [9]. In short, pure bacterial cultures were first cultivated on Biolog Universal Growth (BUG) agar and then incubated for 24 hours at 37 °C [23]. In the OmniLog reader, the produced suspensions were inoculated into Biolog GEN III microplates, which comprise 94 phenotypic tests (71 carbon source utilization assays and 23 chemical sensitivity assays). The microplates were then incubated for 22 hours at 33 °C.

Antibiogram Pattern of *Aeromonas hydrophila*

Following the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the Kirby-Bauer disc diffusion method was used to evaluate the isolates of *Aeromonas hydrophila* for antibiotic susceptibility [24]. Mueller-Hinton Agar was used for the test (Oxoid CM0337, Basingstoke, England). Five milliliters of sterile 0.85% saline solution were aseptically transferred from four to five colonies cultivated on nutritional agar from each confirmed isolate [6].

4. Results

Isolation of *Aeromonas hydrophila*

We recovered *Aeromonas hydrophila* from 32 tissue samples in total, indicating 15.6% prevalence.



Figure 1. Colony morphology of *A. hydrophila*.

Isolation, Identification, and Biochemical Test of *A. Hydrophila*

Five isolates of *A. hydrophila* were discovered in this investigation using the outcomes of seven morphological and biochemical tests. Colonies of presumed *A. hydrophila* were 2-3 mm in diameter, spherical, smooth, and green with dark

cores on *Aeromonas* selective media. The motility, DNase, Voges-Proskauer, oxidase, and indole tests for suspected colonies were all positive. Additionally, they demonstrated an alkaline slope with an acid butt (K/A) in the triple sugar iron (TSI) test, as well as acid generation from glucose. The isolates' citrate utilization was negative.

Table 1. Biochemical result.

ID	Oxidase	VP	TSI	Indole	Citrate	Motility	DNase	G-neg
012	+	+	+	+	-	+	+	+
013	+	+	+	+	+	+	+	+
014	+	+	+	+	+	+	+	+
015	+	+	+	+	-	+	+	+
016	+	+	+	+	-	+	+	+



Figure 2. VP and G-positive result.

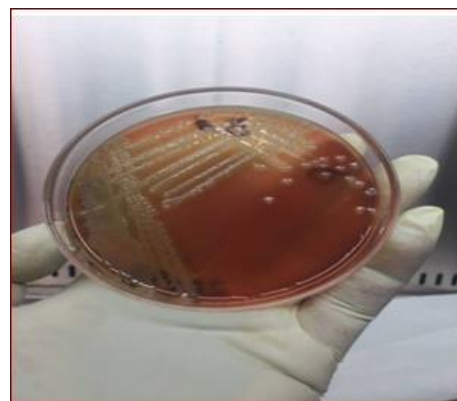


Figure 3. (β) hemolytic.

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Hemolysis Characterization of *Aeromonas hydrophila*

To determine the isolates' importance as a virulent component, their hemolytic activities were determined. *A. hydrophila* exhibited hemolytic (β) activity on blood agar bases that contained 7% whole sheep blood agar. Consequently, $n = 5/32$, or 15.6%, were positive for *A. hydrophila*.

Antibiogram profile

All of the tested isolates were completely sensitive to ciprofloxacin (CIP100%), chloramphenicol (C100%), ceftriaxone (CRO100%), ceftaxime (CTX100%), sulfamethoxazole (SXT100%), tetracycline (TE100%), and gentamicin (CN99%), and resistant to meropenem (MEM), according to the results of the antibiogram assay involving the examined *A. hydrophila* isolates used in the current study.

Confirmation

Aeromonas hydrophila was found in all fish tissue samples, as well as in water and sediment samples taken from the impacted areas of Babogaya Lake, according to the combination of the biochemical profile, morphological traits, and biolog.

5. Discussion

The investigation's findings verified that the main causative agent of the fish mortality outbreak in aquaculture was *A.*

hydrophila [21]. Five (15.6%) of the 32 fish tissue samples tested positive. *A. hydrophila* was well-established in the aquatic ecosystem at the time of the epidemic, as evidenced by the fact that it was isolated from a variety of environmental sources [15]. β -hemolytic activity was detected by hemolysis tests, suggesting the isolates' potential for pathogenicity [20]. *A. hydrophila* demonstrated hemolytic (β) ($n = 5/32$) or 15.6% and tested positive on blood agar bases containing 7% whole sheep blood agar. Additionally, PCR targeting the *ahI* gene and Biolog phenotypic characterization offered unquestionable proof of the pathogen's identity [13]. The finding of *E. coli* O157 in two samples further raises the possibility that the outbreak was made worse by fecal contamination and inadequate environmental hygiene [3]. Rising water temperatures, nitrogen loading, and organic pollution are examples of environmental stressors that probably contributed significantly to the favorable conditions that allowed *A. hydrophila* to proliferate [1]. These elements, along with the bacterium's opportunistic behavior, can cause outbreaks in stressed fish populations very quickly, especially in shallow and semi-closed systems like crater lakes [7] (Armstrong *et al.*, 2016). This epidemic emphasizes the necessity of managing fish health and water quality together, particularly in areas where aquaculture coexists with other human-caused stresses.

6. Conclusion

According to the study, the primary pathogen causing the fish mortality outbreak in Babogaya Lake was *A. hydrophila*. The bacterium's significance in the outbreak is highly supported by its broad prevalence in fish, water, and sediment samples, as well as by virulence indicators such as β -hemolysis and the *aerA* gene. The finding of *E. coli* O157 suggests multiple sources of microbial danger in the lake, further emphasizing the presence of fecal pollution. This outbreak emphasizes how susceptible aquatic systems, such as Babogaya Lake, are to environmental deterioration and the consequent risk of fish population illness onset. Better environmental management, timely disease detection, and routine monitoring are essential for preserving fish health and averting future occurrences of this kind.

7. Recommendations

Based on the findings of this investigation, the following actions are recommended:

1. Implement regular monitoring of water quality and fish health in Babogaya Lake, including bacterial load and temperature fluctuations.
2. Promote good aquaculture practices, including reduced fish stocking density, improved feeding, and biosecurity measures.
3. Control and manage sources of pollution, such as agri-

cultural runoff, untreated sewage, and waste disposal into the lake.

4. Train local fish farmers on disease recognition, early reporting, and proper handling of sick or dead fish.
5. Establish a local aquatic animal health surveillance program under the regional livestock and fisheries authority.
6. Raise community awareness on the public health risks associated with waterborne pathogens like *A. hydrophila* and *E. coli* O157. Further research is needed on antimicrobial resistance patterns of *A. hydrophila* in the region to guide treatment strategies in aquaculture.

Abbreviations

AHI	Animal Health Institute
BUG	Biolog Universal Growth
C	Chloromphenicol
CIP	Ciprofloxacin
CIX	Ceftaxime
CN	Gentamicine
CLSI	Clinical Laboratory Standard Institute
CRO	Ceftriaxone
K/A	Alkaline/Acid
MAS	Motile Aeromonas Septicemia
MEM	Meropenem
PCR	Polymerase Chain Reaction
SXT	Sulfamethoxazole
TE	Tetracycline
TSI	Triple Sugar Iron
VP	Voges-Proskauer

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Abdella, B., Shokrak, N. M., Abozahra, N. A., Elshamy, Y. M., Kadira, H. I. and Mohamed, R. A., 2024. Aquaculture and *Aeromonas hydrophila*: a complex interplay of environmental factors and virulence. *Aquaculture International*, 32(6), pp. 7671-7681.
- [2] Abdel-Latif, H. M. and Khafaga, A. F., 2020. Natural co-infection of cultured Nile tilapia *Oreochromis niloticus* with *Aeromonas hydrophila* and *Gyrodactylus cichlidarum* experiencing high mortality during summer. *Aquaculture Research*, 51(5), pp. 1880-1892.
- [3] Ajekiigbe, V. O., Ogieuhi, I. J., Anthony, C. S., Bakare, I. S., Anyacho, S., Ogunleke, P. O., Fatokun, D. I., Akinmeji, O., Ruth, O. T., Olaore, A. K. and Amusa, O., 2025. Consumer behavior and its role in *E. coli* outbreaks: the impact of fast-food preparation practices and hygiene awareness. *Tropical Medicine and Health*, 53(1), p. 27.

- [4] Akhtar, N., Ishak, M. I. S., Bhawani, S. A., & Umar, K. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water*, 13(19), 2660. <https://doi.org/10.3390/w13192660>
- [5] Ammar, A. M., Mohamed, B. E., & Gharib, A. A. E. A. (2023). Motile Aeromonads as a Nile Tilapia bacterial infection: A review on prevalence, molecular characterization, effect on immune response and alternatives control measures. *Zagazig Veterinary Journal*, 51(2), 112-128. <https://doi.org/10.21608/zvjz.2023.275599>
- [6] Arefa, N., Sarker, A. K., & Rahman, M. A. (2021). Resistance-guided isolation and characterization of antibiotic-producing bacteria from river sediments. *BMC Microbiology*, 21, 1-12. <https://doi.org/10.1186/s12866-021-02117-4>
- [7] Armstrong, R., Hughes, R. and Gangvik, E. eds., 2016. *Handbook of the Unknowable*. TEKS.
- [8] Bakiyev, S., Smekenov, I., Zharkova, I., Kobegenova, S., Sergaliyev, N., Absatirov, G., & Bissenbaev, A. (2022). Isolation, identification, and characterization of pathogenic *Aeromonas hydrophila* from critically endangered *Acipenser baerii*. *Aquaculture Reports*, 26, 101293. <https://doi.org/10.1016/j.aqrep.2022.101293>
- [9] Bisi-Johnson, M. A., Adedeji, A. A., Sulaiman, A. A., Adefisoye, M. A., & Okoh, A. I. (2023). Isolation and genotypic characterization of extended-spectrum beta-lactamase-producing *Escherichia coli* O157:H7 and *Aeromonas hydrophila* from selected freshwater sources in Southwest Nigeria. *Scientific Reports*, 13(1), 10746. <https://doi.org/10.1038/s41598-023-37580-2>
- [10] Degefu, F., Herzig, A., Jirsa, F. and Schagerl, M., 2014. First limnological records of highly threatened tropical high-mountain crater lakes in Ethiopia. *Tropical Conservation Science*, 7(3), pp. 365-381.
- [11] Dissasa, G., Lemma, B., & Mamo, H. (2022). Isolation and identification of major bacteria from three Ethiopian Rift Valley lakes live and processed fish, and water samples: Implications in sanitary system of fish products. *BMC Veterinary Research*, 18(1), 439. <https://doi.org/10.1186/s12917-022-03516-y>
- [12] Dugan, P., Sugunan, V. V., Welcomme, R. L., Bn C., Brummett, R. E., Beveridge, M. C.,... & Chimatiro, S. (2013). Inland fisheries and aquaculture. In *Water for food, water for life* (pp. 459-484). Routledge.
- [13] Fahy, S., O'Connor, J. A., Sleator, R. D. and Lucey, B., 2024. From species to genes: a new diagnostic paradigm. *Antibiotics*, 13(7), p. 661.
- [14] Fernndez-Bravo, A., & Figueras, M. J. (2020). An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. *Microorganisms*, 8(1), 129. <https://doi.org/10.3390/microorganisms8010129>
- [15] Grilo, M. L., Sousa-Santos, C., Robalo, J. and Oliveira, M., 2020. The potential of *Aeromonas* spp. from wildlife as antimicrobial resistance indicators in aquatic environments. *Ecological Indicators*, 115, p. 106396.
- [16] Kebede, S., 2012. *Groundwater in Ethiopia: features, numbers and opportunities*. Springer Science & Business Media.
- [17] Kerigano, N. K., Chibsa, T. R., Molla, Y. G., Mohammed, A. A., Tamiru, M., Bulto, A. O., Wodaj, T. K., Gebreweld, D. S. and Abdi, A. K., 2023. Phenotypic, molecular detection and antibiogram analysis of *Aeromonas hydrophila* from *Oreochromis niloticus* (Nile Tilapia) and Ready-To-eat fish products in selected Rift Valley lakes of Ethiopia. *BMC veterinary research*, 19(1), p. 120.
- [18] Kumar, S., Paul, T., Sarkar, P., & Kumar, K. (2025). Environmental factors affecting aquatic animal health. In *Management of Fish Diseases* (pp. 171-188).
- [19] Mondal, S. and Palit, D., 2022. Challenges in natural resource management for ecological sustainability. In *Natural resources conservation and advances for sustainability* (pp. 29-59). Elsevier.
- [20] Nawar, S., Rashid, M. T., Ahmed, A., Hossain, M. M. and Afzal, A. B., 2021. A study of prevalence and pathogenic activity of bacteria in the air of Dhaka City and their antimicrobial resistance pattern. *American Journal of Molecular Biology*, 11(02), p. 51.
- [21] Rasmussen-Ivey, C. R., Hossain, M. J., Odom, S. E., Terhune, J. S., Hemstreet, W. G., Shoemaker, C. A., Zhang, D., Xu, D. H., Griffin, M. J., Liu, Y. J. and Figueras, M. J., 2016. Classification of a hypervirulent *Aeromonas hydrophila* pathotype responsible for epidemic outbreaks in warm-water fishes. *Frontiers in microbiology*, 7, p. 1615.
- [22] 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(3).
- [23] Woźniak, M., Gałzka, A., Tyskiewicz, R. and Jaroszuk-cisł, J., 2019. Endophytic bacteria potentially promote plant growth by synthesizing different metabolites and their phenotypic/physiological profiles in the Biolog GEN III MicroPlateTM Test. *International Journal of Molecular Sciences*, 20(21), p. 5283.
- [24] Yang, Y., Lu, Q. B., Liu, M. J., Wang, Y. X., Zhang, A. R., Jalali, N., Dean, N. E., Longini, I., Halloran, M. E., Xu, B. and Zhang, X. A., 2020. Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. *medrxiv*, pp. 2020-02.