E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(1): 01-09

www.fisheriesjournal.com
Received: 04-10-2024
Accepted: 10-11-2024

Joseph M Ndegwa
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi, Kenya

Lucy W Njagi
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi, Kenya

Isaac R Mulei
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi, Kenya

Philip N Nyaga
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi, Kenya

Daniel W Wanja
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine and Surgery, Egerton University, P.O. Box 536-20115, Egerton, Kenya

Shimaa E Ali
[1] Norwegian Veterinary Institute, Fish Health Research Group, P.O. Box 64,1431 AS, Norway
[2] Department of Hydrology, Veterinary Research Institute, National Research Centre, Dokki, Egypt

Jérôme Delamare-Debouteville
WorldFish Headquarters, Jalan Batu Maung, Batu Maung, Bayan Lepas, Penang 11960, Malaysia

Bryson B Kimemia
Kenya Medical Research Institute (KEMRI), Raila Odinga way, Off Mbagathi Road, P.O Box 54840-00200, Nairobi, Kenya.

Corresponding Author:
Joseph M Ndegwa
Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Kangemi, Nairobi, Kenya

Conventional and molecular characterization of an *Aeromonas* isolate recovered from an aquaculture farm with high fish mortality in Kenya

Joseph M Ndegwa, Lucy W Njagi, Isaac R Mulei, Philip N Nyaga, Daniel W Wanja, Shimaa E Ali, Jérôme Delamare-Debouteville and Bryson B Kimemia

DOI: https://doi.org/10.22271/fish.2025.v13.i1a.3008

Abstract
Bacterial diseases can pose a significant challenge to aquaculture in Kenya, leading to significant economic losses. A fish farm in Narok County, Kenya, reported mass mortality among its reared fish which included Nile tilapia, catfish and ornamental fish. This study aimed to characterize an *Aeromonas* isolate recovered from the farm and assess its potential role in the outbreak. Water samples (n=10) were collected randomly from different points within the fish ponds using sterile universal bottles and transported to the bacteriology laboratory at the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. The bacterial isolate was identified using conventional culture methods, biochemical assays, Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF), BD Phoenix machine tests, and Whole Genome Sequencing. Based on morphological, biochemical, and MALDI-TOF analyses, the isolate was identified as *Aeromonas veronii* and further confirmed as *Aeromonas veronii* biovar *Sobria* using the BD Phoenix system. The genome size of the isolate was approximately 4.6 Mb with a GC content of 59%. Phylogenetic analysis showed that the isolate clustered with fish isolates from South America and Asia and with human isolates from Asia and Australia. The study underscores the need for more studies on this bacterium's pathogenicity and development of an autogenous vaccine for the prevention of its bacteriosis.

Keywords: *Aeromonas veronii* biovar *sobria*, morphological, biochemical. MALDI-TOF, phylogenetic

Introduction
The aquaculture and fisheries sector in Kenya contributes approximately 0.8% of the country's Gross Domestic Product (GDP) and plays a crucial role in employment and livelihood creation [1]. Over 500,000 individuals are employed directly, with more than 2 million others benefiting individuals [1]. Recognized as a pillar for economic growth and food security, aquaculture is integrated into Kenya's Vision 2030 strategy and other national policy frameworks as a critical tool for improving nutrition, eradicating poverty, and generating employment opportunities [2].
Despite its growing importance, fish production faces numerous challenges, including disease outbreaks caused by bacterial pathogens, which can result in chronic or acute disease outbreaks [3, 4]. Bacteria from the family *Enterobacteriaceae* are known to act as opportunistic pathogens, infecting fish when environmental stressors compromise their immune defenses [5]. Stress factors such as poor water quality, high temperatures, and high-water organic content in aquaculture systems significantly increase the risk of disease outbreaks [6, 7]. This is particularly relevant in fish farms practicing intensive aquaculture, where environmental stressors often prevail, and the bacterial isolate used in this study was obtained.
In Kenya, limited studies have documented bacterial infections in fish, and most efforts have concentrated on isolating bacterial pathogens without undertaking comprehensive molecular characterization [8]. Therefore, to produce fish that are fit for human consumption and a reliable income stream for local communities, it is crucial to ensure that fish stocked in Kenyan fish farms are healthy [9].

Whole-genome sequencing (WGS) has emerged as a powerful tool for advancing the identification and characterization of bacterial pathogens. It provides critical insights into bacterial taxonomy, virulency factors, molecular epidemiology, and phylogenetic relationships [10]. Pathogenic bacteria often rely on a combination of virulence factors -such as adhesion molecules, invasion mechanisms, exotoxins, capsular polysaccharides, siderophores, endotoxins, and other enzymes—to infect their hosts, evade immune defenses, and establish disease [11]. Rather than operating in isolation, these virulence factors work synergistically to enhance bacterial pathogenicity. Therefore, microbe identity and classification are vital parts of transmittable contagion management.

The genetic determinants encoding these virulence traits have become essential for assessing the pathogenic potential of microbial pathogens [10]. Although *Aeromonas* species are frequently isolated from capture fisheries and aquaculture systems, their molecular characterization in Kenya has not been adequately explored. This study aims to fill the gap by conducting a comprehensive molecular analysis of an *Aeromonas* isolate from a Kenyan fish farm.

Materials and Methods

Ethical approval

All procedures done in this study involving experiments concerning live fish were approved and permitted by the Biosafety, Animal Use and Ethics Committee (BAUEC) of the Faculty of Veterinary Medicine, University of Nairobi, Kenya. (REF: FVM BAUEC/2023/424).

Source of the bacterial isolate

Study site

The *Aeromonas* isolate used in this study was recovered from pond water (fish mortalities had already occurred when the farm was visited hence water samples of the ponds were collected instead of fish samples) of a fish farm located in Narok County, Kenya, within the southern region of the Great Rift Valley. The area lies between latitudes 0° 50' - 1° 50' South and longitude 35° 28' - 36° 25' East. Narok County experiences a Marine west coast climate with warm summers (Cfb classification), receiving an average annual rainfall of 177.54 millimeters (6.99 inches) over approximately 226.91 rainy days, accounting for 62.17% of the year [12].

The farm employs mixed farming practices including pig production, layer-chicken rearing, fish farming (Nile tilapia, ornamental fish and catfish), a beef feedlot and crop cultivation. It reported unusual mortalities of more than 80% of its stocked fish from unknown causes. The farm operates intensively with 142 earthen and concrete ponds lined with liners. All the fish ponds were affected by the fish mortalities. Water for fishponds is sourced from a borehole.

Sample collection and bacterial isolation.

Pond water was collected from different points across ten (10) fishponds using sterile universal bottles. The samples were transported in a cool box packed with ice to the bacteriology laboratory at the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for analysis. A loopful of each water sample was aseptically streaked onto freshly prepared nutrient agar, blood agar and tryptone soy agar. Sub-cultures of the isolates were made on tryptone soy agar to obtain pure colonies. The purified colonies were subsequently stored in nutrient agar slant and tryptone soy broth for future use.

Characterization of the bacterial isolate.

Conventional identification of *Aeromonas* isolate.

Colony morphology and Gram staining.

The stored isolates were revived on tryptone soy agar, blood agar and McConkey agar and incubated at 37^0 C for 24 hours. Thereafter the colony morphology and Gram staining characteristics of the bacteria were examined and documented following the protocols by Schaperclaus *et al*. [13] and Tripathi *et al*. [14].

Biochemical identification

Biochemical tests, including sugar fermentation, indole production, methyl red (MR), Voges-Proskauer (VP), citrate utilization, triple sugar iron (TSI), catalase, and oxidase tests, were performed to identify the isolate, following the protocol described by Austin *et al*. [15].

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Analysis

MALDI-TOF analysis was conducted at the International Livestock Research Institute (ILRI), in Nairobi, Kenya. A single bacterial colony was picked using a sterile toothpick and transferred to a target spot on the designated smear area within a circular boundary on the MALDI target plate. The sample was mixed with the matrix solution and allowed to air-dry. Under vacuum conditions, the mixture of sample and matrix crystallized and was subsequently irradiated with a short laser pulse. Laser-induced desorption and ionization enabled mass spectrometric detection, with the organism's identity displayed on the system's interface [16].

Biovar Identification using the BD Phoenix System

Identification of the biovar using BD Phoenix™ machine (BD, USA) was done at Central Investigation Veterinary laboratories (CVL) in Nairobi, Kenya. The Gram stain of the *Aeromonas* isolate was first confirmed before selecting the appropriate Phoenix panel for inoculation. The procedure of identification of the biovar was done as described by Junkins *et al*., [17]. The organism identification appeared on the Phoenix Report Form with a probability percentage from the Phoenix database based on the substrate reaction profile [17].

Whole Genome Sequencing

DNA extraction

Genomic DNA was extracted from the bacterial isolate using the Gene-JET Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., USA) following the manufacturer's instructions.

Preparation of library and sequencing

The extracted DNA sample was quantified using the Qubit™ 4 Fluorometer (Catalog Number Q33226, Publication Number MAN0017209) (Thermo Fisher Scientific Inc., USA). Illumina DNA Prep Kit protocol (Illumina, Inc., USA) was followed for library preparation. For cost efficiency of the library preparation, our DNA sample was added to other samples not relevant to this study. Sequencing was performed using illumina 300-cycle version 2 chemistry set to generate 150-bp paired-end reads.

Analysis of genome sequence data

The contigs from the *Aeromonas veronii* (A4) isolate were first analysed using QUAST to generate the assembly metrics, assessing the overall quality of the genome assembly [18]. The

identity of genomic contigs was identified through ribosomal multi-locus sequence typing (rMLST) using the PubMLST platform [19]. The genome was then annotated using PROKKA [20], which identified open reading frames (ORFs) coding sequences, ribosomal RNA sequences, transfer RNAs (tRNAs), and CRISPR loci [20].

Genome completeness was confirmed using BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis, confirming the presence and completeness of essential, order-specific genomic markers [21]. Virulence factor and AMR genes were determined using ABRICATE, using the Virulence Factors Database (VFDB) [22]. The identified virulence factors were further grouped into functional subsystems via the Bacterial and Viral Bioinformatics Resource Centre (BV-BRC) platform [23].

Phylogenetic analysis was conducted based on core-genome single nucleotide polymorphisms (SNPs) from the study isolate and other *A. veronii* isolates from the BV-BRC database. SNP alignments were generated using Parsnp (Version 2.0.5) [24].

A maximum-likelihood phylogenetic tree was inferred from these alignments using IQ-TREE 2, with 1000 bootstrap replicates to assess the geo-phylogenetic relationships [25].

Results

Overall findings on bacterial isolation and source of *Aeromonas* isolate

A total of seven Gram-negative bacteria were recovered from pond water (Long after the mortality event) from the Narok fish farm, where abnormal mortalities of fish had been reported. The identified bacteria included *Aeromonas*, *Flavobacterium*, *Edwardsiella*, *Serratia*, *Citrobacter*, *Micrococcus* and *Enterobacter* species. For this study, the *Aeromonas* isolate was selected for further investigation. This is because the fish had presented with signs of motile aeromonad septicaemia prior to the mortalities.

Conventional Identification of the *Aeromonas* isolate

Colony morphology characteristics

The isolate exhibited distinct colony morphology across different culture media. On blood agar, the colonies appeared creamy white, raised, medium-sized, and displayed beta-hemolytic. On MacConkey agar, the colonies were creamy white, raised, medium-sized, and non-lactose fermenting. On Tryptose soy agar, the colonies were creamy-white, raised, and medium in size (Figure 1 A-C). The isolate was Gram-negative short rods as shown in the arrow (Figure 1D).

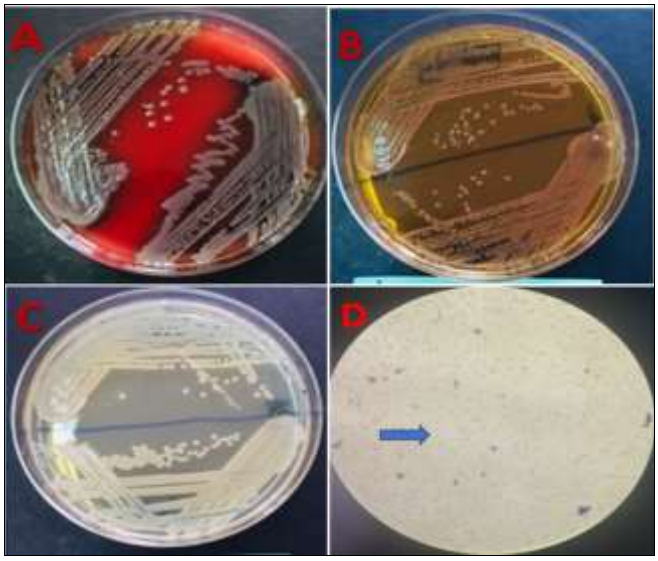

Fig 1: *Aeromonas* isolate on different media. (A) *Aeromonas* isolate on Blood Agar. (B) *Aeromonas* isolate on MacConkey agar. (C) *Aeromonas* isolate on TSA (D) Gram-staining of *Aeromonas* isolate showing Gram-negative short rods (arrow)

Biochemical reactions: The isolate tested positive for indole, Voges-Proskauer and citrate but negative for methyl red and urea (Figure 2A). On Triple Sugar Iron (TSI) agar, both the slant and butt exhibited acidic reactions with gas production (Figure 2B). Effervescence during the catalase test confirmed a positive result. Additionally, the oxidase test yielded a positive result, indicated by the formation of a purple color on the oxidase discs.

Fig 2: (A) Some biochemical reactions of *Aeromonas* isolate (arrow); (B) A*eromonas* isolate reaction on TSI; acid slant, acid butt with gas production (arrow)

Sugars utilization

The isolate metabolized glucose with gas production, while sucrose and mannitol were utilized but without gas production. However, the *Aeromonas* isolate failed to utilize lactose. The conventional biochemical tests identified the isolate as *Aeromonas veronii*. A detailed summary of the biochemical test results for the isolate is presented in Table 1 below.

Table 1: Biochemical characteristics of the *Aeromonas* isolate

Observed and Tested Characteristics	Characteristics of *Aeromonas* experimental isolate	Reference characteristics of *Aeromonas* species[a]
Gram stain	-	-
Cell morphology	Short rods	Short rods
Growth on BA	+, creamy white, raised, medium, beta-hemolytic colonies	+
Growth on McConkey agar	+, creamy white, raised, medium, non-lactose fermenter colonies	+
Growth on TSA	+, creamy-white, raised, medium colonies	+
Catalase	+	+
Oxidase	+	+
Indole	+	+
Methyl red test	-	-
Voges Proskauer	+	+
Citrate	+	+
Urea	-	-
TSI	+, acid slant, acid butt with gas production	+, acid slant, acid butt with gas production
Sugars utilization		
Lactose	-	-
Glucose	+, with gas production	+, with gas production
Sucrose	+, without gas production	+, without gas production
Mannitol	+, without gas production	+, without gas production

Legend: +: positive -: negative [a] Austin and Austin, [15]. Bacterial Fish Pathogens

Species and biovar identification: MALDI-TOF and BD Phoenix System

The *Aeromonas* isolate was identified as *Aeromonas veronii* using the MALDI-TOF system. Further analysis with the BD Phoenix system confirmed the isolate as *Aeromonas veronii* biovar *sobria*.

Whole genome sequence of *Aeromonas veronii* biovar *sobria*

The taxonomic identification of the *Aeromonas veronii* biovar *sobria* is summarized in Table 2. The genome was sequenced to a total length of 4,609,841 base pairs, with a GC content of 58.58%.

Table 2: Taxonomic identification (Ribosomal Multi-Locus Sequence Typing) of *Aeromonas veronii* biovar *sobria*

Contigs	78
Genome Length	4,609,841bps
GC Content	58.58%
Contig L50	15
Contig N50	115,560 bps
Transfer RNA (tRNA)	102
Ribosomal RNA (rRNA)	10
Coding Sequences (CDS)	4,333
Hypothetical Coding Sequences (CDS)	966
CheckM Completeness	100%
Complete BUSCOs	365 (99.7%)
Fragmented BUSCOs	1 (0.3%)
Missing BUSCOs	0 (0%)
Hypothetical proteins	969
Proteins with functional assignments	3364
Proteins with EC number assignments	1020
Proteins with GO assignments	850
Proteins with Pathway assignments	729
Proteins with Subsystem assignments	1414

Virulence Factors

The *Aeromonas veronii* isolate identified in this study harbours several important virulence genes known for causing infections in both aquatic organisms and humans. Prominent among these are the Type III secretion systems and two-component system response regulator, which play critical

roles in pathogenicity and host adaptation.

Antimicrobial Resistance Genes

The antimicrobial resistance (AMR) genes found in the *A. veronii* isolate show resistance to multiple classes of antibiotics, raising concerns in aquaculture. The primary AMR genes identified include: β-Lactam Resistance and Fluoroquinolones resistance genes. The isolate also harboured mutations in the UhpT (Hexose phosphate transport protein) and the Tu (Translation elongation factor) genes that are the targets for Fosfomycin and Elfamycin antibiotics respectively.

Phylogenetic analysis

Fish Isolates

The grouping of *A. veronii* (A4) isolate with other fish-derived strains indicates that the bacterium possesses genomic characteristics that enhance its adaptation and survival in aquatic environments. Notably, the A4 isolate clustered closely with a strain from South America (Brazil) while the broader clade included additional isolates from South America (Peru) and Asia (China and Bangladesh) (Figure 3).

Human Isolates

Although closely related to fish isolates, the proximity of the human-derived *A. veronii* strains to the fish strain on the phylogenetic tree indicates potential zoonotic capabilities. Geographically, this study fish A4 isolate clustered with human isolates from Asia (Taiwan and Japan) and Australia, further supporting the bacterium's ability to cross different host species (Figure 4).

These phylogenetic clusters show the ubiquitous nature of *A. veronii* and suggest a stable genetic makeup, contributing to its persistence across diverse geographic regions and host environments.

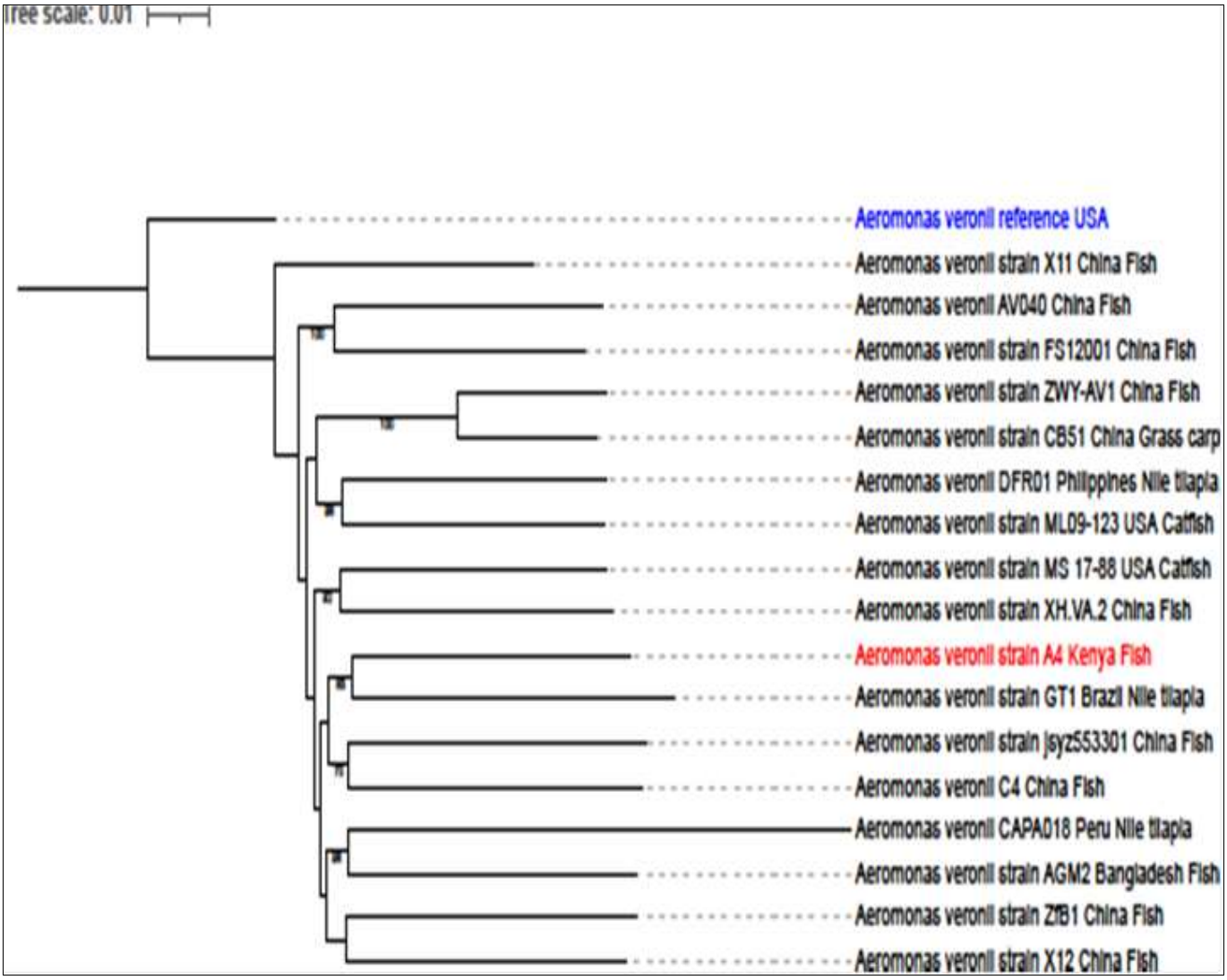

Fig 3: Phylogenetic tree of *Aeromonas veronii* biovar *sobria* (A4) in comparison with other isolates from fish

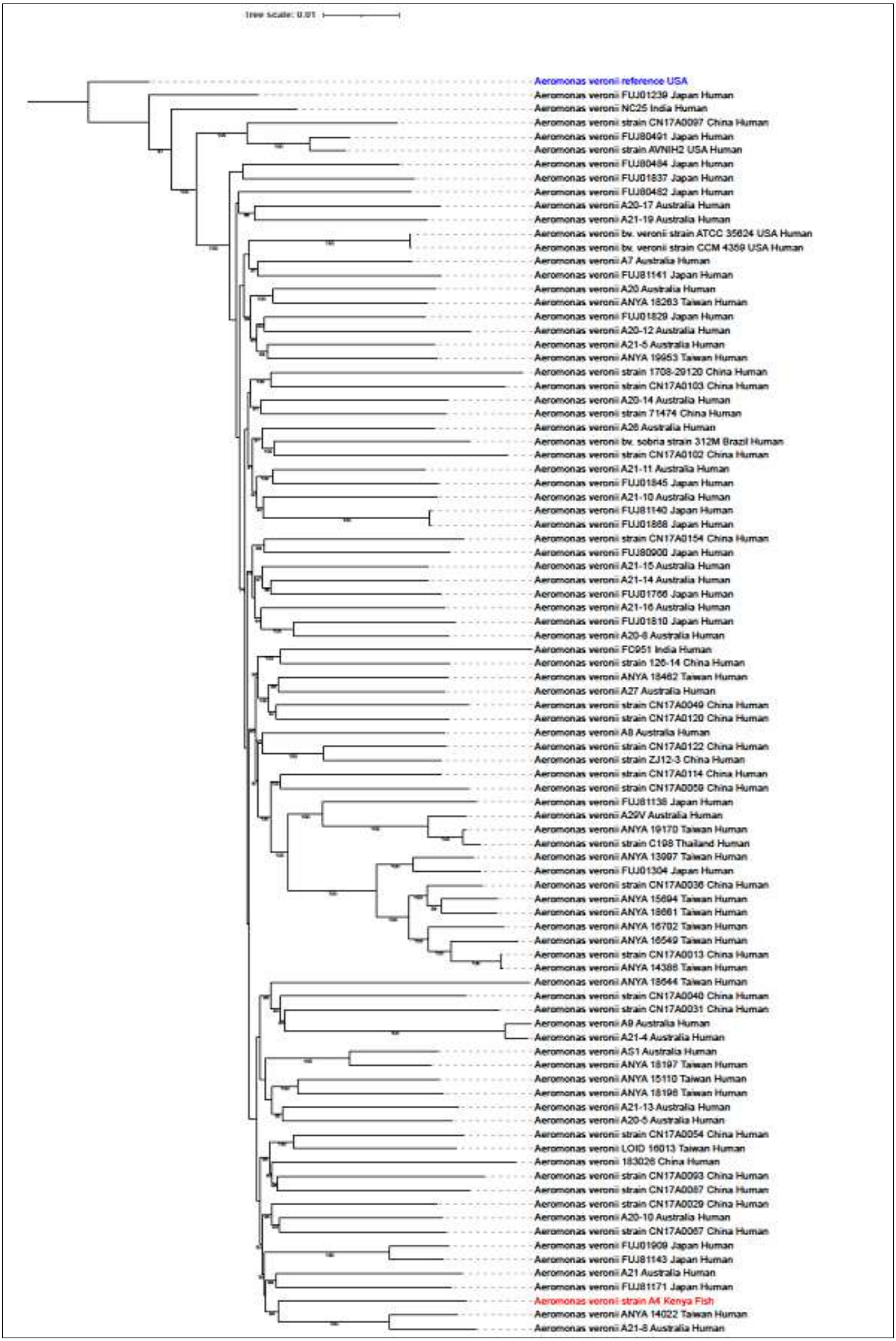

Fig 4: Phylogenetic tree of *Aeromonas veronii* biovar *sobria* (A4) in comparison with human isolates

Discussion

The bacterial isolate recovered in this study was identified as *Aeromonas veronii*, a Gram-negative rod, that tested catalase and oxidase-positive and fermented glucose, sucrose, and mannitol, but not lactose. These characteristics are consistent with prior descriptions by Martin- Carnahan and and Joseph [26], Austin and Austin [27] and Eissa *et al*; [28]. Further confirmation was achieved using MALDI-TOF and BD Phoenix system, which identified the isolate as *A. veronii* biovar *sobria*. The BD Phoenix machine uses a calorimetric oxidation-reduction indicator to detect the organism's growth through turbidometric analysis [17].

The genome of *Aeromonas veronii* (Designated A4) was sequenced, revealing a genome size of approximately 4.6 Mb with a GC content of 59%, consistent with the *A. veronii* reference genome [29]. This bacterium is known to harbour a broad range of virulence factors, including genes involved in adhesion, toxin production, and immune evasion, which make them versatile and adaptable to both aquatic organisms and humans [30]. Notably, *A. veronii* has been isolated from both healthy and diseased fish, showing its ability to persist in hosts and evade immune responses of fish [31].

Key virulence systems identified in the *A. veronii* (A4) genome include the Type III secretion system (T3SS) and two-component systems (TCS). The T3SS is critical for bacterial pathogenicity, enabling the injection of effector proteins directly into host cells [32]. This facilitates bacterial manipulation of host cell functions, suppression of immune responses, and establishment of infection [33].

In addition, TCS plays a pivotal role in environmental sensing and adaptation, enabling *A. veronii* to respond to fluctuating environmental conditions in fish farms [34]. These systems allow the bacterium to detect changes in temperature, pH, oxygen levels, and the presence of pollutants, ensuring survival and persistence in aquaculture environments [35]. The combined activity of T3SS and TCS enhances the pathogen's ability to infect fish and adjust to environmental stress, contributing to high virulence and survival within aquaculture systems.

The zoonotic potential of *A. veronii* poses additional risks to public health, as it can infect multiple hosts, including humans [36]. While environmental conditions in fishponds—such as high nutrient levels and warm temperatures—promote the expression of virulence genes that support colonization of fish, studies show that isolates from fish can also cluster phylogenetically with human strains [37]. This overlap suggests that genetic elements shared between fish and human isolates facilitate cross-host infections [38]. Recent research further highlights the public health risk posed by dual-host strains, which can infect fish and humans [39].

The rapid expansion of global fish farming has heightened the risk of infectious diseases, with intensive farming practices creating stressful conditions that increase fish susceptibility to pathogens [40]. *Aeromonas* spp., including *A. veronii*, have emerged as leading contributors to high mortality in freshwater aquaculture, especially under conditions of environmental stress [41, 42]. This may explain the isolation of *A. veronii* from the aquaculture farm in Narok County, Kenya, where environmental stress likely facilitated the pathogen's proliferation. Continuous monitoring of environmental parameters such as water quality is essential for mitigating infections and reducing fish mortality [38, 43].

This study provides valuable insights into the characterization of *A. veronii* isolated from a fish farm in Kenya. The findings underscore the importance of targeted management strategies, including regular water quality monitoring and early detection systems, to control potential infections caused by *A. veronii*. These measures are essential to prevent outbreaks and mitigate economic losses in aquaculture.

Conclusions and Recommendations

A. veronii biovar *sobria* may have caused the abnormal mortality event in an aquaculture farm in Narok County, Kenya. Intensive fish farming, characterized by high stocking density and accumulation of high organic waste in the environment, contributes to fish stress, creating favorable conditions for disease outbreaks caused by *A. veronii* biovar *sobria*. Further research on the pathogenicity and vaccine development against this bacterium is essential to develop effective mitigation strategies and enhance the management of its associated bacteriosis in Kenya.

Data Availability

Sequencing data generated by this study has been deposited to the NCBI SRA database under the Bio-Project Accession number PRJNA1156617. The assembled genome is available under the Whole Genome Shotgun project accession number JBHFVN010000000 at DDBJ/ENA/GenBank.

Conflict of Interest

The authors declare that there are no conflicts of interest

Acknowledgements

This work was carried out as part of the AHA project (Increased Sustainability in the Aquaculture Sector in Sub-Saharan Africa, through Improved Aquatic Animal Health Management, RAF-19/0051), which was funded by the Norwegian Agency for Development Cooperation (Norad). The research was conducted by the University of Nairobi in collaboration with WorldFish and the Norwegian Veterinary Institute.

References

1. Obiero K, Munguti J, Njiru J, David M. Overview of aquaculture in Kenya. In: Munguti *et al*. (eds.), State of Aquaculture in Kenya, Chapter 1, pp 1-9, Laxpress Services, Nairobi, Kenya, 2017.
2. Kenya's Aquaculture Brief 2017; Status, Trends, Challenges and future outlook.
3. Austin B, Stobie M, Robertson P. Citrobacter freundii: the cause of gastro-enteritis leading to progressive low-level mortalities in farmed rainbow trout, *Oncorhynchus mykiss* Walbaum, in Scotland. Bull Eur Assoc Fish Pathol. 1992;12:166-168.
4. Baeck GW, Kim JH, Choresca Jr C, Gomez DK, Shin SP, Han JE, *et al*. Mass mortality of doctor fish (*Garra rufa* obtusa) caused by *Citrobacter freundii* infection. J Vet Clin Pathol. 2009;26:150-154.
5. Rajasekaran P. Enterobacteriaceae group of organisms in sewage-fed fishes. Adv Biotechnol. 2008;8:12-14.
6. Sekar V, Santiago T, Vijayan K, Alavandi S, Raj V, Rajan J, *et al*. Involvement of *Enterobacter cloacae* in the mortality of the fish, *Mugil cephalus*. Lett Appl Microbiol. 2008;46(6):667-672.
7. Zheng D, Mai K, Liu S, Limin C, Liufu Z, Xu W, *et al*. Effect of temperature and salinity on virulence of *Edwardsiella tarda* to Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquac Res.

2004;35:494-500.
8. Wanja DW, Mbuthia PG, Waruiru RM, Mwadime JM, Bebora LC, Nyaga PN, *et al*. Bacterial pathogens isolated from farmed fish and source pond water in Kirinyaga County, Kenya. Int J Fish Aquat Stud. 2019;7(2):34-39.
9. Akoll P, Mwanja WW. Fish health status, research and management in East Africa: Past and present. Afr J Aquat Sci. 2012;37(2):117-129.
10. Donkor ES. Sequencing of bacterial genomes: principles and insights into pathogenesis and development of antibiotics. Genes. 2013;4:1-18.
11. Sharma AK, Dhasmana N, Dubey N, Kumar N, Gangwal A, Gupta M, *et al*. Bacterial virulence factors: secreted for survival. Indian J Microbiol. 2017;57:1-10.
12. Narok County Government. Proceedings. July 2019.
13. Schaperclaus W, Kulow HM, Schreckenbach K. Fish Diseases. Vol. I. A. A. Balkema / Rotterdam; 1992.
14. Tripathi N, Sapra A. Gram Staining. [Updated 2023 Aug 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562156/
15. Austin B, Austin DA. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 6th ed. Springer, New York; 2016.
16. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem. 2015;61(1):100-111.
17. Junkins A. Comparison of BD Phoenix™ AP Workflow with Vitek 2. J Clin Microbiol. 2010;48(5):1929-1931.
18. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics (Oxford, England). 2013;29(8):1072-1075. https://doi.org/10.1093/bioinformatics/btt086
19. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018;3:124. https://doi.org/10.12688/wellcomeopenres.14826.1
20. Grant JR, Enns E, Marinier E, Mandal A, Herman EK, Chen C, *et al*. Proksee: In-depth characterization and visualization of bacterial genomes. Nucleic Acids Res. 2023;51(W1):W484-W492. https://doi.org/10.1093/nar/gkad326
21. Manni M, Berkeley MR, Seppey M, Simao FA, Zdobnov EM. BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. arXiv. 2021. https://doi.org/10.48550/arXiv.2106.11799
22. Seemann T. Tseemann/abricate [Perl]. https://github.com/tseemann/abricate (Original work published 2014).
23. Olson RD, Assaf R, Brettin T, Conrad N, Cucinell C, Davis JJ, *et al*. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): A resource combining PATRIC, IRD and ViPR. Nucleic Acids Res. 2023;51(D1):D678-D689. https://doi.org/10.1093/nar/gkac1003
24. Kille B, Nute MG, Huang V, Kim E, Phillippy AM, Treangen TJ. Parsnp 2.0: Scalable core-genome alignment for massive microbial datasets. Bioinformatics. 2024;40(5):btae311. https://doi.org/10.1093/bioinformatics/btae311
25. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, *et al*. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37(5):1530-1534. https://doi.org/10.1093/molbev/msaa015
26. Martin-Carnahan A, Joseph SW. Aeromonas. In: Brenner, Krieg, Staley, Garrity (Eds.), Bergey's Manual of Systematic Bacteriology. Vol 2. 2nd ed. Williams and Wilkins, New York; c2005.
27. Austin B, Austin DA. Aeromonadaceae representatives (Motile aeromonads). In: Austin B, Austin DA, eds. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 5th ed. Springer, New York; c2012. p. 119-146.
28. Eissa IAM, Maather El-lamei, Mona Sherif, Desuk E, Mona Zaki, Bakry M. *Aeromonas veronii* biovar sobria, a causative agent of mass mortalities in cultured Nile tilapia in El-Sharkia governorate, Egypt. Egyptian Life Sci J. 2015, 12(5).
29. *Aeromonas veronii* genome assembly ASM869370v1. NCBI. Available from: https://www.ncbi.nlm.nih.gov/data-hub/assembly/GCF_008693705.1/
30. Gonçalves Pessoa RB, de Oliveira WF, Marques DSC, dos Santos Correia MT, de Carvalho EVM M, Coelho LCBB. The genus *Aeromonas*: A general approach. Microb Pathog. 2019;130:81-94. https://doi.org/10.1016/j.micpath.2019.02.036
31. Abernathy JW, Zhang D, Liles MR, Lange MD, Shoemaker CA, Beck BH. Whole-genome sequencing and annotation of *Aeromonas veronii* isolates from channel catfish. Microbiol Res Ann. 2023;12(2):e01231-22. https://doi.org/10.1128/mra.01231-22
32. Zhao YH, Shaw JG. Cross-talk between the *Aeromonas hydrophila* type III secretion system and lateral flagella system. Front Microbiol. 2016;7:1434. https://doi.org/10.3389/fmicb.2016.01434
33. Frey J, Origgi FC. Type III Secretion System of *Aeromonas salmonicida* Undermining the Host's Immune Response. Front Mar Sci. 2016;3:130. https://doi.org/10.3389/fmars.2016.00130
34. Wang Y, Gong J, Guan Y, Zhao Z, Cai Y, Shan X. OmpR (TCS response regulator) of *Aeromonas veronii* plays a major role in drug resistance, stress resistance, and virulence by regulating biofilm formation. Microb Pathog. 2023;181:106176. https://doi.org/10.1016/j.micpath.2023.106176
35. Tomás JM. The Main *Aeromonas* Pathogenic Factors. ISRN Microbiol. 2012;2012:256261. https://doi.org/10.5402/2012/256261
36. Guo K, Sun Y, Tang X, Zhou X, Jiang M, Yang Q, *et al*. Pathogenicity and inactivated vaccine treatment of *Aeromonas veronii* JW-4 on crucian carp. Microb Pathog. 2023;183:106315. https://doi.org/10.1016/j.micpath.2023.106315
37. De Silva Lads, Wickramanayake Mvks, Heo GJ. Virulence and antimicrobial resistance potential of *Aeromonas* spp. associated with shellfish. Lett Appl Microbiol. 2021;73(2):176-86. https://doi.org/10.1111/lam.13489
38. Martino ME, Fasolato L, Montemurro F, Rosteghin M, Manfrin A, Patarnello T, *et al*. Determination of microbial diversity of *Aeromonas* strains on the basis of multilocus sequence typing, phenotype, and presence of putative virulence genes. Appl Environ Microbiol. 2011;77(16):5423-5435. https://doi.org/10.1128/AEM.00708-11
39. Pang M. Editorial: *Aeromonas* spp.-transmission,

pathogenesis, host-pathogen interaction, prevention, and treatment. Front Microbiol. 2023;14:1320343. https://doi.org/10.3389/fmicb.2023.1320343
40. Doan HV. The LD50 of Asian catfish (*Pangasius bocourti*, Sauvage 1870) challenge to pathogen *Aeromonas hydrophila* FW52 strain. Pensacola News J. 2013;75:287-93.
41. Plumb JA, Hanson LA. Health Maintenance and Principal Microbial Diseases of Cultured Fishes. 3rd ed. Iowa: Blackwell Publishing Ltd.; c2011. p. 492.
42. Song X, Zhao J, Bo J, Liu Z, Wu G, Gong C. *Aeromonas hydrophila* induces intestinal inflammation in grass carp (*Ctenopharyngodon idella*): An experimental model. Aquaculture. 2014;434:171-178.
43. Marinho RGB, Tavares-Dias M, Dias-Grigório MKR, Neves LR, Yoshioka ETO, Boijink CL, *et al*. Helminths and protozoans of farmed pirarucu (*Arapaima gigas*) in eastern Amazon and host-parasite relationship. Arq Bras Med Vet Zootec. 2013;65:1192-1202.