Phenotypic and molecular detection of Aeromonads infection in farmed Nile tilapia in Southern highland and Northern Tanzania

Alexanda Mzula [a,b,*], Philemon N. Wambura [b,c], Robinson H. Mdegela [a], Gabriel M. Shirima [a]

[a] Department of Global Health and Biomedical Sciences, School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
[b] College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania
[c] National Ranching Company (NARCO), Ministry of Livestock and Fisheries Development, Tanzania

A B S T R A C T

Aeromonads disease outbreaks are now becoming a common phenomenon in freshwater farmed fish worldwide. In Tanzania, the aquaculture field is increasingly growing save to sustain food protein demand and strengthen household income. To avoid losses that tilapia fish farmers might account, information on magnitude of infection and characteristics of the aetiological agent is vital. This study aimed to establish the prevalence of aeromonads infection in farmed tilapia and assess pond and fish health management practices. A cross sectional study was carried out between February 2017 and October 2018 and a total of 816 whole fish samples were aseptically collected from 32 ponds in Ruvuma, Mbeya, Iringa and Kilimanjaro regions. During sampling, water quality parameters were taken and questionnaires to assess the knowledge of farmers were also provided. Isolation and identification of bacteria was conducted using conventional biotyping and molecular techniques. A total of 201 (80.4%) of 250 isolates that were conventionally identified were confirmed to be aeromonads by amplification of 820 bp rpoD gene, making the overall prevalence of 24.6% (201, n = 816). Sequencing of rpoD gene and phylogenetic analysis revealed two aeromonads species, *Aeromonas hydrophila* and *Aeromonas veronii*. To the best of our knowledge this is the first report to establish the prevalence of aeromonads in apparently healthy farmed tilapia in Southern highlands and Northern zone of Tanzania. In addition it was observed that farmers were lacking proper knowledge and awareness on pond management practices and fish health management. In conclusion, the infection rate of aeromonads in apparently health tilapia coupled with lack of proper knowledge and awareness on pond and fish health management by fish farmers in the study area poses risk of diseases outbreaks in their farms in future. Therefore, it is recommended that the farmers should be trained on basic pond and fish health management and control strategies.

1. Introduction

Aeromonads as disease causing agents are now becoming common culprit causing outbreaks in farmed fish worldwide (Bebak et al., 2015; Harikrishnan and Balasundaram, 2005). Aeromonads are gram negative, rod shaped facultative bacterium which cause various diseases in fish named as haemorrhagic septicaemia, dropsy, epizootic ulcerative syndrome, haemorrhagic enteritis, and red body disease (Abdelhamed et al., 2017; Igbinosa et al., 2012). These bacterial species are ubiquitous in the aquatic environment but now have become a challenging pathogen of cultured fish (Chaix et al., 2017; De Jagoda et al., 2014; Janda and Abbott, 2010; Joseph et al., 2013). Nile tilapia (*Oreochromis niloticus*) is one among wide range of fish species infected by aeromonads

(Baumgartner et al., 2017).

According to recent taxonomy, the genus *Aeromonas* is currently consisting of more than 30 genospecies (Erdem et al., 2011). The phenotypic identification of these species is difficult because of its complexity in using growth and biochemical characteristics as it brings confusions especially to closely related species and strains (Beaz-Hidalgo et al., 2010; Chandran et al., 2002; Puthucheary et al., 2012).

Twenty four years back, before the use of molecular tools the only *Aeromonas* species recognised using a profile of sugars, API systems and Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) were *A. hydrophila*, *A. sobria*, *A. caviae*, *A. veronii* and *A. salmonicida*. Consensus is yet to be reached on assigning the *Aeromonas* strains to the recognised species using conventional

---

biochemical characteristics. The use of housekeeping genes in identification of *Aeromonas* species has recently gained attention to most scientists. These housekeeping genes have high discriminatory and resolving power and upon precise identification of eromonads at genus level, a phylogenetic analysis of either one of them could be used to reveal genospecies. Some of these housekeeping genes employed in inferring the taxonomy of the genus aeromonas include but not limited to *gyrB, rpoD, recA, dnaJ, gyrA, dnaX* and *atpD* (Zhou et al., 2019).

Despite the known contributions of other species of the genus *Aeromonas* in causing diseases in fish, *A. hydrophila* is the main cause of disease outbreaks in fresh water farmed fish contributing to food insecurity and economic loss worldwide (Aboyadak et al., 2015; Baumgartner et al., 2017). Aeromonads diseases in fish farms are accelerated by several factors including variations in physical-chemical parameters of pond water. The important physical-chemical parameters are the increased turbidity, temperature, salinity, pH, water conductivity and low dissolved oxygen (FAO, 2018; Jacobs and Chenia, 2007; Najiah and Laith, 2014). These environmental factors induce stresses that predispose fish to infections and diseases (Camus et al., 1998). It has been well acknowledged that semi-intensive and intensive fish farming coupled with poor management can result into aeromonads disease outbreaks (Najiah and Laith, 2014).

In Tanzania, the aquaculture field is increasingly growing and it has become an attractive venture to most of people to sustain food protein demand and strengthen household income. It is largely driven by the availability of water and land and therefore fresh water fish farming industry is well established in Southern highlands, Northern zone and Lake Zone due to existence of lakes and rivers (MLFD, 2013). Despite these opportunities, the subsector is challenged by feed resources, sources of fingerlings, knowledge and awareness, water quality and diseases.

The recent outbreak of *A. hydrophila* in Tanzania occurred in 2009 at Mtera hydroelectric power dam and caused substantial loss of wild tilapia (*Oreochromis niloticus*) (Shayo et al., 2012). The same aetiological agent was isolated in the same area in 2012 (Shayo et al., 2012). Despite the reported outbreaks and few sporadic cases of unknown aetiology with clinical signs similar to haemorrhagic septicaemia in tilapia farms in Southern highlands of Tanzania, systematic surveillance of aeromonads infections in farmed fish has not been explored. To avoid losses that tilapia fish farmers might encounter, information on magnitude of infection and characteristics of the aetiological agent is vital. The objective of this study was to establish the prevalence of aeromonads infection in farmed tilapia and to assess pond and fish health management practices in Southern highlands and Northern zones of Tanzania, so as to establish information and knowledge that will assist in providing proper mitigation towards establishment of sustainable aquaculture production in the country.

2. Material and methods

2.1. Study site and sampling procedure

A cross sectional study was carried out between February 2017 and October 2018. A total of 816 whole fish samples were aseptically collected from 32 randomly selected ponds in Ruvuma, Mbeya, Iringa and Kilimanjaro regions (8 in each region). The sample size of fish specimens was derived and determined from the method developed by Ossiander (1973). HI9829 portable meter (HANNA Instruments, Woonsocket, and U.S.A) was used to collect pond water quality data; water turbidity, temperature, water salinity, conductivity, dissolved oxygen and pH were recorded from each sampled pond. From each pond, fish were sampled by scooping using small sized fish net. Morphometric parameters (weight and length were recorded before dissection using digital balance and simple ruler, respectively). Fish were aseptically dissected on the spot and the selected organs such as; liver, kidney, spleen and gills were collected and stored in bijou bottles containing Cary-Blair transport medium, placed in a cool box containing ice packs

and transported to the microbiology laboratory at Sokoine University of Agriculture (SUA) for microbiological analysis within 7 days and later at Nelson Mandela African Institution of Science and Technology (MN-A-IST) for Molecular analysis.

2.2. Ethics statement

Sampling of fish and all dissections has been carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European and the National Institutes of Health – Office of Laboratory Animal Welfare policies and laws and the Tanzania Animal Welfare Act of 2008 was complied. This study also complied with the ARRIVE guidelines. Implementation of ethical issues was under the supervision of the Kibong'oto Infectious Diseases Hospital (KIDH), the Nelson Mandela African Institution of Science and Technology (NM-AIST) and Centre for Educational Development in Health Arusha (CEDHA), Health Research Ethics Committee (KNCHREC).

2.3. Assessment of knowledge and practices on fish health and pond management

Alongside with fish samples collection, semi structured questionnaires was administered to each pond owner to gather information related to fish bacterial diseases, farming systems and general management.

2.4. Culture, isolation and identification

Internal organs (liver, heart and kidneys and gills) collected were cultured in MacConkey, Blood agar, *Aeromonas* isolation agar medium (M88)) and Tryptic soy agar supplemented with 5% defibrinated sheep blood. (All culture media manufactured by HiMedia Laboratories Pvt. Ltd. of Mumbai, India). The inoculated plates were incubated at 28 °C for 24–48 hours. The classical identification of bacterial colonies and biotyping was performed as described by Abbott et al. (2003) and Deen et al. (2014) with modification. Briefly, the isolates were tested for 21 phenotypic characteristics in conventional bases. The biochemical tests used to study the phenotypic characteristics included; Raffinose, Lactose, Maltose, Mannose, D-Mannitol, Melibiose, Sucrose, Citrate, Urea, Indole, Catalase, Motility, Ampicillin Resistance, m-Inositol, oxidase, Nitrate, Trehalose, Dulcitol, Cellobiose, and Xylose. All isolates suggestive to aeromonads were stored in cryovials containing 20% glycerol Tryptic soy broth at -20 °C for further molecular typing.

2.5. Molecular genotyping and identification

The genomic DNA was isolated using the thermal extraction method as described by Carriero et al. (2016). Briefly, 1.0 mL of the Tryptic broth culture was pelleted, washed and resuspended by vortexing in Nuclease Free water (Sourced from Inqaba biotech, Hatfield, South Africa), placed in a water bath at 95 °C for 5 min and immediately transferred to ice for 5 min. This procedure was then repeated once more and the suspension centrifuged at 10 000 g for 10 min. The total genomic DNA was spectrophotometrically quantified using NanoDrop^{TM} Lite Spectrophotometer (Thermo Scientific, Waltham, U.S.A) and stored at -20 °C until further use.

PCR amplification and sequencing of RNA polymerase sigma factor gene (*rpoD*) (820 bp) was done according to Carriero et al. (2016) with some modifications as follows; The amplification used the following set of primer *rpoD*70F ACGACTGACCCGGTACGCATGTA (Yamamoto et al., 2000) and *rpoD*11R ATGCTCATGCGRCGGTTGAT (Martinez-Murcia et al., 2011). The reaction mix included 3.0 µL of 10–50 ng of genomic DNA, 12.5 µL of 2X OneTaq Quick Load Standard Buffer (New England BioLab, U.K, sourced from Inqaba biotech, Hatfield,South Africa), 0.5 µL of each primer (0.2 µM) and 8.5 µL Nuclease free water to give a final volume of 25 µL. The reaction mixture was subjected to a PCR regimen of

35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 minute preceded by an initial denaturation step at 95 °C for 3 min and followed by terminal extension at 72 °C for 3 min. The amplified product was gel electrophoresed on 1.5% agarose TBE gel and viewed in a gel documentation system (E-box CX5.TS Epi-illumination, Collegien, France).

The nucleotide sequences of PCR product was determined by Sanger method using ABI 3500 Genetic analyzer (Applied Biosystem ™, Foster City, California, U.S.A) according to manufacturer's instructions and protocol.

Sequence assembly was performed using BioEdit v7.0.5 software. The sequence comparison was performed by BLASTING the sequences in www.ncbi.nlm.nih.gov/ BLAST. Isolates were identified at the species level through alignment of the rpoD gene sequences from this study and type strains reference sequences from the gene bank using Clustal W followed by phylogenetic construction in MEGA X software (Kumar et al., 2018).

2.6. Determination of prevalence

The prevalence was derived based on infection status. Fish were regarded to have been infected when aeromonads were isolated from the Kidney, Spleen or Liver. Fish were grouped in terms of weight (g) into three categories based on FAO classification as 1–10 g (Fingerlings), 11–15 g (Sub-adults) and >26 g (Adults). The prevalence based on these groups was established.

2.7. Data analysis

Descriptive statistical analysis was conducted using Graph pad Prism 5 software. A chi-square of independent variables was carried out to determine the association between fish size groups developed based on fish weight and infection status using a Social Science Statistics program (https://www.socscistatistics.com/tests/chisquare2/Default2.aspx).

The sequence comparison was performed by BLASTING the sequences in www.ncbi.nlm.nih.gov/ BLAST.

3. Results

3.1. Pond water quality parameters of the surveyed ponds

Assessment of water quality parameters in fish ponds showed slightly variation in the four geographical regions. However, significant variation in temperature and turbidity water parameters were observed between the four regions ($p < 0.05$) (Table 1).

Table 1
Mean physical-chemical parameters in fish ponds of four study regions.

REGION	Temperature (°C)	DO (mg/L)	pH	Turbidity (NTU)	Conductivity (µS/cm)
Ruvuma	24.9 ± 0.5[a]	6.5 ± 0.5[a]	6.9 ± 0.1[a]	10.7 ± 1.1[b]	182.3 ± 49.8[a]
Mbeya	26.2 ± 0.4[c]	7.7 ± 0.5[a]	7.0 ± 0.3[a]	18.7 ± 0.2[b]	139.6 ± 32.7[a]
Iringa	25.1 ± 0.4[b]	7.4 ± 0.5[a]	6.7 ± 0.3[a]	33.0 ± 2.3[a]	143.4 ± 32.7[a]
Kilimanjaro	25.7 ± 0.2[b]	6.8 ± 0.5[a]	6.6 ± 0.1[a]	16.1 ± 1.0[b]	174.6 ± 39.3[a]
Preferred range	20 to 30	5 to 8	6 to 9	30 to 80	150 to 500
Stressful range	<12, >35	<5, >8	<4, >11	<12,>80	-

Note: The same letter in superscript within the column indicate no significant difference ($P \geq 0.05$).

3.2. Knowledge and practices

Thirty two (32) fish farmers were interviewed using the semi-structured questionnaire and 87.5% (28/32) were male. Their age ranged from 27 to 65 years with an average of 39.7 ± 1.5 years. Majority of these fish farmers (75%, 24/32) had primary and secondary (31.3%, 10/32) education. The remaining quarter had attended training up to college level.

These fish farmers had an experience in fish farming industry ranging from 1 to 11 years with an average of 4.6 ± 0.4 years. They own earthen ponds ranging from 90 to 864 m² in size with an average pond size of 454 m², with stocking density ranging from 150 to 10,000 fish per pond. Monoculture is the most practiced fish culture system by farmers (68.8%, 22/32), whereas 9.4% (3/32) fish farmers practice polyculture and 21.9% (7/32) of them practice both monoculture and polyculture.

3.3. Pond management practices at the study areas

Majority of the farmers (81.2%) reported to fertilize their ponds by using cow dung (69.2%) and poultry manure (11.5%). These farmers apply the fertilizing material either directly from the source (50%) or dry them first before use (50%). Out of those who fertilize their pond 50% spread the fertilizing material on the surface of the pond water. Sixty eight percent have reported to change water and clean their ponds different circumstances. It was observed that most of farmers stoked their ponds above the recommended stocking rate (Table 2).

3.4. Awareness and knowledge about pond management practices and fish health

Few farmers (28.1%, 9/32) mentioned to have previously acquired diseases in their farms between May and August (66.7%). Other time interval responded by these farmers were September to December (22.2%) and January and April (11. 1%). Out of 32 farmers, 18 (56.3%) experienced fish death in their farms prior to commencement of this study and Ruvuma was the leading region (Fig. 1). Haemorrhages, slow swimming, pope-eye, and reddening were the leading clinical signs mentioned and identified by farmers in all study areas (Fig. 2). According to the respondents, 47% could not manage to state the reasons for

Table 2
Pond management practices performed by fish farmers in the study areas.

| Practice	Category	Frequency	%
Stocking rate	Above recommended (2fish/m²)	24 (n = 32)	75
	Recommended (≤2fish/m²)	8 (n = 32)	25
Pond fertilization	Yes	26 (n = 32)	81.2
	No	6 (n = 32)	18.8
	Cow dung	18 (n = 26)	69.2
	Urea and DAP	1 (n = 26)	3.9
	Poultry manure	3 (n = 26)	11.5
	cow dung and poultry manure	4 (n = 26)	15.4
Fertilizer application	Reduce pond water and apply	13 (n = 26)	50.0
	Spread over the surface	13 (n = 26)	50.0
	Direct from the source	4 (n = 26)	50.0
	Dry	4 (n = 26)	50.0
Change water and cleaning ponds	Yes	22 (n = 32)	68.8
	No	10 (n = 32)	31.2
Circumstances of changing and cleaning	Long stay	7 (n = 26)	26.9
	Smelling	9 (n = 26)	34.6
	Too greenish (dark green)	9 (n = 26)	34.6
	Experience oxygen deficiency	8 (n = 26)	30.8
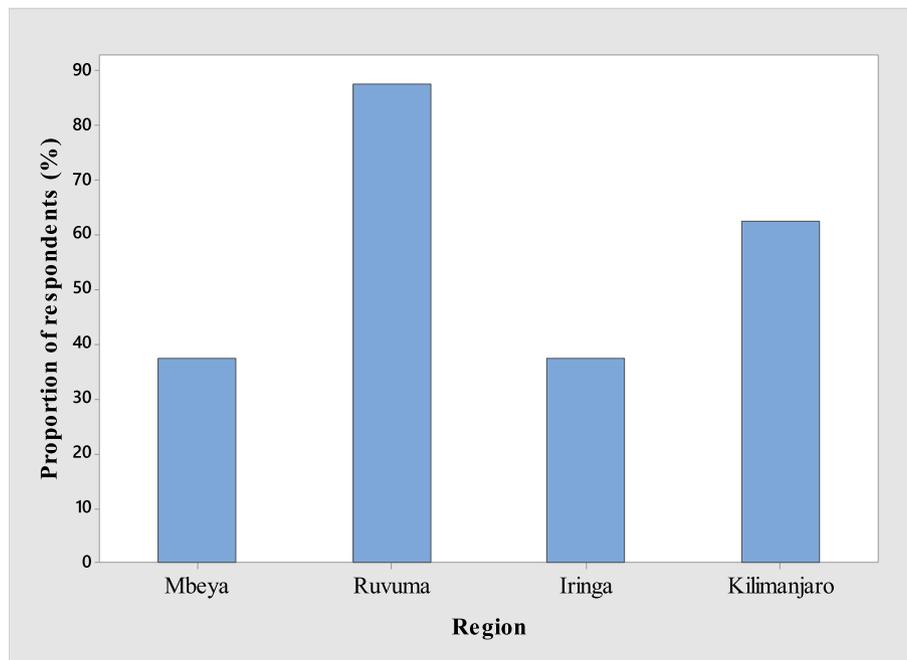
Fig. 1. Proportion of respondents who experienced mortality in their fish farms in the four regions.
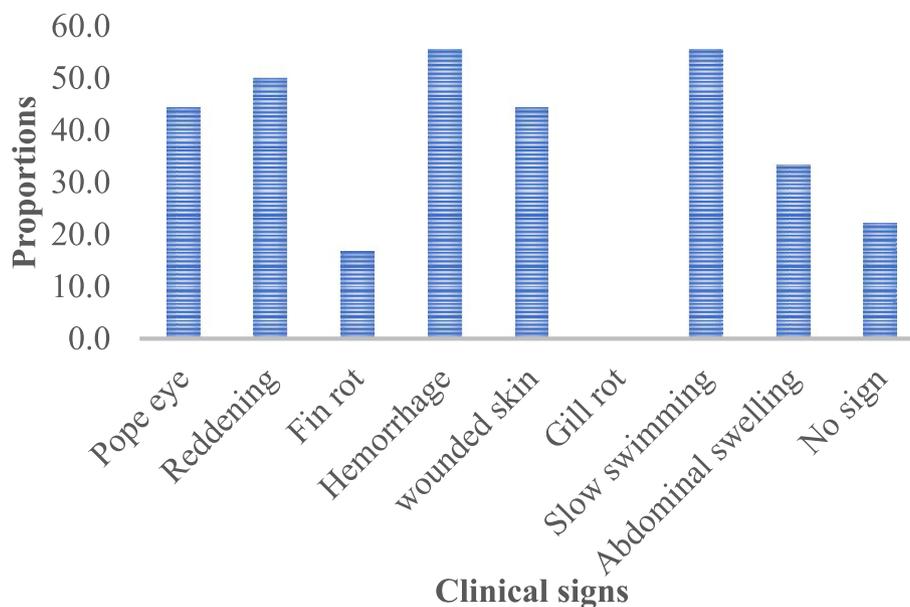

Fig. 2. Proportion of fish farmers who reported to have seen the clinical signs in their farms.

mortality whereas, 18.8% mentioned low oxygen concentration, 12.5% bird injury, 6% due to transportation and 9.4% reported due to inadequate water and feeds supply.

Despite the fact farmers reported infections and mortalities in fish, majority (84.4%, 27/32) of respondents confessed ill-informed about

control methods. However, small proportion uses other methods including antibiotics (9.4 %), herbs (6.3%) and separate infected fish (6.3%).

3.5. Morphometric parameters of fish

Weight and length parameters of fish sampled displayed variability with weight ranged from 10-220g and length ranged from 2-15cm. Fish were grouped in categories of "fingerlings", "sub-adults" and "adults" as adopted from FAO (Table 3).
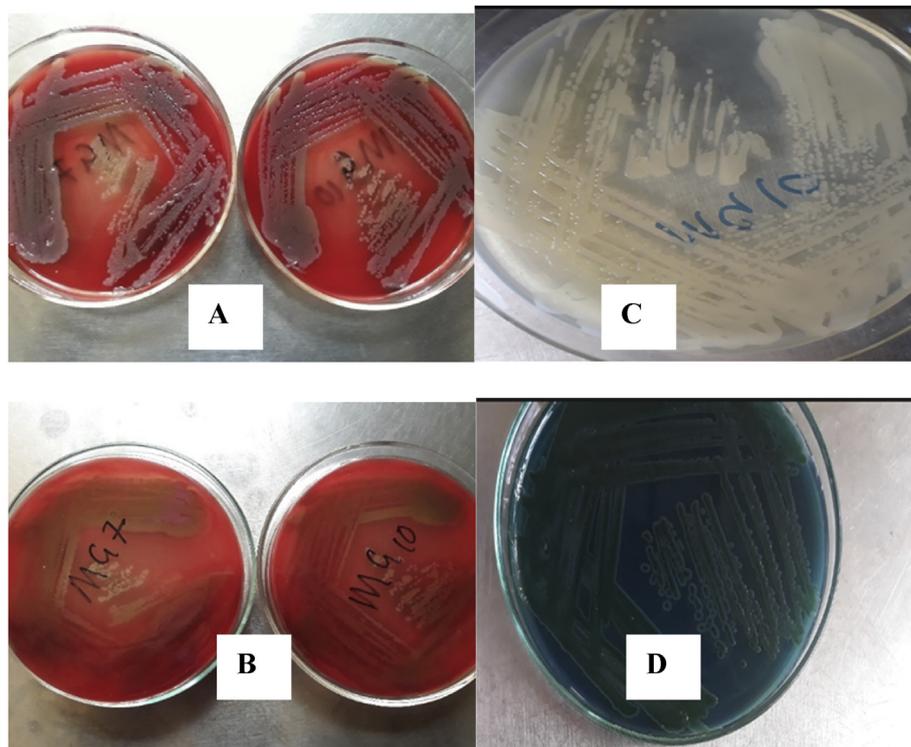
3.6. Culturing, isolation and conventional identification

The bacterial colonies assumed to be aeromonads had medium,

Table 3
Fish groups based on weight and length.

Weight(g)	Category (size)	No of fish	Percentage (%)
1–10	Fingerlings	379	46.5
10–25	Sub adults	231	28.3
>25	Adults	206	25.2
Total		**816**	**100**

Fig. 3. Colonial morphologies of aeromonads in different media. A and B are Blood Agar with B showing β-hemolytic characteristics, C is Tryptic Soy Agar (TSA)and D is *Aeromonas* Isolation Agar (M884).

greyish in colour with β-haemolytic colonies in Blood agar; relatively small and pale colonies (non-lactose fermenter) on MacConkey agar; smooth, shining, creamy colonies on Tryptic soy agar (TSA) and dark green, opaque with dark Centre colonies on *Aeromonas* isolation medium (M884) (Fig. 3). Upon staining, bacteria ware seen to be gram negative, rod shaped, in singles and few in pairs.

All suspected aeromonads colonies when subjected to different biochemical tests gave reactions which are characteristic to the genus. The bacteria produced positive reaction to catalase, Oxidase, D-glucose, Citrate, Arabinose and Mannose (Table 4).

Table 4
Biochemical sugar profile of the *Aeromonas* species.

Biochemical test/ Bacteria	*Aeromonas spp*
Catalase	+
Oxidase	+
m-Inositol	-
Raffinose	-
Lactose	-
Xylose	-
Cellobiose	+/-
Maltose	+
Mannose	+
D-Mannitol	+
Melibiose	-
Sucrose	+
Citrate	+
Urea	-
Indole	+
Motility	+/-
Ampicillin^R	+
Nitrate,	+
D-sorbitol	-
Trehalose	+
Dulcitol	-
Salicin	+/-

3.7. Prevalence of aeromonads infection in freshwater farmed tilapia

Bacteriological testing of 816 apparently healthy tilapia fish were done from 32 fresh water ponds in Songea Municipality (Ruvuma region), Mbarali District (Mbeya Region), Mafinga Township (Iringa Region) and Rombo District (Kilimanjaro Region). Out of the 816 fish samples, 250 (30.6%) were identified to have been naturally infected with aeromonas.

A conventional PCR for identification of aeromonads was done by amplifying the RNA polymerase gene sigma 70 domain (*rpoD* gene). A total of 201 (80.4%) out of 250 isolates that were conventionally identified using biochemical sugars confirmed to be aeromonads by amplification of 820 bp *rpoD* gene (Fig. 4), making the overall molecular prevalence of 24.6% (201, n = 816), higher in Iringa and Mbeya and least in Ruvuma (Fig. 5A). aeromonas spp ware highly isolated in gills (40%, 135/339) and less isolated in Kidney (17%, 57/339) (Fig. 5B).
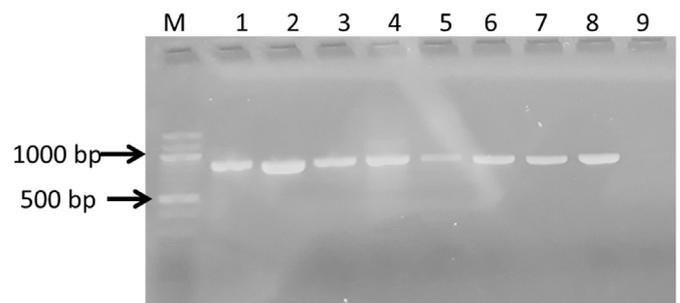
Fig. 4. PCR amplification of *rpoD* gene (820 bp) from aeromonads isolates in this study. Lane 1–7 are representative bacterial isolates from fish collected at Ruvuma, Mbeya Iringa, and Kilimanjaro, lane 8 is the positive control, Lane 9 is a negative control and Lane M, is DNA size marker (100 bp DNA ladder).
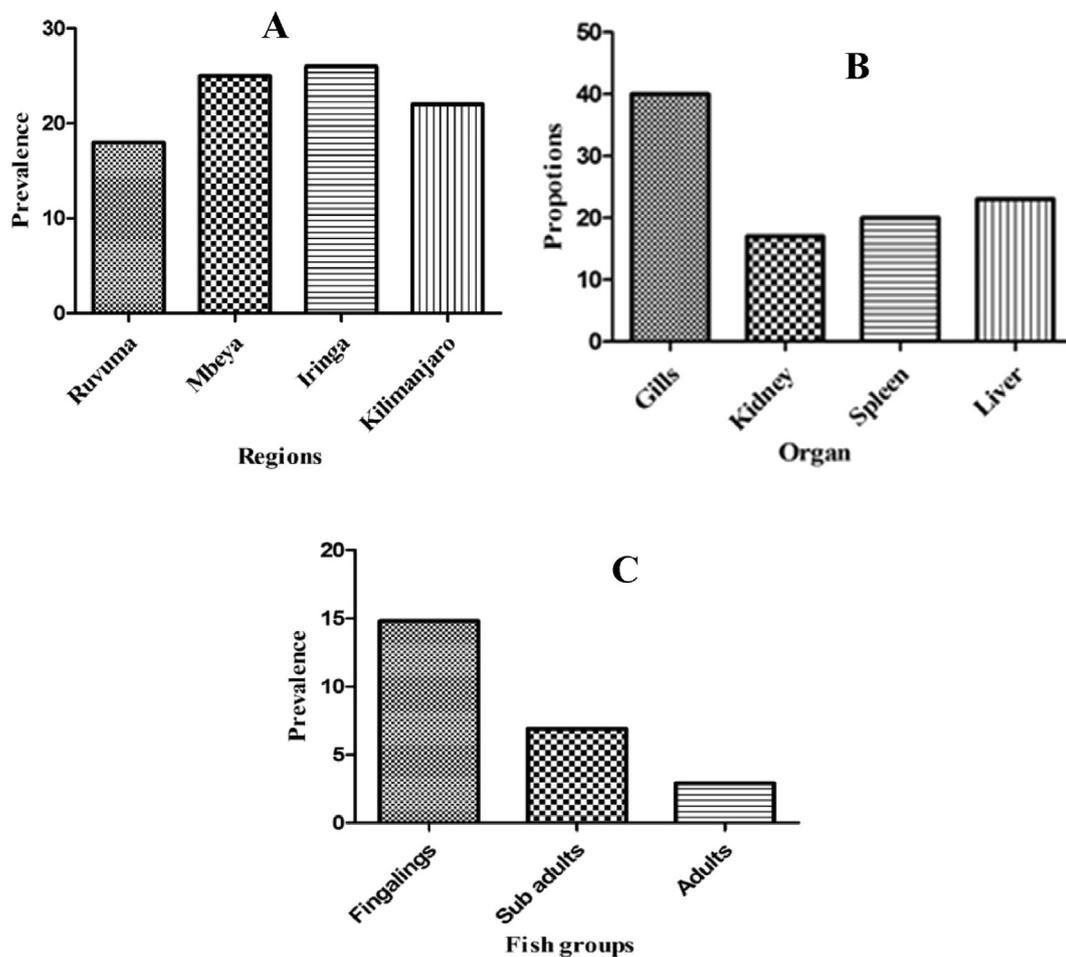
Fig. 5. Prevalence of aeromonas spp based on geographical regions (A), fish internal organs (B) and fish groups (C).

When the relationship between fish groups (fingerlings, sub adults and adults) and infection of *Aeromonas* spp was tested using χ2 test, it was observed that being infected or not infected is dependent on fish groups. There was a significant association between infection status with and fish size group ($p < 0.05$). The prevalence based on fish size groups was high in fingerlings and low in adults (Fig. 5C).

3.8. Phylogenetic analysis

The *rpoD* gene from the isolates displayed sequence homologue of 97–99 % with several *rpoD* sequences of aeromonas spp from the Gene Bank. The phylogeny grouped the isolates from this study into the clusters of *A. hydrophila* and *A. veronii* in relation to reference sequences from the gene bank (Fig. 6).

4. Discussion

Aeromonads disease outbreak has become an important limiting factor to sustainable fish farming worldwide (Ibrahem et al., 2008). These diseases are accelerated by poor physical-chemical pond water parameters as well as poor pond management practices. In this study, there were no significant variations of most of the assessed physical-chemical water parameters in fishponds between all four regions, and that all the parameters were within the desirable range. However, the study reports the occurrence and identification of aeromonads for the first time in farmed tilapia in Southern highland and Northern Tanzania to an overall prevalence of 24.6% with no disease outbreak reported in all farms at the point in time. The prevalence is close to that reported by Deen et al. (2014) in Egypt. As it was explained by

Lio-Po et al. (2001) that disease occurrence in fish farms is a function of the pathogen, host and the environment, the absence of stressful environment could be the reason for the absence of the disease at the time, when this study was carried out. The two *Aeromonas* species identified from farmed tilapia in this study (*A. hydrophila* and A. veronii) are the known important etiological agents of diseases outbreaks in the freshwater tilapia farms and detection of these bacterial species in the kidney, the liver and spleen of apparently healthy fish are not startling because they are ubiquitous of the aquatic environment. The high proportion of infection in gills in comparison to other organs is due to the exposed nature of the organ to microbiota (Mwega et al., 2017). Identification of members of the family *Aeromonadaceae* in apparently healthy fish collaborates with a previous report by Omeje and Chukwu (2014), they found these bacteria in both apparently healthy and diseased fish. Even though they have been detected in apparently healthy fish, they remain to be a potential risk to disease outbreaks when ponds management practices are totally poor.

It is well-known that aeromonads affects all age and size of fish (Camus et al., 1998), however, our findings revealed that fingerlings are highly infected in agreement with what has been explained by Camus et al. (1998). The outbreaks of aeromonads diseases are seasonal based and highly experienced in summer (Ibrahem et al., 2008), similar findings have been observed in this study where fish farmers reported previous outbreaks to have been occurred between May and August. The findings from interviewed fish farmers on knowledge of pond management practices and fish health management revealed that farmers have inadequacy knowledge and are not aware to some pond management practices (Chenyambuga et al., 2014). High stocking rate and poor ways of fertilizing pond are some of them. Assessment of knowledge and
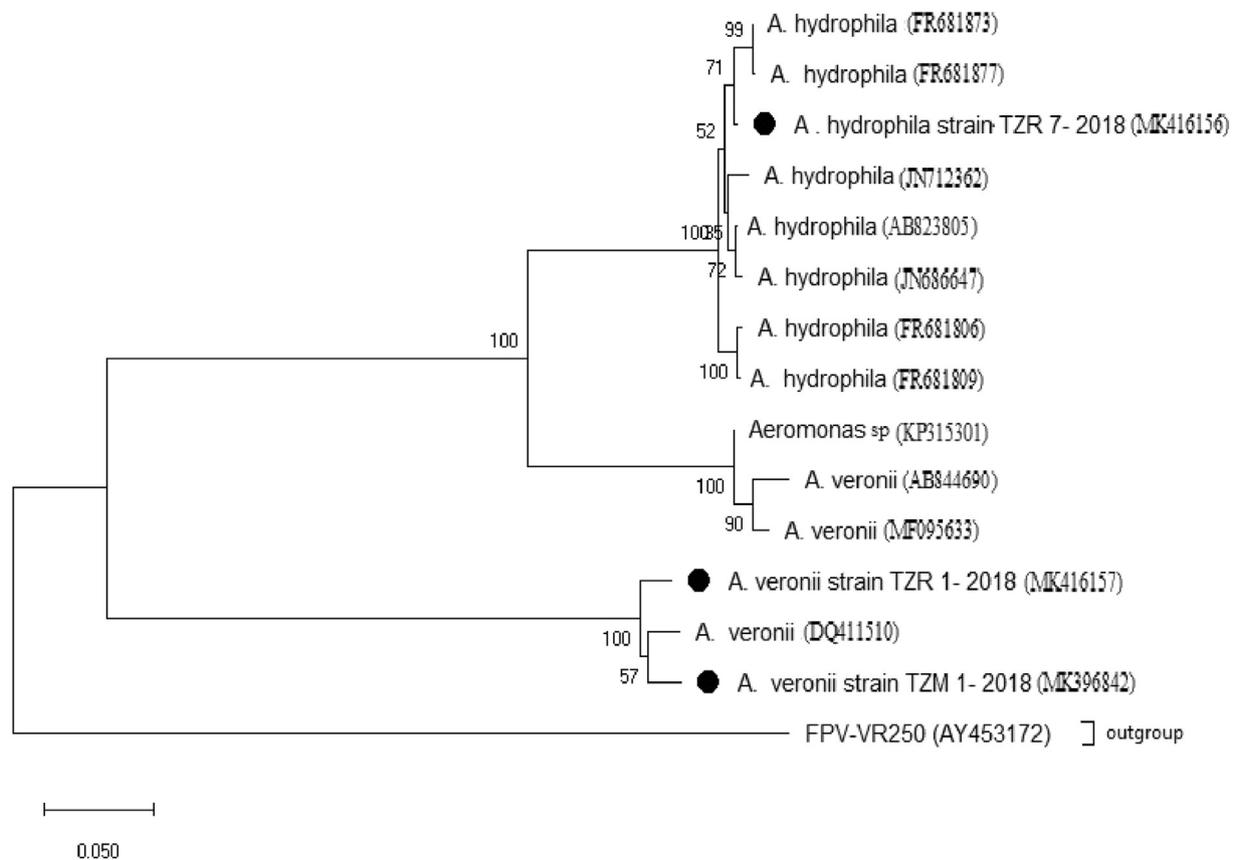
Fig. 6. Phylogenetic tree of representative eromonads isolates from this study (black circle) and closely related taxa from the gene bank. The tree was generated using Neighbor-Joining method (p-distance model), bootstrap values expressed as percentages of 1,000 replication. *Fowlpox virus* (FPV-VR250) saved as an out-group.

awareness on fish health management identified that the majority of farmers lack knowledge on disease diagnosis based on clinical signs; however, farmers from Ruvuma region showed to be familiar with the most common clinical signs. This is because it is this region where farmers reported to have experienced fish mortalities in their farms. One of the most common methods for managing diseases on fish farms is the application of antibiotics (Chitmanat et al., 2016). It was observed from this study that the majority of fish farmers didn't know any method of managing, and controlling fish diseases while few of them mentioning antibiotics as one of the methods. Biosecurity measures, good pond management practices coupled with other fish disease control methods such as disease treatment and vaccination are of paramount importance towards sustainable aquaculture. While these are greatly implemented in developed countries, in developing countries like Tanzania efforts must be made to train farmers who majority of them are sole peasant farmers with primary education on biosecurity measures and pond management practices and on potential risks of bacterial diseases if the same are not employed.

4.1. Conclusion

The infection rate of aeromonads in apparently healthy tilapia coupled with limited knowledge and awareness on proper pond management practices and fish health management by fish farmers in the study area poses the risk of disease outbreaks in their farms. Therefore, it is recommended that the farmers should be trained on basic pond and fish health management and control strategies while striving for best control method to complement such as the use of simple, autogenous vaccines based on accurate typing and evidence-based definition of the epidemiological unit because it is the most viable approach both

regarding efficacy and economic feasibility especially in Low and Middle-Income Countries (LMIC).

Declarations

Author contribution statement

Alexanda Mzula: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Philemon N. Wambura, Robinson H. Mdegela: Conceived and designed the experiments.

Gabriel M. Shirima: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

References

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

Abdelhamed, H., Ibrahim, I, Baumgartner, W., Lawrence, M.L., Karsi, A., 2017. Characterization of histopathological and ultrastructural changes in channel catfish experimentally infected with virulent Aeromonas hydrophila. Front. Microbiol. 8 (AUG), 1–15.

Aboyadak, I.M., Ali, N.G.M., Goda, A.M.A.S., Aboelgalagel, W.H. , Salam, A., 2015. Molecular detection of Aeromonas hydrophila as the main cause of outbreak in Tilapia farms in Egypt. J. Aquacult. Mar. Biol. 2 (6), 2–5.

Baumgartner, W.A., Ford, L., Hanson, L., 2017. Lesions caused by virulent Aeromonas hydrophila in farmed catfish (Ictalurus punctatus and I. punctatus × I. furcatus) in Mississippi. J. Vet. Diagn. Investig. 29 (5), 747–751.

Beaz-Hidalgo, R., Alperi, A., Buján, N., Romalde, J.L., Figueras, M.J., 2010. Comparison of phenotypical and genetic identification of Aeromonas strains isolated from diseased fish. Syst. Appl. Microbiol. 33 (3), 149–153.

Bebak, J., Wagner, B., Burnes, B., Hanson, T., 2015. Farm size, seining practices, and salt use: risk factors for Aeromonas hydrophila outbreaks in farm-raised catfish, Alabama, USA. Prev. Vet. Med. 118 (1), 161–168.

Camus, A.C., Durborow, R.M., Hemstreet, W.G., Thune, R.L., Hawke, J.P., 1998. Aeromonas bacterial infections - motile Aeromonad septicemia. South. Reg. Aquacult. Cent. 478 (1), 1–4.

Carriero, M.M., Mendes Maia, A.A., Moro Sousa, R.L., Henrique-Silva, F., 2016. Characterization of a new strain of Aeromonas dhakensis isolated from diseased pacu fish (Piaractus mesopotamicus) in Brazil. J. Fish Dis. 39 (11), 1285–1295.

Chaix, G., Roger, F., Berthe, T., Lamy, B., Jumas-Bilak, E., Lafite, R., Petit, F., 2017. Distinct Aeromonas populations in water column and associated with copepods from estuarine environment (Seine, France). Front. Microbiol. 8 (JUL), 1–13.

Chandran, M.R., Aruna, B.V., Logambal, S.M., Michael, R.D., 2002. Immunisation of Indian major carps against Aeromonas hydrophila by intraperitoneal injection. Fish Shellfish Immunol. 13, 1–9.

Chenyambuga, S.W., Mwandya, A.Lamtane, HA., Madalla, N., 2014. Productivity and marketing of Nile tilapia (Oreochromis niloticus) cultured in ponds of small-scale farmers in Mvomero and Mbarali districts, Tanzania. Livest. Res. Rural Dev. 26 (3). Retrieved from. http://www.lrrd.org/lrrd26/3/chen26043.htm.

Chitmanat, C., Lebel, P., Whangchai, N., Lebel, L., 2016. Tilapia diseases and management in river-based cage aquaculture in northern Thailand. J. Appl. Aquac. 28 (1), 9–16.

De Jagoda, S.S.S., Wijewardana, T.G., Arulkanthan, A., Igarashi, Y., Tan, E., Kinoshita, S., et al., 2014. Characterization and antimicrobial susceptibility of motile aeromonads isolated from freshwater ornamental fish showing signs of septicaemia. Dis. Aquat. Org. 109 (2), 127–137.

Deen, A. E. N. El, Dorgham, M., Hassan, A.H.M., Hakim, A.S., 2014. Studies on Aeromonas hydrophila in cultured Oreochromis niloticus at kafr El sheikh governorate , Egypt with reference to histopathological alterations in some vital organs. World J. Fish Mar. Sci. 6 (3), 233–240.

Erdem, B., Karİptaş, E., Çİl, E., Işik, K., 2011. Biochemical identifi cation and numerical taxonomy of Aeromonas spp . isolated from food samples in Turkey. Turkish J. Biol. 35, 463–472.

FAO, 2018. Manual on Polyculture and Integrated Fish Farming in Bangladesh. Retrieved November 10, 2018, from Hazard Management website: http://www.fao.org/do crep/field/003/ac375e/AC375E05.htm.

Harikrishnan, R., Balasundaram, C., 2005. Modern trends in Aeromonas hydrophila disease management with fish. Rev. Fish. Sci. 13 (4), 281–320.

Ibrahem, M.D., Mostafa, M.M., Arab, R.M.H., Rezk, M., 2008. Prevalence of Aeromonas hydrophila infection in wild and cultured Tilapia Nilotica (O. Niloticus). In: 8th International Symposium on Tilapia in Aquaculture, pp. 1257–1271.

Igbinosa, I.H., Igumbor, E.U., Aghdasi, F., Tom, M., Okoh, A.I., 2012. Emerging Aeromonas species infections and their significance in public health. Sci. World J. 2012, 1–13.

Jacobs, L., Chenia, H.Y., 2007. Characterization of integrons and tetracycline resistance determinants in Aeromonas spp. isolated from South African aquaculture systems. Int. J. Food Microbiol. 114 (3), 295–306.

Janda, J.M., Abbott, S.L., 2010. The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev. 23 (1), 35–73.

Joseph, A.V., Sasidharan, R.S., Nair, H.P., Bhat, S.G., 2013. Occurrence of potential pathogenic Aeromonas species in tropical seafood, Aquafarms and mangroves off Cochin coast in South India. Vet. World 6 (6), 300–306.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular Evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549.

Lio-Po, G.D., Lavilla, C.R., Cruz-lacierda, E.R., 2001. Health management in aquaculture. In: C.-L., E.R., Lio-Po, Gilda D., Lavilla, Celia R. (Eds.), *Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center*. TIGBAUAN, ILOILO. Aquaculture Department Southeast Asian Fisheries Development Center Tigbauan, Iloilo, Philippines ALL.

Martinez-Murcia, A.J., Monera, A., Saavedra, M.J., Oncina, R., Lopez-Alvarez, M., Lara, E., Figueras, M.J., 2011. Multilocus phylogenetic analysis of the genus *Aeromonas*. Syst. Appl. Microbiol. 34 (3), 189–199.

MLFD, 2013. Fisheries Sector Development Programme: Fisheries Sector Development Programme. In: Fisheries Sector Development Programme.

Mwega, E., Tuntufye, H., Colquhoun, D., Mutoloki, S., Mdegela, R., Evensen, Ø., Wasteson, Y., 2017. Isolation and Characterization of Flavobacteriaceae from Nile Tilapia in Tanzania. Retrieved July 27, 2018, from World Aquaculture Society Meetings website. https://www.was.org/meetings/ShowAbstract.aspx?Id=47279.

Najiah, M., Laith, A.R., 2014. Aeromonas hydrophila: antimicrobial susceptibility and histopathology of isolates from diseased catfish, Clarias gariepinus (Burchell). J. Aquac. Res. Dev. 05 (02), 1–7.

Omeje, V.O., Chukwu, C., 2014. Prevalence of Aeromonas hydrophila isolates in cultured and feral Clarias gariepinus of the Kainji lake area, Nigeria. Niger. Vet. J. 35 (1), 948–955.

Ossiander, F.J., Wedemeyer, G., 1973. Computer program for sample sizes required to determine disease incidence in fish populations. J. Fish, Res. Board Can. 30, 1383–1384.

Puthucheary, S.D., Puah, S.M., Chua, K.H., 2012. Molecular characterization of clinical isolates of Aeromonas species from Malaysia. PLoS One 7 (2), 1–7.

Shayo, S.D., Mwita, C.J., Hosea, K., 2012. Ulcerative Aeromonas infections in Tilapia (Cichlidae: Tilapiini) from Mtera hydropower dam, Tanzania Shayo. Open Access Sci. Rep. 1 (2), 1–7.

Yamamoto, S., Kasai, H., Arnold, D.L., Jackson, R.W., Vivian, A., Harayama, S., 2000. Phylogeny of the genus Pseudomonas: intrageneric structure reconstructed from the nucleotide sequences of gyrB and rpoD genes. Microbiology 146 (10), 2385–2394.

Zhou, Y., Yu, L., Nan, Z., Zhang, P., Kan, B., Yan, D., Su, J., 2019. Taxonomy, virulence genes and antimicrobial resistance of Aeromonas isolated from extra-intestinal and intestinal infections. BMC Infect. Dis. 19 (1), 1–9.