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Original research

Prevalence of *Aeromonas* species in *Clarias gariepinus* and water in different culture facilities from fish farms in Kwara State, Nigeria

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ABSTRACT

Aeromonas species are associated with diseases on fish farms leading to mortality and economic losses. In order to determine the prevalence and diversity of *Aeromonas* species from water and *Clarias gariepinus* cultured in the different holding facilities; five hundred and seventy *six Clarias gariepinus* fish and one hundred and eight water samples were randomly sampled from fish farms in Kwara State. The samples were clinically and bacteriologically examined and confirmed using Oxiod rapid microbat identification test kits for Gram-negative bacteria, Microbact 24E (MB24E). The overall prevalence of *Aeromonas* species from water from the different cultured facilities revealed water sampled from earthen ponds had the highest prevalence of 66.67 % followed by concrete tanks with a prevalence of 41.66 % and the least prevalence of 30.56 % was recorded for water in plastic tanks. A similar pattern was recorded in *Clarias gariepinus* from the different holding facilities showing that earthen ponds had the highest prevalence of 40.10 % when compared with concrete tanks 28.65%, and to plastic tanks 20.83 %. Four varying diversities of *Aeromonas* species comprising of *Aeromonas hydrophila, Aeromonas caviae, Aeromonas veronii biovar sobria*, and *Aeromonas veronii biovar veronii were* isolated from both water and fish from the different holding facilities. From this study, cultured *Clarias gariepinus* were susceptible to *Aeromonas* species irrespective of the culture facilities. Therefore, there is need for proper management practices and adherence to biosecurity measures to prevent the outbreak and spread of diseases on the farms.

KeyWords: Aquaculture; culture facility; fish disease; prevalence

INTRODUCTION

Fish farming has developed rapidly due to the global decline in wild fish stocks, mostly caused by overfishing and climate change (Adah *et al*., 2021; Naylor *et al*., 2021). Fish farming has established itself as a significant component of the Nigerian economy and a viable strategy for increasing global fish production (Wuyep & Rampedi, 2018; Adah *et al.*, 2022). Fish is an excellent source of affordable, high-quality protein, particularly in developing nations; because of the rising need for animal protein, which has led to the intensification and rearing of fish in various culture facilities (Obiero, *et al*., 2019; Kaleem & Sabi, 2020).

Fish farming will unavoidably face substantial challenges as the need for inexpensive protein sources rises, driving fish production to become more intensive and, in turn, increasing the frequency and severity of disease outbreaks (Adah *et al*., 2021). However, the challenges due to the presence of pathogenic organisms especially bacteria, have limited its effective production and availability of a readily cheap source of protein (Afolabi, *et al*., 2020). Disease at any stage of the fish culture is a significant setback that usually leads to great consequences on the economic viability of fish farms and on the yield of protein for human consumption (Tavares-Dias & Martins, 2017; Elsheshtawy, *et al*., 2019).

Gram-negative bacteria like *Aeromonas* species are the most common causes of morbidity and mortality leading to great economic losses in the aquaculture industry (Mailafia *et al*., 2021; Pepi & Focardi, 2021). *Aeromonas* species are ubiquitous bacteria which are commonly isolated from freshwater ponds and different holding facilities (Adah *et al.*, 2021). They inhabit the gastrointestinal tracts of fish and have been considered important pathogens of aquatic animals, causing significant economic losses in the aquaculture industry worldwide (Pessoa, *et al*., 2019; Silva *et al*., 2019) and can be transmitted to humans (Fowoyo & Achimugu, 2019). Diseases caused by *Aeromonas* species

range from acute rapid fatal septicaemia to latent infection and have been referred to as haemorrhagic septicaemia, brown patch disease of the skin, and fin rot (Chen *et al*., 2019; Hossain *et al*., 2019; Raj *et al*., 2019). Consequently, it is important to examine and detect the occurrence, trends and distribution of *Aeromonas* species from water and fish for human consumption from different parts of the world. This study aimed to isolate *Aeromonas* species from water and *Clarias gariepinus* (*C. gariepinus*) cultured in the different holding facilities in Kwara State, North Central Region of Nigeria.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Kwara State, North Central Nigeria. Kwara State connects the northern and southern areas of Nigeria. The State is located in Nigeria's North Central geopolitical zone and has geographic coordinates of longitude 5° 00'E and latitude 8° 30'N with a total area of 13,947.27 square miles (35,705 km^2). The State is bordered to the east by Kogi State and bordered to the south by Oyo, Osun, and Ekiti States. Kwara State is bordered to the north by Niger State, and the Republic of Benin to the west (Adam *et al*., 2022). A cross-sectional study was carried out using a multistage random sampling of 36 operational *C. gariepinus* grow-out farms reared in the different holding facilities comprising 12 each of earthen ponds, plastic and concrete tanks respectively. Fish and water samples were sampled from the fish farms based on the availability and willingness of the farmers to participate in the study.

BACTERIAL ISOLATION AND IDENTIFICATION FROM WATER

A total of one hundred and eight water samples (200 mL) each from the different culture facilities in triplicates were taken in sterile labelled containers for bacteriological analysis. The bottles were placed in an ice box to keep the temperature below 10˚C and were analyzed within 4 hours post-collection. The membrane filtration technique was used based on recommendations for the bacteriological examination of water (Hay *et al*., 1994). Water samples were filtered through a sterile 0.45 µm pore-size membrane filter of 47 mm diameter and the filter was placed into a kryo bottle containing alkaline peptone water.

FISH SAMPLE COLLECTION

Five hundred and seventy six *C. gariepinus* were sampled from thirty-six active fish farms. *C. gariepinus* in various stages of development (fingerlings, growers, adults, and brood stocks) were stocked on the fish farms. Diseased and healthy fish, were randomly collected live from the farms and transported in plastic receptacles containing water from the culture facility to the Fish Clinic of the Veterinary Teaching Hospital University of Ilorin, Kwara State for further diagnostic procedures and examination.

CLINICAL AND POSTMORTEM EXAMINATION OF THE FISH SAMPLES

The sampling technique was carried out in accordance with the standards for fish disease diagnosis and aquatic animal health monitoring (Austin, 2019; Tavornapanich *et al.*, 2020). All the samples of the fish obtained were evaluated clinically and a postmortem examination was carried out as described by Austin (2019). Samples from the skin, gills, gastrointestinal tracts, kidney, liver and spleen were collected aseptically as described by Austin (2019).

AEROMONAS ISOLATION AND IDENTIFICATION FROM FISH

Based on the observations on the fish farms, bacteria were isolated from both diseased and apparently healthy fish. Portions of the skin, gills, liver, heart, and spleen were weighed aseptically and put in separate labelled kryo bottles containing 20 mL of alkaline peptone water (Oxoid®, UK) as the pre-enrichment broth and incubated at 37 °C for 24 h. Growth in the selective pre-enrichment cultures were transferred with a sterile loop and inoculated onto the selective *Aeromonas* agar (Oxoid®, UK) supplemented with ampicillin (10mg/L) (Oxoid®, UK) and incubated at 37 ˚ C for 24 h after which dark green, opaque with dark centres colonies which were presumptive for *Aeromonas* species were streaked on MacConkeys agar (MCA) plates and incubated at 37˚ C for 24 h for lactose fermentation activity (Austin, 2014). After this, Gram reaction, oxidase and catalase tests were also determined for the presumptive *Aeromonas* species using standard methods (Austin & Austin, 2016; Ganesan *et al*., 2023).

BIOCHEMICAL IDENTIFICATION OF *AEROMONAS* SPECIES

All suspected colonies were collected and examined for phenotypic and biochemical characteristics. The biochemical characterization of the *Aeromonas* isolates was assessed by conventional biochemical tests such as citrate test, hydrogen sulphide, indole test, methyl red test, motility test, sugar (glucose, inositol, and mannitol) urease test, Voges Proskauer test. (Adah *et al.*, 2021) Additionally, the evaluation of Gram staining, colonial features, motility, oxidase, and catalase activities, as well as growth on various agar and in response to various temperature conditions was determined according to Pitt & Barer, (2012) and Tavornapanich *et al.*, (2020).

Furthermore, the *Aeromonas* species were confirmed using Oxoid rapid microbat identification test kits for Gram-negative bacteria, Microbact 24E (MB24E) (Oxoid® Ltd, Basingstoke, England. United Kingdom). It consisted of dehydrated substrates for 24 different biochemical placed in

the wells of a microtitre tray. Sterile normal saline was prepared and 5 ml dispensed into each test tube. Using a sterile loop, 1-3 colonies of the culture were picked from nutrient agar (Oxoid® Ltd, UK), emulsified in 5 ml sterile saline, mixed thoroughly and incubated at 37 ± 2 ˚C for 4 hours. The Microbact 24E plate was placed in a holding tray and the wells were exposed by cutting the end tag of the sealing strip and slowly peeling backward and using a sterile Pasteur pipette, four drops of the bacterial suspension were added to each well and the substrates were over laid with mineral oil in the appropriate wells i.e 1, 2, 3, 20 and 24. The plates were incubated at 37 ± 2 ˚C for 48 hours. After 48 hours, the adhesive seal was peeled and Nitrate, Kovacs, Voges - Proskauer reaction (VP) and tryptophan deaminase (TDA) reagents were added to wells 7, 8, 10 and 12 respectively and colour changes of the different tests were observed and recorded in the booklet which was transcribed into an 8 digit code and organisms were interpreted as stipulated by the manufacturers using the microbact software version Microbact TM 200 identification package V2.03 (Windows TM) which is a computer identification software which immediately gave the probable identity of the *Aeromonas* species (Mailafia *et al*., 2021).

STATISTICAL ANALYSIS

Data were summarized using Microsoft Excel 2013. Data of the isolation of the *Aeromonas* species were expressed using simple percentage and the prevalence of the different *Aeromonas* species from the different holding facilities were compared using the chi-square. Values of $P < 0.05$ were considered significant.

RESULTS

The overall prevalence of *Aeromonas* species from *C. gariepinus* from the different holding facilities showed that earthen ponds had the highest prevalence of 77 (40.10 %), when compared with concrete tanks 55 (28.65%), and to plastic tanks 40 (20.84 %). The prevalence of *Aeromonas* species isolated from water from the different culture facilities revealed that water sampled from earthen ponds had the highest prevalence of 24 (66.67%) followed by concrete tanks 15 (41.67%) and the least prevalence of 11 (30.56%) recorded for water in plastic tanks (Figure I). There was significant difference in the prevalence of *Aeromonas* species from both water and fish ($P < 0.05$). A total of 50 and 172 *Aeromonas* species with a prevalence of 46.29 % and 29.86 % from water and fish respectively were isolated from the different earthen ponds, plastic and concrete tanks with four different phenospecies (Figure II). Four different phenospecies of *Aeromonas* were isolated from the water with the highest prevalence of 29 (26.85 %) for *Aeromonas hydrophila* followed by 12 (11.11%) for *Aeromonas caviae*, then 7 (6.48 %) *Aeromonas veronii* biovar *veronii*, and the least was observed for *Aeromonas veronii sobria* 2 (1.85%) from the study area. (Figure II). The prevalence of the different species of *Aeromonas* from fish revealed that the highest prevalence was of 115 (19.96 %) for *A. hydrophila* followed by prevalence of 30 (5.2%) for *A. caviae*, then 15 (2.60%) *A. veronii sobria*, and the least was observed for *A. veronii bv* veronii 12 (2.08%) from the study area (Figure II).

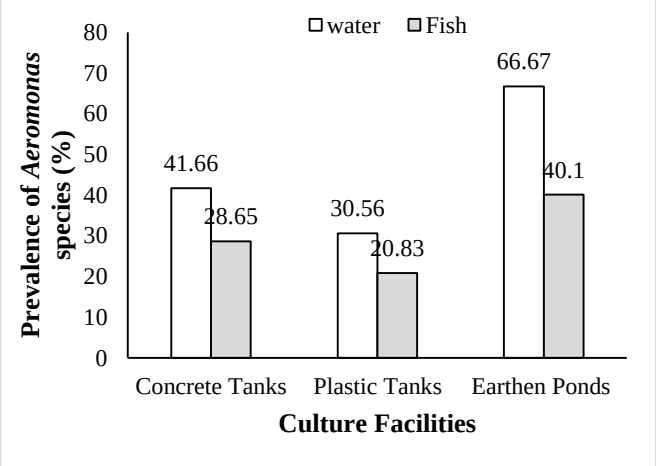

Figure I: Overall Prevalence (%) of *Aeromonas species* from water (N=36) and fish (N =192) each from the different culture facilities. N= number of samples

PREVALENCE OF THE DIFFERENT *AEROMONAS*

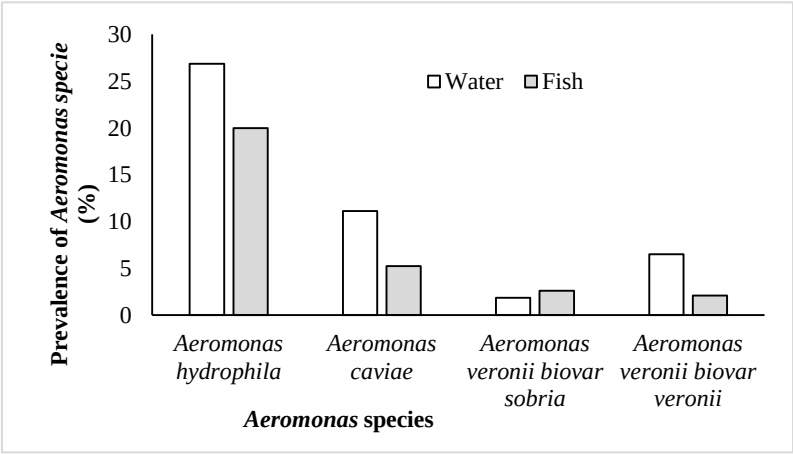

Figure II: Overall Prevalence (%) of phenospecies of *Aeromonas* from water (N=108) and fish (N=576). N; number of samples

SPECIES OBTAINED FROM WATER IN THE DIFFERENT HOLDING FACILITIES

The prevalence of the different species of *Aeromonas* isolated from the water samples revealed that *A. hydrophila* was highest in all the holding facilities; prevalence of 15 (41.67 %) was observed in earthen ponds followed by the prevalence in concrete tanks 8 (22.22 %) and the least prevalence of 6 (16.66%) recorded in plastic tanks. The prevalence of *A. caviae* was highest in concrete tanks 5 (13.89 %), followed by the prevalence of 4 (11.10 %) obtained in earthen ponds and the least prevalence of 3 (8. 33 %) was recorded in plastic tanks. The prevalence of *A. veronii biovar sobria* was highest in earthen ponds 2 (5.58 %), however, 0 % prevalence was recorded in both concrete and plastic tanks respectively. The highest prevalence of 3 (8.33 %) for *A. veronii biovar veronii* was recorded in earthen ponds. The prevalence of 2 (5.56 %) for *A. veronii biovar veronii* was similar in both concrete and plastic tanks respectively. The prevalence of *A. hydrophila* differed significantly with (P <0.04) between the different holding facilities (Table I). There was a significant difference in the overall prevalence of *Aeromonas* species obtained from all the holding facilities, however only the prevalence of *Aeromonas* species obtained within the different concrete tanks and earthen ponds differed significantly in the study area (Table I).

PREVALENCE OF THE DIFFERENT SPECIES OF *AEROMONAS* FROM FISH FROM THE DIFFERENT HOLDING FACILITIES

The highest prevalence of 56 (29.27 %) of *A. hydrophila* was recorded in catfish obtained from the earthen ponds followed by the prevalence of 35 (18.23 %) obtained from concrete tanks and the least prevalence of 24 (12.50 %) was recorded in catfish obtained from plastic tanks.The highest prevalence of 12 (6.25 %) of *A. caviae* was recorded in catfish obtained from earthen ponds, which was followed by the prevalence of 10(5.21 %) and 8 (4.17 %) recorded in catfish obtained from concrete and plastic tanks respectively. The highest prevalence of 6 (3.13%) of *A. veronii biovar sobria* was recorded in *C. gariepinus* obtained from earthen ponds, followed by the prevalence of 5(2.60 %) recorded in *C. gariepinus* cultured in concrete tanks, then the least prevalence of 4 (2.08%) recorded in *C. gariepinus* reared in plastic tanks. The prevalence of *A. veronii biovar veronii* from the fish was highest in concrete tanks 5(2.60%) followed by plastic tanks 4 (2.08 %) and the least prevalence of 3 (1.56 %) was recorded in fish reared in earthen ponds. The prevalence of *A. hydrophila* differed significantly (P < 0.01) between the different holding facilities (Table II). The overall prevalence of *Aeromonas* species differed significantly (P<0.001) from the different culture facilities. *Aeromonas* species obtained within the different all the culture facilities differed significantly from the study area (Table II).

Table I: Distribution of *Aeromonas* species from water isolated from the different holding facilities

Species	**Holding facilities**			$\chi^{2\,a}$	**p-value** [a]
	Concrete Tanks N (%)	**Plastic Tanks** N (%)	**Earthen Pond** N (%)		
Aeromonas hydrophila	8 (22.22)	6 (16.66)	15 (41.67)	6.31	0.04¥
Aeromonas caviae	5 (13.89)	3 (8.33)	4 (11.11)	0.56	0.75
Aeromonas veronii* biovar *sobria	0 (0.0)	0 (0.0)	2 (5.56)	4.07	0.13
Aeromonas veronii* biovar *veronii	2 (5.56)	2 (5.56)	3 (8.33)	0.31	0.85
Overall Prevalence	15 (41.67)	11(30.56)	24(66.67)	9.91	< 0.01¥
$\chi^{2\,b}$	10.94	7.38	22		
p-value [b]	0.01¥	0.06	< 0.01¥		

N = Number of isolates χ^2 = Chi-square value ¥ = Significant at p < 0.05; [a] = between row; [b] = between column

Table II : Distribution of *Aeromonas* species from *Clarias gariepinus* isolated from the different holding facilities

Species	**Holding facilities**			$\chi^{2\,a}$	**p-value** [a]
	Concrete Tanks N (%)	**Plastic Tanks N (%)**	**Earthen Pond N (%)**		
Aeromonas hydrophila	35 (18.23)	24 (12.50)	56 (29.17)	17.23	< 0.01¥
Aeromonas caviae	10 (5.21)	8 (4.17)	12 (6.25)	0.84	0.66
Aeromonas veronii* biovar *sobria	5 (2.60)	4 (2.08)	6 (3.13)	0.41	0.81
Aeromonas veronii* biovar *veronii	5 (2.60)	4 (2.08)	3 (1.56)	0.51	0.77
Overall prevalence	55 (28.6)	40 (20.8)	77 (40.1)	17.22	<0.001¥
χ^2	48.47	28.69	106.40		
p-value [b]	<0.01¥	<0.001¥	<0.001¥		

N = Number of isolates χ^2 = Chi-square; value¥ = Significant at p<0.05; [a] = between row [b] = between column

DISCUSSION

Aeromonas species are widely distributed in the aquatic environment and are responsible for several diseases in fish farms. The presence of different species of *Aeromonas* in water in holding facilities predisposes fish to disease (Guerra *et al*., 2022; Majeed *et al*., 2023). The prevalence of 46.29 % recorded in this study was higher than the prevalence of 12.5% recorded by El-Gohary *et al.* (2020) from pond water in Nile Tilapia Fish Farms in Egypt, but was lower than the prevalence of 53.3 % obtained by Adah *et al.* (2021) from the water in the different cultural facilities in Kaduna State, Nigeria. The difference in the geographical locations may have caused variations in how the pathogen, host, and environment interacted and could have been attributed to the variations of the prevalence in this study (Ben Hamed, *et al*., 2018).

The increased prevalence obtained from the earthen ponds when compared to concrete and plastic tanks may be due to the increased abundance of bacteria associated with the increased trophic state of earthen ponds. This association tends to be stronger in finer textured sediment which is strongly influenced by the type and quantity of clay minerals and organic matter present promoting the increase and persistence of the bacteria in the environment as seen in earthen ponds but which differs in both concrete and plastic tanks (Hassard *et al*., 2016; Luo, *et al*., 2019)

One of the most significant constraints on fish productivity is outbreaks of *Aeromonas* disease on the fish farms (Nhinh *et al*., 2021; Reda *et al*., 2021) and knowledge about the prevalent *Aeromonas* species is needed for management and prevention of diseases on the farm. In this present study, the overall prevalence of *Aeromonas* species recorded from *C. gariepinus* reared in the different culture facilities in the study area was 29.86%, this is however higher than the prevalence of 19.6% reported by Adah *et al* .(2021) in Kaduna State, Nigeria. Nonetheless, it was lower than the reports of Perretta *et al*. (2018) who obtained a prevalence of 35.5% from fish in Uruguay, and El-Gohary *et al*. (2020) from Egypt with a prevalence of 33.3%. The variability in the prevalence of *Aeromonas* species observed may be due to the different species of fish, holding facilities, sampling methods, geographic locations, and management practices.

Conversely, higher prevalence rates of *Aeromonas* species from water and fish, is a signal that aeromoniasis is on the increase and appropriate measures need to be put in place to control and prevent this disease in fish farms. In this study, the *Aeromonas* species were also isolated more from water than fish this shows that *Aeromonas* species is a natural inhabitant and is widely distributed in in aquatic environment, this is consistent with the findings of Wamalla *et al*. (2018) and Adah *et al*. (2021) .

The fact that different species of *Aeromonas*, were found to be isolated from *C. gariepinus* in the current investigation suggests that the fish may be susceptible to disease. Similar results from Pakistan have been reported in earlier investigations (Ali *et al*., 2016).

The prevalence of the different *Aeromonas* species found in fish and water varied among the holding facilities that were sampled in this study. This could be as a result of the varying types of ponds, water sources, and management techniques used in the various fish farms (Elsheshtawy *et al*., 2020).

In this study, four different *Aeromonas* species (A. *hydrophila*, A. *caviae*, A. *veronii sobria*, and A. *veronii veronii*) were isolated with A. *hydrophila* being the most dominant species this is consistent with the findings of Perretta *et al*. (2018) and Borella *et al*. (2020). However, it is different from the results of Ashiru *et al*. (2017) and Grilo *et al*. (2021) who opined differences in the most prevalent of the phenospecies of *Aeromonas* from the fish farm. This finding is most likely as a result of the diverse *Aeromonas* species present and their ability to adapt to the aquatic environment successfully leading to their widespread distribution. It is worthy of note that these *Aeromonas* species isolated in this study are important pathogens of fish associated with varying diseases in fish farms and are also of public health interest (El-Gohary *et al*., 2020; Borella *et al*., 2020; Adah *et al*., 2021).

In conclusion, this study highlighted the prevalence and diversity of *Aeromonas* species isolated from different culture facilities. The distribution of the *Aeromona*s species is a public health concern, hence proper management practice and biosecurity measures need to be observed and adhered to in fish farms in other to mitigate the occurrence and spread of disease outbreak.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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