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Prevalence and Risk Factors Associated with *Aeromonas hydrophila* infection in *Clarias gariepinus* and Pond Water from Fish Farms in Kaduna State, Nigeria.

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Abstract

Clarias gariepinus remains important among fish species, and its farming in Africa has contributed immensely to fast growth in pisciculture. However, the successful rearing of fish is hampered by the occurrence of disease. The study was carried out to establish the prevalence, risk factors associated with, and antibiotic susceptible patterns of *Aeromonas hydrophila* isolates from *C. gariepinus* obtained from fish farms in the study area in Kaduna state, Nigeria. Two hundred and fifty-five (255) fish samples with their respective pond water from 30 randomly selected fish farms in Kaduna state were examined for the prevalence of *Aeromonas hydrophila*. The prevalence of *A. hydrophila* was 19.6% (50/255) and 53.3% (16/30) in fish and pond water respectively. *A. hydrophila* infected fish exhibited erosions and haemorrhages on the skin and fin and degeneration of the fin and barbell. The age, weight, and holding facilities were significantly associated with the prevalence of *A. hydrophila*. Multidrug resistance (MDR) ability ranging from two to seven commonly used antibiotics and twelve resistant patterns was also displayed by the isolates. The presence of *A. hydrophila* with associated MDR characteristics portends public and aquatic health hazards and, therefore, needs active surveillance and monitoring.

KeyWords: Aquaculture, Antibiotics, *Clarias gariepinus*, management practise, multi-drug resistance

1. Introduction

Wild fish stock is declining worldwide mainly due to overfishing and climate change, paving the way to the rapid development of fish farming. Fish farming is the world's fastest thriving sector of animal production, involving the use of water (FAO, 2017). Fish takes a prominent place as a source of protein compared to other protein sources and is estimated to provide at least 50% of total animal protein intake in developing countries (Ugwem *et al*., 2011; Obiero *et al.*, 2019). However, the successful rearing of *Clarias gariepinus (C. gariepinus)* is hampered by the occurrence of disease, which at any stage of the fish culture will have a great consequence on the economic viability of fish farms and the yield of protein for human consumption (Babek *et al*., 2015; Opiyo *et al*., 2018).

Aeromonas hydrophila (A. hydrophila) is known to be one of the most important bacteria associated with disease in marine, freshwater and cultured fish (Pękala-Safińska, 2018), and infection by *A. hydrophila* has been recognized for many years and has been associated with brown patch skin disease, tail and fin rot, motile aeromonad septicaemia and haemorrhagic septicaemia which can lead to huge mortality among wild and cultured fishes (Plumb and Hanson, 2011; Bebak *et al.,* 2015).

Aeromonas hydrophila is a ubiquitous Gram-negative bacterium, facultatively anaerobic, oxidase-positive, and glucose-fermenting bacteria belonging to the family *Aeromonadaceae* (Hussain *et al*.,2014; Stratev and Odeyemi, 2015), which is commonly isolated from freshwater ponds and inhabits the gastrointestinal tract and are considered to be emerging bacterial pathogens (Igbinosa *et al.,* 2012). More so, *A. hydrophila* has also been reported to cause zoonotic diseases leading to intestinal and extra-intestinal diseases in humans such as septic arthritis, diarrhoea (traveller's diarrhoea), gastroenteritis, skin and wound infections, meningitis, and fulminating septicaemia (Salunke *et al*.,2015).

Diseases associated with *A. hydrophila may have led to an increase in antibiotics application in the fish farms* to manage infections and mixtures of antibiotics in feeds resulting in antibiotic resistance among pathogenic bacteria. This is a more challenging problem in developing countries (Wegener and Frimodt-Moller, 2000). Reports on the prevalence of *A. hydrophila* from *C. gariepinus* and pond water in Kaduna state, Nigeria are scanty. Hence, this study is undertaken to ascertain the prevalence, risk factors associated with, and antibiotic susceptible patterns of *A. hydrophila* isolates from *C. gariepinus* and pond water from fish farms in the study areas.

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2. Materials and method

2.1. Study Location

The study was conducted in Kaduna State, where 4 Local Government Areas (LGAs) including Sabo-Gari, Kaduna-South, Kaduna North, and Zaria LGAs were chosen by random sampling.

Kaduna State is situated in the North -Central region of Nigeria (with Kaduna town as its capital) and shares common borders with Zamfara, Katsina, Niger, Kano, Bauchi, and Plateau States and to the South-West, the Federal Capital Territory, and Abuja. The global location of the State is 10°20′N, 7° 45′E 10.333°N. The State occupies an area of approximately 48,473.2 square kilometres and has a population of more than 6 million people (KSGC, 2017).

2.2. Sample Collection

A stratified random sampling method was employed, in which four Local Government Areas (LGAs) and thirty fish farms were selected comprising 40 concrete ponds, 26 earthen ponds, and 22 plastic tanks making a total of 88 holding facilities within which fish and water were selected. A range of 5-15 fish/ponds were selected based on the stocking density and water samples were collected from 2-6 holding facilities based on the number of holding facilities on the farms (Table 1). A total of two hundred and fifty-five fish samples were conveyed in a plastic receptacle with a cover having the pond water. Also 88 water samples each measuring 500 ml were collected from ponds using sterile bottles and transported under the cold chain to the Veterinary Microbiology laboratory of Ahmadu Bello University Zaria for analyses.

Table 1. Distribution of selected fish holding facilities in 4 LGAs in Kaduna State.

LGA	NO OF FARMS	EARTHEN POND	CONCRETE POND	PLASTIC TANK
Sabo Gari	5	0	16	3
Kaduna North	11	14	0	10
Kaduna South	8	5	12	6
Zaria	6	7	12	3
TOTAL	30	26	40	22

Clarias gariepinus samples were examined clinically, by taking history and conducting antemortem, and post-mortem procedures after which each fish was tagged (Kwaga *et al.,* 1988; (Kwaga *et al.,* 1988; Austin and Austin, 2012). The age, sex, and gross lesions were observed and recorded. Each live fish was sacrificed (by brain spiking to minimise suffering) and placed on a clean stainless tray dorsally, and swab (sterile cotton wool soaked in 70% alcohol) was used to clean the fish from the operculum to the abdominal area to reduce bacterial load.

2.3. Bacterial culture and isolation of Aeromonas hydrophila

The isolation of *Aeromonas hydrophila* followed the standard procedure described by Cowan and Steel (1974). For each fish, the gastrointestinal tract was excised and macerated, and 10% of it was inoculated into a test tube containing enrichment broth (Alkaline peptone water) pH,

8.6. The glass tubes were incubated at 37^{0}C for 18-24 hours. This was then subcultured onto MacConkey agar. Also, water samples collected from each farm were pooled and centrifuged at 3,000 rpm for 10 minutes. The sediment was inoculated into alkaline peptone water and incubated at 37^{0}C for 18-24 hours and later subcultured onto MacConkey agar (Buchanan and Gibbons, 1974).

Biochemical tests were carried out ~~for~~ on the isolated Gram-negative bacteria (non-lactose fermenters) included catalase test, citrate utilization test, haemolysis of sheep Red Blood cells, hydrogen sulphide production, indole test, methyl red test, oxidase test, sugar fermentation tests (glucose, sorbitol, sucrose, lactose, rhamnose, and galactose), urease test, and Voges -Proskauer test.

All the chemicals used for biochemical tests were set according to manufacturer instructions (Difco®, Laboratories, USA and Oxoid®, London, UK) and the results were interpreted using the manual for bacteria identification (Cowan and Steel, 1974) and online ABIS Advanced Bacteriological Identification Software (ABIS, 2017).

2.4. Antibiotic sensitivity of the bacterial isolates

The antimicrobial susceptibility of 50 and 16 *Aeromonas hydrophila* isolates from fish and pond water, respectively, were ascertained using the disc diffusion method. The antibiotics were selected based on their common use in the fish farms and these included Ampicillin (10 µg), Chloramphenicol (10 µg) Gentamycin (10 µg), Oxacillin (5µg), Penicillin (10 units), Streptomycin (10 µg), Tetracycline (30 µg), and Vancomycin (5 µg). The susceptibility test was carried out on Muller Hinton agar using antibiotic-impregnated discs. Zones of inhibition were compared with reference strain (ATCC 646) and interpreted as sensitive, intermediate, and resistant (CLSI, 2011).

2.5. Statistical Analysis

Data from this study were loaded into Microsoft Office Excel version 2016 for establishing the frequencies and percentages (%). Chi-square test was used to assess the discrete variables at a 95% confidence interval at $p < 0.05$ was considered to ascertain the associations of potential risk factors with the isolate on of *A. hydrophila in the study area*. The Statistical Package for the Social Sciences (SPSS, Chicago, Illinois, USA) for windows version 22.0 was used for all analysis, and p-value < 0.05 was considered significant in all the analyses.

3. Results

3.1. Clinical manifestations and prevalence rate of Aeromonas hydrophila in C. gariepinus

The *Clarias gariepinus* samples comprised of 200 clinically sick and 55 apparently healthy ones aged between 4 -24 weeks and measuring 15-42 cm in length, 3.8-12 cm in width, and 200-1000g in weight. Among the sick *Clarias gariepinus*, observations such as anorexia and sluggish movements were observed. On physical examination, one or more of the following were observed, which include: exophthalmia (protrusion of the eyeball), erosions, and severe haemorrhages on the skin, eyes, barbels, and fin. Fin rot, white spot, oedema petechiation, and hyperemia of the abdomen. Post-mortem examination

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of the sick fish revealed pale gills, congestion of liver, kidney, and spleen, distended gallbladder, and yellowish-green mucoid in the intestine.

The highest isolation rate of *A. hydrophila* was 33.3 % (9/27) obtained from sick fish in Sabo Gari LGA, while the least isolation rate of 13.4 % (9/67) was obtained from Kaduna South LGA. More so, among the apparently healthy fish, the isolation rate was up to 23.1 % (3/13) in Kaduna South LGA. However, there was no significant difference (*P* > 0.05) within the different locations sampled. The prevalence of *A. hydrophila* was 28.1 %, 20.7 %, 20 %, and 15 % obtained from *C. gariepinus* gotten from Sabo Gari (9/32), Zaria (12/58), Kaduna North (17/85), and Kaduna South (12/80) respectively (Table 2). A total of 50 *A. hydrophila*, 43 (21.5 %) from sick (n= 200) and 7 (12.7 %) from apparently healthy (n= 50) were obtained from *Clarias gariepinus* respectively given a total prevalence rate of 19.6 % (50/255), but there was no significant difference (*P* > 0.05) between the fish sampled (Table 2).

The prevalence of *A. hydrophila* infection increases with the age, weight, and length of *C. gariepinus*. The likelihood of infection with *A. hydrophila* in female *C. gariepinus* was 1.41 times when compared to their male counterpart. *C gariepinus* managed semi-intensively were 1.46 times more likely to be infected with *A. hydrophila* when compared to *C. gariepinus* raised in the intensive system, while sick *C. gariepinus* were 1.87 times likely to be infected with *A. hydrophila* when compared with the healthy ones. There was a higher infection tendency of 2.98 of *A. hydrophila* among *C. gariepinus* raised in earthen ponds and 2.70 times likely to occur in fish raised in a concrete tank. There was no significant difference in the prevalence of *A. hydrophila* from the different sampled Local Government Areas.

However, the prevalence was higher in *C. gariepinus* sampled in Sabo Gari LGA. The association between age, (1-3, 4-6, and 7-9months), weight (201-400g and 401-600g), and holding facilities (concrete and earthen ponds) with the prevalence of *A. hydrophila* was statistically significant at p < 0.05. The prevalence rate of 24 %, 22.2 %, and 9.5 % was obtained from *C. gariepinus* in earthen ponds, concrete ponds, and plastic tanks, respectively, although there was no significant difference p > 0.05 (Table 3).

Table 2. Prevalence of *Aeromonas hydrophila* in sick, apparently healthy fish and pond water obtained from four LGAs in Kaduna State.

LGA	NFS	No. of *A. hydrophila* isolate (%)	*P*-Value (NFS)	NFS	No. of *A. hydrophila* isolate (%)	NPWS	No. of *A. hydrophila* isolate (%)	*P*-Value (NPWS)
Sabo Gari				32	9 (28.1)	5	2 (40.0)	
Apparently healthy	5	0 (0.0)	0.16					
Sick	27	9 (33.3)						
Kaduna North				85	17 (20.0)	11	7 (63.7)	
Apparently healthy	22	3 (13.6)	0.39					
Sick	63	14 (22.2)						0.56
Kaduna south				80	12 (15.0)	8	5 (62.5)	
Apparently healthy	13	3 (23.1)	0.37					
Sick	67	9 (13.4)						
Zaria				58	12 (20.7)	6	2 (33.3)	
Apparently healthy	15	1 (6.7)	0.12					
Sick	43	11 (25.6)						
Total NFS								
Apparently healthy	55	7 (12.7)	0.14					
Sick	200	43 (21.5)						
Total	255	50 (19.6)		255	50 (19.6)	30	16 (53.3)	

Key: NFS: Number of fish sampled; NPWS: Number of pooled pond water sample

Table 3. Prevalence and risk factors associated with *A. hydrophila* infection in *C. gariepinus* from 4 LGAs in Kaduna State.

Risk Factors	N	Prevalence (%)	OR (95% CI)	*P*-Value
Sex				
Female	105	24 (22.86)	1.41 (0.75 - 2.64)	0.28
Male [a]	150	26 (17.33)	1.00	
Weight(g)				
0-200	44	8 (18.18)		
201-400	96	10 (10.42)	0.08 (0.02 - 0.34)	<0.01*
401-600	53	12 (22.64)	0.20 (0.04 - 0.85)	0.03*
601-800	35	9 (25.71)	0.24 (0.05 -1.07)	0.06
801-1000	12	5 (41.67)	0.49 (0.08 -2.81)	0.43
> 1000 [a]	10	6 (60.00)	1.00	
Total Length (cm)				
< 20	20	2 (10.00)	0.31 (0.04- 1.38)	0.14
21-30	45	7 (15.56)	0.52 (0.18 - 1.41)	0.20
31-40	72	14 (19.44)	0.67(0.29-1.59)	0.66
41-50	65	13 (20.00)	0.70(0.29- 1.67)	0.42
51-60 [a]	53	14 (26.42)	1.00	
Age (months)				
1-3	70	8(11.43)	0.07(0.02- 0.23)	0.0001*
4-6	85	9(10.59)	0.07 (0.02- 0.21)	0.0001*
7-9	56	10(17.86)	0.12 (0.04-0.38)	0.0001*
10-12	23	10(43.48)	2.36(0.68- 8.59)	0.18
>12 [a]	20	13(65.00)	1.00	
Management system				
Semi- intensive	75	18 (24.00)	1.46 (0.75 -2.80)	0.2624
Intensive [a]	180	32 (17.78)	1.00	
Health Status				
Sick	200	43 (21.50)	1.87 (0.82 - 4.77)	0.14
Apparently healthy [a]	55	7 (9.09)	1.00	
Local Government Areas				
Sabo Gari	32	9 (28.13)	1.49 (0.53 - 4.11)	0.44
Kaduna North	85	17 (20.00)	0.96 (0.42 - 2.25)	0.92
Kaduna South	80	12 (15.00)	0.68 (0.28 - 1.67)	0.40
Zaria [a]	58	12 (20.69)	1.00	
Holding facilities				
Concrete ponds	117	26 (22.22)	2.70 (1.08 - 7.60)	0.03*
Earthen ponds	75	18 (24.00)	2.98 (1.13 - 8.73)	0.03*
Plastic tanks [a]	63	6 (9.52)	1.00	

[a] = Reference category; OR = Odds Ratio; CI = Confidence Interval; * = Significant *P* < 0.05

3.2. Prevalence of A. hydrophila in Pond Water collected from the sampled location

Aeromonas hydrophila isolation rates of 63.7 %, 62.5 %, 40.0 %, and 33.3 % were obtained from pond water gotten from Kaduna North (7/11), Kaduna South (5/8%), Sabo Gari (2/5), and Zaria LGAs (2/6), respectively. The total isolation rate was 53.3% (16/30) from the pooled pond water (Table 2). The isolation rate of 60% (6/10) was obtained from pooled pond water collected from concrete and earthen ponds while 40% (4/10) was obtained from pooled pond water collected from plastic tanks (Table 2).

The prevalence of *A. hydrophila* was higher in pond water sampled from Kaduna North LGA when compared to Sabo Gari, Kaduna South, and Zaria LGAs. The prevalence was higher and was 2.16 likely to occur in pond water sampled from the earthen and concrete pond when compared to pond water samples from plastic tanks. Consequently, ponds semi intensively managed was 1.48 likely for *A. hydrophila* to occur when compared with ponds intensively managed. There was no significant difference (p > 0.05) in the prevalence of *A. hydrophila* from water samples (Table 4).

Table 4. Prevalence and risk factors associated with *A. hydrophila* infection in pond water from 4 LGAs in Kaduna State.

Risk	N	Prevalence (%)	OR (95% CI)	*P*-Value
Local Government Areas				
Sabo Gari	5	2 (40.00)	1.30 (0.09 - 19.43)	0.85
Kaduna North	11	7 (63.63)	3.24 (0.39 - 35.49)	0.29
Kaduna South	8	5 (62.50)	3.04 (0.32 - 37.36)	0.35
Zaria [a]	6	2 (33.33)	1.00	
Holding facilities				
Concrete ponds	10	6 (60.00)	2.16 (0.35 - 14.71)	0.42
Earthen ponds	10	6 (60.00)	2.16 (0.35 - 14.71)	0.42
Plastic tanks [a]	10	4 (40.00)	1.00	
Management system				
Semi- intensive	10	6 (60.00)	1.48 (0.31- 7.70)	0.63
Intensive [a]	20	10 (50.00)	1.00	

OR=Odds Ratio, CI=Confidence Interval, a Reference category;

3.3. Antibiotic susceptibility of the Aeromonas hydrophila *isolates.*

The susceptibility test of *Aeromonas hydrophila* indicated that gentamicin had the highest sensitivity (66.7 %, 44/66) followed by chloramphenicol (48.5 %, 32/66), streptomycin (15.2 %, 10/66), oxytetracycline and tetracycline (6.1 % 4/66), respectively, and none to vancomycin, penicillin, and ampicillin. All the *A. hydrophila* isolates were found to be resistant to penicillin (100 %, 66/66), oxytetracycline (93.9 %, 62/66), vancomycin (92.4 % ,61/66) ampicillin (69.7 %, 46/66) tetracycline (60.6%, 40/66), chloramphenicol (36.4 %,

24/66), streptomycin (30.3 %, 20/66) and gentamicin (9.1 %, 6/66) respectively. There was a statistical significance p < 0.01 between the sensitive, resistant, and intermediate *A. hydrophila* (Table 5). Multidrug resistance (MDR) was displayed *by A. hydrophila* isolates to the antibiotics mostly used in pisciculture in Kaduna state, Nigeria. Multidrug resistance of *A. hydrophila* isolates ranged between 2 – 7 different antibiotics used, twelve different MDR patterns were observed and the prevalence of MDR among the *A. hydrophila* ranged between 3.0 % - 15.2 % (Table 6).

Table 5. Antibiotics susceptibility of *Aeromonas hydrophila* isolated from fish and pond   water

Antibiotic	Sensitive (%)	*P*-Value	Intermediate (%)	*P*-Value	Resistant (%)	*P*-Value
Ampicillin	0 (0.0)		20 (30.3)		46 (69.7)	
Chloramphenicol	32 (48.5)		10 (15.2)		24 (36.4)	
Gentamycin	44 (66.7)		16 (24.2)		6 (9.1)	
Oxytetracycline	4 (6.1)		0 (0.0)		62 (93.9)	
Penicillin	0 (0.0)	<0.01*	0 (0.0)	<0.01*	66 (100.0)	<0.01*
Streptomycin	10 (15.2)		36 (54.5)		20 (30.3)	
Tetracycline	4 (6.1)		22 (33.3)		40 (60.6)	
Vancomycin	0 (0.0)		5 (7.6)		61 (92.4)	
Total	94 (17.8)		108 (20.5)		326 (61.7)	

* = Significant *P* < 0.05

Table 6. Multidrug resistance patterns of *Aeromonas hydrophila* isolated from fish and pond water

Resistance patterns	Number of *Aeromonas hydrophila* isolates	Prevalence (%)	*P*-Value
PEN, OXA,	5	7.6	
PEN, OXA, VAN, TET	10	15.2	
PEN, OXA, VAN, AMP,	4	6.1	
PEN, OXA, VAN, STR, AMP	5	7.6	
PEN, OXA, VAN, GEN, STR	2	3.0	
PEN, OXA, VAN, TET, CHL	3	4.5	
PEN, OXA, VAN, TET, AMP	10	15.2	0.25
PEN, VAN, GEN, AMP, CHL	4	6.1	
PEN, OXA, VAN, TET, AMP	6	9.1	
PEN, OXA, VAN, TET, AMP, CHL	4	6.1	
PEN, OXA, VAN, STR, AMP, CHL	6	9.1	
PEN, OXA, VAN, TET, STR, AMP, CHL	7	10.6	

PEN: Penicillin; AMP: Ampicillin; CHL: Chloramphenicol; GEN Gentamycin; OXA: Oxacillin STR: Streptomycin; TET: Tetracycline; VAN: Vancomycin

4. Discussion

In this present study, the prevalence rate in *C. gariepinus* was higher than that of Mailafia (2003), who reported a prevalence of 11.6 % in fish from wild sources. These differences could be due to different geographical locations, seasons of the year, species of fish, isolation methods, quality of water, and management practices. Isolation and identification of *A. hydrophila* in apparently healthy *C. gariepinus* were similar to the report of Omeje and Chukwu (2014), where *A. hydrophila* was isolated in both healthy and diseased fish. Consequently, the isolation in apparently healthy *Clarias gariepinus* may lead to the outbreak of disease when the water quality and management practices of fish farms become unfavorable for production. The variation of the prevalence rates in the different locations may have been contributed by the differences in the interaction of the pathogen, host, and the environment (Raman *et al.*, 2013). Isolation of *A. hydrophila* in ponds water and *C. gariepinus* in this study showed that it is a natural inhabitant of the culture system in the selected fish farms (Shiranee *et al.*, 1993). The isolation of *Aeromonas species* in pond water has been described to be an indication of the presence of fish disease on a farm (Noga, 2000). The higher prevalence of *A. hydrophila* in the earthen ponds could be because most of the farms sourced their water from the natural water bodies' that are already contaminated with animal and human activities especially the dumping of refuse which invariably get into the ponds. This development is suitable for the propagation and multiplication of several microorganisms including *A. hydrophila*. More so, *A. hydrophila* is saprophytic and thus their prevalence of *A. hydrophila* is ensuing upon environmental fluctuations and changes (Okpokwasili and Ogbulie, 1999).

It has been documented that A. hydrophila causes disease in both cultured and wild fish and can cause clinical signs to the host tissue. Which may be in the form of haeamorrhages and inflammation (Goharriz *et al.,* 2015; Stratev *et al.*, 2015). Our findings were similar to the clinical manifestation of *A. hydrophila* found in *Clarias gariepinus* and other fish species (Omeje and Chukwu, 2014; Kumar *et al.*, 2016). Anyanwu *et al* (2014) and El-Bouhy *et al. (2015)* reported earlier that motile Aeromonads are associated with erosive and heamorrhagic ulcerative skin lesions observed in the present study. Damage to the fish skin has been recognized as a portal of entry for many bacterial pathogens (Long *et al.*, 2014). The different patterns of clinical and pathological manifestations expressed in diseased fish caused by *A. hydrophila* as observed in this study may be due to the difference in individual *C. gariepinus* susceptibility to *A. hydrophila* (Baumgartner *et al.*, 2017; Mohamed, 2018). The prevalence of *A. hydrophila* in *C. gariepinus* in our study was less compared to the 30.5 % prevalence rate reported by Omeje and Chukwu (2014), and 31.7 % by Omeje and Chukwu (2012).

There was no bias in the infection of *A. hydrophila* among the *C. gariepinus* based on sex, weight, and length. It has been documented that *Aeromonas* species affects fishes of all ages and sizes (Camus *et al.*, 1998), but in our study, the isolation rate was in older fish when compared with younger fish. This is contrary to the reports by Mzula *et al.,* 2019 who opined that the infection

of *A. hydrophila* was higher in fingerlings than in older fish, our observation could be attributed to the fact that older fish have stayed longer in the different holding facilities increased the exposure to the *A. hydrophila.* More so, they are bigger in size and this provides a larger surface area for the infection to multiply in number than smaller ones.

Management practise of the fish farms seems to contribute significantly to the isolation of *A. hydrophila* from *C. gariepinus*. In line with this, it was observed that *C gariepinus* raised in earthen ponds, which are semi intensively managed were more prone to the infection than those raised in concrete and plastic tanks intensively managed. Our findings could be attributed to the high presence of elevated pollution levels and anthropogenic activities in earthen ponds which often causes the *A. infections* in *C. gariepinus*, and so the vulnerability of *C. gariepinus* to pathogenic infections is enhanced (Dar *et al.*, 2016).

A. hydrophila isolates in this study showed a high level of multidrug resistance and were resistant to oxytetracycline, vancomycin, ampicillin, and tetracycline in which Gentamicin had the highest sensitivity. The result obtained in this present study is similar to that reported by Nahar *et al.* (2016) and Odeyemi and Ahmad (2017). Antibiotic application on fish farms is often practiced as a means of managing diseases on fish farms (Chitmanat *et al.*, 2016), and this practice leads to the increased development of resistance of *A. hydrophila* infection against the antibiotics used (Nahar *et al.*, 2016). This resistance of *A. hydrophila* to routinely used antimicrobial agents is a budding problem in pisciculture (Dias *et al.,* 2012), and the spread of antibiotics resistance is of great concern because *A. hydrophila* is also a zoonotic pathogen (Janda and Abbott, 2010). The excessive and indiscriminate use of penicillin, amoxicillin, oxytetracycline, and vancomycin may have predisposed this current finding. Nahar *et al.* (2016), reported that *Aeromonas hydrophila* showed marked levels of resistance against chloramphenicol, penicillin, amoxicillin, metronidazole, sulphamethoxazole-trimethoprim, and amikacin.

The diversity of antibiotic resistance pattern exhibited by *A. hydrophila* encountered in this present study reflects the diversity among the isolates and the challenge of multidrug resistance (MDR) seems to affect many pets, livestock, and aquatic animals and its consequence might be detrimental (Lee and Wendy, 2011; Igbinosa *et al.,* 2012; Daodu *et al.,* 2017). MDR might also reflect the consequence of the irrational use of antibiotics used in fish farms.

5. Conclusion

Aeromonas hydrophila is present in pond water and *C. gariepinus* from selected fish farms in Kaduna state harboring multidrug-resistant *Aeromonas hydrophila* which constitute a potential public health risk and may affect aquatic health. The unregulated antibiotic usage in the aquatic industry in Nigeria has to be keenly scrutinized and monitored from time to time to determine the spread and increase of bacterial resistance. The detection of *A. hydrophila* in fish suggests that strict hygiene procedures

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and proper cooking before consumption of fish is essential to safeguard consumers.

Acknowledgment

Statement of Animal Right

All regulations and international standard involved in the use of animal were duly followed.

Conflict of interest

The Authors hereby declare that there was no conflict of interest.

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