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Omeje VO
Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

Okolo CC
Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

Eze DC
Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

Corresponding Author:
Omeje VO
Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

Pathogenicity (LD_{50}) and antibiotics sensitivity tests of *Aeromonas hydrophila* isolated from fishes of the Kainji Lake area

Omeje VO, Okolo CC and Eze DC

Abstract
This study investigated the pathogenicity and antibiotics sensitivity of *Aeromonas hydrophila* isolated from the Kainji Lake Area. Feral fishes were collected from sampling stations in the Lake Kainji using stratified random sampling and they included 240 *Oreochromis niloticus*, 120 *Clarias gariepinus* and 120 *Heterotis niloticus* while cultured 144 *Oreochromis niloticus*, 128 *C. gariepinus* and 128 *H. niloticus* were collected from 8 homestead fish farms within Kainji Lake area. A total of 18 *A. hydrophila* isolates proportionately and randomly selected were used to test for pathogenicity. The *in vitro* antibiotic susceptibility test of the isolates was determined using disc diffusion method. The pathogenicity of the isolates showed that 27.78% was virulent, 22.22% moderately virulent while 50% was avirulent strains of the *A. hydrophila*. Eighty percent (80%) of the virulent strains were isolated from cultured fish samples while 20% were from feral fishes. Isolates were most susceptible to gentamycin (73.3%), nitrifurantoin (66.7%), nalidixic acid (63.3%) and co-trimoxazole (53.3%) and most resistant to ampicillin (86.7%) and colistin (83.3%). Isolates from the cultured fishes were more resistant to antibiotic than the feral isolates. High percentage of antimicrobial resistance and emergence of multiple drug resistance among the *A. hydrophila* strains was observed.

Keywords: Virulence, *Aeromonas hydrophila*, antibiotic, drug resistance, Kainji Lake

1. Introduction
Aeromonas hydrophila and other motile aeromonads are among the most common bacteria in freshwater habitat throughout the whole world and they frequently cause disease among cultured and feral fishes [1]. Motile aeromonads septicemia, a systemic fish disease is caused by *A. hydrophila* resulting in heavy mortalities in farmed and wild fishes [2]. The organism is responsible for many disease situations which could either be chronic, per acute or acute, the severity of which is dependent on a number of factors. Some of the factors that determine the severity of *A. hydrophila* infections just like most diseases caused by microorganism include the virulence of the invading microorganism, the physiologic and genetic makeup of the host fish and the degree of environmental stress exerted on the fish population [3]. The effect of the disease is not only mortalities but also loss of growth, reduction in fecundity and loss due to control measures. *Aeromonas hydrophila* is a ubiquitous Gram negative, motile, rod shaped bacterium which can be commonly isolated from freshwater ponds and is also a normal inhabitant of the gastrointestinal tract of fish [4]. In tropical aquaculture, it is considered to be a major economic problem [5]. It is zoonotic and has the potential to be a foodborne pathogen [6]. *Aeromonas hydrophila* has been associated with several disease conditions in fish, including tail rot, fin rot, and haemorrahagic septicaemia. Disease has been recognized as one of the major limiting factors to fish production. The frequency and severity of disease outbreaks have been increasing with increasing aquacultural activities and international fish trade [7]. The rapid development of fish culture in sub-Saharan Africa may therefore not achieve the desired impact due to disease problems except if efforts are directed towards curtailing fish mortalities due to disease outbreaks.
As *A. hydrophila* is one of the most important pathogens of Freshwater fishes [8] a lot of reports are available concerning the pathogenic mechanism of the bacterium [9]. Information on the pathogenicity of *A. hydrophila* in freshwater fishes in sub-Saharan Africa is however very

scanty. This study is aimed at filling that gap. The success of aquaculture production depends on prompt diagnosis, treatment and management of infection any time there is an outbreak. Until a suitable vaccine becomes available commercially, antimicrobial compounds will be invaluable for preventing heavy losses which may result from outbreaks of aeromonads septicaemic disease. In this respect, several agents have been found to be suitable for control of the disease. It is important to run antibiotic sensitivity tests prior to using antibiotics for controlling *Aeromonas* outbreaks. Many strains of *Aeromona*s are resistant to commonly-used antibiotic [10], and it is important to determine which drug should be used before spending time and money on an ineffective product. In this study, antibiotic sensitivity test were conducted with isolated bacteria to determine which drug(s) were effective to control the pathogen. Even though disease prevention in fish is always preferable to cure, treatment of infected stock may eventually have to be undertaken. The problem of drug resistant in fish farming is increasingly receiving close attention. It is imperative to recognize the need to use drugs that are effective in treating a particular infection effectively while at the same time avoid using those that can result in resistance. Apart from the debilitating effect of developing antibiotic resistant bacteria in a fish farm, resistant *A. hydrophila* strain of fish origin can be a source of a possible transfer of resistance to humans [6]. Therefore, stringent precautions are necessary to prevent misuse of antibiotics which could enable resistant bacterial populations to develop in the aquatic environment. The objective of this study therefore was to investigate the pathogenicity and sensitivity of the isolated *A. hydrophila* to a variety of commonly used antibiotics in the study area.

2. Materials and Methods

Aeromonas hydrophila, the causative agent of motile aeromonads septicaemia infection was isolated from cultured and feral fishes of the Kainji Lake area. Fish for the study were collected from 6 sampling stations within Kainji Lake and 8 homestead fish farms within the Kainji Lake area which were selected following stratified random sampling. Fishes collected from sampling stations include 240 *Oreochromis niloticus* (40 fish/station), 120 *Clarias gariepinus* (20 fish/station), and 120 *Heterotis niloticus* (20 fish/station). Fishes collected from 8 homestead fish farms include 144 *O. niloticus* (18 fish/farm), 128 *C. gariepinus* (16 fish/farm) and 128 *H. niloticus* (16 fish/ farm). Isolation and identification of the bacteria from the intestines using morphological characteristics and biochemical tests were carried out following standard procedures [11, 12].

2.1 Pathogenicity test

The isolates identified as *A. hydrophila* were tested for virulence according to the method adopted by Beher *et al*. [13] A total of 18 isolates proportionately and randomly selected (3 isolated from cultured tilapa, 3 from feral tilapia, 3 from cultured *C. gariepinus*, 3 from feral *C. gariepinus*, 3 from cultured *H. niloticus* and 3 from feral *H. niloticus*.) were used for the study. The test fish include *Clarias gariepinus* juveniles of mean standard length 20.6±0.19cm (18.2 – 23.0), average weight of 83.5 ± 0.68g (70.2 – 96.8) and juveniles of *O. niloticus* of standard length 12.8±0.31cm (11.2 – 13.7) and average weight 36.5±0.32g (28.5 – 43.2). The fish were obtained from the hatchery complex of National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Nigeria. The *C. gariepinus* juveniles were all from the same parent stock. Seven groups (representing the graded doses of the bacteria) of 12 fish per dose each of *C. gariepinus* and *O. niloticus* were set up to test each of the isolates. The fish for the different replicates were separately stocked in 40.5L (30cmX30cmX45cm) glass aquaria containing air stones and acclimated to laboratory condition for two weeks. During the acclimation, the *C. gariepinus* were fed 6% of their body weight three times a day using a commercial catfish feed (Coppens®) while the tilapia were equally fed 6% of their body weight three times a day with NIFFR compounded tilapia feed of 25% crude protein. Water quality parameters were monitored daily and recorded. Water quality was maintained by continuous aeration, dissolved oxygen and ammonia levels were within the normal range recommended for fish culture [14]. The mean water temperature was 26.5 ± 1.8 ^{0}C, the dissolved oxygen was 6.8 ± 0.5mg/l and pH 7.6 ± 0.23. There was no filtration system in the aquaria tanks therefore the tanks were cleaned daily by siphoning. The fish were weighed and measured to the nearest g and cm respectively. Different concentrations of bacteria were made in physiological saline by serial dilution. Each fish were injected intra-peritoneal (ip) using 1ml tuberculin syringe and a 26 gauge needle with logarithmically decreasing dilutions of *A. hydrophila* isolates. The doses were 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 10^9 CFU (0.1ml)/ fish. The time from injection until death of each fish were recorded. Observations on the specific mortality of the infected fish were made up to the fifth days post infection and the LD_{50} calculated using the method of Reed & Muench [15] from the result obtained. If the LD_{50} was from 10^3 - 10^6 the isolates was classified as virulent, when it was from 10^6 - 10^7, moderately virulent, but if it was from 10^7 - 10^9, it was classified as avirulent [16].

2.2 Antibiotic Sensitivity tests

The susceptibility of the isolates to the following antibiotics: tetracycline, ampicillin, streptomycin, colistin, gentamicin, cotrimoxazole, nalidixic acid, and nitrofurantoin were tested using disc diffusion methods. A total of 30 isolates proportionately and randomly selected (5 isolated from cultured *O. niloticus*, 5 from feral *O. niloticus*., 5 from cultured *C. gariepinus*, 5 from feral *C. gariepinus*, 5 from cultured *H. niloticus* and 5 from feral *H. niloticus*) were used for the antibiotic sensitivity test. The antibiotic sensitivity disc used was manufactured by Abtek biological Ltd, UK. The disc had the following concentration of each of the antibiotic as follows; Tetracycline (TET) 25µg, Ampicillin (AMP) 25 µg, Streptomycin (STR) 25 µg, Colistin (COL) 25 µg, Gentamicin (GEN) 10 µg, Cotrimoxazole (COT) 25 µg, Nalidixic acid (NAL) 30 µg, and Nitrofurantoin (NT) 200 µg. The method employed in the sensitivity tests were modified from Bauer *et al*. [17] and the diameter of the zone of clearance was compared with the interpretive chart of performance standards for antimicrobial disc susceptibility tests. Based on that, each drug was classified as R for Resistant, I for Intermediate and S for Sensitive (Susceptible).

3. Results

Water quality parameters were relatively constant throughout the duration of the experiment. Water temperature was 26.5 ± 0.8 (varied from 23.2 to 30.6), the dissolved oxygen was 6.2mg/l (varied from 5.6 to 7.0) and pH 7.6 (varied from 7.2 to 8.4).

3.1 Pathogenicity (LD_{50}) of the isolates

The dose of the isolates at which 50% mortality of the fish occurred within the stipulated time was taken as the LD_{50}. Table 1 represents the LD_{50} of one of the isolates (strain CF3 which signifies feral *Clarias gariepinus*).

Table 1: Determination of LD_{50} of *A. hydrophila* strain CF3 in *C. gariepinus*

Injected dose CFU/ fish/ml	No. of fishes challenged	No. of dead fishes	Mortality %	LD_{50}
10^9	12	11	91.7	
10^8	12	9	75	
10^7	12	8	66.7	
10^6	12	6	50.0	10^6
10^5	12	5	41.7	
10^4	12	4	33.3	
10^3	12	2	16.7	

The LD_{50} of each of the isolates as calculated are as shown in Table 2 and based on the LD_{50}, the isolates were classified as virulent, moderately virulent or avirulent.

Table 2: LD_{50} of 18 strains of *A. hydrophila* isolated from fishes of the Kainji Lake and environs.

Strain №	LD_{50} in *C. gariepinus*	LD_{50} in *O. niloticus*	Virulence
TF1	$10^{6.2}$	$10^{6.3}$	moderate
TF2	$10^{7.4}$	$10^{7.4}$	avirulent
TF3	$10^{7.8}$	10^8	avirulent
TC1	10^4	10^4	virulent
TC2	$10^{6.6}$	$10^{6.4}$	moderate
TC3	$10^{7.8}$	$10^{7.8}$	avirulent
CF1	10^8	$10^{8.2}$	avirulent
CF2	$10^{6.8}$	10^7	moderate
CF3	10^6	10^6	virulent
CC1	$10^{7.6}$	$10^{7.7}$	avirulent
CC2	10^4	$10^{4.2}$	virulent
CC3	$10^{5.4}$	$10^{5.6}$	virulent
HF1	$10^{7.6}$	$10^{7.8}$	avirulent
HF2	$10^{8.2}$	$10^{8.4}$	avirulent
HF3	$10^{6.8}$	$10^{6.8}$	moderate
HC1	$10^{8.2}$	$10^{8.3}$	avirulent
HC2	$10^{5.6}$	$10^{5.8}$	virulent
HC3	10^8	$10^{8.2}$	avirulent

The result of the LD_{50} of *A. hydrophila* indicates that out of the 18 strains tested, 5 (27.78%) were virulent, 9 (50%) were avirulent while 4 (22.22%) were classified as moderately virulent. Out of the five strains that are virulent 4 (80%) were isolates from the cultured fish while only one was from the wild fish samples.

3.2 Antibiotics Sensitivity of the isolates

The results of the antibiotic sensitivity of the different isolates of *A. hydrophila* (Table 3) indicated that out of 30 isolates tested, 43.3% were sensitive to tetracycline while 40% showed intermediate response to the antibiotics, and 16.7% were resistant to tetracycline. Out of 30 isolates, 86.7% were resistant while 10% showed partial sensitivity while only 3.3% was sensitive to ampicillin. 53.3% of the isolates were sensitive to Co-trimoxazole, 33.3% showed intermediate response while 13.3% were outright resistant to Co-trimoxazole. 73.3% of the isolates were sensitive to gentamycin while 23.3% showed intermediate response and only 3.3% was resistant to gentamycin. Similarly, 63.3% of the isolates were sensitive to nalidixic acid, 30% intermediate while 6.7% were resistant to the antibiotics. Also 66.7% of the 30 isolates were sensitive to nitrofurantoin antibiotics while 26.7% showed intermediate response while 6.7% were resistant. 83.3% of the isolates tested were resistant to colistin, 13.3% showed intermediate response while only 3.3% of the isolates was sensitive to colistin antibiotics. Out of the 30 isolates tested, 33.3% were sensitive to streptomycin, 46.7% showed intermediate response while 20% of the isolates were resistant to streptomycin. Eight isolates (26.7%) were resistant to 4 or more antibiotic indicating multiple drug resistance.

Table 3: Sensitivity of *Aeromonas hydrophila* isolates to antibiotic

Isolate	TET	AMP	COT	GEN	NAL	NT	COL	STR
TF1	S	R	S	S	S	S	R	S
TF2	S	R	S	S	I	S	R	S
TF3	I	R	S	S	S	I	S	I
TF4	I	R	I	S	S	S	I	S
TF5	S	I	S	S	S	S	R	S
TC1	S	R	R	S	S	S	R	I
TC2	I	R	I	S	S	S	R	S
TC3	R	R	S	S	I	S	R	S
TC4	I	R	S	S	S	S	R	I

TC5	I	R	S	S	S	S	R	I
CF1	I	R	R	I	S	S	R	R
CF2	S	I	S	S	I	S	I	I
CF3	S	R	S	S	I	I	R	R
CF4	S	R	S	S	S	S	I	I
CF5	I	R	I	S	S	S	R	S
CC1	I	R	S	S	I	I	R	I
CC2	I	R	I	I	S	R	R	I
CC3	R	R	I	I	I	S	R	R
CC4	R	R	R	I	S	I	R	R
CC5	S	R	R	S	I	S	R	R
HF1	S	I	I	S	S	I	R	I
HF2	I	R	S	S	I	I	R	S
HF3	S	R	S	I	S	S	R	I
HF4	S	S	S	S	S	S	I	S
HF5	I	R	I	S	S	I	R	I
HC1	R	R	I	S	R	S	R	R
HC2	I	R	S	I	I	S	R	I
HC3	S	R	I	I	S	I	R	I
HC4	S	R	S	S	S	R	R	S
HC5	R	R	I	R	R	S	R	I

Key: S- sensitive; I- intermediate; R- resistant; TF- feral tilapia; TC- cultured tilapia; CF- feral clarias; CC- cultured clarias; HF- feral haterotis, HC- cultured heterotis Relative sensitivity of *A. hydrophila* isolated from cultured and feral fishes to different antibiotics were as shown in Table 4.

Table 4: Sensitivity pattern of *Aeromonas hydrophila* isolates to different antibiotic

	TET	**AMP**	**COT**	**GEN**	**NAL**	**NT**	**COL**	**STR**
№ (%) Sensitive	13 (43.3)	1 (3.3)	16 (53.3)	22 (73.3)	19 (63.3)	20 (66.7)	1 (3.3)	10 (33.3)
№ (%) Intermediate	12 (40)	3 (10)	10 (33.3)	7 (23.3)	9 (30)	8 (26.7)	4 (13.3)	14 (46.7)
№ (%) Resistant	5 (16.7)	26 (86)	4 (13.3)	1 (3.3)	2 (6.7)	2 (6.7)	25 (83.3)	6 (20)
№ (%) Cultured sensitive	4 (26.7)	0 (0%)	6 (40)	9 (60)	8 (53.3)	10 (66.7)	0 (0%)	3 (20)
№ (%) Cultured intermediate	6 (40)	0 (0%)	6 (40)	5 (33.3)	5 (33.3)	3 (20)	0 (0%)	8 (53.3)
№ (%) Cultured resistant	5 (33.3)	15 (100)	3 (20)	1 (6.7)	2 (13.3)	2 (13.3)	15 (100)	4 (26.7)
№ (%) Feral sensitive	9 (60)	1 (6.7)	10 (66.7)	13 (86.7)	11 (73.3)	12 (80)	1 (6.7)	7 (46.7)
№ (%) Feral intermediate	6 (40)	3 (20)	3 (20)	2 (13.3)	4 (26.7)	3 (20)	4 (26.7)	6 (40)
№ (%) Feral resistant	0 (0)	11 (73.3)	2 (13.3)	0 (0%)	0 (0%)	0 (0%)	10 (66.7)	2 (13.3)

Out of the 15 isolates from cultured fish tested, 26.7% were sensitive to tetracycline while 60% of the isolates from feral fishes were sensitive. All the isolates from cultured fish (100%) were resistant to ampicillin while 73.3% from the feral fish were resistant. Similarly, 40% of the isolates from cultured fish were sensitive to Co-trimoxazole while 66.7% of the isolates from feral fish were sensitive. While 60% of the isolates from culture facilities were sensitive, 86.7% from the feral fish were sensitive to gentamycin. Also 53.3% and 73.3% of isolates from culture facilities and feral fish respectively were sensitive to nalidixic acid. Similarly, 66.7% and 80% were recorded for cultured and feral fish respectively in antibiotics sensitivity test of nitrifurantoin. While all (100%) of the isolates from the cultured fish were resistant to colistin, 66.7% from the feral fish were resistant. Whereas 26.7% of the isolates from cultured fish were resistant to streptomycin, only 13.3% of those of feral fish were resistant.

4. Discussion

Several studies have assessed the virulence (LD_{50}) of different strains of *A. hydrophila* [8, 18]. Virulence is inversely related to the size of the effective dose (LD_{50}), that is the smaller the dose required to kill, the greater the virulence. Results of the pathogenicity studies show that 27.8% of the isolates were virulent, 50% avirulent while 22.2% were moderately virulent and 80% of the virulent isolates were from cultured fish. Since the bacterium can be commonly isolated from freshwater ponds [4, 19] and is also a normal inhabitant of the gastrointestinal tract of fish [20. 21], high percentage of avirulent strain among fishes is not unexpected. The pathogenicty of *A. hydrophila* for experimentally infected *O. niloticus, C. gariepinus* and *H. niloticus* may be attributed to the production of extracellular enzymes and lethal toxins [22, 23]. Production of endotoxins, extracellular enterotoxins, haemolysins, cytotoxins, proteases, ability to adhere to cells and possession of certain surface proteins have also been attributed to the pathogenicity of motile aeromonads [24]. Variations in the production of virulence factor among the various strains of *A. hydrophila* have been reported [18]. The high percentage of the avirulent strain among the *A. hydrophila* isolated from Kainji Lake fishes may be the reason why most of the fish that yielded *A. hydrophila* did not show any sign of infection. It was also observed that a virulent isolate from a particular fish species will infect others from different species signifying cross infectivity. The implication of this is that when an infected fish is introduced into a culture facility, all the fish in that facility are at risk irrespective of the species. The fact that up to 80% of the virulent strains come from culture facilities lends credence to the fact that because of human activities in the environment of the fish such as indiscriminate application of antimicrobials, mutation of the bacterium do occur giving rise to more virulent strains.

Antibiotics refer to substances produced by microorganisms or to similar substances (produced wholly or partly by chemical synthesis) which in low concentration inhibits the growth of other micro-organisms [25]. When a bacteria

population adapt to the presence of antibiotics, sensitive cells are gradually replaced by cells that carry the genes for resistance [26]. The emergence of bacterial isolates that are resistant to an antibiotics agent represents a continuing ecological battle to achieve a natural host – parasite balance [27]. As new antibiotics are developed and used, resistant strains may develop. Because of the increasing rate of antibiotic resistance by *A. hydrophila* [28, 29], antibiotic sensitivity test was performed in order to determine the sensitivity of *A. hydrophila* to different antibiotic that are available within the study area. All the isolates exhibited greatest sensitivity to gentamycin (73.3%), followed by nitrifurantoin (66.7%), nalidixic acid (63.3%) and co-trimoxazole (53.3%) respectively. Similarly the isolates exhibited greatest resistance to ampicillin (86.7%) followed closely by colistin (83.3%). This is in agreement with Kaskhedikar & Chhabra [30] who reported that all *A. hydrophila* isolates they tested for antibiotic sensitivity were resistant to penicillin (ampicillin family) and polymixin (colistin family) antibiotic. They also reported 100% sensitivity to gentamycin, chloramphenicol, nitofurantoin and nalidixic acid. High percentage of antimicrobial resistance and emergence of multiple drug resistance among the *A. hydrophila* strains was observed. Out of total isolates, 10% were resistant to five antimicrobial drugs, another 10% to four drugs, and 16.7% to three drugs. Therefore a total of 36.7% of the isolates were resistant to three or more drugs. It is instructive to note that most of the isolates that exhibited multiple drug resistance were from the cultured fish. The frequent use of antibiotics in fish farms for chemotherapy, prophylaxis and to enhance growth could have accounted for resistance to commonly used antibiotics since it is apparent that resistance to antibiotics may be due to indiscriminate use [21]. Restriction of the use of drugs in aquaculture to control fish disease will aid in minimizing the development and spread of resistant plasmid (R- factor) carrying micro-organisms that may confer drug resistant to otherwise susceptible bacteria species [31, 27] ultimately leading to public health hazards. Environmental areas of heavy human impact appear to be associated with a higher incidence of antibiotic-resistant strains of aeromonads [32, 10]. The problems associated with indiscriminate use of antibiotics have been reported in aquaculture [33]. While none of the isolates from the feral fish were out rightly resistant to tetracycline, 33.3% of those from the culture fish were resistant. The increasing antibiotic resistance among *A. hydrophila* also has the potential of causing health problems in human beings. These characteristics make it to be an emerging pathogen posing several threats to humans [34]. Only two of the isolates were resistant to none of the antibiotics and these were isolates from the feral fish. This finding is in agreement with the report of Adnair & Turutoglu [35] who opined that because of frequent use of Oxytetracycline by fish farmers in Turkey, most of the *A. hydrophila* they isolated from carp *Cyprinus carpio* were resistant to oxytetracycline. Similar observations were noted in the present study where sensitivity to tetracycline was 26.7% among isolates from cultured fish and 60% from those of the feral fish. According to Chopra & Roberts [36] the fact that tetracyclines are chelating agents means that they may be inactivated in the bowel by dietary calcium and magnesium ions. Thus water hardness may also have relevance to the efficient use of tetracyclines in fish [35].

There is the need to constantly monitor the susceptibility pattern of bacterial pathogen to commonly used antibiotics for effective treatment of such important fish diseases. Although certain bacteria are susceptible in-vitro to a particular agent, use of such drug may be inappropriate either on pharmacological grounds or because other less toxic agents are preferred [37]. Fish infected by resistant strains of *A. hydrophila* can serve as reservoir hosts [38]. Based on the result of the antibiotics susceptibility test, it is recommended that gentamycin should be the drug of choice when an outbreak of *A. hydrophila* infection is suspected in a fish farm. Nitrofurantoin which showed greatest effect against the isolates second only to gentamycin has doubt expressed against it. According to Wolf and Dunbar [39], although nitrofurans are highly effective *in vitro*, it is not effective *in vivo* in control of fish diseases. Therefore *in vitro* susceptibility testing does not always predict clinical outcome. This could be due to the absorption and excretion characteristics which largely determine the efficacy of drugs *in vivo*. Nalidixic acid has been shown to be effective and well tolerated in the treatment of Gram negative organisms. Smith [40] reported that goldfish (*Carassius auratus*) were fully protected against laboratory challenge with *A. hydrophila* by a single dose of nalidixic acid. Co-trimoxazole though placed 4th in the sensitivity test results has been used in fish culture for control of bacterial diseases. According to the United State Pharmacopeial Convention [41], the only potentiated sulphonamide to be licensed in the United States for fisheries use was sulphadiazine – trimethroprim combination co-trimoxazole (Tribrissen, Wellcome). Also according to Smith [41], sulphadiazine – trimethroprim combination Romet 30 (Roche) became available for control of furuncolosis in trout and salmon with indicated in-vitro activity against *Aeromonas hydrophila*, *Vibrio angullarium* and *Yesinia ruckeri.*

5. Conclusion

The bacteria isolated from the cultured fish were more resistant to antibiotic which imply the abuse or indiscriminate use of antibiotic in most fish farms. The antibiotics sensitivity results indicate that gentamycin and nalidixic acid as well as cotrimoxazole, chloramphenicol and nitrofurantoin should be suitable for those cases of *Aeromonas hydrophila* infection in which antimicrobial therapy is necessary.

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