Antibiotics-resistance pattern of bacteria isolated from fish ponds in Ikorodu, Lagos State, Nigeria

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Abstract: Aquacultures are practised in different water confinements which include plastic ponds, concrete and earthen and are known to harbour pathogens. In aquaculture (fish rearing) the use of antibiotics is on the increase where they are used as disease eradicators and as growth promoters. This study sought to investigate the antibiotic resistance pattern of bacteria isolated from fish ponds in Ikorodu, Lagos State, Nigeria. A total number of ten water samples were collected from five different sampling points at depth 1.5 m within the ponds, close to the outlets and at the outlets from the cat-fish and tilapia-fish ponds. These samples were serially diluted, inoculated and the pure isolates were subjected to antibiotics sensitivity testing using Kirby- Bauer's disc diffusion method. Based on the cellular, morphological and biochemical characterization nine bacterial isolates were identified and isolates found to show multiple resistances to antibiotics were further identified by molecular analysis using 16SrRNA gene detection and sequencing. The Antibiotic susceptibility test showed that the isolates were resistant to ceftazidime, cefuroxime, nitrofurantoin, ampicillin, amoxycillin, clavulanate. gentamicin, ciprofloxacin and all the isolates were susceptible to ofloxacin. The molecular analysis revealed that the organisms which showed multiple resistances to antibiotics were *Azotobacter chroococcum*, *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. In conclusion this study has revealed the need for good management of aquaculture facilities in order to avoid zoonotic diseases. Also, monitoring of antibiotics usage in fish ponds management should be given high priority to avoid resistant genes from being transferred to other bacteria of human clinical significance.

Key word: Aquaculture, pathogens, antibiotics, zoonotic, pond.

INTRODUCTION

According to FAO (2007), fish is the most important single source of high-quality protein providing about 16% of the animal protein consumed by the world's population. In Africa, fish constitutes about 17% of animal protein consumed (Allison *et al.,* 2009). Fish is preferred as a protein source as it can be consumed by all cadres of individuals regardless of their religious beliefs and nutritional preferences. Fish has a nutrient profile superior to all terrestrial meats (Feldhusen *et al.,* 2000; Fisher *et al*., 2017). Fish is known to contain low fat and low cholesterol and to be highly digestible making them suitable to the infants, children, and elderly (Sapkota *et al.,* 2008). It is a good source of sulphur and essential amino acids such as lysine, leucine, valine and arginine. Fish contains thiamine and a rich source of Omega-3 polysaturated fatty acids, fat soluble vitamins (A, D and E) and water soluble vitamins (B complex) and minerals (calcium, phosphorus, iron, iodine and selenium). High content of polyunsaturated (Omega –III) fatty acid is important in lowering blood cholesterol level and high blood pressure (Zárate *et al.,* 2019). Fish for consumption could be cooked at a temperature above 100°C for more than 20 minutes while some barbecued fishes are cooked below these conditions thereby exposing consumers to pathogenic microbes which were able to survive the cooking conditions temperature.

Africa's fast-growing human population outstrips growth in fish supply, and most of the continent's wild fish populations are fully exploited (Cai and Leung, 2017). Several efforts have been made both in developing and developed countries to meet this demand for fish. However, it has been forecasted that the demand for fish will grow beyond levels that can be sustained (Vignesh *et al.,* 2011). To meet the much needed demand for animal proteins, make profits and create jobs, people engage in fish aquaculture; therefore, aquaculture production must more than double by 2050 to satisfy the projected fish demand (Cai and Leung, 2017).

A fish pond (earthen, plastic, concrete, tarpaulin) is a type of aquaculture usually filled with fresh water, fairly shallow and is usually non-flowing. Fish ponds have been referred to be self-contained ecosystems which are often teeming with rich vegetable and diverse organisms which include bacteria, fungi, protozoa, algae and phytoplankton, periphyton and biofilms (Olukunle and Oyewumi, 2017). However, fish farmers face huge loss as a result of infections by pathogenic bacteria, among the common fish pathogens which are *Staphylococcous* sp., *Aeromonas* sp., *Salmonella* sp., *Shigella* sp., *Enterococcus faecalis, E. coli, Yersinia* sp, *V. cholera* and other *Vibrios* (Schmidt *et al.,* 2000; Novoslavskij *et al*., 2015). Others are *Pseudomonas* sp. and *Streptococcous* sp. Diseases caused by these pathogenic bacteria include white- skin, haemorrhagical septicaemia, furunculosis and so on (Ponnerassery *et al.,* 2012). The risk of bacterial infections among fish is high, therefore, heavy amount of antimicrobials are used in fish feed for preventive and curative purposes in aquaculture facilities worldwide (Sapkota *et al.,* 2008). Antibiotics in fish farming and other animal food production is widely believed to contribute to the dramatic increase in numbers of antibiotic-resistant bacterial strains now threatening human health and has adverse impact on fish and human therapy (Smith *et al.,* 2003; Apenteng *et al.,* 2017). As a result of the non-hygienic and stressful conditions present in aquaculture facilities like fish pond, fish and related products are a potential health risk to humans and the environment since they harbour important human pathogenic bacteria on or inside them (Smith *et al.*, 2003; Gufe *et al.,* 2019). The safety of eating fish from contaminated fish ponds cannot be guaranteed. Therefore, there is a need to study the bacterial isolates from fish ponds and their antibiotic resistance pattern against some clinically used antibiotics which form the focus of this study. This is of paramount importance in order to know the potential risk posed to human health by consuming fish from contaminated ponds and the clinically importance of such bacteria isolated from such ponds.

MATERIALS AND METHODS

Description of study area: This study was conducted in Lagos State University of Science and Technology (LASUSTECH) formerly (Lagos State Polytechnic) Ikorodu campus, located at about 26 km North-East of the city of Lagos, along Sagamu road between Latitude 6.6463^{o} N and Longitude 3.5179 ^{o} E.

Sample Collection: Pond water sample were aseptically taken from the ponds using sterile screw cap bottles. A total number of ten water samples were collected from five different sampling points at depth 1.5 m within the catfish and tilapia ponds, close to the outlets and at the outlets from the cat-fish and tilapia-fish ponds of the fisheries Department of LASUSTECH. The water samples were transported to the laboratory in an ice-packed container for microbiological analysis within 8 hours of collection.

Bacterial analysis of pond water samples: Samples from different sampling pints of the same pond were homogenized and 1 ml each of the water samples was transferred into 9 ml of sterile normal saline and then serially diluted. One millilitre of the stock solution, dilutions 10^{-3}, 10^{-5} and 10^{-7} of each sample was inoculated on Tryptic soy agar using pour plate and streaking method. Each analysis was performed in duplicates and the plates were incubated aerobically at 37^{0}C for 48 hrs. Total colonies on the plates were counted and recorded as colony forming unit per ml (cfu/ml).Pure cultures of bacterial isolate were obtained by sub-culturing on TSA agar and then stored on nutrient agar slant at 4°Cin the refrigerator for further identification.

Identification and characterization of the bacterial isolates: Bacterial isolates were characterized on the basis of their cultural characteristics, colonial morphology, microscopy and biochemical profiles which include Gram staining, motility test indole

test, urease test, glucose fermentation, lactose fermentation, H_2S production, gas production, catalase test, oxidase test and spore forming test (Cheesbrough, 2006).

Antibiotics Susceptibility/Resistance Test: Susceptibility of the isolated bacteria to antibiotics was tested using Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966) and Mueller Hinton agar and the interpretation of the results was based on the national committee for Clinical and Laboratory Standards institute (CLSl, 2007) criteria as sensitive, intermediate and resistant. Bacterial isolates that showed multiple resistance pattern were subjected to molecular analysis.

DNA extraction procedure of some bacterial isolates: One hundred micro litres (100µl of specimen) was added into a micro-centrifuge tube, and then 500µl of the Lysis Buffer Vortex was also added. It was then incubated at 56ºC for 10 min and centrifuge at 10,000 rpm for 1 minute. After spinning, 200 µl of absolute ethanol was added to the tube; the mixture was transferred into the spin column and then centrifuged at 10,000 rpm for 30 sec. The flow-through was discarded and the collection tube was blotted on a tissue paper. 500 µl of wash buffer 1 was added to the spin column and centrifuged at 10,000 rpm for 30 sec. The flow-through was discarded and the collection tube was blotted on a tissue paper. 500 µl of wash buffer 2 was added to the spin column and centrifuged at 10,000 rpm for 1 min. The flow-through was discarded and the collection tube was blotted on a tissue paper. The spin column was centrifuged again at 12,000 to 14,000 rpm for 3 mins to remove all traces of ethanol. The spin column was placed into another micro-centrifuge tube; fifty micro litre (50 µl) elution buffer or nuclease-free water was added to the centre of the column. It was then incubated at room temperature for 1 to 2 mins. It was centrifuged at 10,000 rpm for 1 min to elute the DNA. DNA was stored at -20 or -80 °C.

QIAquick PCR Purification Kit Protocol using a microcentrifuge: This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions. For cleanup of other enzymatic reactions, follow the protocol as described for PCR samples or use the MinElute Reaction Cleanup Kit. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

PCR Amplification of the 16SrRNA Gene Amplified at 430bp:Polymerase chain reaction was carried out to amplify the 16SrRNA gene of the bacteria using the primer pair 799F AACMGGATTAGATACC and 1193R ACGTCATCCCCACCTTCC. The PCR was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. Polymerase chain reaction was performed in 25 µl of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 25pMol of each primer (StabVida, Portugal), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Techne 3 Prime thermal cycler for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95°C; 1 minute at 61°C and 1 minute 30 Seconds at 72°C. This was followed by a final extension step of 10 minutes at 72°C. The amplified product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100 bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker (Adzitey *et al.* 2012).

Sequencing:All PCR products were purified with QIAquick PCR Purification kit and sent

to Epoch Life science (USA) for Sanger sequencing. The corresponding sequences were identified using the online blast search at http://blast.ncbi.nlm.nih.gov/Blast.cgi

RESULTS

This study investigated the antibiotics resistance pattern of microorganisms isolated from fish ponds in LASUSTECH, Ikorodu, Lagos State, Nigeria. The study revealed that seven bacterial isolates CP1, CP2, CP3, CP4, CP5 and CP6 grew on Tryptic soy agar from the catfish pond, while and three bacterial isolates TP1, TP2 and TP3 grew on TSA from the tilapia pond. The total viable counst of the bacterial isolates revealed that TP3 had highest counts in all the dilution concentrations, followed by TP1 while CP3 had the lowest counts among the bacterial isolates. The total viable counts are more in tilapia ponds than in catfish pond; the total viable counts decreased with the decrease in the concentrations of the water samples. Based on cellular, morphological and biochemical characterization, the isolates were identified to be CPI: *Azotobacter* sp, CP2: *Corynbacter* sp. CP3: *Bacillus* sp, CP4: *Bacillus anthracis,* CP5: *Paenibacillus* sp, CP6: *Bacillus subtilis*, TP1: *Proteus* sp, TP2: *Pseudomonas* sp, TP3: *E. coli.* Those organisms that showed multiple resistance to the antibiotics used were identified molecularly and the results revealed that *Pseudomonas aureginosa* had the highest resistant profile, *Azotobacter chroococcum*, *Proteus mirabilis* and *Escherichia coli* all showed resistance to five out of the eight antibiotics used for the susceptibility/resistance test.

DISCUSSION

Findings from this work on the antibiotics resistance pattern of microorganisms isolated from fish ponds in LASUSTECH, Ikorodu, Lagos State, Nigeria demonstrated that there was presence of antibiotics-resistant bacteria (*Azotobacter chroococcum*, *Proteus mirabilis Pseudomonas aeruginosa,* and *Escherichia coli*) in the catfish and tilapia ponds. This finding agrees with a former report that fish ponds have been referred to be self-contained ecosystems which are often teeming with rich vegetable and diverse organisms (Olukunle and Oyewumi, 2017). Also there are a number of important studies that indicate that the bacterial flora in the environment surrounding aquaculture sites contain an increased number of antibiotic-resistant bacteria (Sørum, 2006), and that these bacteria harbour new and previously uncharacterized resistance determinants (Saga *et al.,* 2005).

Table 1: Total viable counts of bacterial isolates from cat fish and tilapia ponds water samples (cfu/ml)

S/N	Sample codes	Stock Solution	Dilution 10^{-3}	Dilution 10^{-5}	Dilution 10^{-7}
1	CP1	17.1	10.5	7.1	2.5
2	CP2	10.4	5.2	3.4	0
3	CP3	5.3	2.3	1.3	0
4	CP4	3.4	1.2	0	0
5	CP5	10.1	5.3	3.3	0
6	CP6	15.2	5.2	3.2	0
		61.5	**29.7**	**18.3**	**2.5**
8	TP1	40.7	30.5	15.2	3.3
9	TP2	40.1	30.1	10.1	2.2
10	TP3	45.3	33.3	15.3	4.1
		126.1	**93.9**	**40.6**	**9.6**

Table 2: Biochemical characterization of the bacterial isolates from catfish and tilapia pond water samples

Isolate	Glucose	Lactose	H2s	Gas	Motility	Indole	Urease	Citrate	oxidase	Catalase	Coagulase	Mannitol	Spore	Pigmentation	Vokes	Gram stain
CP1	+	+	-	+	+	+	+/-	-	-	+	ND	-	-	-	-	GNB
CP2	+	-	-	-	+	-	-	+/-	-	+	ND	+	-	-	-	GPB
CP3	+	+	-	-	-	-	+	-	-	+	ND	-	-	-	-	GPB
CP4	+	+	-	-	-	-	+	-	-	+	ND	-	+	-	-	GPB
CP5	+		- -	-	+	-	+	-	-	+	ND	--	+	-	+	GPB
CP6	+	-	-	-	+	-	-	-	-	+	ND	+	+	-	-	GPB
TP1	+	+	-	-	-	+	+	+	-	+	ND	+	-	-	+	GNB
TP2	+	+	-	+	+	+/-	-	-	-	+	ND	-	-	-	-	GNB
TP3	+	-	-	+	+	+	-	+	+	+	ND	+	-	+	+	GNB

Key: Suspected organisms: CPI: *Azotobacter* sp, CP2: *Corynbacter* sp. CP3: *Bacillus* sp, CP4: *Bacillus anthracis*, CP5: *Paenibacillus* sp, CP6: *Bacillus subtilis*, TP1: *Proteus* sp, TP2: *Pseudomonas* sp, TP3: *E. coli*

Table 3: Antibiotic susceptibility/resistance profile of the bacterial isolates with diameter one of inhibition in Millimetre (mm)

S/N	Sample code	CAZ	CRX	GEN	CIP	NIT	AMP	OFL	AUG
1	CP1	R(10)	R(10)	S(17)	S(23)	R(10)	R(8)	S(20)	R(2)
2	CP2	S(20)	S (23)	S(15)	S(21)	S(23)	S(20)	S(17)	S(19)
3	CP3	S(19)	S(24)	S(16)	S(22)	S(24)	S(19)	S(17)	S(18)
4	CP4	S(20)	S(25)	S(17)	S(21)	S(23)	S(19)	S(19)	S(20)
5	CP5	S(21)	S(23)	S(18)	S(20)	S(23)	S(18)	S(17)	S(19)
6	CP6	S(23)	S(24)	S(15)	S(21)	S(23)	S(20)	S(17)	S(19)
7	CP7	S(19)	S(23)	S(15)	S(22)	S(24)	S(17)	S(18)	R(4)
8	TP1	S(20)	R(4)	S(16)	R(6)	R(8)	R(2)	S(17)	R(2)
9	TP2	R(6)	R(8)	R(2)	R(4)	S(23)	R(3)	S(17)	R(2)
10	TP3	R(3)	R(4)	S(16)	R(5)	S(23)	R(6)	S(17)	R(2)

R- Resistant, S- Sensitive,CAZ- Ceftazidime 30 ug, CRX- Cefuroxime-30ug, GEN-Gentamicin- 10 ug, CPR- Ciprofloxacin- 5ug, NIT- Nitrofurantoin- 300ug, AMP –Ampicillin –10ug,OFL- Ofloxacin- 5ug, AUG- Amoxicillin/Clavulanate- 30ug CPI: *Azotobacter* sp **CP2***: Corynbacter* sp, **CP3***: Bacillus* sp, **CP4***: Bacillus anthracis* **CP5:** *Paenibacillus* sp **CP6***: Bacillus subtilis,* **CP7:** *Micrococcus* sp **TP1:** *Proteus mirabilis* **TP2***: Pseudomonas aeroginosa,* **TP3**: *Escherichia coli*

In this study a decrease in the total viable counts of the organisms relative to the decrease in concentration of the water samples was observed. It also revealed that the decrease in concentration of the water samples through serial dilution led to the decrease in the microbial population. *Escherichia coli* had the highest counts in all the dilution concentrations, followed by *Proteus mirabilis*, while *Azotobacter chroococcum* and *Pseudomonas aureginosa* had the lowest counts among the bacterial isolates. This agrees with a previous report of Pillay (1990) and Bostock *et al.* (2010) that fish living in natural environment are known to harbour pathogenic enterobacteriaceae . Fakorede *et al*. (2019) also reported that *E. coli* is known to survive well in aquatic environments, and are highly adept at horizontal gene transfer, a notorious vehicle for antibiotic resistance dissemination. *Escherichia coli* present in fish are considered as an indicator of feacal contamination and it signifies a more positive assumption of hazard than the other coliform bacteria (Hansen *et al.* 2008).

The findings of the biochemical characterization of the organisms confirmed the report that Gram negative rod shaped bacteria inhabited a cultured population of aquatic environment where fishes live (Udeze *et al.,* 2012). The antibiotic susceptibility/resistance findings revealed that all the isolates were resistant to cefuroxime, ampicillin and amoxicillin but all the isolates were susceptible to ofloxacin. Six bacteria (*Corynbacter* sp, Bacillus sp, *Bacillus anthracis, Paenibacillus* sp, *Bacillus subtilis and Micrococcus* sp) out of the ten bacterial isolates were susceptible to all the eight antibiotics used. *Pseudomonas aureginosa* had the highest resistant profile, *Azotobacter chroococcum*, *Proteus mirabilis and Escherichia coli* all showed resistance to five out of the eight antibiotics used for the susceptibility/resistance profile.

Pseudomonas aeruginosa is known to be susceptible to antibiotics such as ceftazidime, ciprofloxacin and gentamicin (Pang *et al*., 2019; Bassetti *et al*., 2020). In this study *Pseudomonas aureginosa* had the highest resistant profile showing resistance to six (ceftazidime, cefuroxime, gentamicin, ciprofloxacin, ampicillin and amoxicillin/clavulanate) out of the eight antibiotics used but sensitive to cefuroxime and nitrofuratoin. This is a strong indication that this multiple resistance to antibiotics could have been acquired by this strain of *Pseudomonas aeruginosa*. This correlates with former reports that acquired resistance is a type of plasmid-mediated resistance (Foster, 2017). Through plasmid-mediated transduction, transformation, and insertion of drug-resistant genes, excessive β-lactamase can be produced; leading to bacteria resistance. Foster (2017), Haaber *et al*.(2017) and Neuhauser *et al*. (2003) reported that *Pseudomonas sp.* strains demonstrated high rate of resistance to several antibiotics. Environmental and clinical *Pseudomonas aeruginosa* strains showed multiple resistances to drugs and these phenotypes are known as one of the most significant causes of nosocomial infections (Neuhauser *et al*., 2003). The results of the antibiotic susceptibility/resistance profile of *Pseudomonas aeruginosa* strains isolated from clinical and environmental samples indicated that 45% of the strains from clinical samples are resistant to more than three antimicrobial agents from different classes, while 37% of the strains from environmental samples were resistant to more than three antibiotics from different classes (Shokoohizadeh, 2018).

This study revealed that *Azotobacter Chroococum* showed resistance to five (ceftazidime, cefuroxime, nitrofurantoin, ampicillin and amoxicillin/clavulanate) out of the eight antibiotics used but was sensitive to gentamicin, ciprofloxacin and ofloxacin. This strongly indicated that this multiple resistance is typical of this *Azotobacter chroococcum* strain. This agrees with former reports that out of 117 strains of *Azotobacter chroococcum* investigated over 95% of the isolates were resistant to 10 µg ml/l concentration of

tetracycline, erythromycin, chloramphenicol and ampicillin (Saxena, *et al*., 2019). Seventy percent of the strains showed resistance to trimethoprim, streptomycin, rifampicin, nalidixic acid and kanamycin and 8% of the strains were resistant to 400 µg ml/l concentration of tetracycline, streptomycin, kanamycin, chloramphenicol and ampicillin. *Azotobacter chroocuccum* strains in this previous study demonstrated a high intrinsic resistance to the ten antibiotics (Sindhu *et al*., 1989). *Azotobacter chroococcum* usually aids the diversity and distribution of conjugative plasmids among several *E coli* strains which demonstrated multiple resistances to antimicrobial agents (Cernat *et al*., 2002).

In this study *Proteus mirabilis* was resistant to five antibiotics (cefuroxime, ciprofloxacin, ampicillin, amoxicillin/clavulanate and nitrofurantoin) out of the eight antibiotics used, but was sensitive to ceftazidime, gentamicin and ofloxacin. This partially agrees with the report by Okonkwo (2010) that strains of *Proteus mirabilis* which showed up to 100% resistance to ampicillin and tetracycline but sensitive to gentamicin, ciprofloxacin and ofloxacin are about 10%. Therefore it suggested that the multiple resistance of *Proteus mirabilis* to the various antibiotics is typical and also acquired. *Proteus mirabilis* is usually involved in colonization, contamination and it's seldom isolated in serious infections (Rozalski, 1997). *Escherichia coli* is the most frequently isolated enterobacteriaceae species in hospitals, followed by *Proteus mirabilis.* Wild-type strains of *Proteus mirabilis* are known to be sensitive to β-lactams, which are mostly enzyme mediated and the resistance are acquired (Decousser *et al*., 1999).

This study revealed that *E. coli* showed resistance to five (ceftazidime, cefuroxime, ciprofloxacin, ampicillin and amoxicillin/clavulanate) out of the eight antibiotics used but was sensitive to gentamicin, nitrofurantoin and ofloxacin. This strongly indicates that this multiple resistance is typical of *E.coli* confirming a previous report that *E. coli* is susceptible to nitrofuratoin, gentamicin and ciprofloxacin and resistant to ampicillin, amoxicillin, tetracycline, trimethoprim, cefuroxime, ceftriaxone and ciprofloxacin (Wu *et al*., 2022).The resistance of *E. coli* against antibiotics has been on the increase since the first cases were reported. *Escherichia coli* has now been included along with the other enterobacteriaceae in the World Health Organization's list of the twelve families of bacteria that pose the greatest threat to human health (WHO, 2017). *Escherichia coli*'s contribution to the menace of antimicrobial resistance phenomenon can be viewed under two major complementary perspectives. These two perspectives are i) the infections caused by strains of *E. coli* which are resistant to multiple drugs are increasing in number; and the potential of these strains of *E. coli* the to transfer their traits for genetic resistance to other bacteria (Galindo-Méndez, 2020). These two attributes that have made *E. coli* a key player in the antibiotic resistance menace globally because of its ability to transmit easily from animals to human and among humans through the feacal-oral route (Galindo-Méndez, 2020). Secondly, the microorganism's capability to colonize the gut of humans and animals allow it to be in close association with other several bacteria. This association grants *E. coli* the dual role of donating genetic material to other bacteria and acquisition of resistance genes from other microorganisms (Manu *et al*., 2011; Galindo-Méndez, 2020).

CONCLUSION

This study has revealed the presence of antibiotics-resistant bacteria such as *Pseudomonas aureginosa, Proteus mirabilis, Azotobacter chroococum* and especially *Escherichia coli* in fish ponds. There is therefore, an urgent need for good management of aquaculture facilities in order to avoid zoonotic diseases. Also, monitoring of antibiotics usage in fish ponds should be given high priority to avoid

resistant genes from being transferred to other bacteria of human clinical significance.

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