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Microbial Profile and Antibiotic Susceptibility of Microbes Isolated from Wastewater from Some Fish Ponds in Lagos, Nigeria.

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Abstract

Aquaculture is the fastest growing food industry in the world. It can be practiced on a small, medium or large scale. The proliferation of fish ponds and the indiscriminate discharge of waste water from aquaculture into the environment without adequate treatment are of a great environmental and public health concern. Waste water were collected from seven fish farms around Lagos, the samples were analyzed for total heterotrophic bacteria, fungi, total coliform, faecal coliform and other bacteria of public health importance. The antibiotic susceptibility of the isolates obtained was also carried out. A total of about one hundred and twenty-nine (129) bacteria isolates were obtained. The total heterotrophic bacterial count ranged between 1.00 x 10^4 and 2.32 x 10^6cfuml^{-1} while the total fungi count ranged between 1.31 x 10^4 and 6.25 x 10^4cfuml^{-1}. The identified bacterial isolates were the genera *Pseudomonas*, *Micrococcus*, *Bacillus*, *Staphylococcus* and Family Enterobacteriacea. The fungal isolates encountered include *Aspergillus* sp, *Mucor* sp, *Rhizopus* sp, *Penicillium* sp and *Fusarium* sp. The isolates obtained exhibited varying levels of resistance (7.14 – 100%) to all the fourteen antibiotics tested, however, the resistance of the isolates to Cloxacillin (100%) and Erythromycin (85.7%) is noteworthy. The microbiological quality of the fish pond effluents and the level of multiple antibiotics resistance demonstrated by the isolates suggest a need for comprehensive waste management system in our aquaculture practice.
Key words: Fish, fish ponds, waste water, microorganisms, antibiotic susceptibility, aquaculture.

Introduction

As the world fish stock diminishes, the limitation in capture fisheries production has necessitated aquaculture which involves the production of fish in a controlled environment. Indeed, in the past two decades, aquaculture has become the fastest growing food industry in most countries including Nigeria (Amosu *et al*., 2017). Fish and fishery products are important to the human population all over the world as they are a preferred source of animal protein which is relatively cheaper and has little or no religious bias (Reilly and Kaferstein, 1999; FAO, 2002; Grigorakis and Rigos, 2011; Saremi *et al*., 2013; Phillips *et al*., 2014**).**

Aquaculture can be practiced using concrete ponds, earthen ponds, fibre glass tanks and plastic tanks; however concrete tanks are being used in most places nowadays. These range from small scale for home use subsistence to large scale commercial systems (Jensen and Greenlees, 1997; Onome and Ebinimi, 2010; Ugwumba, 2010). Water is a very important and an indispensable component of aquaculture; it creates the natural ecosystem for fish metabolism. Unfortunately, it serves as a major source of bacterial contamination mostly through the waste water which contains uneaten feed, faeces and a lot of microorganisms. The proliferation of these fish ponds therefore raises concerns over the possible environmental and public health effects of the increasing waste water produced, particularly the indiscriminate discharge into the environment without proper treatment (Cao *et al*., 2007; Fafioye, 2011; Vaz-Moreira and Nunes, 2014; Danba *et al*., 2015).

Microorganisms are naturally found in fish pond water and sediment. As integral components of the ecosystem, the non-pathogenic group of these organisms plays the beneficial role of nutrient recycling, organic matter degradation and utilization as probiotic. Meanwhile, a few of the pathogenic/opportunistic group might be present to create hazardous public health risks (Anand *et al*., 2010; Macedo *et al*., 2011). The use of antibiotics as prophylactic and therapeutic measure for bacterial infection in aquaculture is a frequent practice (Romero *et al*., 2012) The indiscriminate use of these antibacterial drugs and other synthetic chemotherapeutic agents to treat fish diseases and /or as feed additives has resulted in an increase in populations of antibiotic-resistant pathogenic bacteria as well as resistant plasmids in food-producing animals, fish, and water microflora. This is also another issue of environmental and public health importance since some antibiotic resistant bacteria persist in the environment more than the antibiotic itself (Ogbondeminu and Olayemi, 1993; Grigorakis and Rigos, 2011, Gao *et al*., 2012).

Fish pond wastewater is usually discharged into water bodies such as lagoon, rivers, streams and lakes hence; the quality of the waste water including the volume of the discharge, the chemical and microbiological content affects the receiving water body. Consequently, fish pond wastewaters are potential source of water pollution (Boyd, 2003, Akpor and Muchie, 2011**).** These water bodies have also been reported as an important reservoir of antibiotic-resistance, which could facilitate the exchange of resistance genes between pathogenic and non-pathogenic bacteria (Baquero *et al.*, 2008; Rizzo *et al.*, 2013; Agwu, 2013).

Lagos is one of the coastal states in Nigeria and it is also an industrialized state. There are lots of fish ponds around as fish farming can be practiced on a small, medium or large scale. There is a dearth of information on the microbial composition and the antibiotic susceptibility profile of bacteria from fish-farms' waste water around Lagos. There has been some reports on the abundance of bacteria isolates from fish ponds (Ogbondeminu, 1993; Ajayi and Akonai, 2003; Fafioye, 2011; Danba *et al.,* 2015). This work was therefore embarked upon to ascertain the different groups of microorganisms in aquaculture effluents from Lagos, the antibiotic susceptibility patterns and the impact such might have on the environment. The knowledge from this study will help in highlighting negative impact and proffering solutions concerning waste management and water quality standards.

Materials and Methods

Sample collection

Water samples were aseptically collected from seven fish ponds directly into sterile glass containers, placed in an ice box and transported to the laboratory for microbiological analysis. The samples were collected over a period of two months and analyzed within four hours of collection. Ponds A, B and C were plastic tanks; D, E and F were earthen ponds; while G was a concrete pond.

Isolation of heterotrophic bacteria and fungi

The isolation of heterotrophic bacteria was carried out according to the method of APHA (2005). This was by making a ten-fold serial dilution of the water samples in sterile distill water and 0.1ml aliquot of each dilution were inoculated on nutrient agar (Oxoid) using the spread-plate method cultured in duplicates. The plates were then incubated at 35°C for 24 hrs. For fungi, the aliquots were inoculated on potato dextrose agar (Oxoid) plates and incubated at room temperature for five to seven days.

Enumeration of total and faecal coliforms

The samples were analyzed for total and faecal coliforms using the Most Probable Number Method (Five Tubes). The samples were inoculated in brilliant green broth and incubated at 35°C for total coliform and 44°C for faecal coliform for 48 hrs. The positive tubes were then sub-cultured on eosine methylene blue (EMB) agar at 35°C for 24 hrs.

Isolation of Salmonella and Shigella species

Salmonella and *Shigella* species were isolated by inoculating aliquots of the water samples on salmonella/ shigella Agar (SSA) (Oxoid) plates. The plates were incubated at 35°C for 24 hrs.

Isolation of Vibrio species

Vibrio species were isolated by inoculating 0.1 ml aliquot samples on thiosulphate citrate bile salt (TCBS) agar plates, the plates were incubated at 35°C for 24 hrs.

Identification of Isolates

The isolates obtained were identified using cultural/macroscopic characteristic, microscopic characteristics and biochemical characteristics. The identities of the bacterial isolates were determined according to standard procedure with the use of the Bergy's Manual of Systematic Bacteriology (Krieg and Holt, 1994), while that of fungal isolates was done following the method of Barnet and Barry (1972).

Antibiotic susceptibility testing

The susceptibility of the bacterial isolates to eleven (11) antibiotics was determined using the disc diffusion method. After 18 hrs, culture of pure isolates in tryptone soy broth, cells were harvested, washed in phosphate buffer (0.05 M, pH 7.0) and re-suspended in normal saline at a density of about 10^6 cfu ml^{-1}. Aliquot (0.5 ml) suspensions were uniformly spread on Muller

Hinton agar plates and allowed to stand for 5 to 10 minutes prior to the placement of the antibiotic discs on the surface of duplicate plates. The inoculated plates were incubated at room temperature (28 ± 2 °C) for 18 to 24 hrs alongside agar plates inoculated only with bacterial test isolates, without the introduction of antibiotic disks, which served as controls. After incubation, the zones of inhibition observed around the antibiotic discs were taken as indications of sensitivity while growth around the drugs indicated resistance. The antibiotics tested, with their respective concentrations given in parentheses, were: Ceftrazidime: **CAZ** (30µg); Cefuroxime: **CRX** (30µg); Gentamicin: **GEN** (10µg); Cetriaxone: **CTR** (30µg); Erythromycin: **ERY** (50µg); Cloxacillin: **CXC** (30µg); Ciprofloxacin: **CPR** (5µg); Ofloxacin: **OFL** (5µg); Augmentine: **AUG** (30µg); Nitrofurantoin: **NIT**(300µg); Ampicillin: **AMP** (10µg).

Multiple Antibiotic Resistance (MAR) Indexing

Multiple antibiotic resistance (MAR) values for each isolate were calculated by summing the number of antibiotics to which the isolate was resistant and dividing that by the total number of antibiotics assayed (Middleton and Ambrose, 2005). The MAR values for each fish pond was further determined by summing the MAR values of individual isolates and dividing by the total number of isolates tested per fishpond.

Statistical Analysis

The mean and standard deviations of microbial counts were derived using Microsoft Excel; while the analysis of variance (ANOVA) was determined using Prism version 5.03 computer software programmes (GraphPad Software**,** San Diego, CA. USA). Tests were carried out at 5% significance level.

Results

The population of the different groups of culturable bacteria and heterotrophic fungi found in the various fish ponds is presented in Table 1. The total heterotrophic bacterial count ranged between 1.00 x 10^4 and 2.32 x 10^6cfuml^{-1}, with Pond C having the highest number while Pond E had the least value. The population of the *Samonella / Shighella* sp. also varied, but not significantly, with a relatively low count of 7.50 x 10^1 and 5.25 x 10^2 cfuml^{-1} observed in Ponds G and F respectively and highest count of 2.78 x 10^5cfuml^{-1} in Pond C while other ponds count ranged from 1.00 x 10^4 to 3.79 x 10^4 cfuml^{-1}. *Vibrio* sp. were detected only in Ponds A (3.04 x 10^4 cfuml^{-1}) and B (1.40 x 10^3 cfuml^{-1}). Likewise, heterotrophic fungi were not detected in three ponds (E, F and G) but had a population range of 1.31 x 10^4 to 6.25 x 10^4 cfuml^{-1} in the other four ponds. The most probable number (MPN) of total coliforms in the ponds was between 150 to 1800/100 ml. The MPN of Faecal coliform on the other hand was between 30 and 150/100 ml.

Presumptive identification of bacteria and fungi Isolates

A total of 129 bacteria isolates were obtained. Out of these isolates, 103 (79.84%) were gram negative, while 26 (20.16%) were gram positive. Among these bacteria were members of *Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp., *Stapylococcus* sp., *Salmonella* sp., *Vibrio* sp., *E. coli* and Family Enterobacteriacea. The fungi species encountered include *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp,, *Penicillium* sp. and *Fusarium* sp. A good number of bacteria of public health importance such as *Vibro* spp., *Salmonella* sp., *Shigella* sp. and *Escherichia coli* were also noted, and their presence in the different pond wastewater are presented in Table 2.

Table 1: Population of Microbial Groups in Fish Ponds

FISH POND	Heterotrophic bacteria ($cfuml^{-1}$) ±SD	Salmonella/ Shigella ($cfuml^{-1}$) ±SD	*Vibrio* sp. ($cfuml^{-1}$) ±SD	Heterotrophic Fungi ($cfuml^{-1}$) ±SD	Total coliform (MPN/ 100ml)	Feacal coliform (MPN/ 100ml)
A	3.80×10^5 ±2.72	3.79×10^4 ±6.0	3.04×10^4 ±3.17	1.65×10^4 ±1.16	350	130
B	1.37×10^5 ±1.01	2.87×10^4 ±2.05	1.40×10^3 ±9.89	1.31×10^4 ±4.18	150	33
C	2.32×10^6 ±2.55	2.78×10^5 ±1.39	ND	6.25×10^4 ±9.19	150	30
D	1.34×10^5 ±1.27	1.34×10^4 ±1.27	ND	2.94×10^4 ±4.07	1600	130
E	1.00×10^4 ±0.0	1.00×10^4 ±0	ND	ND	1800	150
F	1.57×10^5 ±3.78	5.25×10^2 ±2.33	ND	ND	1100	35
G	9.37×10^4 ±1.52	7.5×10^1 ±7.7	ND	ND	350	130

ND- Not detected

Table 2: Presence of some Bacteria of Public Health Importance in Fish Pond Water

Pond	*Salmonella* sp.	*Vibrio* sp.	*E. coli*
A	+	+	+
B	+	+	+
C	+	+	+
D	+	-	+
E	+	-	+
F	+	-	+
G	+	-	+

+ indicates growth or presence; - indicates no growth or absence

Antibiotics resistance among the bacterial isolates from the various fish pond waste water

The bacterial isolates from Pond A wastewater resisted five of the eleven antibiotics tested. All the isolates resisted Cloxacillin, while 86.6% and 60% of the isolates resisted Erythromycin and Ampicillin respectively. A lower resistance was observed for Cetriaxone (13.3%) and Augmentine (6.7%). Conversely, the isolates from Pond B exhibited varying levels (7.14 – 100%) of resistance to all the eleven antibiotics. A similar multiple resistance to thirteen antibiotics was also observed in the isolates from Pond C wastewater. The resistant isolates for six of the antibiotics (Ceftrazidime, Cefuroxime, Cetriaxone, Erythromycin, Cloxacillin, Ampicillin) was above 50%, while Augmentine and Nitrofurantoin were resisted by 40 and 20% of the isolates respectively. It was noteworthy that all isolates from Pond C were susceptible to Gentamicin. Multiple antibiotic resistances were noted in a good number of the bacterial isolates from the three earthen ponds D, E and F. In Pond D, all the isolates were susceptible to Ciprofloxacin and Oflaxacin but resisted Augmentine, 6.6% were resistant to Gentamicin and Nitrofurantoin; while 66.7 to 93.3% resisted the other six antibiotics. Furthermore, among the isolates from Pond E, 80% were resistant to Erythromycin and Cloxacillin, 60% resisted Ampicillin; while ≤ 30% resistance was observed in the other antibiotics with exception to total susceptibility to Ceftrazidime, Gentamicin, Ciprofloxacin and Oflaxacin. In the same way, the isolates in Pond F showed susceptibility to Gentamicin and Nitrofurantoin and very low (≤ 35%) resistance to most of the antibiotics apart from erythromycin and cloxacillin which had 88 and 100% resistance respectively. The number of isolates from the concrete fish pond which resisted cloxacillin (100%), erythromycin (84.62%) and ampicillin (62%) were also high. Nevertheless, they were all susceptible to gentamicin, ciprofloxacin and oflaxacin while a few (≤ 46%) resisted the other antibiotics. Two-way analysis of variance of the level of antibiotic resistance among the isolates in each pond indicated a significant difference ($p < 0.0001$) between the percentage resistances of the isolates to the different antibiotics. Also, there was significant variation ($p = 0.0002$) in the percentage resistance of the isolates from the different fish ponds. The Bonferroni posttest that compared each of the ponds against the other further showed no significant variation ($p > 0.5$) in the antibiotic resistance among the isolates in Ponds A and B which are plastic ponds. However, the resistance to cefuroxime observed for the isolates in the third plastic Pond C, varied significantly ($p < 0.01$) with the resistance from Pond A. Similarly, the isolates from Pond A and the earthen Pond D also exhibited a significant different ($p < 0.05$) resistance to Cefuroxime, Ceftrazidime and Augmentine. Furthermore, the resistance to Augumentine among isolates from Pond B and Pond D also varied significantly ($p< 0.05$). The variation in the resistance of the isolates from the earthen ponds (D, E, F) was also noteworthy, Pond D and E had $p < 0.05$ for Ceftrazidime. Between the isolates from Ponds D and F, resistance to Augumentine showed $p< 0.01$ while resistance to Ampicillin varied with $p < 0.05$. Interestingly, the resistance of the isolates from the concrete pond to all the tested drugs did not show any significant variation from what was noted in all the other pond types.

Multiple antibiotics resistance (MAR) values

The highest MAR value (0.52) was noted for the earthen Pond D while the plastic Pond A had the least value (0.24). The MAR value for the other plastic Ponds, C and B was also high (0.46 and 0.33 respectively) as depicted in Table 3. Furthermore, considering the MAR value for the individual isolates, the isolates which resisted all the tested antibiotics resulting in 1.00 MAR value was isolated from the plastic pond B. On the contrary, some isolates from Pond E were susceptible to all the antibiotics thus showing 0 MAR value, while Ponds A, B, D and G had isolates that resisted only one drug and so had 0.09 MAR value.

Discussion

The abundance of heterotrophic bacteria in all the fish ponds in this study, further ascertain that bacteria are essential part of the pond ecosystem (Vaz-moreira and Nunes, 2014; Vasile *et al*., 2017). There was however, an obvious varying bacterial count among the different ponds which

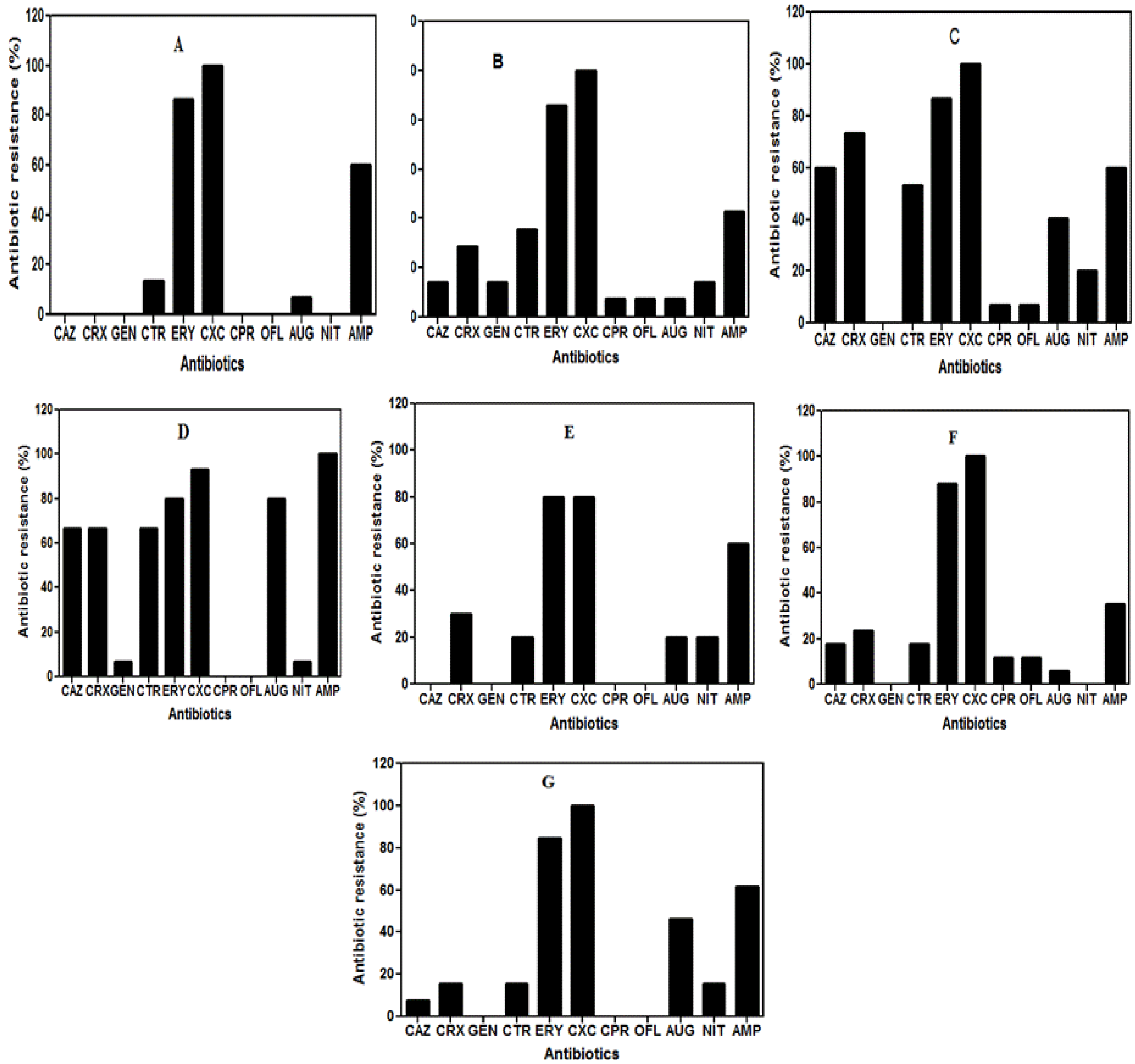

Figure 1: Percentage of the *bacterial isolates resistant to specific antibiotic during the study.* Ceftrazidime (CAZ); Cefuroxime: (CRX); Gentamicin: (GEN); Cetriaxone(CTR); Erythromycin(ERY); Cloxacillin (CXC); Ciprofloxacin (CPR); Ofloxacin: (OFL); Augmentine (AUG); Nitrofurantoin (NIT); Ampicillin (AMP)

was expected since each pond had a different management system and water supply. It had earlier been established that the management system employed in a fish pond as well as the source of water determines to a great extent the microbial load of the pond ecosystem. In addition, pond constant aeration and low depth results in high exposure to sunlight and consequently low bacterial count (Apun *et al*., 1999; Vasile *et al*., 2017). According to the study by Qin *et al*. (2016), different types of feeds used in fish ponds also influence the bacterial abundance. Discharge of these waste waters into water bodies will consequently affect the microbial community (Jensen and Greenlees, 1997) leading to elevation of bacterial counts of these water bodies receiving the wastewater.

Table 3: Multiple Antibiotics Resistance (MAR) values of bacteria isolated from the different fish ponds

POND	**Range of MAR**	**S**tandard Deviation	**MAR Value**
A	0.09 - 0.36	0.082	0.24
B	0.09 -1.00	0.23	0.33
C	0.18 -0.636	0.177	0.46
D	0.09 - 0.818	0.164	0.52
E	0 - 0.636	0.198	0.28
F	0.18 - 0.636	0.128	0.28
G	0.09- 0.55	0.13	0.29

MAR = Multiple antibiotics resistance index.

The bacteria isolated were predominantly gram negative bacteria (79.84%) (*E. coli*, *Salmonella* sp, *Shigella* sp. and *Vibrio* sp.) which coincides with the abundance of five genera of gram negative bacteria (*Escherichia, Shigella, Salmonella, Pseudomonas and Enterobacter*) in selected cat fish ponds in Kano metropolis, Nigeria as reported by Danba *et al*. (2015). Furthermore, Macedo *et al*. (2011) also reported fish pond effluent with high concentrations of thermo tolerant coliforms, total coliforms and heterotrophic bacteria. The presence of faecal coliforms suggests feacal pollution of the fish ponds. Feacal contamination of the ponds can be through water supply and animal manure used for fertilization (Reilly and Kaferstein 1999; Kay *et al*., 2008). In addition, feacal contamination of ponds might also come from visiting birds and possibly from fish feaces (Andleeb *et al*., 2014). *Salmonella/Shigella* and *Vibrio* species were also present in some of the fish pond wastewater studied. The presence of these pathogenic bacteria such as *E. coli, Salmonella* sp. and *Vibrio* sp in the pond are of great public health importance particularly when the waste water is discharged into water bodies without adequate treatment, since they are causative agents of conjunctivitis, enteritis, diarrhoea, cholera, typhoid fever and abdominal pains which can also lead to death (Jensen and Greenlees, 1997; Fafioye, 2011).

The frequency and volume of antibiotic used in an environment had been directly related to antibiotic resistance; however, an unpredictable correlation of resistance to antibiotic usage has also been noted. Expectedly, the persistent use of antibiotics in aquaculture has warranted their high occurrence in fish pond environments and concomitant resistance among microbes in this environment (Tendencia and De La Peña 2001**;** Lee *et al*., 2005; Hoa *et al*., 2011). The bacteria isolates from this study exhibited antibiotic resistance to a good number of antibiotics. The most resisted drug being Erythromycin and Cloxacillin. These are among the most commonly used antibiotics for treatment of a broad range of bacterial infection, hence suggests possible abuse of these drugs. Increase in resistance of fish pathogens to a number of antibiotics and the presence of resistance genes in mobile plasmids and integrons in some pathogenic bacteria species (*Aeromonas, Yersinia, Photobacterium, Edwardsiella* and *Vibrio)* had been reported (Miller and Harbottle, 2018). Therefore, the observed resistance of the bacterial isolates to Ciprofloxacin and Oflaxacin were comparable, certainly because the two antibiotics belong to the same class of fluoroquinoes. Contrarily, although Cloxacillin and Ampicillin belong to the same class of β-lactams antibiotics, it was noted that resistance of the organisms to Cloxacillin was higher than resistance to Ampicillin; this could be attributed to more frequent use of the former. When the fishpond wastewater are discharged into water bodies, these resistant organisms enter the microbial communities and disrupts the physiology of indigenous microorganisms by increasing the number of resistant organisms through the transfer of resistance genes and consequently reducing susceptibility to antibiotics. Hence, the occurrence of these resistant organisms in the environment is becoming another issue of public health concern (Martinez, 2009; Hoa *et al*., 2011; Gao *et al*., 2012; Agwu, 2013). Romero *et al*. (2012) had noted the transfer of plasmid-borne resistance gene from a fish pathogen *Aeromonas salmonicida* to *Escherichia coli*, and also from a fish pathogen *Vibrio anguillarum* to *Vibrio cholera* the causative agent of cholera in humans.

A very high multiple antibiotic resistance (MAR) index was exhibited by the some isolates from this study. It is however noteworthy that comparable high MAR index had been noted earlier among microorganisms from Lagos Lagoon. Ajayi and Akonai (2003) reported 60% of isolates tested particularly enteric bacteria including *Klebsiella spp.*, *Enterobacter spp.* and *Escherichia* exhibiting multiple antibiotics resistance. Agwu (2013) encountered heterotrophic bacteria with MAR value of 0.57, while MAR value of 0.75 was also observed among *Escherichia coli* of the same Lagoon (Agwu and Oluwagunke, 2014). The occurrence of these isolates with high MAR values in the fishpond waste water further affirms that these isolates in the water body originated from sewage and wastewater discharged into the water body.

With the rapid spread of fishpond to supplement the depletion of capture fishery resources, the treatment of effluents prior to discharge will be of great help to the environment. Water recycling systems can be applied in ponds to reduce the quantity of effluent discharged into natural waters. Furthermore, the use of effluents to irrigate crops can serve as a good cost effective treatment option (Cao *et al*., 2007; Edwards, 2015). Other measures such as appropriate placing of nets or shades over ponds to avoid feacal contamination by birds and through rainfalls, sterilization of fish feed and manure including adequate pond water treatment and hygiene of pond workers has been proposed as measures to assist the microbiological quality of fish ponds (Andleeb *et al*., 2014). Although monitoring the use of antibiotics in fish farming is expedient, the use of probiotics, essential oils and phage therapy should also be considered as options for treatment of bacterial infections while vaccination can be used as a prophylactic measure (Lee *et al*., 2005; Romero *et al*., 2012). The implementation of these measures might seem a herculean task, however, a well-thought out strategy for adequate and suitable waste treatment, strict and regular monitoring of wastewater quality against stipulated standards and proper legislation will accomplish this challenge.

Conclusion

Considering the fact that these fish pond effluents will end up in our natural water bodies, the microbiological quality of the fish pond effluents reported in this study in addition to the level of multiple antibiotics resistance demonstrated by the isolates suggest that there is an urgent need for comprehensive waste management system in our aquaculture practice.

References

Agwu, O. (2013). Antibiotic resistance among heterotrophic bacteria in Lagos Lagoon, Nigeria. *African Journal of Aquatic Science*, 38(3): 331–336. http://doi.org/10.2989/16085914.2013.806886

Agwu, O. A., and Oluwagunke, T. O. (2014). Pattern of Multiple Antibiotics Resistance among Surface Water *Escherichia coli*, *Advances in Life Sciences*, 4(5), 213–219. http://doi.org/10.5923/j.als.20140405.01

Ajayi, A.O. and Akonai, K. (2003). Antibiotic Sensitivity Profile of Microorganisms Encountered in the Lagos Lagoon, Nigeria. *African Journal of Biomedical Research*, 6, 79–84.

Akpor, O.B. and Muchie, M. (2011). Environmental and public health implications of wastewater quality. *African Journal of Biotechnology*, 10(13): 2379–2387. http://doi.org/10.5897/AJB10.1797

Amosu, A.O., Hammed, A.M.. Togunde, K.A. Joseph, O.O. and Adekoya, A. (2017). Possible Aquaculture Development in Nigeria: Evidence for Commercial Prospects. *Journal of Agricultural Science and Technology* B7: 194- 205. doi: 10.17265/2161-6264/2017.03.007

Anand G., E., Das, S., Chandrasekar, K., Arun, G., and Balamurugan, S. (2010). Monitoring of Total Heterotrophic Bacteria and *Vibrio Spp*. in an Aquaculture Pond. *Current Research Journal of Biological Science*, 2(1): 48–52.

Andleeb, S., Maryam, M., and Aslam, A. (2014). Original Article Quantitative analysis of a fish pond for coliform bacterial content, *Punjab University Journal of Zoology*. 29(1):33–39.

APHA (2005). Standard methods for the examination of water and waste water. American Public Health Association, Washington D.C. p1000

Apun, K., Yusof, A. M., and Jugang, K. (1999). Distribution of bacteria in tropical freshwater fish and ponds. *International Journal of Environmental Health Research* 9: 285–292.

Barnet, H.L. and Barry, B.H. (1972). Illustrated genera of imperfect fungi. 3rd Ed. Burgess Publication Co., Minneapolis.

Baquero, F., Martínez, J. L and Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* 19: 260–265. https://doi.org/10.1016/j.copbio.2008.05.006

Boyd, C. E. (2003). Guidelines for aquaculture effluent management at the farm-level. *Aquaculture*, 226(1–4): 101–112. http://doi.org/10.1016/S0044-8486(03)00471-X

Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., and Diana, J. (2007). Environmental Impact of Aquaculture and Counter measures to Aquaculture. Pollution in China, *Environmental Science and Pollution Research International* 14(7), 452–462.

Danba, E.P., David, D.L. Wahedi, J.A., 3Buba, U., Bingari, M. S., & Umaru, F.F., Ahmed, M.K., Tukur, K.U., Barau, B.W., Dauda, U.D. and Thomas, T. L. (2015).

Microbiological Analysis of Selected Catfish Ponds in Kano. *IOSR Journal of Agriculture and Veterinary Science*, 8(8): 74–78. http://doi.org/10.9790/2380-08817478

Edwards, P. (2015). Aquaculture environment interactions: Past, present and likely future trends. *Aquaculture*, 447: 2–14. http://doi.org/10.1016/j.aquaculture.2015.02.001

Fafioye, O. O. (2011). Preliminary studies on water characteristics and bacterial population in high yield Kajola fish ponds. *Journal of Agricultural Extension and Rural Development*, 3(3): 68–71. Retrieved from http://www.jwildlifedis.org/doi/10.7589/0090-3558-41.2.334

FAO 2002. Food and Agricultural Organisation Publication year report, 2002. FAO Rome p81-94.

Gao, P., Mao, D., Luo, Y., Wang, L., Xu, B., and Xu, L. (2012). Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Research*, 46(7): 2355–2364. http://doi.org/10.1016/j.watres.2012.02.004

Grigorakis, K. and Rigos, G. (2011). Aquaculture effects on environmental and public welfare - The case of Mediterranean mariculture. *Chemosphere*, 85(6): 899–919. http://doi.org/10.1016/j.chemosphere.2011.07.015

Hoa, P. T. P., Managaki, S., Nakada, N., Takada, H., Shimizu, A., Anh, D. H., and Suzuki, S. (2011). Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. *Science of the Total Environment*, 409(15): 2894–2901. http://doi.org/10.1016/j.scitotenv.2011.04.030

Jensen, G. L., and Greenlees, K. J. (1997). Public health issues in aquaculture. Revue Scientifique et Technique (International Office of Epizootics), 16(2), 641–651.

Kay, D., Crowther , J., Stapleton, C.M., Wyer, M.D., Ewtrell, L.F., Edwards, A., Francis, C.A., McDonald, A.T., and Watkinson, J. (2008). Feacal indicator organisms concentrations in sewage and treated effluents. *Water Resources* 42: 442- 445.

Krieg, N.R. and Holt, J.G. (1994). Bergy's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore Ltd. USA.

Lee, T. X., Munekage, Y., and Kato, S. I. (2005). Antibiotic resistance in bacteria from shrimp farming in mangrove areas. *Science of the Total Environment*, 349(1–3): 95–105. http://doi.org/10.1016/j.scitotenv.2005.01.006

Macedo, C. F., Amaral, L. A., and Sipaúba-, L. H. (2011). Microbiology quality in continuous water flow fish ponds. Qualidade microbiológica em viveiros de criação de peixes com disposição seqüencial Ciências Agrárias, Londrina, 32 (2):701-708. http://doi.org/10.5433/1679-0359.2011v32n2p701

Martinez, J.L. (2009). Environmental pollution by antibiotic and antibiotic resistance determinants. *Environmental Pollution* 157: 2893- 2902.

Middleton, J. H., and Ambrose, A. (2005). Enumeration and Antibiotic Resistance Patterns of Fecal Indicator Organisms Isolated from Migratory Canada Geese (*Branta canadensis*). *Journal of Wildlife Diseases*, 41(2): 334–341. http://doi.org/10.7589/0090-3558-41.2.334

Miller R. A. and Harbottle H. (2018). Antimicrobial Drug Resistance in Fish Pathogens. *Microbiology Spectrum* 6(1): ARBA-0017-2017. doi:10.1128/microbiolspec.ARBA-0017-2017.

Ogbondeminu, F.S. and Olayemi, A. B. (1993). Antibiotic Resistance in Enteric Bacterial Isolates from fish and water media. *Journal of. Aquaculture in the Tropics*, 8: 207–212.

Onome, A.D. and Ebinimi, A. (2010). Comparative assessment of water quality parameters of fresh water earthen and stagnant concrete tanks for fish production in Port Harcourt Nigeria. *International Journal of Science and Nature*. 11: 34-37.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Frills, C., Jones, R., Nigutagale, C., Preston, R. and Waddell, J. (2014). Does the use of antibiotics in food animals pose a risk to human health? A critical review of public data. *Journal of Antimicrobial Chemotherapy* 53: 28-52.

Qin, Y., Hou, J., Deng, M., Liu, Q., Wu, C. and Ji, Y. (2016). Bacterial abundance and diversity in pond water supplied with different feeds. *Scientific Reports*. 1- 13. http://doi.org/10.1038/srep35232/

Reilly, A. and Kaferstein, F. (1999). Food safety and products from aquaculture. *Journal of Applied Microbiology Symposium Supplement*, 85, 249S–257S.

Rizzo L., Manaia C., Merlin C., Schwartz T., Dagot C., Ploy M.C., Michael I. and Fatta-Kassinos D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total Environment* 447: 345–360. https://doi.org/10.1016/jscitotenv.2013.01.032

Romero, J., Feijoo, C.G. and Navarrete, P. (2012). Antibiotics in Aquaculture – Use, Abuse and Alternatives, Health and Environment in Aquaculture, Dr. Edmir Carvalho (Ed.), ISBN: 978-953-51-0497-1, InTech, Available from: http://www.intechopen.com/books/health-and-environment-in-aquaculture/antibiotics- in-aquaculture-use-abuse-and-alternatives-

Saremi, A., Saremi, K., Sadeghi, M. and Sedghi, H. (2013). The effect of aquaculture effluents on water quality parameters of Haraz River. *Iranian Journal of Fisheries* Sciences 12(2): 445–453.

Tendencia, E. A. and De La Peña, L. D. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture*, 195(3–4): 193–204. http://doi.org/10.1016/S0044-8486(00)00570-6

Ugwumba, O.C. (2010). Profitability and technical efficiency of catfish production in Anambra State Nigeria. *Ph.D Thesis*. Department of Agricultural Economics and Extension, Delta State University, Abraka, Nigeria. pp 63.

Vasile, M. A., Metaxa, I., Plăcintă, S., Mogodan, A., Petrea, Ș. M., and Platon, C. (2017). Preliminary study on bacteriological and physicochemical water profile of cyprinid fish, AACL Bioflux, 10(1), 103–112.

Vaz-moreira, I. and Nunes, O. C. (2014). Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome, *FEMS Microbiology Review* 38(4), 761–778. http://doi.org/10.1111/1574-6976.12062