BIU Journal of Basic and Applied Sciences 4(1): 115 – 126, 2019.

ISSN: 2563-6424

ANTIBIOTICS SUSCEPTIBILITY PROFILE OF BACTERIA ISOLATED FROM FISH PONDS IN BENIN CITY

LUCKY OMOROGIUWA
Department of Biological Sciences (Microbiology Unit), Benson Idahosa University, Benin City, Nigeria
*Email: lomorogiuwa@biu.edu.ng

ABSTRACT
Fish farming is a recognized means of livelihood in Nigeria; however, the intensive use of antibiotics to prevent infection and increase fish yield calls for concern. This practice may lead to the growth of antibiotics resistant bacteria which contains resistant genes that can be transferred to human pathogens, causing treatment- resistant illness. This study investigated the antibiotics susceptibility profile of bacteria isolated from five fish ponds in Benin City using standard microbiological procedures. Isolates were identified using morphological characteristics and conventional biochemical tests, while antibiotic susceptibility test was determined by the Kirby-Bauer disc diffusion method. Results showed that total aerobic count of the ponds ranges from minimum 7.85 x 10^4 cfu/ml recorded in Ekewan fish pond to maximum 135.06 x 10^4 cfu/ml recorded in Sapele Road fish pond. Bacteria species isolated were *Staphylococcus aureus, Bacillus* spp *and Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp and *E. coli.* Frequency of occurrence and percentage distribution of the isolates showed that *Bacillus* spp had the highest while *Klebsiella* spp had the lowest. Community fish pond recorded the highest number of isolates while Adesuwa fish pond recorded the lowest number of isolates. Antibiotics susceptibility patterns showed that majority of the isolates were resistant to perfloxacin (85.18%) and amoxicillin (74.81%). It is suggested that the prophylactic and indiscriminate use of antibiotics may be a predisposing factor in the development of antibiotics resistance among the bacteria isolates. This may pose a threat to human population by transferring these resistant genes to other bacteria of human clinical significance.

KEYWORDS: Bacteria isolates, Susceptibility, Antibiotics, Fish ponds, Frequency

INTRODUCTION

Fish has a nutrient profile superior to all terrestrial meats (Feldhusen *et al*., 2000). 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*., 2017)

As the population of humans increase, the demand for fish will also grow flanking it (FAO, 2007). 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 (Vignesha *et al*., 2011). To meet the much needed demand for animal proteins, make profits and create jobs, people engage in fish aquaculture. A fish pond is a type of aquaculture usually filled with fresh water, fairly shallow and is usually non-flowing. Tidal ponds, reservoirs, storage tanks, raceway and fish farm tanks are not included (Wilcox, 1985). Fish ponds have been referred to be self-contained ecosystems which are often teeming with rich vegetable and diverse organisms (Olukunle and Oyewumi, 2017). However, fish farmers face huge loss as a result of infections by pathogenic bacteria, among the common fish pathogens are *Staphylococcous* sp*., Aeromonas* sp*., Salmonella* sp., *Shigella* sp., *Enterococcus faecalis, E. coli, Yersinia* sp*, V. cholera and* other Vibrios (Schmidt *et al*., 2000). Others are *Pseudomonas* sp. and *Streptococcous* sp. Diseases caused by these pathogenic bacteria include white- skin, haemorrhagical septicaemia, furunculosis etc. (Ponnerassery *et al*., 2012).

For optimum production, fish farmers use antibiotics for both prophylactic and remedial purposes. According to Cabello *et al*. (2006), large amount of antibiotics is used in the fish industry to avoid infection in developing countries. According to Vignesha *et al*. (2011) most antibiotics used in fish aquaculture are non-biodegradable and remain in the aquaculture environment for long periods of time. This may lead to increase in population of bacteria that can survive in the presence of these antibiotics, thereby acquiring resistance which can passed to their progenies. Beyond increased resistance in the ecosystem, these resistant bacteria can be transferred to humans leading to the development of infectious diseases which are difficult to treat and thereby pose public health risk.

Monitoring antibiotics resistant bacteria in animals reared for the purpose of human consumption is paramount for the regulation of resistance in animals and man; this will help to detect trends and changes of resistance pattern (WHO, 2001). According to World Health Organization for Animal Health (OIE), regular surveillance of resistant microorganisms in aquatic is necessary (Smith *et al*., 2013). Hafsat *et al*. (2015), reported the presence of multidrug resistant bacteria in fresh fish in Maiduguri. Olukunle and Oyewumi (2017) reported that the physicochemical parameters of two fish ponds investigated in Akure where within the recommended range for fish production. According to Dunba *et al*. (2015), *Staphylococcus aureus*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Shigella* spp, *Enterococcus faecalis* and *Enterobacter aerogenes* were found in fish pond in Taraba State. The aim of this research is to isolate, identify as well determine the antibiotics

susceptibility profile of bacteria species isolated from fish ponds in Benin City.

MATERIALS AND METHOD

Study Area

This research was conducted in the metropolitan area of Benin City (Lat. 6º 17' to 7º 12′N and Long. 5° 15' to 5° 41'E), the capital of Edo State in southern Nigeria. The average precipitation rate and relative humidity are 79.2mm and 87% respectively. Benin City experiences all year round rainfall which peaks around July, with a mean annual rainfall of 2980 mm.

Sample Collection.

Water samples were aseptically collected from five fish ponds within Benin City namely; Ekewan fish pond, GRA fish pond, Sapele Road fish pond, Adesuwa fish pond and Community fish pond. These fish ponds contained fishes of various sizes and ages, most of them were active and they range from two months to ten months. Community fish pond was constructed with large plastic container while the others were constructed with concrete, the average fish stock and depth ranged from 2000-3500 and 6-7 feet respectively. Samples were collected with a sterile bottle at a depth of 14 cm two times a day; morning and evening. The water samples were transferred to the laboratory for analysis.

Sterilization

The work bench was sterilized by cleaning with a wet swab (cotton wool soaked with 75% alcohol). The solid and liquid media were sterilized by autoclaving at 121°C for 15minutes. Glass wares such as conical flask and test tubes were sterilized in oven at 160°C for one hour. The inoculating loop and inoculating needle were sterilized by holding the nichrome wire in flame until it glowed red. The glass wares were allowed to cool before being used while the media was left to cool to 45°C.

Sample Preparation

The samples were shaken properly and diluted serially to sixth dilution using saline peptone water as the diluent; this diluent is used for maximum recovery of bacteria from a sample. About9ml of peptone water was put into test tubes (15ml) and autoclaved at 121°C for 15minutes and allowed to cool. Using sterile pipette sterilized at 160°C for 1hour in the oven, 1ml of pond water from each bottle was diluted to the sixth dilution. About 1ml of aliquot of each dilution was inoculated on nutrient agar, MacConkey agar, and Eosin Methylene Blue (EMB). Nutrient agar was used to determine the total aerobic bacterial heterotrophic count, while MacConkey agar and Eosin Methylene Blue (EMB) were used to isolate coliforms. Culturing of the bacteria was carried out using the pour plate method for MacConkey agar and Nutrient agar and streak plate method for the EMB agar. This was done in triplicate and was then incubated for 24-48 hours at 37°C.

Isolation and Identification of Isolates

After incubation, the morphology of colonies which appeared on different agar plates were observed. Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types onto freshly prepared nutrient agar plates. Discrete colonies, which developed on the plates were transferred into a nutrient agar slant and preserved in the refrigerator at 4ºC. Identification of isolates were done based on their motility, colonial morphology and

biochemical properties (Cheesbrough, 2006)

Identification of Isolates

Identification of isolates was done by cultural and microscopic analysis as well as conventional, physiological and biochemical test. Distinct colonies were picked from incubated plates, purified by sub culturing before being examined microscopically for Gram reaction cell morphology, pigmentation and biochemical analysis included.

Gram Staining Process

Smears of each colony that were picked were made on a grease free slide, after which the slides were flooded with crystal violet for 30 seconds. The stains were rapidly washed off under the tap, and then the water was tipped off. The slides were covered with iodine for 30seconds and washed off with clean water. The smears on the slides were decolourized rapidly using alcohol, which was immediately washed off again. The slides were placed on a rack for the smears to air dry. After drying, each slide was examined under the microscope.

Antibiotics Susceptibility Tests

Antibiotics susceptibility test were performed for the colonies by using the disc diffusion method as described by National Committee for Clinical Laboratory Standards (NCCLS, 1997), with antibiotics containing disc. Antibiotics used include Ofloxacin (30 µg), Chloramphenicol (30 µg), and Ciprofloxacin (10 µg), Amoxicillin (30µg), Augmentin (30µg), Gentamycin (10 µg), Perfloxacin (30 µg), Streptomycin (30 µg), and Erythromycin (19 µg). Respective colonies were streaked on nutrient agar, the disc were then transferred into the plate with a sterile forceps and incubated at 37^0C for 24 - 48 hours. The zone diameters for each antibiotic disc were translated in prefixed susceptibility (S) and resistant (R) categories by referring to the clinical laboratory standards (NCCLS, 1999). Resistance was recorded when there was no zone of inhibition around the respective disc and sensitivity was recorded when there was presence of inhibition.

RESULTS

Total Aerobic Bacteria Count

Table 1 shows the total aerobic bacteria count expressed in cfu x 10^4 obtained from the five fish ponds at different times of the day (morning and evening). In the morning, the mean total aerobic bacteria count ranged from minimum 8.43 x 10^4 cfu/ml recorded in Ekewan fish pond to maximum 115.17 x 10^4 cfu/ml recorded in Sapele road fish pond pond. In the evening, the mean total aerobic bacteria count ranged from minimum 7.25 x 10^4 cfu/ml recorded in Ekewan fish pond (GRA) to maximum 155.00 x 10^4 cfu/ml recorded in Sapele road fish pond. The average total aerobic bacteria count recorded in this analysis is 54 x 10^4 cfu/ml.

Table 1: Total Aerobic Bacteria Count

POND LOCATION	MEAN COUNT x 10^4 ±SD		
	MORNING	EVENING	AVERAGE
Ekewan Fish Pond	9.68 ± 1.05	10.77 ± 2.4	10.23 ± 1.73
G.R.A Fish Pond	100.3 ± 2.08	106. 8 ± 6.33	103 ± 4.21
Sapele Road Fish Pond	115.17 ± 1.04	155.00 ± 7.82	135.06 ± 4.43
Adesuwa Fish Pond	8.43 ± 1.60	7.25 ± 0.92	7.85 ± 1.26
Community Fish Pond	14.77 ± 4.07	13.25 ± 3.44	14.01 ± 3.76
			54.14 ± 1.47

Bacteria Isolated from the Fish Ponds

Table 3 showed the bacteria species isolated, they belonged to six genera. *Staphylococcus aureus, Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., and *E. coli* were present in Ekewan fish pond, Sapele road fish pond and Community fish pond. However, G.R.A fish pond had the above bacteria species except *Klebsiella* sp. while Adesuwa fish pond had the above isolates except *E. coli and Klebsiella* sp.

Table 3: Bacteria Species Isolated from the Fish Ponds

POND LOCATIONS	BACTERIA ISOLATES
Ekewan Fish Pond	*Staphylococcus aureus, Bacillus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. *and E. coli.*
G.RA Fish Pond	*Staphylococcus aureus, Bacillus* spp., *Pseudomonas* spp., *Proteus* spp. *and E. coli.*
Sapele Road Fish Pond	*Staphylococcus aureus, Bacillus* spp ., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp. *and E. coli.*
Adesuwa Fish Pond	*Staphylococcus aureus, Bacillus* spp., *Pseudomonas* spp, *Proteus* spp.,
Community Fish Pond	*Staphylococcus aureus, Bacillus* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., *and E. coli.*

Frequency and Percentage Distribution of Bacteria

Table 4 showed the frequency of occurrence of bacteria isolated from the five fish ponds. A total of 135 isolates belonging to six genera were recorded namely; *Staphylococcus aureus* (36), *Bacillus* sp. (40), *E. coli* (13), *Proteus* sp. (24), *Pseudomonas* sp. (18), *Klebsiella* sp. (4). *Bacillus* sp. had the highest 40 (29.63%), while *Klebsiella* sp. had the lowest 4 (2.96%). Community fish pond recorded the highest number of isolates (40) while Adesuwa fish pond recorded the lowest number of isolates (18)

Table 4: Frequency and Percentage Distribution of Bacteria

Isolates	Frequency of occurrence (no/percentage)					Total
	Ekewan Fish pond	G.R.A Fish Pond	Sapele Road Fish Pond	Adesuwa Fish Pond	Community Fish Pond	
Staphylococcus aureus	6 (28. 60%)	7 (24.14%)	9 (33.30%)	4 (22.22%)	10 (25.00%)	36(26.67%)
Bacillus sp.	3 (14.30%)	11 (37.93%)	7 (25.90%)	5 (27.78%)	14 (35.00%)	40(29.63%)
E. coli	3 (14.30%)	5 (17.24%)	3 (11.10%)	0 (0%)	2 (5.00%)	13(9.63%)
Proteus sp.	5 (23.80%)	2 (6.90%)	4 (14.80%)	6 (25.00%)	7 (17.50%)	24(17.78%)
Pseudomonas sp.	3 (14.30%)	4 (12.50%)	2 (7.40%)	3 (12.50%)	6 (15.00%)	18(13.33%)
Klebsiella sp.	1 (4.76%)	0 (0%)	2 (7.40%)	0 (0%)	1 (2.50%)	4(2.96%)
Total	21	29	27	18	40	135

Table 5: Antibiotics Susceptibility Patterns of Bacteria Isolated from Fish Ponds

Pond location	Isolates	AM	OFX	PEF	CPX	AU	GEN	S	E	CH
ESEOSA FISH POND	6	5(83.33%)	5(83.33%)	3(50%)	5(83.33%)	6(100%)	6(100%)	4(66.7%)	4(66.7%)	3(50%)
Staphylococcus aureus										
Bacillus sp.	3	0(0%)	0(0%)	0(0%)	3(100%)	3(100%)	0(0%)	0(0%)	0(0%)	2(66.6%)
E. coli	3	0(0%)	1(33.33%)	1(33.33%)	3(100%)	0(0%)	1(33.3%)	0(0%)	0(0%)	2(66.6%)
Proteus sp.	5	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	5(100%)	0(0%)	0(0%)	3(60%)
Pseudomonas sp.	3	0(0%)	0(0%)	1(33.33%)	2(66.67%)	0(0%)	0(0%)	0(0%)	0(0%)	1(33.33%)
Klebsiella sp.	1	0(0%)	0(0%)	1(100%)	1(100%)	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)
EMMA FISH POND										
Staphylococcus aureus	7	7(100%)	5(7.4%)	1(14.25%)	7(100%)	7(100%)	6(85.7%)	5(71.5%)	5(71.46%)	5(71.46%)
Bacillus sp.	11	0(0%)	5(45.45%)	0(0%)	6(59.54%)	10(90.9%)	0(0%)	11(100%)	0(0%)	1(100%)
E. coli	5	0(0%)	2(40%)	0(0%)	0(0%)	0(0%)	2(40%)	0(0%)	0(0%)	0(0%)
Proteus sp.	2	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	0(0%)	0(0%)	0(0%)	2(100%)
Pseudomonas sp.	4	0(0%)	0(0%)	0(0%)	2(50%)	2(50%)	1(75%)	0(0%)	2(50%)	1(25%)
Dr SAM FISH POND										
Staphylococcus aureus	9	8(88.90%)	6(60.66%)	1(11.11%)	3(33.3%)	0(0%)	5(55.6%)	3(33%)	1(11.11%)	5(55.55%)
Bacillus sp.	7	2(28.57%)	7(100%)	1(14.28%)	7(100%)	7(100%)	2(28.57%)	6(85.7%)	0(0%)	7(100%)
E. coli	3	0(0%)	1(33.33%)	1(33.33%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Proteus sp.	4	0(0%)	2(50%)	2(50%)	2(50%)	2(50%)	0(0%)	1(25%)	0(0%)	4(100%)
Pseudomonas sp.	2	0(0%)	1(50%)	1(50%)	1(50%)	0(0%)	0(0%)	1(50%)	0(0%)	2(100%)
Klebsiella sp.	2	1(50%)	0(0%)	0(0%)	2(100%)	2(100%)	0(0%)	0(0%)	0(0%)	0(0%)
OSULA FISH POND	4	0(0%)	2(50.0%)	4(100%)	4(100%)	4(100%)	4(100%)	4(100%)	2(50%)	2(50%)
Staphylococcus Aureus										

Bacillus sp.	5	1(20%)	0(0%)	0(0%)	3(60.0%)	5(100%)	0(0%)	0(0%)	0(0%)	5(100%)
Proteus sp.	6	0(0%)	3(50.0%)	0(0%)	3(50.0%)	0(0%)	6(100%)	0(0%)	0(0%)	3(50%)
Pseudomonas sp.	3	1(33.3%)	0(0%)	3(100%)	3(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
COMMUNITY FISH POND										
Staphylococcus aureus	10	6(60%)	5(50.0%)	0(0%)	6(60.0%)	10(100%)	5(50%)	5(50%)	10(100%)	10(100%)
Bacillus sp	14	6(42.86%)	10(78.5%)	0(0%)	10(71.4%)	8(57.1%)	0(0%)	5(38%)	4(28.6%)	0(0%)
E. coli	2	0(0%)	1(50.0%)	0(0%)	2(100.0%)	1(50%)	0(0%)	2(100%)	0(0%)	14(100%)
Proteus sp.	7	0(0%)	0(0%)	0(0%)	2(28.6%)	0(0%)	0(0%)	0(0%)	0(0%)	5(71.4%)
Pseudomonas sp.	6	0(0%)	6(100%)	0(0%)	2(33.3%)	0(0%)	1(16.6%)	0(0%)	3(50%)	5(83.3%)
Klebsiella sp.	1	1(100%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)

AM Amoxicillin, PEF Perfloxacin, OFX Ofloxacin CPX Ciprofloxacin, AU Augmentin, GEN Gentamycin, S Streptomycin, E Erythromycin, CH Chloramphenicol

DISCUSSION

Intensive use of antibiotics in domestic and commercial fish farming has resulted in the growth of antibiotics resistant bacteria in the aquatic ecosystem. These resistant bacteria can be transferred to humans directly or their resistant genes can be transferred to human pathogens (Hafsat *et al*., 2015.)

The presence of *Staphylococcus aureus, Bacillus* spp, *Esherichia coli, Proteus* spp*, Pseudomonas* spp *and Klebsiella* spp in the fish ponds under study agrees with earlier findings by Danba *et al*., (2015) who reported that these organisms are found in fish ponds. *Staphylococcus aureus* has been linked to bacteria sepsis; osteomyelitis, bacteraemia and otitis, in humans, while *Pseudomonas* spp can cause inflammation (Udeze *et al*., 2012). *E. coli* can cause diseases of the gastrointestinal tract, Bacillus spp can cause food poisoning and deep seated soft tissue infections and systemic infections (Tena *et al*., 2007). *Klebsiella* sp can cause a wide range of community related diseases like *pneumonia* and more generalized infections (Botelho-Nevers *et al*., 2007). *Proteus* spp has been implicated in urinary tract infection and septicemias (Endimiani *et al*., 2005).

The high bacteria load recorded in the fish pond located in Sapele road could be due to the nature of operation of the fish pond as well as maintenance, the owner of this fish pond occasionally apply organic manure on the fish pond. This practice has been associated with buildup of organic matter and bacteria in the pond environment. Sharmila *et al*. (1996), reported that bacteria load in pond ecosystem is greatly influenced by the presence of organic matter. High bacteria load could also be due run-off from roads and surrounding soil which can carry animal wastes into the pond. Birds and dogs which are free roaming animals can also be responsible for a significant source of fecal contamination of ponds (Green *et al*., 2012).

Ekewan fish pond recorded low total bacteria count probably because of good pond management which includes using continuous flow of water. This may prevent the buildup of bacteria load hence the low total aerobic bacteria count. There was a variation in the frequency and percentage distribution of the bacteria isolates. *Bacillus species* and *Staphylococcus aureus* recorded the highest frequency of occurrence and percentage distribution of 40 (29.63%) and 36 (26.67%) respectively. The high frequency of *Staphylococcus aure*us in this study compares with the findings of Dalgaard *et al*. (2006). The high presence of *Staphylococcus aure*us could be from the fish handlers as humans harbour Staphylococcus aureus as a normal flora (Danba *et al*., 2015)

Antibiotics susceptibility test was done for all the 135 isolates, the isolates showed varied resistance patterns to all the antibiotics used, 85.18% of the 135 isolates showed resistance to amoxicillin while 74.18% showed resistance to perfloxacin, this is consistent with earlier findings of Sunder *et al*., (2006). However majority of the isolates were very sensitive to chloramphenicol and ciprofloxacin.

The organisms from different genera also showed variable range of resistance to the antibiotics used, majority of the *E. coli* isolates showed absolute resistance to amoxicillin and a very low resistance to chloramphenicol. This finding is in

agreement with Samuel *et al*. (2011) who reported minimum resistance of *E.coli* isolated from cat fish pond to chloramphenicol. This finding is also supported by reports of Overdevest *et al*. (2011) that antibiotic resistance among Enterobacteriaceae has increased drastically during the past decade.

All the *Pseudomonas Species and Proteus Species* isolated from cat fish pond showed a high resistance to majority of the antibiotics used, this may be associated with the excessive or indiscriminate use of antibiotics (Macpherson *et al*., 1991; Schmiat *et al*., (2000). The high resistance of *Pseudomonas* sp. to antibiotics used in this study is consistent with earlier report by (Al-Jebouri and Al-Meshhadani, 1985), who reported that *Pseudomonas* sp. are known to be highly resistant to antimicrobial drugs. High incident of multiple resistance patterns in bacteria isolates fish have also be reported by (Hafsat *et al*., 2015).

CONCLUSION

Poor pond management could be a major reason for bacteria buildup in the pond ecosystem; some of these bacteria are pathogenic. The indiscriminate use of antibiotics for both prophylactic and remedial purposes can lead to the presence of antibiotic resistant bacteria in the pond environment. Their presence can be a major threat to public health. These bacteria can transfer resistant gene to other bacteria of human clinical significance.

REFERENCES

Al-Jebouri, M. M. and Al-Meshhadani, N. S. (1985). A note on antibiotic resistant *Escherichia coli* in adultman, raw sewage and sewage polluted River Tigris in Mosul, Nineva. *Journal Applied Bacteriology,* 59: 513-518.

Botelho-Nevers, E., Gouriet, F., Lepidi, H., Couvret, A., Amphoux, D., Dessi, G. and Raoult, P., (2007). Chronic nasal infection caused by *Klebsiella* rhinoscleromatis or *Klebsiella* ozaenae: Two forgotten infectious diseases. *International Journal of Infectious Disease*, 11: 423-429.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7): 1137-44.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Cambridge University Press. pp. 62.

Dalgaard, P., Samieia, N. and Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (*Belonebelone*) – effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology*, 101: 80 – 95.

Danba, E. P., David, D. L., Wahedi, J. A., Buba, U., Bingari, M. S., Umaru, F. F., Ahmed, M. K., Tukur, Barau, B.W., Dauda, U.D. and Thomas, T. L. (2015). Microbiological Analysis of Selected Catfish Ponds in Kano Metropolis, Nigeria. *Journal of Agriculture and Veterinary Science*, 8(8): 74-78.

Doyle, E. M. (2007). Microbial food spoilage-losses and control

strategies. Assessment of fish and fish products. *Weekly Report.* 17: 13 -14.

Endimiani, A., Luzzaro, F., Brigante, G., Perilli, M., Lombardi, G., Amicosante, G. Rossolini, M. and Toniolo, A. (2005). *Proteus mirabilis* bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. *Antimicrobial Agents Chemotherapy*, 49(7): 2598-2605.

Green, H. C., Dick, L. K., Gilpin, B. M., Samadpour, M. and Field, K. G. (2012). Genetic markers for rapid PCR based identification of gull, Canada goose, duck and chicken fecal contamination in water. *Applied Environmental Microbiology,* 78(2): 503-510.

Hafsat, A. G., Yaqub, A. G., Abubakar, S., Isa, A. G. and Roy, B. B. (2015). Multi-Drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria. *International Journal of Animal and Veterinary Advances,* 7(3): 49-54.

Feldhusen, F. (2000). The role of sea food bacterial food-borne diseases. *Microbes and Infections,* 2: 1651 – 1660.

McPhearson, R. M., DePoala, A., Zywno, S. R., Motes, M. L. and Guarino, A. M. (1991). Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture,* 99: 203 -211.

Olukunle, O. F. and Oyewumi, O. O (2017). Multi-Drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)* 2(2): 2456-1878.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens X. and Kluytmans, J. (2011). Extended spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerging Infectious Diseases Journal,* 17(7): 1216-1222.

Ponnerassery, S. S., Aliya, A., Nashwa, A., and Saoud, A. (2012). Comparative Pathogenomics of Bacteria Causing Infectious Diseases in Fish. *International Journal of Evolutionary Biology,* 10: 11-36.

Samuel, L., Marian, M. M., Apun, K., Lesley, M. B. and Son, R. (2011). Characterization of *Escherichia coli* isolated from cultured catfish by antibiotic resistance and RAPD analysis. *International Food Research Journal* 18(3): 971-976.

Sarter, S. H., Nguyen, N. K.., Hung, L. T., Lazard, J. and Montet, D. (2007). Antibiotic resistance in Gram negative bacteria isolated from farmed catfish. *Food Control* 18: 1391-1396.

Smith, P.V., Alday-Sanz, J., Matysczak, G., Moulin, C. R., Lavilla, P., Prater, D. (2013). Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. *Revue scientifique et technique,* 32(2): 583-593.

Schmidt, A. S., Bruun, M., Dalsgaard, S. I., Pedersen, K. and Larsen, J. (2000). Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with Danish rainbow trout farms. *Applied and Environmental Microbiology,* 66: 4908- 4915.

Sharmila, R., Abraham T. J. and Sundararaj, V. (1996). Bacterial flora of semi-intensive pond-reared *Penaeusindicus* (H. Milne Edwards) and the environment. *Journal of Aquaculture in the. Tropics*, 11: 193-203.

Sunder, J., Jeyakumar, S. P. S., Ahlawat, P. S., Rai, R. B., Kundu, A., Senalis, S., Chatterjee, R. N., Saha, S. A. and Yadav, S. (2006). Antibiotic resistance pattern of bacterial isolates from fishes of Andaman and Nicobar Islands. *Indian Journal of Fisheries,* 53(2): 231-235.

Tena, D., A´ngel Martı´nez-Torres, J., Pe´rez-Pomata, M.T., Sa´ez-Nieto, J.A., Rubio, V., and Bisquert, J. (2007). Cutaneous infection due to *Bacillus pumilus:* Report of 3 cases. *Clinical Infectious Disease*, 44: 40-42.

Emmerson, M. (1994). Nosocomial Staphylococcal outbreak. *Scandinavian Journal Infectious Disieases Suppl*. 93: 47-54

Udeze, A.O., Talatu, M., Ezediokpu, M. N., Nwanze, J. C., Onoh, C. and Okonko, I. O. (2012). The effect of *Klebsiella pneumoniae* on catfish (*Clarias gariepinus*). Reseacher 4(4): 51-59.

Vignesha, R., Karthikeyanb, B.S., N. Periyasamya, N., and K. Devanathana, K. (2011). Antibiotics in aquaculture: An overview. *Southern Asian Journal of Experimental Biology*, 1:3-13.

WHO (2001). Global strategy for containment of antimicrobial resistance. World Health Organization, Geneva, Switzerland, pp: 1-96.

Wilcox, L. V. (1985). Classification and use of irrigation water. US Department of Agriculture, CIRC 696 Washington DC.

National Committee for Clinical Laboratory Standards (NCCLS) (1997). Performance standard for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals; tentative standard. NCCLS document M31-T. Villanova, PA: NCCLS.

Zárate, R., Nabilel, J., Noemi, T., José, A. P., and Covadonga, R. (2017). Signifcance of long chain polyunsaturated fatty acids in human health. *Clinical Translational Medicine*, 6:25