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Pengujian sensitivitas antibiotik isolat mikroba yang berasal dari panti pembenihan ikan

Assessment of antibiotics sensitivity of microbial isolates from fish hatcheries

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Abstract

The study assessed the antibiotic sensitivity of bacterial isolates from fish hatcheries. The result revealed that bacteria of public health importance were isolated, however, the TVC did not exceed 10^5 CFU/g. A total of 16 isolates were detected. Thirteen (13) antibiotics recorded over 50% resistance, with the highest resistance (100%) observed in amoxicillin clavulanate (AUG). Ofloxime (OFX), gentamycin (GN) and levofloxacin (LBC) recorded the lowest resistance rates of 18.75%, 37.5% and 43.75% respectively. Only these antibiotics with the least recorded resistance had susceptibility ranging between 50% (LBC) and 68.75% (OFX). The overall average resistance of the isolates to antibiotics was 68.75%; susceptibility was 26.67% and intermediate 4.58%. Isolates 15 showed the highest resistance of 100%, followed by isolates 12 and 10 with 93.33% and 93.33% resistance, respectively. A resistance range of 60% to 86.67% was observed in isolates 2, 4, 5, 6, 7, 9, 13, 14, 16, and 17. Isolates 3, 8 and 11 had resistance below 50% ranging between 26.67% and 46.67%. The highest multiple antibiotic resistance index (MARI) was observed in isolate 15 with a MARI of 1. Isolates 10 and 12 had a MARI of 0.93. This was followed in descending order by isolates 2 and 9 (0.86), isolate 5 (0.8), isolate 14 (0.73), isolates 6 and 7 (0.67) and isolates 4, 13, 16 and 17 (0.6). The least MARI recorded were observed in isolates 3 and 11 (0.47) and isolate 8 (0.27), respectively. The emergence of antibiotic resistance on fish farms in the Ijebu-Ode region calls for public health intervention strategies.

Keywords: antibiotic; fish health; isolates; resistance.

1. Introduction

Many fisheries resources have been over-exploited with many currently depleted and unable to support the global demand for seafood. Aquaculture is seen as a key industry in satisfying the growing demand for food for human consumption. Currently, aquaculture supplies more than 50% of all the seafood produced for human consumption, having increased production from 2.6 to 60.4 million tons per year between 1970 and 2010 with a mean annual growth rate of 7.8% (Troell *et al*., 2014). This makes the aquaculture industry the fastest-growing food-production industry in the world (FAO, 2014). However, this production is hampered by unpredictable mortalities that may be due to negative interactions between fish and pathogens.

Globally, antibiotics are not only utilized in human medicine but also in livestock to treat bacterial infections and/or to promote animal growth (Du and Liu, 2012) and aquaculture is not exempt from this practice (Cabello, 2006; Romero *et al*., 2012; Durojaiye *et al*., 2019). Excessive use of antibiotics in aquaculture in many countries has caused problems and concerns due to the development of bacterial resistance, food safety hazards and environmental issues (World Health Organization, 2016). However, the amount of antibiotics used in aquaculture worldwide is very difficult to estimate due to unavailability of information from many countries or due to gaps in available data, making it impossible to have accurate estimates (Heuer *et al*., 2009; Romero *et al*., 2012). Hence, the use of alternative approaches like sensitivity tests will help in evaluating the extent of use or misuse of antibiotics in the aquaculture industry, especially in developing countries like Nigeria where use of antibiotics has been established (Durojaiye and Sule, 2018; Durojaiye *et al*., 2019; Durojaiye *et al*., 2020).

A sensitivity test involves checking the effectiveness of a drug against a bacterium (Lalitha, 2004). The test reveals possible drug resistance in common pathogenic organisms (Tiamiyu *et al.,* 2015). It has been observed that in fish health management, antibiotics are used based on the symptoms without proper diagnosis and sensitivity tests. Several studies have shown that a majority of farmers in the Ijebu-Ode Region, Ogun State, Nigeria, use antibiotics (Durojaiye and Sule, 2018; Durojaiye *et al*., 2019; Durojaiye *et al*., 2020). However, the studies didn't report the rate of use in order to ascertain if

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antibiotics are being abused or not. Hence, this study was carried out to provide accurate information on possible antibiotics abuse.

2. Materials and Methods

2.1. Study area

The study was carried out in Ijebu-Ode Local Government Area of Ogun State. The city is located in South Western Nigeria, with estimated population of 218,600 and is the second largest city in Ogun State after Abeokuta. Fish farming is well established in Ijebu-Ode Local Government and this can be justified by the fact that each area in the Local Government has at least five fish farms including homestead system (preliminary survey).

2.2. Sample collection

Samples of *Clarias gariepinus* fingerlings were randomly collected from hatcheries in three regions (A, B, C) within Ijebu-Ode. A total of 50 samples were collected from each region. The samples were transported live to the laboratory for bacteriological examination in plastic containers filled with water from the hatchery each sample was taken from. To control temperature increase, the plastic containers were placed in insulated coolers filled with ice.

2.3. Sample preparation

Materials such as glass wares and media were sterilized before use. The glass including McCarteny bottles, test tubes, Petri dishes, conical flasks, measuring cylinders, and pipettes were washed with detergent, rinsed thoroughly with clean tap water and allowed to air dry followed by sterilization in a hot air oven at 160^{o}C for an hour. The media used include Salmonella Shigella Agar (SSA), Nutrient Agar (NA), Potato Dextrose Agar (PDA), Tryptone Soy Broth (TSB), Triple Sugar Iron Agar (TSI), Simon Citrate Agar, Gram reagent, Thiosulphate Citrate Bile Salt Agar (TCBS) and Muller Hinton Agar (MHA). These media were prepared according to the manufacturer's specifications.

2.4. Dilution

The fish from each farm were separated from the water using a sieve. The samples were put into a laboratory mortar and poured into a paste. Five (5) gram of the mashed fish from each farm was weighed out using the laboratory weighing scale. Serial dilution of the sample was done. 45ml of distilled was measured into a 100 ml conical flask and sterilized using an autoclave for 15 minutes, 1 atmosphere and 121^{0}C and cooled. The 5g fish paste was introduced into the 45ml sterilized and distilled water, shaken and allowed to stand. This was done to ensure a 1:9 ratio standard for microbial dilution techniques. The objective of the serial dilution is to estimate the concentration (number of colonies) by counting the colonies of the organism cultured from serial dilution and then backtracking the measured count to the unknown concentration).

The concentration (number) of viable microorganisms was estimated from a dilution plate. This was done in duplicates to ensure accuracy. The media used for total Viable Count (TVC) was Nutrient Agar and it was prepared according to the manufacturer's instructions. 1ml of the aliquot (inoculums) was taken from the original concentration (10^{0}) and transferred serially into also already prepared 9ml sterile and cooled water.

2.4 Isolation of microorganisms

The samples were serially diluted and microbes were isolated using the pour plate method (Harrigan and McCance, 1976). Synthetic media were used for the isolation of organisms according to the manufacturer's instructions.

2.5. Enumeration of Total Heterotrophic count

This was done by plating out an aliquot of the serially diluted sample on Nutrient agar in duplicate. The plates were inverted and incubated at 35^{0}C for 18-48hrs. After 24 hours of cultivation, the result was taken for the TVC (Total viable count).

2.6. Enumeration of Total Coliforms

This was done using the most probable Number (MPN) method. This was done to enumerate the number of coliforms in the sample. This was carried out by preparing 10 (Ten) tubes of 10ml single-strength lactose broth. 1 ml of the same was added to five of the tubes while 0.1 ml of the sample was added to a second set of 5 tubes. A 10ml double strength was also prepared in 5 tubes and a 10ml sample was added to each of the five tubes and incubated for 48 hours to check for gas production in the inverted Durham tube in the lactose broth.

2.7. Other organisms' detection of public health importance

The presence of other microbes of public health importance was done by plating out an aliquot of the serially-diluted sample on *Salmonella Shigella* Agar (SSA), Thiosulphate citrate bile salt (TCBS) Agar, for the detection of salmonella and vibrio species, respectively. The morphology and cultural characteristics of the colonies were observed and a subculture was done to contain a pure culture from the mixed culture. The pure culture obtained were stored in TSB (Trypon soy Broth) after which further biochemical test were carried out to further as certain the type of bacteria isolated from the fish. A Gram reaction was done on the pure culture to know if the organisms were Gram-positive or Gram-negative.

2.8. Simon citrate test

This is usually done to differentiate Gram-negative bacteria on the basis of citrate utilization. It is useful for selecting organisms that use citrate as their main carbon and energy source.

2.9. Antibiotics sensitivity test

Randomly selected bacterial isolates were tested for their sensitivity to antibiotics. The antimicrobial activity of commercially available antibiotics on the isolates was compared by growth inhibition using the modified disk-agar diffusion method on Nutrient Agar as recommended by Stukus (1997). Two (2) mls of the pure culture of the isolates in the tryptone soy Broth were taken into a fresh 8ml TSB into Cryo tubes. The isolate grown in the fresh TSB was centrifuged at 4000 rpm for 10 minutes to harvest enough cells. 3mls of already prepared sterile phosphate buffer (0.05M, pH7) was added. The 100ml of the buffer contains 0.05g of Na_2HPO_4 and 0.03g of KH_2PO_4. This was mixed using a vortex mixer to ensure adequate mixing. 700 microlitre (µl) of the vortexed cells were pipette on the already prepared MHA and spread evenly on the petri dish. An antibiotic was dropped on the plate. It was done with so much care not to shift after it had touched the plate and incubated at 35^{0}c for 18-24hrs. The zones of clearance were measured using a plate ruler. The isolates were defined as resistant (R), intermediate (I) or susceptible (S) according to the Clinical and Laboratory Standards Institute, CLSI (2012).

From the results of the sensitivity test, 5 isolates with the highest resistance were subjected to polymerase chain reaction (PCR) after the DNA of the isolates were extracted following the

method of Omoya and Ajayi (2020). The PCR products obtained were sequenced and sequences were compared to the sequences available in the GenBank using the National Center for Biotechnology Information (NCBI), Basic Local Alignment Search Tool (BLAST).

3. Results and Discussion

3.1. Results

3.1.1. Total heterotrophic and Coliform count

Table 1 shows the results of the microbial load of the samples collected. The least microbial load was 1.099 X 10^4 cfu/g (Region B) while the highest was 3.970 X 10^4 cfu/g (Region C) with an average range of 2.139 X 10^4 to 3.921 X 10^4 cfu/g. As indicated in Table 2, microorganisms of public health detected in the samples include Salmonella, Shigella and Vibrio. The three microorganisms were present in all regions sampled except Salmonella (Region C). After 48 hours of incubation, there was no gas production. Hence, the most probable number (MPN) is 0/100ml indicating that no coliform bacteria were detected in the samples.

Table 1
Microbial load of samples (Total Viable Count -cfu/g)

Dilution	Region A	Region B	Region C
10^{-4}	3.219 X 10^4	1.099 X 10^4	3.970 X 10^4
	2.708 X 10^4	3.178 X 10^4	3.871 X 10^4
Average	2.964 X 10^4	2.139 X 10^4	3.921 X 10^4

Table 2
Presence of microorganisms of public health importance.

Parameter Tested	Region A	Region B	Region C
Salmonella	Present	Present	Absent
Shigella	Present	Present	Present
Vibrio	Present	Present	Present

3.1.2. Biochemical and cultural characterisation of isolates

The results for biochemical and cultural characterization of the isolates are presented in Tables 3 and 4 respectively. A total of 16 isolates were detected. All the isolates were Gram-negative representing 5 genera (Shigella, Salmonella, Citrobacter, Vibrio and Pseudomonas). Vibrio had the highest frequency of occurrence (50%), followed by citrobacter (31.25%). Pseudomonas, Shigella and Salmonella occurred at the same frequency (6.25%).

Table 3
Biochemical Characterisation of Isolates from Various Hatcheries

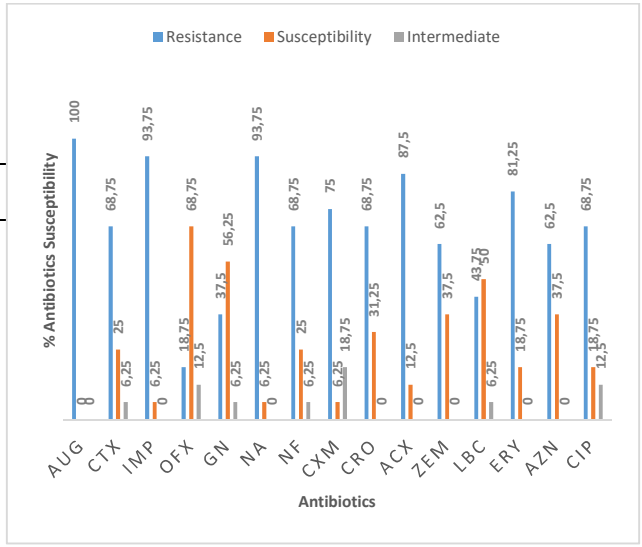
Isolate code	Suspected organism	Gram reaction	Catalase
2	*Shigella spp*	-	-
3	*Salmonella spp*	-	-
4	*Citrobacter spp*	-	-
5	*Vibrio spp*	-	-
6	*Citrobacter spp*	-	-
7	*Vibrio spp*	-	-
8	*Vibrio spp*	-	-
9	*Vibrio spp*	-	-
10	*Citrobacter spp*	-	-
11	*Vibrio spp*	-	-
12	*Vibrio spp*	-	-
13	*Vibrio spp*	-	-
14	*Citrobacter spp*	-	-
15	*Citrobacter spp*	-	-
16	*Vibrio spp*	-	-
17	*Pseudomonas spp*	-	-

Table 4
Cultural characteristics of Isolates.

Isolate Code	Cultural characteristics	Suspected organism
2	Cream-raised circular colony	*Shigella spp*
3	Large Cream colony	*Salmonella spp*
4	Tiny Cream colony	*Citrobacter spp*
5	Small Cream colony	*Vibrio spp*
6	Tiny Cream colony	*Citrobacter spp*
7	Flat Cream colony	*Vibrio spp*
8	Small Cream colony	*Vibrio spp*
9	Black circular colony	*Vibrio spp*
10	Pink circular colony	*Citrobacter spp*
11	Black circular colony	*Vibrio spp*
12	Pink circular colony	*Vibrio spp*
13	Pink circular colony	*Vibrio spp*
14	Black circular colony	*Citrobacter spp*
15	Black circular colony	*Citrobacter spp*
16	Yellow circular colony	*Vibrio spp*
17	Yellow circular colony	*Pseudomonas spp*

3.1.3. Antibiotics sensitivity pattern of isolates

Figure 1 represents the antibiotic sensitivity pattern of all isolates to antibiotics. Fifteen (15) antibiotics were used for the sensitivity test. They are amoxicillin clavulanate: AUG(30µg), imipenem/cilastain: IMP (10/10 µg), cefuroxime: CXM (30 µg), ofloxime: OFX (5 µg), erythromycin: ERY (10 µg), gentamycin: GN (10 µg), azthromcin: AZN (15 µg), cefotaxime: CTX (25 µg), certriaxione: CRO (25 µg), levofloxacin: LBC (5 µg), ciprofloxacin: CIP (5 µg), nalidixic: NA (30 µg), nitrofuration: NF (300 µg), ampiclox: ACX (10 µg) and cefexime: ZEM (5 µg). Thirteen (13) antibiotics recorded over 50% resistance, with the highest resistance (100%) observed in amoxicillin clavulanate (AUG). Ofloxime (OFX), gentamycin (GN) and levofloxacin (LBC) recorded the lowest resistance of 18.75%, 37.5% and 43.75% respectively. Only these antibiotics with the least recorded resistance had susceptibility ranging between 50% (LBC) and 68.75% (OFX). The overall average resistance of the isolates to antibiotics was 68.75%; susceptibility was 26.67% and intermediate 4.58%.



amoxicillin clavulanate: AUG(30µg), imipenem/cilastain: IMP (10/10 µg), cefuroxime: CXM (30 µg), ofloxime: OFX (5 µg), erythromycin: ERY (10 µg), gentamycin: GN (10 µg), azthromcin: AZN (15 µg), cefotaxime: CTX (25 µg), certriaxione: CRO (25 µg), levofloxacin: LBC (5 µg), ciprofloxacin: CIP (5 µg), nalidixic: NA (30 µg), nitrofuration: NF (300 µg), ampiclox: ACX (10 µg) and cefexime: ZEM (5 µg).

Figure 1. Antibiotics Sensitivity Pattern of All Isolates to Antibiotics

The result for the antibiotic sensitivity pattern for individual isolates is presented in Table 5. Isolates 15 showed the highest resistance of 100%, followed by isolates 12 and 10 with 93.33% and 93.33% resistance, respectively. A resistance range of 60% to 86.67% was observed in isolates 2, 4, 5, 6, 7, 9, 13, 14, 16 and 17. Isolates 3, 8 and 11 had resistance below 50% ranging between 26.67% and 46.67%. The highest multiple antibiotic resistance index (MARI) was observed in isolate 15 with MARI of 1. Isolates 10 and 12 had MARI of 0.93. This was followed in descending order by isolates 2 and 9 (0.86), isolate 5 (0.8), isolate 14 (0.73), isolates 6 and 7 (0.67) and isolates 4, 13, 16 and 17 (0.6). The least MARI recorded were observed in isolates 3 and 11 (0.47) and isolate 8 (0.27), respectively. In Table 6, the results for PCR identification of isolates with the highest resistance are presented. The organisms identified are *Enterobacter cloacae, Pseudomonas aeruginosa, Providencia rettgeri, Citrobacter fruendi* and *Enterobacter sp.*

Table 5
Antibiotics Sensitivity Pattern of Individual Isolates to Antibiotics

code	Antibiotic susceptibility															Susceptibility %		
	AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC	ERY	AZN	CIP	R	I	S
2	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	86.67	0	13.33
3	R	S	R	S	S	R	R	I	S	R	S	I	R	R	I	46.67	20	33.33
4	R	R	R	S	I	R	I	I	R	R	R	S	R	S	R	60	20	20
5	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	80	0	20
6	R	R	R	S	S	R	R	R	R	R	R	S	R	S	I	66.67	6.67	26.67
7	R	R	R	S	S	R	S	R	R	R	S	S	R	R	R	66.67	0	33.33
8	R	S	R	S	S	S	S	R	S	S	R	S	S	S	S	26.67	0	73.33
9	R	R	R	I	R	R	R	R	R	R	R	R	R	S	R	86.67	6.67	6.67
10	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	93.33	6.67	0
11	R	S	R	S	S	R	R	S	S	R	S	R	S	R	S	46.67	0	53.33
12	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	93.33	0	6.67
13	R	R	R	S	S	R	S	R	R	R	S	S	R	R	S	60	0	40
14	R	R	R	S	S	R	S	R	R	R	R	R	S	R	R	73.33	0	33.33
15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100	0	0
16	R	I	R	S	R	R	R	I	S	R	S	S	R	R	R	60	13.33	26.67
17	R	S	R	S	R	R	R	R	S	S	S	R	R	S	R	60	-	40

S: Susceptible I: Intermediate R: Resistant

amoxicillin clavulanate: AUG(30µg), imipenem/cilastain: IMP (10/10 µg), cefuroxime: CXM (30 µg), ofloxime: OFX (5 µg), erythromycin: ERY (10 µg), gentamycin: GN (10 µg), azthromcin: AZN (15 µg), cefotaxime: CTX (25 µg), certriaxione: CRO (25 µg), levofloxacin: LBC (5 µg), ciprofloxacin: CIP (5 µg), nalidixic: NA (30 µg), nitrofuration: NF (300 µg), ampiclox: ACX (10 µg) and cefexime: ZEM (5 µg).

Table 6.
PCR Identification of isolates with the highest resistance

S/N	Isolate Number	Organism identified
1.	2	*Enterobacter cloecae*
2.	9	*Pseudomonas aeruginosa*
3.	10	*Providencia rettgeri*
4.	12	*Citrobacter freundi*
5.	15	*Enterobacter sp*

3.2. Discussion

The total viable counts (TVC) observed in this study was lower compared to the findings of Adedeji *et al*. (2012), Hilary *et al*. (2018) and Ogunleye *et al*. (2021). Bacteria of public health importance were isolated, however, the TVC did not exceed 10^5 CFU/g approved by FAO (1979). The reduction in the bacterial load observed in this finding might be related to the use of antibiotics for fish health management in this region as earlier reported by Durojaiye and Sule (2018) and Durojaiye *et al*. (2019).

Antibiotics are administered to prevent various disease and parasitic infections in fish (FAO, 2016). However, antibiotic resistance develops when antibiotics are used indiscriminately (Cabello, 2006). Antibiotic resistance has been reported in fish production (Akinbowale *et al*., 2006; Adedeji *et al*., 2011). Findings from this study revealed that bacteria isolated showed varying degrees of antibiotic resistance with a majority of the isolates having over 50% resistance. The isolates with the highest resistance were identified using PCR. These organisms have varying ranges of pathogenicity in fish, wildlife and humans. *Enterobacter cloacae* and *Enterobacter sp* have been reported to cause blood poisoning, urinary tract infections and meningitis in newborns. *Pseudomonas aeruginosa* is capable of causing diseases in both plants and humans. It has been reported to be a multi-drug resistant pathogen which supports the findings from this study. Hence, treatment of *P. aeruginosa* infections can be difficult. *Providencia rettgeri* and *Citrobacter freundi* are associated with blood infections, hence, their presence and antibiotic resistance is of public health concern.

4. Conclusion

The emergence of antibiotic resistance on fish farms in Ijebu-Ode region calls for public health intervention strategies. There is need to for aggressive awareness campaigns of dangers of antibiotics resistance. In addition, future researches in fish health management should focus on safe alternatives like phytobiotics and synbiotics.

References

Adedeji, O.B., Emikpe, B.O., and Adebisi, T. 2011. Bacterial load on the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus* form Ibadan, South West Nigeria: Public health implicatios. *Journal of Microbiology and Biotechnology Research*, 1(1): 52-59.

Adedeji, O.B., Okerentugba, P.O., Innocent, D.E., Adiele, H.C., and Okonko, I.O. 2012. Benefits, public health hazards and risks associated with fish consumption. *New York Science Journal*, 5(8): 2039-2047.

Akinbowale, O.L., Peng, H., and Barton, M.D. 2006. Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Aquaculture*, 20(2): 108-119.

Cabello, F.C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environment. Microbiol*, 8: 1137-1144.

Clinican Laboratory Standard Institute (CLSI) 2012. Current Clinical and laboratory Standard Institute Criteria for interpretation of susceptibility testing of carbapenems in Enterobacteriaceae. *CLSL document M100*, S22.

Du, L., and Liu, W. 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustain. Dev.,* 32: 309–327.

Durojaiye, A.F., Ojetayo, T.A., Sule, S.O., Balogun, T.E., and Akintuyole, A.S. 2020. Incidence of Diseases in Fish Hatcheries and Control Measures Adopted by Fish Farmers in Ijebu-Ode Region, Ogun State, Nigeria. *FUW Trends in Science and Technology,* 5(1): 083-086.

Durojaiye, A.F., Sule, S.O. and Ojetayo, T.A. 2019. Health Management Practices Adopted in Fish Production at Eriwe Fish Farming Community, Ogun State, Nigeria. *Nigerian Journal of Scientific Research,* 18: 503-511.

Durojaiye, A.F., and Sule, S.O. 2018. A Preliminary Assessment of Aquamedicines used at Eriwe Fish Farm Community, Ogun State, Nigeria. *Proceedings of Fisheries Society of Nigeria 33rd Annual Conference, 28th October-2nd November*, *Ikorodu, Lagos*.

Food and Agriculture Organisation, FAO 1979. CFU g^{-1} as approved by Food and Agricultural Organization (FAO, 1979). http://www.fao.org/3/T0610E/T0610E.pdf.

Food and Agriculture Organisation, FAO 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

Food and Agriculture Organization FAO. 2014. The State of World Fisheries and Aquaculture 2014. Rome. 223 pp www.fao.org/3/a-i3720e.pdf.

Harrigan, W.F., and McCance, M.E. 1976. Laboratory methods in food and diary microbiology (2nd ed). Academic press Inc. London. https://agris.fao.org.

Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I., and Angulo, F. J. 2009. Human health consequences of use of antimicrobial agents in aquaculture. *Clin. Infect. Dis.* 49: 1248–1253.

Hilary, A.O., Musa, O.N., Mercy, M., Joseph, W., and Patrick, M.K. 2018. Microbiological safety of fresh tilapia (Oreochromis niloticus) from Kenyan Freshwater fish value chains. *Journal of Food Protection,* 81(12): 1973-1981.

Lalitha, MK. 2004. Manual on antimicrobial susceptibility testing: Indian Association of Medical Microbiologists. pp. 3-4.

Ogunleye, S.C., Ishola, O.O., Faroyin, O.M., and Adedeji, O.B. 2021. Total aerobic counts from Oreochromis niloticus obtained from selected frms in Ibadan. *Sokoto Journal of Veterinary Sciences,* 19(1): 55-60.

Omoya, F.O., and Ajayi, A.T. 2020. Assessment of the microbial tuality of seafood and effects of salt concentration and temperature on isolated microorganisms. *Journal of Microbiology and Antimicrobials*, 12(1): 17-31.

Romero, J., Feijoó, C. G., and Navarrete, P. 2012. Antibiotics in Aquaculture Use, Abuse and Alternatives. *Rijeka: INTECH Open Access Publisher*.

Stukus, P.E. 1997 Antimicrobial testing. The Kirby-Bauer method (filter paper disk method) in Stukus, P.E (Ed) Investigating microbiology: a laboratory manual for general microbiology. *Oriando Harcourt Brace & Company cap*., 44. P243-247.

Tiamiyu, A.M., Soladoye, M.O., Adegboyega, T.T., and Adetona, M.O. 2015. Occurrence and Antibiotic Sensitivity of Bacterial Strains Isolated from Nile Tilapia, *Oreochromis niloticus* Obtained in Ibadan, Southwest Nigeria. *Journal of Biosciences and Medicines*, 3: 19-26.

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., and Folke, C. 2014. Does aquaculture add resilience to the global food system? *Proc. Natl. Acad. Sci. U.S.A.* 111, 13257–13263.

World Health Organization, WHO. 2016. WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food: Report of a WHO Consultation with the Participation of the Food and Agriculture Organization of the United Nations and the Office International des Epizooties. Geneva: World Health Organization. http://www.who.int/foodsafety/publications/containment-amr/en/