Innovations

Bacterial Pathogens of Farmed Catfish (*Clariasgariepinus*) in some Fish Farms in Calabar Metropolis, Nigeria

[1]Inyang-Etoh, A. P*.,[1]Eteng, S.U.,[2]Inyang-Etoh, P. C., and [1]Ekanem, A. P.

[1]Department of Fisheries and Aquaculture, University of Calabar, Calabar, Nigeria
orcid.org/0000-0002-9716-5658
[2]Department of Medical Parasitology and Entomology, University of Calabar, Calabar, Nigeria

Correspondence Author: **Dr A. P. Inyang-Etoh**

Abstract: *Catfish production is a good source of income in Nigeria but bacterial fish pathogen can make the business a very challenging one. The present studywas to isolate, screen, identify and also determine the antibiotic susceptibility test of isolated bacterial pathogen of farmed catfish (Clariasgarriepinus) in some fish farms in Calabar Metropolis. A total of 20 farms comprising of 10 farms in Calabar Municipality and 10 farms in Calabar South Local Government areas were investigated betweenMarch 2017 to February 2018. A two-stage sampling procedure was adopted for the study. The first stage was the selection of the 10 fish farms by simple random sampling technique in each of the Local Government Areas in Calabar Metropolis. The second was the random sampling of 24 fishes (2 fishes per month) from each farm in each of the two Local Government Areas. Sampled fishes weighing 100-250gramswere collected and taken to the Fish Pathology Laboratory in the Faculty of Oceanographyfor analysis. The samples were sacrificed by cardiac puncture, tissues from skin, gills, gall bladder, stomach, kidney and liver were excised under aseptic conditions, inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24hrs. They were subsequently sub-cultured onto MacConkey and Blood agars, and incubated at 37°C for 24 hrs. Bacterial colonies that grew on the media were picked and molecular identification of the suspected isolates was done using Polymerase Chain Reaction (PCR) analysis. Antibiotic susceptibility testing on the bacteria isolates was done using 6 broad spectrum antibiotics. A total of 28 (5.83%) fishes had bacterial infection comprising of 17(7.08%) for Calabar Municipality and 11 (4.58%) for Calabar South LGA. There was no significant difference in the prevalence of bacterial fish pathogen between the rainy and dry season in Calabar Municipality (P≥0.005) and Calabar*

South (P≥0.005)respectively. Most of the bacterial isolates were 100% sensitive to Levofloxacin, Ceftazidime, Ceftriazone, Cipioflocacin and Gentamicin. This study has shown that Pseudomonasaeroginosa, Alcaligenes faecalis,Aeromonas veronii, Pseudomonas sp, Aeromonas jandaei, Aeromonas sp, Comamonassp, Alcaligenes sp, Peudomonasxiamenensis and Comamonas testosterone are common fish pathogens in fish farms in Calabar metropolis and were most sensitive to Ceftazidime(CAZ),Ceftriaxone (CRO), Ciprofloxacin (CIP) and Gentamicin (CN). It is recommended that fish farmers should do routine random screening of their fish ponds to guard against outbreak of bacterial fish diseases.

Keywords: *Bacterial Pathogens, Farmed Catfish (Clariasgariepinus), Fish Farms, Calabar*

1.0 Introduction

Domestic fish production is vital to the Nigerian economy and its one of the main sources of livelihood as it decreases the rate of unemployment and enhances the Gross Domestic Product (GDP). Catfish has an advantage of requiring less amount of time, space, money and feed consumption, providing food for the populace, allowing for improved protein nutrition, low cholesterol content and low carbohydrate content. It is fast, simple to cook and also tastes great (Anoop *et al*., (2009). African catfish shows greater resistance to diseases and parasites and the fact that catfish is very hardy are those important attributes of catfish against other domesticated fishsuch as tilapia.

Though pond offers the cultivation of fish under a controlled condition, it however harbours great number of microorganisms which includes probiotics and pathogenic. Conditions which enhance bacterial diseases are overcrowding, wounds on fish flesh, occurrence of decayed organic matters in the pond, low dissolved oxygen content in the water. An abrupt increase in fish mortality is usually caused by stressors such as clogged water inflows, sharp decrease in temperature and toxic substances in the water. Majority of fish diseases are opportunistic in nature, they have contact with the fish when they are stressed and the immune system lowered.

Facultative and opportunistic *Aeromonas hydrophila*, *Pseudomonas* spp., and *Myxobacteria* are the most difficult bacteria in African catfish and have been reported to be the main cause of fin rot in African catfish (Naim and Ahmed 2008). Bacteria diseases such as *Pasteurellosis*, bacterial gill disease, peduncle disease (fin root), infectious abdominal dropsy of carp, *Furunculousis*, motile aeromonad disease, *Vibrosis*, *Columnaris*disease and other ubiquitous facultative bacteria, Myxobacterial infection- gill and fin rot, *Mycobacteriosis* of fishes and *Epitheliocystis* are used to enlighten fish farmers on some problems and solutions in fish culturing. The aim of this study is to screen, isolate, identify and also determine the antibiotic susceptibility test of isolated bacterial pathogens of

farmed catfish (*Clariasgariepinus*) in some fish farms in Calabar metropolis, Nigeria.

2.0 Materials and Methods

2.1 Study area

This study was carried out in CalabarMetropolisis which is the capital of Cross River State in South Eastern Nigeria. The city has a land area of about 30,355 square kilometers and has a height of approximately 100m above the main sea level. The Two main rivers which dominate the landscape of Calabar include the Great KwaRiver and Calabar River (Effiong, 2011).Calabar Metropolis comprises of two local government areas which include; Calabar South and Calabar Municipal Council. Calabar Metropolis belongs to the low and swampland of South Eastern Nigeria (Obianuju and Effiong,2015).Calabar Metropolis lies betweenLatitudes 4° 15' & 5° 15'N, Longitudes 8°15' & 8^0 25'E. Calabar has a sub-equatorial climate and is characterized by a double maxima rainfall which starts from April and ends in October, reaching its peak in July and September with an average annual rainfall of about 1830 mm. However, there is rainfall throughout the year but over 80% of the annual rainfall falls over the period stated above. The temperature of the area rarely falls below19°C and average 27°C all year round (NIMET, 2008). The relativehumidity of Calabar metropolis is high, between 80% and 100% (Eze, *et al*. 2010). Calabar metropolis has a population of 503,819 (Cross River State development project 2007).The main economic activities in thearea are fishing, farming and public sector engagements which arebasically government owned ministries, Afangideh, *et al.* (2011).Since Calabar has become a tourist destination for both local and foreign tourists, it is important to ensure that the quality of fish cultured and made available for public consumption are free of bacterial fish pathogens to eliminate the public health problems associated with consumption of farmed catfish which is in popular demand in this area.

2.2 Field sampling

2.2.1 Reconnaissance survey

Prior to the commencement of the study, visits weremade to the study sites for cross-sectional observational study of the major fish ponds in Calabar metropolis. Informed written consent was obtained from owners of the fish pondsin other tomake adequate arrangementswith the farm ownerson how to obtainthe diseased fishand transport themto the laboratory for analysis.

2.2.2 Study duration/sampling regime

This study was conducted from themonth of March 2017 toFebruary 2018. Fish samples were collected once a month between 10amand 12noonon each sampling dayat all study sites with infected fish on each sampling occasion.

2.2.3 Sample collection

Samples were collected from twenty (20) fish farms in Calabar metropolis, Cross River State. The sampling technique that wasadopted comprised two stage sampling procedure. The first stage involved theselection of ten (10) fish farms (by simple random sampling method) in each of the two Local GovernmentAreas of Calabar Metropolis. The second stage involved the random sampling of 24 fishes (2 fishes per month) from each farm in each of the two local government areas, (20 fish farms).

2.2.4 Fish capturing methods

Four hundred and eighty (480) fresh fish samples weighing between 100 to 250 grams were collected from different ponds in Calabarmetropolis using a drag net measuring 15 by 6 meters. Twenty-four (24) fishes each were caught randomly from each of the 20 farms.

2.3 Preservation and transportation of samples to the laboratory

Samples were transported to the laboratory live, using buckets containing same pond water as described by Olaosebikan and Raji (2004).

2.4 Laboratory studies

2.4.1 Measurement of fish length and weight

The weight and length of the fish specimens were measured with electronic balance and measuring board respectively.

2.4.2 Processing of sample

Following external examination of the fishes they were sacrificed by cardiac puncture and the bodycavity slit opened under aseptic conditions to expose theinternal organs. Disinfection of the body surfaces of the fish was done by swabbing with 70% alcohol. Dissecting instrument was conveniently sterilized by dipping in 70% alcohol and flamed before use. An incision was made through the body wall in the mid ventral line opposite the base of the pectoral fin. Blunt ended scissors was used to extend the incision anteriorly to the symphysis of the mandible and posterior to the vent taking care not to puncture the intestine. Underlying viscera was carefully separated and the two sides of the body pinned back to the dissecting surface.

2.4.3 Isolation medium:

Brain Heart Infusion, MacConkey and Nutrient agar were used for the isolation of the bacterial fish pathogen. All the media were prepared according to the manufacturers' instructions (Biolab, Merck, South Africa).

2.4.3.1 Isolation and culture of bacteria

With the help of a culture loop and a heated scalped blade, samples were taken from the skin, kidney, gall bladder, gills, stomach and liver. Inocula of the internal organs for culture were obtained by scaring the exposed scalped blade. A sterile inoculating loop was inserted through the sterilized area and the resultant inocula streaked upon the nutrient agar plate and then the plates was incubated for 48 hours at 37^0C

2.4.3.2 Preparation of pure cultures

To obtain pure cultures of the isolated bacteria, nutrient agar slants was prepared. The bacteria were inoculated on the nutrient agar slants and incubated for 24 – 48 hours at 37°C

2.4.4Identification of isolates

2.4.4.1 Microscopy

Smears were prepared of different types of colonies and stained by Gram's staining method and examined under the binocularmicroscope.

2.4.4.2 Antibiotic susceptibility testing

The Kirby-Bauer disc diffusion method was used for Antibiotic susceptibility testing. The result was read by measuring the diameter of the zone of inhibition with a ruler placed behind the plate. The result was recorded and reported as either "S" for sensitive and "R" for resistant after consulting the Clinical Laboratory Standard Institute (CLSI) (2014) performance standards for Antimicrobial Susceptibility Testing (AST). Bacteria isolates from this study were tested for their susceptibility to 6 broad spectrum antibiotics which includes; Ceftazidime 30*μg* (CAZ), Ceftriazone 30*μg* (CRO), Ciprofloxacin 5*μg* (CIP), Cefuroxine 30*μg* (CXM) Levofloxacin 5*μg* (LEV) and Gentamicin 10*μg* (CN). The Kirby Bauer disc diffusion method (Kirby *et al*. 1996) was used in this study.

The zone of inhibition diameter was measured in millimetre (mm) using standard method (McCasland, and True, 2001). Antibiotics used were classified into sensitive, moderate and resistant.

2.4.4.3 Molecular identification (Bacterial genomic DNA extraction (Boiling method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro-centrifuge tube and stored at -20°C for other downstream reactions.

2.4.4.4 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop1000 spectrophotometer, manufacturedin the United State of America. The softwareof the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the ‘‘measure’’ button.

2.4.4.5 16S rRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3'and 1492R: 5'-CGGTTACCTTGTTACGACT T-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the 2X Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light trans illuminator.

2.5 Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul Big Dye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 1000bp. The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

2.6 Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

2.7Structured Questionnaire

A pre-tested, structured questionnaire was used to collect participants' data which included demographics of fish farmers.

2.8 Statistical analysis

Data analysis was done using the statistical package for social sciences (SPSS) version 20 (IBM/Incorporated Armonk, USA). Results were presented as mean ±Standard deviation and frequencies as percentages. Chi-square distribution was used to calculate associations between variables (bacterial infections and seasons). Student T-test was used to determine the difference between means of the seasonal water quality variables.Probability level of less than or equal to 0.05(≤0.05)was statistically significant. Thiswas usedto determine ifthere is any significant difference of bacterial occurrence between the Calabar-South and Calabar-Municipality, and also between the different fish farms sampled. Comparisons were made at the 95% confidence level ($p \leq 0.05$).

3.0 Results

A total of twenty (20) farms in Calabar metropolis (comprising of ten fish farms in Calabar Municipality and 10 fish farms in Calabar South L.G. As) were investigated by screening, isolating, identifying and also performing the antibiotic susceptibility assay of the pathogenic bacteria isolates of catfish (*Clariasgariepinus*). Table 1shows the distribution of bacterial infections of farmed catfish (*Clariasgariepinus*) in some fish farms in Calabar Metropolis. A total of four hundred and eighty (480) fishes were evaluated, out of which 29 fishes, (6.04%) were infected (comprising of 12 (5%) for Calabar South L.G. A. and 17 (7.1%) for Calabar Municipality). Table 2: shows the distribution of bacterial fish infections according to fish farms in Calabar Municipality Local Government Area. A total of two hundred and forty (240) fishes wereevaluated in the municipality, out of which 17 fishes, (7.08%) were infected within the twelve-month study period. The total number of 6 fishes (2.50%) was infected during the rainy season while 11 fishes (4.58%) were infected in dry season respectively. The difference was not statistically significant ($\chi 2 = 0.667$, df (1), $p \geq 0.05$).

Table 1: Distribution of bacterial infections of farmed catfish (*Clariasgariepinus*) in some fish farms in Calabar Metropolis

Local Government Areas	No. of farms examined	No. of fishes sampled	No. (%) with infection	No. (%) without infection
Calabar South	10	240	12 (5.0)	228 (95.0)
Calabar Municipality	10	240	17 (7.1)	223 (92.9)
Total	20	480	29 (6.04)	451(93.96)

Table 2 Distribution of bacterial fish infections according to fish farms examined in Calabar Municipality

	Farm	No. of fish sampled	Total No. (%) of fish infected	No. (%) of fish infected in rainy season	No. (%) of fish infected in dry season	
1	Fish farm A	24	1(4.17)	0(0)	1(4.17)	
2	Fish farm B	24	3(12.50)	1(4.17)	2(8.33)	
3	Fish farm C	24	0(0)	0(0)	0(0)	
4	Fish farm D	24	3(12.50)	0(0)	3(12.50)	
5	Fish farm E	24	0(0)	0(0)	0(0)	
6	Fish farm F	24	0(0)	0(0)	0(0)	
7	Fish farm G	24	0(0)	0(0)	0(0)	
8	Fish farm H	24	4(16.70)	2(8.33)	2(8.33)	
9	Fish farm I	24	3(12.50)	1(4.17)	2(8.33)	
10	Fish farm J	24	3(12.50)	2(8.33)	1(4.17)	
	Total	**240**	**17(7.08)**	**6(2.50)**	**11(4.58)**	

χ^2=0.667p≥0.05

The distribution of bacterial fish infections according to fish farms examined in Calabar South is shown in Table 3. A total of two hundred and forty (240) fishes were evaluated, out of which 11 fishes, (4.58%) were infected. The total number of 4(1.67%) infected fishes was recorded in rainy season while 7 (2.92%) infected fish was recorded in dry season respectively. The difference was not statistically significant (X2 = 1.000, df (1), P = 0.317).

Table 3 Distribution of bacterial fish infections according to fish farms examined in Calabar South

	Farm	**No. of fish sampled**	**Total No. (%) of fish infected**	**No. (%) of fish infected in rainy season**	**No. (%) of fish infected in dry season**	
1.	Fish farm 01	24	2(8.33)	1(4.17)	1(4.17)	
2.	Fish farm 02	24	0(0)	0(0)	0(0)	
3.	Fish farm 03	24	1(4.17)	0(0)	1(4.17)	
4	Fish farm 04	24	0(0)	0(0)	0(0)	
5.	Fish farm 05	24	1(4.17)	0(0)	1(4.17)	
6.	Fish farm 06	24	5(20.83)	3(12.50)	2(8.33)	
7.	Fish farm 07	24	0(0)	0(0)	0(0)	
8.	Fish farm 08	24	1(4.17)	0(0)	1(4.17)	
9.	Fish farm 09	24	1(4.17)	0(0)	1(4.17)	
10.	Fish farm 10	24	0(0)	0(0)	0(0)	
	Total	**240**	**11(4.58)**	**4(1.67)**	**7(2.92)**	

$\chi^2$1.000 p≥0.05

Table 4 shows the bacteria diversity in the tissue of *C. Gariepinus*in Calabar Municipality Local Government Area using Polymerase chain reaction (PCR). *Pseudomonas Species* was isolated from the liver (37.5%), and skin (14.3%), *Aeromonas veronii* was isolated from the gills (50%) and stomach (33.3%), *Aeromonas Sp*was isolated from the skin (14.3%), *P. aeruginosa* was isolated from the liver (37.5%), gills (50%), skin (42.85%) and stomach (33.3%) respectively. *ComamonasSp* was isolated from the liver (25%), *Alcaligenes faecalis* was isolated from the stomach (33%) and *Comamonastestosteroni* was isolated from the bladder (100%) of *Clariasgariepinus*. The bacteria diversity in the tissue of *Clariasgariepinus* in Calabar South Local Government Area using Polymerase Chain Reaction (PCR) is shown in Table 5. *Alcaligenes Sp* was isolated from the skin (40%), *Alcaligene faecalis* was isolated from the skin (20%) and gills (33.3%)

respectively. *Pseudomonas aeruginosa* was isolated from the skin (40%), liver (100%) stomach (50%), gall bladder (100%) and kidney (100%). *Aeromonas Jandaei* was isolated from the gall bladder (50%) and *Pseudomonas xiamenensis* was isolated from the gills (66.7%) of *Clariasgariepinus*.

Table 4 Bacteria Diversity in the tissue of *C. gariepinus* in Calabar Municipality using PCR

Bacterial Species	**No Liver**	**%**	**No Gills**	**%**	**No Skin**	**%**	**No Stomach**	**%**	**No Gall bladder**	**%**	**No Kidney**	**%**
Pseudomonas sp	3	(37.5)	0	(0)	1	14.3	0	(0)	0	(0)	0	(0)
Aeromonas veronii	0	(0)	2	(50)			2	33.3	0	(0)	0	(0)
Aeromonas sp	0	(0)	0	(0)	1	14.3	0	(0)	0	(0)	0	(0)
P. aeruginosa	3	(37.5)	2	(50)	3	42.85	2	(33.3)	0	(0)	0	(0)
Comamonassp	2	(25)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Alcaligenes faecalis	0	(0)	0	(0)	0	(0)	2	(33.3)	0	(0)	0	(0)
Comamonas testosterone	0	(0)	0	(0)	0	(0)	0	(0)	1	100	0	(0)
Total	8		4		7		6		1			

Table 5 Bacteria diversity in the tissues of *C. gariepinus* in Calabar South using PCR

Bacterial species	**No Skin**	**%**	**No Gills**	**%**	**No Liver**	**%**	**No Gall Bladder**	**%**	**No Stomach**	**%**	**No Kidney**	**%**
Alcaligenes sp	2	(40)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
Alcaligenes faecalis	1	(20)	1	(33.3)	0	(0)	0	(0)	0	(0)	0	(0)
P. aeroginosa	2	(40)	0	(0)	4	100	2	50	2	100	2	100
Aeromonas Jandaei	0	(0)	0	(0)	0	(0)	2	50	0	(0)	0	(0)
P. xiamenensis	0	(0)	2	(66.7)	0	(0)	0	(0)	0	(0)	0	(0)
Total	5		3		4		4		2		2	

The antibiotic susceptibility test result of the isolated bacteriais shown in figure 1. An antibiotic sensitivity test was conducted onbacterialisolates to ascertain the level of resistance. *Aeromonas jandaei*, *Comamonas testosterone* and *pseudomonas stutzeri*were one hundred percent (100%) sensitive to Levofloxacin, (Lev). *Aeromonas veronii* was fifty (50%) sensitive, *Pseudomonas aeruginose* was 93.75% sensitive and *Alcaligenes faecalis*seventy-five percent (75%)sensitive to Levofloxacin. *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Aeromonas veronii*, *Comamonastestosteroni, Pseudomonas xiamenesis* and *pseudomonas stutzeri* were one hundred percent (100%) sensitive to Ceftazidime (CAZ) while *Aeromonas Jandaei*was the lowest which was fifty percent (50%) sensitive. *Alcaligenes faecalis, Aeromonas veronii, Comamonastestosteroni, Pseudomonas xiamenensis* and *Pseudomonas stutzeri* showed one hundred percent (100%) sensitive to Ceftriazone (CRO), *pseudomonas aeroginosa* was eighty-seven-point five percent (87.5%) sensitive and *Aeromonas Jandaei* was the lowest which was fifty percent (50%) sensitive. *Alcaligenes faecalis, Aeromonas veronii, Aeromonas Jandaei, comamonastestosteroni, Pseudomonas xiamenensis* and *Pseudomonas stutzeri* showed one hundred percent (100%) sensitive to Ciprofloxacin (CIP) while *Pseudomonas aeruginosa* showed 93.75% sensitivity.

Alcaligenes faecalis, Aeromonas veronii, comamonastestosteroni, Pseudomonasxiamenensis and *pseudomonas stutzeri* were one hundred percent (100%) sentitive to Gentamicin (CN) and *Pseudomonas aeruginosa* was 93.75% sensitive. *Comamonastestosteroni* and *Aeromonas Jandaei* showed low sensitivity to Cefuroxine (CXM) and were fifty percent sensitive respectively.

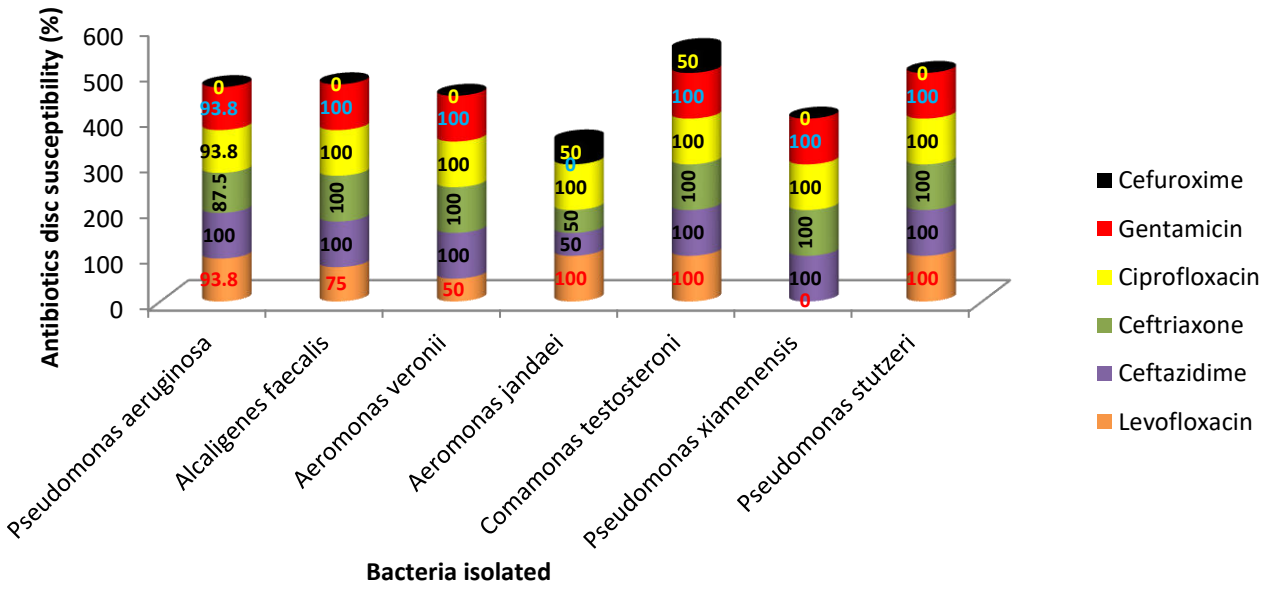

FIG.1: Antibiotic susceptibility testing of the isolated bacteria

4.0 Discussion

The present investigation shows that out of the two hundred and forty (240) fishes studied from each local government area (Calabar Municipality and Calabar South respectively) for a period of twelve months, 7.01% of fishes examined were infected in Calabar Municipality while 5.0% of fishes examined were infected in Calabar south LGA. This report is lower than the findings ofMohanty and Sahoo, (2007) who reported the prevalence of bacterial pathogen in ponds to exceed 5% and 50% respectively in tanks. It is also lower than the report by Aly (2013) in Egypt who reported incidences of *E. tada* in Nile tilapia and African catfish at 34% and 50% respectively. This study agrees with the findings of Efuntoye *et al.,* (2012) who reported the prevalence of bacterium isolated from *C. gariepinus* to be 3.5%. This study disagrees with the findings of Walakira*et al.,* (2014) who reported the prevalence of flavobacteria to be as high as 35% and 55.6% (Tamale *et al.,* 2011) respectively. These differences could be due to variation in seasons and sensitivity and specificity of the techniques used to identify the bacteria. This study also showed shows that the number of fishes infected during rainy season from both Calabar Municipality and Calabar South LGAs were 2.50% and 1.67% respectively while that of dry season was 4.58% and 2.92% respectively. This study disagrees with the findings of Karimi, (2015) who reported that 54.9% bacteria was isolated during dry season while 45.1% bacteria was isolated during the rainy season. This study disagrees with the work of Karimi, (2015) who reported that 6.6% of bacteria were isolated during dry season while 11.0% was isolated during the rainy season respectively. This study shows that the number of bacteria isolated during the dry season was greater than that of the rainy season. The reason adduced for higher number of bacteria isolates during dry season compared to rainy season according to Wemedo (2002) is that during the rainy seasons, lower temperatures reduced microbial activity. Another reason given attributed to this phenomenon is that saturation of soil by rain limits activity by reducing aeration (Marshall and Devinny, 1998). This study shows that there was no significant difference in the prevalence of bacterial fish pathogens between the two seasons in Calabar municipality (P=0.414) and also in Calabar South LGArespectively. The difference was not statistically significant between the two seasons throughout the period of study (P= 0.317).

The results of this present study revealed that various bacteria were present in the skin, gills, liver, gall bladder, stomach and kidney of *C.gariepinus*. Calabar Municipality had the highest percentage (42.85% of *P. Flourescens*biovariant isolated from the skin than Calabar south LGA which had (40%) of the same organism that was isolated from the skin. Calabar south LGA had the highest percentage (50%) of *P. Flourescensbiovariant* isolated from the liver than Calabar Municipality which had (12.5%) of the same organism isolated from the liver. Calabar south had the highest percentage (50%) of *P.*

Flourescensbiovariant isolated from the gall bladder whereas Calabar municipality had 0%. The present study shows that Calabar Municipality had the highest percentage (50%) of *P. alcaligenes* isolated from the gills of *C. gariepinus* than Calabar south LGA which had (33.3%) of the same organism that was isolated from the gills. Calabar municipality had the highest percentage (100%) of *P. alcaligenes* that was isolated from gall bladder whereas Calabar south LGA had (0%). Calabar Municipality had the highest percentage (28.6%) of *P. stutzeri* that was isolated from the skin whereas Calabar South LGA had 0%. Calabar Municipality had the highest percentage (25%) of *P. stutzeri* that was isolated from the gills whereas Calabar South LGA had 0%. Calabar Municipality had the highest percentage (62.5%) of *P. stutzeri* that was isolated from the liver than Calabar south which had (50%) of the same organism that was isolated from the liver. Calabar south had the highest percentage (50%) of *P. stutzeri* that was isolated from the gall bladder whereas Calabar Municipality had 0%. Calabar Municipality had the highest percentage (33.3%) of *P. stutzeri* that was isolated from the stomach whereas Calabar south LGA had 0%. Calabar south had the highest percentage (100%) of *P. Stutzeri*that was isolated from the gall bladder whereas Calabar Municipality had 0%. 28.6% and 33.3% of *Pseudomonas acidovarans* was isolated from the skin and stomach of *C. gariepinus* in Calabar Municipality.

Calabar south had the highest percentage (40%) of *P. aeruginosa* that was isolated from the skin whereas Calabar Municipality had 0%. Calabar Municipality had the highest percentage of *P. aeruginosa* (25%) that was isolated from the gills whereas Calabar South had 0%. Calabar Municipality had the highest percentage of *P. aeruginosa* (25%) that was isolated from the liver whereas Calabar South had 0%. Calabar Municipality had the highest percentage of *P. aeruginosa* (33.3%) that was isolated from the stomach whereas Calabar south had 0%. 66.7% of *Salmonella enterica* was isolated from gills of *C.gariepinus* in Calabar South.

Alcaide *et al.* (2005) reported that *Salmonella sp* is potential pathogens for humans and fish. It is among the most imported cause of human gastro intestinal disease worldwide and many seafood importing countries will not accept products containing these pathogens. (Musefiu*et al.* (2011). Budiati*et al.* 2011, Bremer *et al.* 2003, Kumar *et al.,* 2009; Heinitz *et al.* 2000 and Ponce *et al.* 2008, in their study reported that *Salmonella spp* have been recovered from gills, intestine and whole body of catfish, *C. gariepinus* and sea food in Malaysia and elsewhere. The present study agrees with the findings of Efuntoye*et al.* (2012) who reported two species, *S. typhimurium* and *Salmonella entirica*. This constitutes a food safety problem because catfish could be a potential agent of transfer of these species to unsuspecting consumers. 100% of *P. mendocina* was isolated from the stomach of *C. gariepinus* in Calabar south. 20% of *Morganella morganii* was isolated from the skin of *C. gariepinus* in Calabar South.

The presence of these bacteria in the tissue of *C.gariepinus* could be due to contamination of the water body as previously reported by Adebayo - Tayo *et al.* (2008) and Junaid *et al.,* (2010). This study does not agree with the findings of Ismail *et al.* (2013) who reported that the bacterial organism that was isolated from the skin of catfish (*C.gariepinus*were *Flavobacterium columnare*(31.1%), *Aeromonas hydrophila* (20.3%), *Edwardsiellatarda* (15.4%) and *Pseudomonas spp* (11.6%). Abd EL-Rahman and Elkamel (2007) reported that *Pseudomonas spp* was the third major isolates from affected skin of sharp tooth catfish. In the present study, *Pseudomonas spp* was the first major bacteria isolated, this may be due to the fact that the fish had a confined case of bacterial skin infection as the clinical signs of the diseased fish. This study is not in agreement with the findings of Nwankwo *et al.* (2017) who reported a high diversity of bacteria present in the skin, gills and intestine of *C. gariepinus* to include *Bacillus Spp* (18.6%), *Streptoccusspp* (17.0%) and *Staphylococcus spp* (17.0%) were most prevalent bacterial species. Conversely, *Enterobacter spp,Pseudomonas spp* and *Serratia spp* (5.1% each) were the least. The present investigation does not agree with the finding of Tivkaa*et al.* (2013) who reported a high diversity of bacteria present in the skin, gills and intestine of *C. gariepinus* to include *Escherichia coli* (42%), *Salmonella spp* (75%), *Enterococcus spp* (77%), *Pseudomonas spp* (50%), Serratia spp (17%), *Streptococcus spp* (25%), *Staphylococcus spp* (58%), *vibrio sp* (33%), *Shigella spp* (25%), *Proteus spp* (58%) and the least was *Klebsiella spp* which was (8%).

Bacteria diversity in the tissue of *C. gariepinus*: The results of this study revealed that 37.5% and 14.3% of *Pseudomonas spp* was isolated from the liver and skin of *C. gariepinus* in Calabar Municipality. This study disagrees with the findings of Akinyemi *et al.* (2016) who reported that 5% of *Pseudomonas spp* was isolated from the gills of *C. gariepinus* in yewa river of Ogun state. This study disagrees with that of Nwankwo *et al.* (2017) who reported that 5.1% of *pseudomonas spp*was isolated from the gills of *C. gariepinus in* Port Harcourt.The study showed that 50% and 33.3% of *Aeromonas veronii* was isolated from the gills and stomach of *C. gariepinus*, 14.3% of *Aeromonas spp* was isolated from the skin of *C. gariepinus* in Calabar Municipality while 50% of *Aeromonasjandaei*was isolated from the gall bladder of *C. gariepinus* in Calabar South LGA. The present investigation is not in agreement with the findings of Akinyemi *et al.* (2016) who reported that 5% of *Aeromonas hydrophila* was isolated from the skin of *C.gariepinus.* This study does not agree with the findings of Aya *et al.* (2018) who reported that 4% each of *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* were isolated from the kidney, skin and spleen of *C. gariepinus* respectively. This study does not agree with the findings of Aya *et al.* (2018) who reported that 8%, 4% and 12% of *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas veronii* respectively was isolated from the kidney, liver, skin, gills, and spleen of *Oreochromis niloticus*.

This study showed that 42.85%, 50%, 37.5% and 33.3% of *Pseudomonas aeruginosa* was isolated from the skin, gills, liver and stomach of *C. gariepinus*respectively in Calabar Municipality whereas 4%, 100%, 50%, 100% and 100% of *Pseudomonas aeruginosa* was isolated from the skin, liver, gall bladder, stomach and kidney of *C. gariepinus* respectively in Calabar South LGA. This high diversity of *Pseudomonas aeruginosa* in Calabar south could be attributed to the fact that this organism is more prevalence in Calabar south LGA than in Calabar Municipality. This high diversity of *Pseudomonas aeruginosa* isolated in the present study does not corroborates with the findings of Aya *et al.* (2018) who reported that 4% of *Pseudomonas aeruginosa* was isolated from the liver of *C. gariepinus*. This study shows that 25% of *Comamonasspp* was isolated from the liver of *C.gariepinus* and 100% of *Comamonastestosteroni* was isolated from the gall bladder of *C. gariepinus* all in Calabar Municipality. This study agrees with the findings of Akinyemi *at al.,* (2016) who reported that *Comamonasspp* was found to have the highest frequency found in the gill, gut and skin of *C.gariepinus* with a total of 25%. These bacterial strains found in this study, are in line with the findings of Cipriano and Dove (2011) who discovered about a dozen bacterial species within the genera *Comamonas, Pseudomonas, Alcaligenes, Moraxella*, and *Acinetobacte*r as a rich microbial flora on the skin and mucus of healthy fish prior to the detection of *Aeromonas Salmonicida*. This study shows that 33.3% of *Alcaligenes faecalis* was isolated from the stomach of *C. gariepinus* in Calabar Municipality while 20% and 33.3% of *Alcaligenes faecalis* was isolated from the skin and gills of *C.gariepinus* in Calabar South. 40% of *Alcaligenes spp* was isolated from the skin of *C.gariepinus* in Calabar south LGA.

This study shows that 66.7% of *Pseudomonas xiamenensis* was isolated from the gills of *C. gariepinus in* Calabar south. This study disagrees with the findings of Sowunmi *et al.* (2006) who reported that 4% of *Pseudomonas flourescens* was isolated from the gills of *C. gariepinus*. This study is also not in line with the findings of Akinyemii*et al.* (2016) who reported that 5% of *pseudomonas spp* was isolated from the gills of *C. gariepinus*. According to Cipriano and Dove (2011), human infections caused by pathogen transmitted from fish or the aquatic environment are quite common depending on the season, patients contact with fish and related environment, dietary habits and the immune system status of the exposed individual.

The identity between the query and the reference sequences is the basic principle of molecular identification and phylogenetic reconstruction (Wattoo, *et al.* (2016). The traditional identification of bacteria on the basis of phenotypic characteristics is generally not as accurate as identification based on genotypic methods. Comparison of the bacterial 16S rRNA gene sequence has emerged as a preferred genetic technique in bacterial taxonomy at various levels due to its presence in almost all bacteria, its stability over times and the large sequence

length (1,500 bp) for informatics purposes (Patel, 2001, Adekambi, *et al*. 2004; Fanti, *et al.* (2004). In the current study, the 16S rRNA gene sequence of the 29 bacteria isolates were compared with the known sequences on the NCBI data bank. All the isolates except p4 were unambiguously identified to species level according toShinwari, *et al.* (2014).

Antibiotic susceptibility test result of the isolated bacteria: The present investigation shows that *Aeromonas jandaei, Comamonastestosteroni, Pseudomonas stutzeri, Alcaligenes faecalis, Aeromonas veronii* and *Pseudomonas xiamenensis* were 100% sensitive to Cipro floxacin.This study agrees with the findings of Ghaly *et al.* (2015) who reported that *Aeromonas Spp* showed strong susceptibility to Ciprofloxacin. Ashiru *et al.* (2011) reported that *Aeromonas spp (Aeromonas Sobria, Aeromonas Caviae and Aeromonas hydrophila*) were all highly sensitive to ciprofloxacin. This study is in line with the findings of Elsayed *et al.* (2018) who reported that Ciprofloxacin and nalidixic acid were highly effective against *Aeromonas hydrophila* and these results also agrees with (Grande *et al.* 2018). This study agrees with the findings of Elsayed *et al.* (2018) who reported that Ciprofloxacin and rifampicin were very effective against *Pseudomonas Spp* and these results is also similar to that presented by (Lee and Wendy 2017). This study agrees with the findings of Nakade (2012) who reported that Ciprofloxacin was highly sensitive to *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia.* This study is not in line with the findings of Ekelemu*et al.* (2016) who reported that *Pseudomonas spp, Serratia spp* and *Aeromonas spp* were 30% sensitive to Ciprofloxacin followed by Gentamycin which was 25% sensitive to *Pseudomonas spp,Serratiaspp*and *Aeromonas spp*. The present investigation is in line with the findings of Akani *et al.,* (20018) who reported that *Pseudomonas spp, vibrio spp and staphylococcus* were 87%, 88.4% and 82.44% respectively sensitive to Ciprofloxacin.

This study showed that *Aeromonas jandaei, Comamonastestosteroni* and *Pseudomonas stutzeri* were 100% sensitive to Levofloxacin. This study is not in line with the findings of Karlowsky*et al.* (2000) who reported that *Pseudomonas aeruginosa* was 27% resistant to Levofloxacin. The present investigation agrees with the findings of Madubuike*et al.* (2014) who reported that all the *Aeromonas spp* were susceptible to gentamicin, ciprofloxacin, ceftazidime and levofloxacin. This study is in line with the findings of Madubuike*et al.* (2014) who reported that all the *Aeromonas spp* (*Aeromonas hydrophila, Aeromonas Caviae* and *Aeromonas Sobria*) were 100% sensitive to Levofloxacin. This study shows that *Alicaligenesfeacalis, Aeromonas veronii*, *Comamonastestosteroni, pseudomonas xiamenensis* and *pseudomonas stutzeri* were 100% sensitive to Gentamicin. This study is in line with the findings of Wemala*et al.* (2018) who reported that *Aeromonas species* showed 100% susceptibility to Gentamycin. This study is in line with the findings of Rahman *et al.* (2011) who reported that *Pseudomonas fluorescens* was sensitive to Gentamycin. This study does not agree with the

findings of Ekelemu*et al.* (2016) who reported that *Pseudomonas spp, Serratia spp*and *Aeromonas spp* were 100% resistant to Cefuroxime and Ceftazdime.

4.1 Conclusion

A total of 29 (6.04%) fishes had infection comprising of 17(7.01%) for Calabar Municipality and 12 (5.0%) for Calabar South LGAs respectively. *Pseudomonas aeroginosa, P. xiamenensis Pseudomonas spp, Aeromonas veronii, Aeromonas jandaei, Aeromonas spp, Alkaligenes faecalis, Alkaliqenesspp, Comamonastestosteroni,* and *other Comamonasspp* were the bacteria isolated from the catfishes examined. There was no statistically significant difference in the prevalence of bacterial fish pathogens between rainy and dry seasons in Calabar Municipality (P≥0.05) and Calabar South LGAs (P≥0.05). Most of the bacterial isolates were 100% sensitive to Levofloxacin, Ceftazidime, Ceftriazone, Cipiofloxacin and Gentamicin. Different size and age groups of fish stocking should be avoided. Stocking of healthy and disease-free fingerlings should be maintained properly. Proper stocking density of healthy fish, their right feed in optimum doses and right feeding time should be maintained. Moreover, preventative actions are required to minimize the potentials for common outbreak drivers such as high stocking density, injuries following transport, closed rearing methods and open exchange pathways that leads to infection and spread of bacterial diseases of fish.

5.0 *References*

1. *Abd El-Rahman, A. A. & Elkamel, A.(2007).Bacterial causes of skin lesions in some freshwater fish. Alex. Journal Veterinary Medicine, 25, (1): 25- 30*
2. *Adebayo-Tayo, B. C., Oniladeand, H. & Patrick, A. A. (2008) Mycofloral of smoke-dried fishes sold in Uyo, eastern Nigeria. World Journal of Agricultural Science. 4(3) 346 – 350.*
3. *Afangideh, A. I., Njar, G. N., Ewa, E. E., Eli, H. D. & Iwara, A. I. (2011) Assessment of water quality status of borehole in Calabar South Local Government Area, Cross River State. International Journal of Biosciences, 1 (5): 71-76*
4. *Akani, N. P. Williams, J. O. & Njoku, O. R. (2018). Bacteriology and sensitivity pattern of some bacterial isolates from clarias gariepinus sold in different markets in Port Harcourt Metropolis.World Journal of Pharmaceutical and Medical Research,4(8), 85-91.*
5. *Akinyemi, A. A., Ekelemu, J. K., Oyelakin, O. O., Oloyede, A. R., & Green, B. M. (2016). Molecular characterization of bacteria associated with African catfish (clarias gariepinus) (burchell, 1822) from yewa-mata Station on Yewa*

River by 16s rrna gene sequencing method. Global Journal of Bio-science and Biotechnology. G.J.B.B., vol.5 (3), 295-300.

6. *Alcaide, E., Blasco, M.D. & Esteve, C. (2005) Occurrence of drug-resistant bacteria in two European eel farms, Applied and Environmental Microbiology, 71(6), 3348 – 3350.*
7. *Aly,S.M. (2013).A Review of Fish Diseases in the Egyptian Aquaculture Sector. Working Report. [www.livestockfish.cgiar.org] site visited on 20th June 2017.*
8. *Anoop, K. R., Sundar, K. S. G., Khan, B. A & Lal, S. (2009). Common moorhen Gallinula chloropus in the diet of the African catfish Clarias gariepinus in Keoladeo Ghana National Park, India. Indian Birds 5(2):22-23.*
9. *Ashiru, A.W., Uaboi-Egbeni, P. O., Oguntowo, J. E. & Idika, C. N. (2011) Isolation and antibiotic profile of Aeromonas species from Tilapia Fish (Tilapia nilotica) and Catfish (Clarias betrachus) Pakistan Journal of Nutrition 10 (10): 982-986.*
10. *Bremer, P. J., Fletcher, G. C. & Osborne, C. (2003). Salmonella in Seafood. New Zealand Institute for Crop and Food Research Limited.,New Zealand.*
11. *Budiati,T. G. Rusul, A. F. M. Alkarkhi, R. Ahmad, & Arip, Y.T. (2011). Prevalence of Salmonella spp. From catfish (Clarias gariepinus) by using improvement isolation methods. International. Conference. Asia Agric.Animal. International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE,) 13: 71-75.*
12. *Cipriano, R.C. & Dove, A. (2011) Far from superficial: microbial diversity associated with the skin and mucus of fish. Journal of Aquatic Animal Health 4:114-118.*
13. *Efiong J. (2011). Changing pattern of land use in the Calabar river catchment, Southeastern Nigeria. Journal of Sustain;able Development, 4(1):92-102.*
14. *Efuntoye, M. O., Olurin, K. B. & Jegede, G. C. (2012): Bacterial flora from healthy Clarias gariepinus and their antimicrobial resistance pattern. Advance Journal of Food Science and Technology 4(3): 121-125.*
15. *Ekelemu, J. K., Akinyemi, A. A., Oyelakin, O. O., Oloyede, A. R. & Agbasi, V. O. (2016). Molecular characterization and antibiotic resistance profile of bacteria associated with Clarias gariepinus (Burchell, 1822) from Ebute Iboro Location on Yewa River.American Journal of Experimental Agriculture 11(5): 1-8.*
16. *Elsayed Mahmoud Ezzat, Essawy Mahmoud, Shabana Iman Ibrahim, Abou El-Atta Mohamed & EL-Banna Noha.(2018):Studies on bacterial pathogens in some marine fishes in EL-Mansoura, Egypt.American Journal of Agricultural and Biological Sciences 2018, 13 (1): 9.15*
17. *Eze,E.B. & Efiong, J. (2010). Morphometric parameters of the Calabar river basin: Implication for hydrologic processes. Journal of Geography and Geology, 2(1):18-26.*

18. *Fanti, F., E. Tortoli, L. Hall, G. D. Roberts, R. M. Kroppenstedt, I. Dodi, S. Conti, L. Polonelli & Chezzi, C. (2004). Mycobacterium parmense sp. International Journal of Systematics and Evolutionary Microbiology, 54:1123–1127.*
19. *Felsenstein, J. (1985). Confidence limits on pylogenies with a molecular clock. Systematic Zoology, 34:152-161.*
20. *Ghaly, F. M., Dina, M. Baraka, S., Mahmoud, H., Sohair, S., Abd EL-Salam, S., Awad, M. & Abeer, A. E. (2015): Efficacy of ciprofloxacin and clove extract on bacterial infection of Clarias gariepinus. Middle East Journal of Applied Sciences, 5(1): 01-09,*
21. *Grande, B. M. J., Romero, J. L., Perez, P.R. Cobo, M.A. & Galvez, et al., (2018). Analysis of potential risks from the bacterial communities associated with air-contact surfaces from tilapia (Oreochromis niloticus) fish farming.Journal of Environmental Research, 160: 385-390.*
22. *Heinitz, M. L., Ruble, R. D., Wagner, D. E. & Tatini, S. R. (2000). Incidence of Salmonella in fish and sea food. International Journal of Food Microbiology, 63: 579-592.*
23. *Ismail,S., Mohamad, M. W., Abd Al-Azeem; Mahmoud, M., Mahmoud, A., Mokhtar, M. & Abd-El A. (2013). Microbiologystudies on the affections of skin in sharp tooth catfish (Clarias gariepinus) Assiut VeterinaryMedical Jounal. 59 (137): 1-9.*
24. *Junaid, S. A., Olauboin, F. & Olabode, A. O. (2010). Mycotic contamination of stock fish sold in Jos, Nigeria. Journal of Yeast and Fungal Research, 1(7): 136 – 141.*
25. *Jukes, T. H. & Cantor, C. R. (1969) Evolution of protein molecules. In Munro H. N, editor, Mammalian protein metabolism, Academic Press, New York. pp.21-132.*
26. *Karimi, R. D. (2015). The bacterial flora of tilapia (Oreochromis Niloticus) and carfish (Clarias gariepinus) from eathen ponds in Sagana fish farm and Masinga dam. An M. Sc. Thesis submitted to the School of pure and Applied Sciences, Kenyatta University, pp72.*
27. *Karlowsky, J. A., Kelly, L. J., Thornsberry, C., Jones, M. E., Evangelista, A. T. & Critchley, I. A. (2002) Susceptibility to fluoroquinolones among commonly isolated Gram-negative bacilli in(2000): TRUST and TSN data for the United States. Tracking Resistance in the United States Today. The Surveillance Network.International Journal of Antimicrobial Agents 2002;19(1):21–31*
28. *Kirby, W., Bauer, A.W., Sherris, J.C. & Truck, M. (1996). Antibiotic susceptibility testing by a standard single disc method. American Journal of clinical pathology, 45:493-496*
29. *Kumar, R., Surendran, P. K. & Thampuran, N. (2009). Distribution and genotypic characteristics of Salmonellar serovars isolated from tropical seafood of Cochin, India. Journal Appllied Microbiology, 106: 515-524.*

30. *Lee,S.W. & Wendy, W. (2017). Antibiotic and heavy metal resistance of Aeromonas hydrophila and Edwardsiella tarda isolated from red hybrid tilapia (Oreochromis spp.) coinfected with motile Aeromonas septicemia and edwardsiellosis. Veterinary World, 10: 803-807.*
31. *Madubuike U. A., Kennedy, F. C. & Vincent, S. S. (2014).Antibiogram of aerobic bacteria isolated from skin lesions of African catfish cultured in Southeast, Nigeria.International Journal of Fisheries and Aquatic Studies; 2(1): 134-141.*
32. *Marshall, T. R., & Devinny, J. S. (1998). The microbial ecosystem in petroleum wasteland treatment. Water Science and Technology, 20:285-291.*
33. *McCasland, B. & True, K. (2001). Zone diameter standards. Bacteriology, Chapter 5. In: National Wild Fish Health Survey(NWFHS) Laboratory Procedure Manual 10 June 2001. California-Nevada Fish Health Centre, Anderson California, 1-28*
34. *Mohanty, B. R. & Sahoo, P. K. (2007). Edwardsiellosis in fish: a brief review. Journal of Biosciences 32: 1331-1344.*
35. *Musefiu,T. A., Obuko, E.B. & Bolarinwa, A. O.(2011) Isolation and identification of aerobic bacteria flora of the skin and stomach of wild and cultured Clarias gariepinus and Oreochromis niloticus from Ibadan, Southwest Nigeria. Journal of Applied Sciences Research, 7(7), 2011, 1047 – 1051.*
36. *Naim, U. & Ahmed, H. A. (2008). Bacterial flora of polycultured common carp (Cyprinus carpio) and African catfish (Clarias gariepinus). International Aquatic Research, 4:10.*
37. *Nakade, D. B. (2012).Antibiotic sensitivity of common bacterial pathogens against selected Quinolones.International Science Congress Association. (ISCA) Journal of Biological Sciences. Vol. 1(1): 77-79,*
38. *Nigerian Meteorological Agency (NIMET) (2008). Daily measurements of meteoroligical parameters. Nimet Office, Margaret Ekpo International Airport, Calabar, Cross River State, Nigeria.*
39. *Nwankwo,C. E. & Akani, N. P. (2017). Bacterial flora of Clarias gariepinus from some selected fish ponds in Port Harcourt.Journal of Environment and Biotechnology Research, Vol. 6, No. 2, Pages 208-212.*
40. *Obianuju, E. A. & Efiong, J. (2015) Implication of open occupation dumpsite on groundwater quality in Calabar metroolis, Nigeria. Journal of geography, Envieonment and earth sicnece International 2(3)117-125, Article no. JGSSI, 215.011*
41. *Patel, J. B. (2001) 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. Molecular Diagnosis, 6: 313-321*
42. *Ponce, E., A.A. Khan, C. M. Cheng, C. Sumage-West & C.E. Cerniglia, (2008). Prevalence and characterization of Salmonella enterica serovar weltevreden from imported seafood. Food Microbiology., 1: 29-35.*

43. *Rahman M. M., M. J. Foysal & M. Alam, (2011). Antibiotic sensitivity and in-vitro antimicrobial activity of plant extracts to Pseudomonas fluorescens isolates collected from diseased fish. International Journal of Natural Sciences. 1 :(4), 82-88.*
44. *Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.*
45. *Shinwari, Z., Khansa, J. & Nadia, B. Z. (2014). Molecular systematics of selected genera of subfamily Mimosoidae-Fabaceae. Pakistanian Journal of Botany, 46(2): 591-598*
46. *Sowunmi, A. A., Okunubi, M. A., & Efuntoye, M.O. (2006). Occurrence of bacteria in gills and buccal cavity of Clarias gariepinus (Burchell, 1822) and Tilapia zillii (Gervais) from Lekki lagoon, Southwest Nigeria. World Journal of Biological Research 001: 1 pp14-17.*
47. *Tamale, A.,Ejobi, F., Rutaisire, J.,Isyagi, N., Nakavuma, J., Nyakarahuka, L & Amenlen, D.(2011) Prevalence of Columnaris, ecto-parasite and fungal conditions in selected fish farms. In: International conference on agro-biotechnology, bio-safety and seed systems in developing Countries. www.scifode-foundation.org.*
48. *Tivkaa, J. A. & Sampson, U. N. (2013). Bacterial flora of african catfish (Clarias gariepinus) harvested from ponds in Uyo South-South Nigeria. Journal of Environmental Science,Toxicology and Food Technology (IOSR-JESTFT). 5(3): 72-76.*
49. *Walakira J, et al. (2014) Common fish diseases and parasites affecting wild and farmed tilapia and catfish in central and western Uganda. Uganda Journal of Agricultural Sciences; 15(2):1-11*
50. *Wemala, S. P., Mugimba, K. K.., Mutoloki, S., Evensen, Ø., Mdegela, R., yarugaba, D. K. & Sørum, H. (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. Fisheries and Aquatic Sciences.21:6, pp 1-10.*
51. *Wattoo, J. I., Muhammad, Z. S., Muhammad, S. S., Amina, A., Amir, H.& Mushtaq, A. S. (2016). DNA Barcoding: Amplification and sequence analysis of rbcl and matK genome regions in three divergent plant species. Advances in life Sciences, 4(1):03-07*
52. *Wemedo, O. (2000). Seasoned effect on the bacterial and fungal population of an oil 58 field waste water-polluted soil in Nigeria. Nigeria River States University of Science and Technology, Port Harcourt*