Incidence of *Plesiomonas* specie in Catfish (*Clarias gariepinus*)and Tilapia Fish (*Oreochromis niloticus*) Sold in Selected Markets in Lagos State

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Abstract: *Plesiomonas* sp is a facultative anaerobic, gram negative, oxidase positive, non-sporing, motile bacilli, that has been implicated in food and water borne diseases. This experiment was conducted to determine the incidence of this bacteria in catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*) sold in selected markets in Lagos State. A total number of 64 samples (fish) were collected from different locations between the months of August and September, 2018. 10g from each sample was serially diluted in 90 ml of sterile distilled water and 1 ml from 10^{-3} dilution was inoculated in duplicate on solidified inositol brilliant green bile agar. Plateswere incubated at 35°C for 24-48hr. Isolates were Identified based on culturalcharacteristics, Gram staining and biochemical characterization. The total viable count showed that catfish from location D (Iyana-Iba market) had the highest microbial load of 9.2×10^4 cfu/g while the lowest microbial load for catfish was recorded in location B (Igando market) with 1.0×10^4 cfu/g. The highest and lowest microbial load for tilapia samples were observed in location G (Oyingbo market) with 2.5×10^4 cfu/g and 0.7×10^4 cfu/g respectively. Antibiotics susceptibility test conducted showed that most of the isolates were multi-drug-resistant with multiple antibiotic resistance index above 0.2 risk limit. Hemolysis test also revealed varying zones of clearing confirming their ability to produce hemolysin. *Plesiomonas* sp is an organism of concern because of its ability to cause intestinal infections and could be a public health threat if ignored.

Key words: *Plesiomonas* sp, seafood and antibiotic resistance.

INTRODUCTION

There is continuous emergence of food borne diseases globally. There has been widespread occurrence of food-borne diseases especially in under developed countries. Unhygienically prepared food products from either animal or plant origin serves as sources to food-borne disease outbreaks. There has been an increase in the quality as well as safety of food produced in under developed counties over time due to poor knowledge of food hygiene, improper cooking, temperature abuse, cross contamination and poor facilities for storage (Olumide and Bamidele, 2016).

Fish is a well-known food commonly regarded as a cheap source of protein in under developed countries. It is also known as a better source of protein vitamins and minerals. It is a very important component of diet globally. Naturally, there are bacteria on the skin, gut and slime of living fish, which act as normal flora and do not cause harm to humans upon consumption (Karimi, 2015).

One of the best aquaculture species is the African catfish (*Clarias gariepinus*) which have qualities such as its ability to feed on almost anything, ability to survive harsh pond rearing conditions, high fecundity, fast growth and resistance to diseases, and also the ability to survive outside water through the aid of atmospheric oxygen.(Enyidi and Nduh-Nduh, 2016). Tilapias (*Oreochromis niloticus*) are also recognized as Cichlid fish in many African countries. They were found wildly in great rivers and lakes of the continent during the 20th century. After the 2nd world war, the knowledge of breeding tilapia in ponds was introduced which has now turned into a common practice in aquaculture in several parts of the world. Tilapia has been proven to be less recognized as just an African fish but fully

recognized as an international fish, which also pointed out that it may eventually become one among the most important fishes in the world (Hussain, 2004).

The emergence of diseases associated with the consumption of meat have led to the very high demand for fish. The use of organic waste and the harvesting of fish from untreated water sources have contributed to the large population of pathogens in sea foods particularly in fishes thereby resulting in increase in food borne infections (Janda *et al*., 2016).

Plesiomonas shigelloides is a facultative, anaerobic, gram negative rod, oxidase positive, non-sporing, motile bacilli. It has been found to cause food and water borne diseases. Identifying this organism has also been a very difficult attempt due to its similarity with the family *Photobacterium, Vibro, Aeromonas,* and *Enhydrobacter* (Gonzalez-Rey *et al.,* 2000) However, through current molecular studies, it has been observed to be related to the genus *Proteus* within the family Enterobacteriacea. This bacterium is also an aquatic organism and has been recently recognized as a potential human and animal pathogen. The primary reservoir to which this organism is mainly found are fresh and estuarine water, majorly in temperate climate (Gonzalez-Rey *et al*., 2000).

The virulence of *Plesiomonas shigelloides* is known to be comparatively low but it can be deadly in people with weakened immunity whenever they get infected, even if clinical symptoms of immunodeficiency are not seen. Reports have also shown that this pathogen uses heme as an iron source. It has also been reported to be beta lactamase positive and involved in the production of proteic/lipopolysaccharidic complex toxins responsible for both cytotoxicity and enteropathogenicity (Martins *et al.,* 2010)

There are also reports of isolation of this bacterium from salt water fish, fresh water fish, ornamental aquaria and aquarium. Marine mammals like cetaceans, pinnipeds, sea otters and bottlenose dolphins, water fowls and other mammals have also been linked with *Plesiomonas shigelloides* (Janda *et al*., 2016). Although its relation or linkage to human infections possess very limited data due to under reporting.

Some studies noted that *Plesiomonas shigelloides* can be isolated from asymptomatic individuals (Rajagoplan*et al.*, 2014). It was also reported that this bacterium may take advantage of the disruption of the gut microbiota and physiology due to the concurrent presence of related pathogens thereby starting a pathology in the gut of human. For instance, when *Vibrio cholerae* releases enterotoxins, the normal gut microbiota may be removed thereby enabling *Plesiomonas shigelloides* to establish an infection (Escobar *et al*., 2012).

The use of antibiotics intensively on animal farms has been attributed to the emergence and spread of antibiotic resistant genes among many bacterial general and species.(Matsuyama *et al*., 2015; Nain *et al*., 2015; Wamala*et al.,* 2018).The involvement of this pathogen in disease outbreak is greatly under reported in this part of the world.This study aimed at determining the incidence of *Plesiomonas* sp in cat fish and tilapia sourced from different fish markets in Lagos mainland and its pattern of resistance to selected antibiotics.

MATERIALS AND METHODS

Sample Collection

A total number of 64 fishes (32 live cat fish and 32 fresh tilapia) were collected from different locations; Ojuelegba, Oyingbo, Igando, Ikotun, Iyana-paja, Ilepo, Makoko, Ebute Meta and Bariga all in Lagos, Nigeria between the months of August and September, 2018.The fishes weredissected using sterile scalpel blades and theguts were removed and processed following standard microbiological guidelines.

Microbiological Evaluation of Bacterial Load

10g of the each gut was macerated in 90ml of sterile distilled water and 10 fold serial dilution was carried out. 1ml from 10^{-3} dilution factorwas plated in duplicate on Inositol brilliant green agar which was

prepared according to manufacturer's instruction and incubated for 24-48 hours at 35°C. Distinct colonies were subcultured to obtain pure isolates as described by(Nain *et al*., 2015).

Identification of Isolates

Pure cultures of the isolated bacteria were identified using their distinctive cultural and morphological characteristic on media such as margins, shape, consistency, elevation and color.Microscopic examination of the isolates was done byGram staining as described by UK standard for microbiology investigation, 2015.

Biochemical test such as oxidase, lysine, adonitol, ractinose ornithine, indole ,glucose, manitol, xylose, ONPG, urease, vogesproskauer reaction, citrate, TDA, gelatin, malonate, inositol, arginine sorbitol, rhamnose, sucrose, lactose,hydrogensulphide arabinose and salicin were done on the bacterial isolates using Microbact 24E kit for proper identification and characterization. Motility test was also done to observe the movement ability of the organism using the SIM medium according to the UK standard for microbiology investigation, 2015.

TEST FOR HEMOLYSIS

It was done using blood agar base with the addition of the required amount of sheep blood. The agar base was sterilized before the addition of the sheep blood. The isolates were then point inoculated on the agar plate and incubated at 35 ^{0} C for 24 hours (Khan *et a*l., 2018; Russell and Bribo, 2006). This was conducted to check the ability of the isolates to lyse red blood cells.

Antibiotics Susceptibility Test

This was used to determine the resistance pattern of the isolates following Clinical Laboratory Standard Institute (CLSI) guidelines. Isolates were tested with 8 antibiotics; Cefuroxime (30 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Oflaxacin (5 µg), Augmentin (30 µg), Nitrofurantin (300 µg) and Ciprofloxacin (5 µg). A 0.5 McFarland turbidity was used as standard for each bacterial isolate emulsified in sterile normal saline. 0.1ml of the bacteria suspension was inoculated on Mueller Hinton agar. Plates were allowed to dry for 15 mins and antibiotic disc were placed aseptically on each inoculated plate. The plates were incubated for 24 hours at 35°C as described by (Matsuyama *et al*., 2015).Inhibition zones were measured and translated using CLSI, 2018 guidelines. Furthermore, the isolates were grouped as sensitive, intermediate and resistant to each antibiotic. Multiple antibiotic resistance (MAR) index was determined by the number of antibiotics each isolate was resistant to divided by the total number of antibiotics tested. Calculated values greater than 0.2 implies that the bacterial isolate may be linked to antibiotics abuse. *Escherichia coli* ATCC 25922 standard typed strain was used as a control (Adinortey *et al*., 2020)

RESULTS

The cultural characteristics of the colonies on Inositol brilliant green bile agar appeared as pink, smooth, opaque, circular, raised and mucoid colony. The results of the bacterial colony count were also determined and recorded in cfu/g. From location A (Ikotun market), the total viable count ranged from $1.8x10^4$ to $11.1x10^5$cfu/g for cat fish samples, while the tilapia samples had no growth recorded. Location B (Igando market) total viable count ranged between $1.0x10^4$ and $5.0x10^4$cfu/g for Cat fish samples while tilapia samples recorded no growth. Location C (Ile-epo market) total viable count ranged from $2.3x10^4$ to $8.05x10^4$cfu/g for cat fish samples, while tilapia samples recorded no visible growth. Location D (Iyana-iba market) total viable count ranged between $4.3x10^4$ and $9.2x10^4$cfu/g for cat fish samples while tilapia samples recorded no visible growth. In location E (Makoko market) total viable count was lesser as only one growth was recorded from one of the cat fish samples which is $9.0x10^4$cfu/g while tilapia samples recorded no visible growth. Location F (Ojuelegba market) recorded total viable

count ranging from $6.7x10^4$ to $7.0x10^4$cfu/g for cat fish samples while tilapia samples recorded no visible growth. location G (Oyingbo market) had a total viable count of $5.7x10^4$cfu/g from one of the cat fish samples while tilapia samples ranged between $0.7x10^4$ and $2.5x\ 10^4$cfu/g. location H (Ebute-meta market) had viable count from only one of the catfish samples with $6.9x10^4$cfu/g. Generally high bacterial load was observed in all the catfish samples from all the locations while tilapia recorded very low bacterial count in all the samples.(Table 1). The T-test analysis showed that there is significant difference between the isolates from cat fish and tilapia fish ($P<0.05$).

Morphological identification of the isolates determined through Gram staining showed that they were Gram negative rods and Biochemical characterization using Microbact 24E showed the organism to be motility, nitrate, oxidase, lysine, glucose, manitol, H_2S, xylose, urease, lactose,V. P, citrate, gelatin, malonate, inositol, adonitol, rhamnose, raffinose sorbitol, sucrose, arabinose and arginine positive but reaction to orinthine, ONPG, indole, TDA and salicin were negative. These results confirmed the isolates to be *Plesiomonas* sp. Hemolysis test on blood agar base showed varying zones of clearing confirming the ability of the isolates to secrete hemolysin, a compound that lyses the red blood cells. The result from the antibiotics susceptibility test carried out on the isolates using 8 antibiotics; CAZ (30µg), CRX (30µg), GEN (10µg), CXM (5µg), OFL (5µg), AUG (30µg), NIT (30µg) and CRO (5µg) showed that most of the isolates were multi drug resistant i.e they are resistant to more than 2 antibiotics (Table 2)

Multiple Antibiotics Resistance index revealed 5 resistance phenotypes. All the isolates had MAR index higher than 0.20 (Figure 1).

Table 1: Total colony count of fish samples from various locations.

LOCATION	MEAN COLONY COUNT OF FISH(CFU/g)	
	CAT FISH	TILAPIA
A	$1.8x10^4$ - $11.1x10^5$	0
B	$1.0x10^4$ - $5.0x10^4$	0
C	$2.3x10^4$ to $8.05x10^4$	0
D	$4.3x10^4$ - $9.2x10^4$	0
E	0- $9.0x10^4$	0
F	$6.7x10^4$ - $7.0x10^4$	0
G	0- $5.7x10^4$	$0.7x10^4$ - $2.5x\ 10^4$
H	0- $6.9x10^4$	0

A =Ikotun market, B = Igando market, C = Ile-epo market, D= Iyana-iba market
E = Makoko market, F = Ojuelegba market, G = Oyingbo market
H =Ebute-meta market

Table 2: Antibiotics Susceptibility Test for Catfish and Tilapia showing Inhibition Zones Measured in mm.

S/N	Isolates code	CAZ (30µg)	CRX (30µg)	GEN (10µg)	CXM (5µg)	OFL (5µg)	AUG (30 µg)	NIT (300 µg)	CRP (5 µg)
1	CFI	0	0	11	0	0	0	11	0
2	CFII	29	0	0	0	0	0	0	15
3	CFIII	20	20	21	0	0	0	0	0
4	CFIV	10	0	0	0	17	0	0	28
5	CFV	0	0	14	0	0	0	18	0
6	CFVI	29	0	19	20	8	0	0	24
7	CFVII	0	0	0	0	14	0	20	9
8	CFVIII	0	0	20	0	29	0	20	20
9	CFIX	30	0	0	0	0	16	29	7
10	CFX	15	21	10	23	12	0	16	0
11	CFXI	23	22	12	0	21	0	17	16
12	CFXII	30	0	15	0	13	0	15	30
13	TPI	0	7	0	8	24	0	12	26
14	TPII	0	0	11	0	8	0	10	0

Keys: CF= Catfish, TP=Tilapia
CXM- Cefixime, CRX- Cefuroxime, OFL- Ofloxacin, AUG- Augmentin, GEN- Gentamicin, NIT- Nitrofurantin, CRP- Ciprofloxacin. CAZ- Ceftazidime

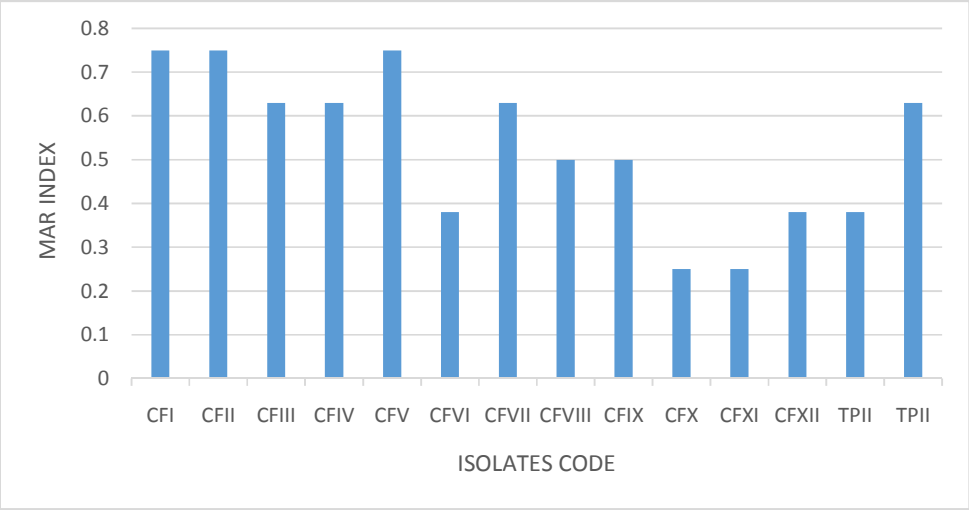

Fig 1: Multiple Antibiotics Resistance (MAR) index of the isolates.
This figure shows the resistance pattern of the isolates calculated using the formula MAR=a/b. Where a represents the number of antibiotics to which the isolates were resistant and b represents the total number of antibiotics the isolates were exposed to (Adinortey *et al.,* 2020)

DISCUSSION

Fish is a well-known food commonly regarded as a cheap source of protein in developing countries. The prevalence of pathogenic bacteria in sea foods has become an issue of great concern worldwide. This experiment was conducted to determine the prevalence rate of *Plesiomonas* sp in cat fish

(*Clarias gariepinus*) and tilapia (*Oreochromis niloticus*) collected from different locations between the month of August and September 2018 using the standard microbiological techniques. The results for cultural characteristics of the isolates were similar for both samples and the morphological characteristics showed the isolates to be Gram negative rods as described by (Shigematsu *et al.,* 2001). Biochemical characterization using Microbact 24 E showed the isolates to be positive to most of the tests and negative to few which confirmed the isolates to be *Plesiomonas* sp and supported by the work of (Wamala *et al*., 2018). The bacteria also have the ability to secrete hemolysin which is a compound that lyses the red blood cell. This is one of the virulent factors exhibited by this organism and this agrees with the findings of (Janda *et al*., 2016). The total bacteria count recorded showed the highest microbial load in cat fish sample from location A (Ikotun market) with $11.1x10^5$cfu/g and the lowest as $1.0x10^4$cfu/g from location B (Igando market). The tilapia samples majorly recorded no growth except for samples obtained from location G (Oyingbo market) with the highest microbial load recorded as $2.5x10^4$cfu/g and the lowest as $0.7x10^4$cfu/g this is in line with the work of (Wamala *et al*., 2018).This organism is considered an opportunistic pathogen that is widely distributed in aquatic environments but usually under reported. This can also be link to poor quality of pond water or fish feeds. The T-test analysis showed that there is significant difference between the colony count from cat fish and tilapia ($P<0.05$) which implies that this organism is more prevalent in cat fish than tilapia. Table 2 result shows majority of the isolates were multi drug resistant i.e they are resistant to more than 2 antibiotics many of which were resistant majorly to CXM, AUG, CRX and CRP which implies that this drugs cannot be prescribed or administered to control this organism as supported bythe work of (Baron *et al*., 2017).

Figure 1 reveal varying values and positions in the MAR index chart .MAR is a cost effective and efficient procedure in antibiotic susceptibility testing. This bacteria is an enteric opportunistic pathogen in humans and animals which can be linked to fecal contamination. MAR index values that are greater than 0.2 indicates high risk of contamination where antibiotics are often used. It can also be used as a marker to confirm the risk of pollution that may lead to life threatening issues. Generally, most of the isolate identified in all the locations recorded MAR index greater than 0.2.This implies that these isolates have been exposed to regular antibiotics. Thus optimization of antimicrobial drugs in aquaculture is necessary (Adenaike *et al.,* 2016 Adinortey *et al.,* 2020)

The ability of the bacterium to resist multiple drugs may result from frequent exposure to antibiotics in aquaculture and this can lead to public health threat if these fishes are consumed without proper preparations.

CONCLUSION

This study reveals a high prevalence of antibiotics resistant *Plesiomonas* sp in live cat fish sold in selected markets in Lagos, Nigeria. This may be linked to poor quality of pond water or fish feeds and the frequent use of antibiotics in aquaculture. This organism is considered an opportunistic pathogen that is widely distributed in aquatic environments but usually under reported.Therefore, it is of paramount importance to encourage best farming practices and retailing to prevent bacterial contamination. In addition, sea foods should be properly cooked before consumption.

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