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BACTERIOLOGY AND SENSITIVITY PATTERN OF SOME BACTERIAL ISOLATES FROM *CLARIAS GARIEPINUS* SOLD IN DIFFERENT MARKETS IN PORT HARCOURT METROPOLIS

***N. P. Akani, J. O. Williams and O. R. Njoku**

Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, P. M. B.5080. Rivers State.

***Corresponding Author: N. P. Akani**

Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, P. M. B.5080. Rivers State.

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ABSTRACT

Bacteriology and sensitivity pattern of some bacterial isolates from *Clarias gariepinus* sold in different markets in Port Harcourt Metropolis was investigated. Ten *C. gariepinus* from three markets (Mile 1, Mile 3 and Iwofe) were obtained and analyzed using standard methods. Results revealed no difference ($p \geq 0.05$) in the bacterial types from the 3 markets. Total heterotrophic bacteria ranged between 8.04±0.30 $Log_{10}CFUg^{-1}$(skin) and 8.14±0.18 $Log_{10}CFUg^{-1}$(intestine); Total Staphylococcal count, 4.92±1.17$Log_{10}CFUg^{-1}$(intestine) and 5.79±0.40$Log_{10}CFUg^{-1}$(skin); Total Pseudomonads count, 2.84±2.08$Log_{10}CFUg^{-1}$(intestine) and 3.73±1.98$Log_{10}CFUg^{-1}$ (skin); Total Vibriod count, 4.93±2.11$Log_{10}CFUg^{-1}$(intestine) and 5.78±0.80$Log_{10}CFUg^{-1}$(skin); Total coliform count, 7.96±0.37$Log_{10}CFUg^{-1}$(intestine) and 8.14±0.33$Log_{10}CFUg^{-1}$(skin). Two hundred and eighty (280) isolates were identified to belong to six genera namely; *Bacillus* spp (7.86%), *Escherichia coli* (12.86%), *Klebsiella* spp (15.00%), *Pseudomonas* spp (16.79%), *Staphylococcus* spp (22.86%) and *Vibrio* spp (24.63%) with *Bacillus* spp. being the least and *Vibrio* spp the highest. Susceptibility test conducted revealed that the organisms were generally sensitive to ciprofloxacin; *Vibrio* spp 61(88.4%), *Pseudomonas* spp 29(87.9%) and *Staphylococcus* 14(82.4%). Resistance to streptomycin was 53 (76.8%) and 26 (78.8%) for *Vibrio* spp and *Pseudomonas* spp. respectively. *Staphylococcus* spp was not susceptible to most of the antibiotics tested and showed 100% resistance to Zinnacef. The presence of increasing resistance of these potential pathogens can lead to re-emerging diseases of fish, economic loss and public health hazards. Ciprofloxacin can therefore be used as a drug of choice for the treatment of infections of *C. gariepinus* due to these pathogens.

KEYWORDS: *Clarias gariepinus*, bacteriology, antibiotics, susceptibilty pattern.

INTRODUCTION

Fish is a major source of food and global income. [1] It belongs to the class, Pisces. The usefulness of fish can never be exaggerated as it is known to be a low fat food, excellent source of protein, vitamins and minerals. According to,[2] over the years, agriculture has shown high interest in fish farming due to the importance of fish as a cheap source of protein, since beef meat including goat meat are expensive and beyond the reach of an average Nigerian citizen.

Clarias gariepinus (Burchell, 1822) also known as African sharp tooth fish is a species of catfish of the family Clariidae, the air breathing catfish. The species have the ability to survive and grow with an artificial and natural food, it has the ability to grow to a large size within a short period of time, it also has high yield potentials, with the ability to tolerate low dissolved oxygen and other aquatic conditions.[3]

C. gariepinus has been reared for many years in Africa with mixed success.[4] This is likely to be based on the fact that they live in microorganism-rich environment and are constantly exposed to attack or are vulnerable to pathogenic and opportunistic organisms.[5] The habitat of fish being aquatic is very challenging with fish constantly in contact with a great range of pathogenic and non pathogenic microorganisms.[6] Factors like congestion, periodic handling, high or sudden changes in temperature, poor water sanitation and malnutrition contribute to physiological changes in fish such as stress or immune suppression, thus, intensify their susceptibility to infection. Moreover, overcrowding of fish and poor water sanitation enhance the spread of pathogens thereby increasing the mortality rate.[7, 8, 9] Degradation in the environment due to pollution in aquatic habitat and poor culture conditions of some fish ponds has enhanced the presence of these pathogenic agents with bacteria known to be among the microorganisms of farmed catfishes and also among the

highly encountered cause of diseases in stressed warm aquaculture.[10] A number of bacterial pathogens have been reported to cause *Clarais* diseases worldwide. The presence of bacteria in *Clarais* could play diverse roles; some of which are beneficial while some could lead to post harvest spoilage or adverse health conditions.[11] Bacteria that infect fish belong to three groups: gram positive for example, *Staphylococcus aureus*, gram negative for example, *Pseudomonas* spp, and acid fast bacteria and several workers have conducted investigations on these bacteria.[12]

Knowledge about antibiotic susceptibility of bacteria is vital for the proper management of the diseases they cause. Worldwide use of antibiotics in aquaculture and the potential transmission of resistant bacteria between terrestrial and aquatic environments have been reported.[8] Veterinary drugs are usually used in aquaculture to prevent or treat disease outbreaks in order to prevent economic losses due to the above mentioned reasons. The veterinary drugs and other antimicrobial drugs are always administered as additives in fish food or sometimes as baths injections and can also be used as prophylaxis (prevent occurrence of disease), therapeutics (for treatment) or growth promoters.[13] Notwithstanding, administration of veterinary drugs is becoming reduced due to the fact that they present numerous side-effects for the environment and health safety. For example, the development of resistant bacteria strains has been noticed due to massive use of antibiotics.[14,15] The search for newer antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to common synthetic drugs.[16] Emergence of resistant strains of pathogenic microorganisms has also continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use and this therefore is the essence of this research. The objective of this study is to ascertain the bacteriology and sensitivity pattern of some bacteria isolated from *C.gariepinus* sold in different markets in Port Harcourt metropolis.

MATERIALS AND METHODS

2.1 Sample collection

Ten life adult *C.gariepinus* were obtained from mile 1, mile 3 and iwofe markets in Ikwerre local government and Obi/akpor area of Rivers State. The specie was chosen because of its availability all year round, ease of maintenance in laboratory condition and relative sensitivity (high level of tolerance). The samples were packed in a sterile container containing tap water and the mouth of the trough covered with a net and then transported to the Department of Applied and Environmental Biology Laboratory, Rivers State University Nkpolu for Microbiological analysis. The fish were killed and dissected in order to collect samples of the skin, gill and intestine tissues in sterile petri-dishes for bacteriology.

The gills, skin and intestine of the fish were soaked in water and forcefully agitated to dislodge the present microorganisms.

2.2 Enumeration and isolation of the bacteria

Enumeration of bacterial populations was done as described by.[17] The method used was the ten-fold serial dilution method.

One gram of each tissue (skin, gills and intestine) was aseptically introduced in 9ml of sterile distilled water giving an initial dilution of 1:10ml. Subsequently the serial dilutions were made by adding 1.0ml of the last dilution to 9.0ml fresh diluents. Finally 0.1ml of appropriate dilution (10^{-3} and 10^{-5}) were inoculated in duplicate onto sterile solidified Nutrient agar (NA), Mannitol Salt agar (MSA), Cetrimide (CA), Thiosulphate citrate bile salt agar (TCBS) and Mackonkey agar (MA) and evenly spread out with a sterile glass spreader. These were incubated at 37^{o}C for 24 hours and observed for growth. Plates with colonies between 30 to 300 were counted and representative colonies were sub-cultured onto sterile Nutrient agar (NA) plates to obtain pure cultures for further characterization.[18]

2.2.2 Identification of bacterial isolates

Identification of the bacterial isolates was done based on the method described by Buchann and Gibbon, Cowan, Cruickshank *et al* and Cheesebrough respectively.[19,20,21,22] These tests include Grams reaction, motility, catalase, oxidase, citrate, coagulase, indole production and fermentation of the following sugars: glucose, lactose, mannitol, sucrose, and fructose.

2.3. Antimicrobial Susceptibility

The antibiogram was done with the disk diffusion method[23] using Mueller-Hinton agar. Initially, an emulsion of sample in saline solution was prepared by adjustment to the 0.5 McFarland turbidity standard, equivalents to 1×10^{8}CFU mL^{-1}.[24] The susceptibility of the organisms were tested in relation to several families of antibiotics, including Pefloxacin (PEF;10µg), Gentamycin (CN;10µg), Ampiclox (APX;30µg), Zinnacef (Z;20µg), Amoxicillin (AM; 30µg), Rocephin (R;25µg), Ciprofloxacin(CPX; 10µg), Streptomycin (S;30µg), Septrin (SXT; 30µg), Erythromycin (E; 10µg), Oxacyline (OX; µg), Augmentin (AU; 30µg), Tarivid (OFX; 10µg), Chloramphenicol (CH; 30µg) and Sparfloxacin (SP;10 µg). Using sterile tweezers, commercially available antibiotic disks (Laborclin) were placed individually on the surface of Mueller-Hinton and incubated for 24hours at 37^{o}C. After 24 hours of incubation at 35^{o}C, the zones of inhibitions were read and recorded as "susceptible," "intermediate," or "resistant" as recommended by CLSI.[24]

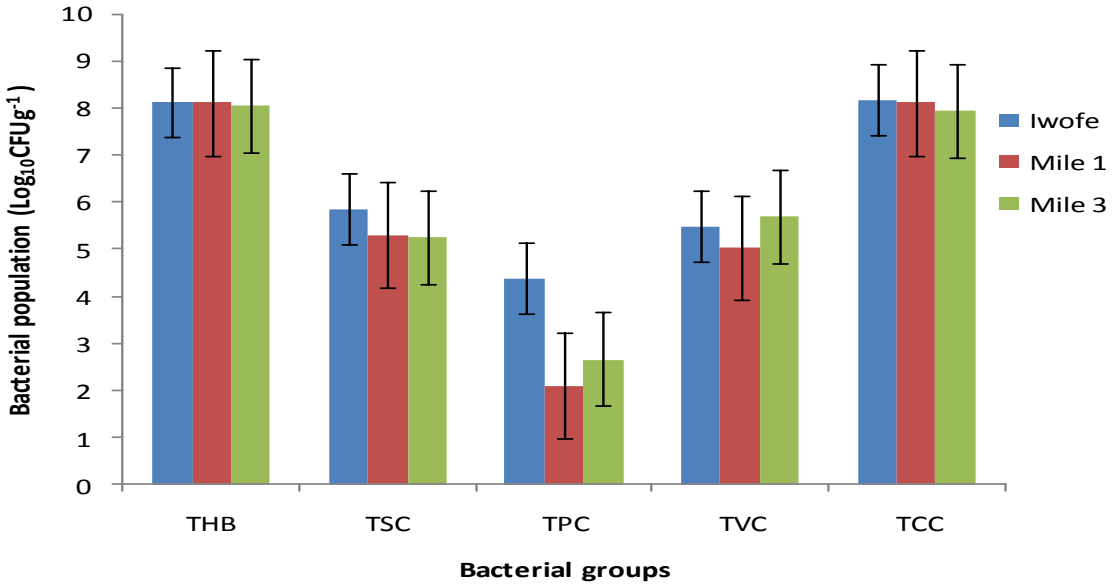
Statistical Analysis

Statistical analysis was carried out on the data obtained during the study. Analysis of variance (ANOVA) and

Student Newman Keul's (S-N-K) test was used to test for significance and mean separation respectively. This was done using a computer based program – SPSS version 22.

3. RESULTS AND DISCUSSION

Tissues (Skin, gills and intestine) of *Clarias gariepiuns* obtained from different markets were subjected to antibiogram to determine their susceptibility pattern. The genera isolated were identified as *Staphylococcus* spp, *Pseudomonas* spp, and *Vibrio* spp. Results of the bacterial population counts from different parts (skin, gills and intestine) of *Clarias gariepinus* from various markets sampled during the study periods are presented in Table 1 and 2. No difference ($p \geq 0.05$) was observed between the markets as well as different fish parts sampled.



Key: THB=Total Heterotrophic Bacteria, TSC=Total Staphylococcal Count, TPC= Total Pseudomonads Count, Total Vibriod Count, TCC=Total Coliform Count

Fig. 1: Variation in bacterial population in relation to the various bacterial groups obtained from the sampled *Clarias gariepinus*

Result of bacterial population in relation to the bacterial group shows that total heterotrophic bacterial (THB) counts and total coliform counts (TCC) were higher in all the sampled locations ranging between $8.05\pm0.04Log_{10}CFUg^{-1}$(Mile 3) to $8.12\pm0.07Log_{10}CFUg^{-1}$(Iwofe) and $7.95\pm0.29Log_{10}CFUg^{-1}$(mile 3) to $8.17\pm0.09Log_{10}CFUg^{-1}$(Iwofe). Followed by total Staphylococcal counts (TSC) and total Vibriod count (TVC) ranging between $5.25\pm0.35Log_{10}CFUg^{-1}$ (Mile 3) to $5.85\pm0.33Log_{10}CFUg^{-1}$(Iwofe) and $5.03\pm0.72Log_{10}CFUg^{-1}$(Mile 1) to $5.69\pm0.23Log_{10}CFUg^{-1}$(Mile 3).Total Pseudomonads Counts (TPC) were lower ranging from $2.11\pm0.88Log_{10}CFUg^{-1}$(Mile 1) to $4.39\pm0.64Log_{10}CFUg^{-1}$(Iwofe). There was no difference ($p \geq 0.05$) in the group of the organisms in relation to the markets except for *Pseudomonas*. This could be due to some predisposing factors which may enhance the proliferation of *Pseudomonas*. Such factors may include: physical stress such as excess of pH, injuries or damage to the skin mostly caused by cannibalism among *Clarias gariepinus*, reduced dissolved oxygen, presence of toxic substances in the water, malnutrition, overcrowding of the fishes in the pond and poor sanitation.[8,7,9] All the sampled *C. gariepinus* tissues obtained from Iwofe market had higher bacterial loads compared to other markets. This could probably be because Iwofe market is located in a very busy part of Obio/Akpor Local Government Area where daily activities go on and are likely to increase the microbial load. Fumes from the vehicles generated in Iwofe Motor Park, dust raised by the vehicles and individuals, aerosols from the dumpsites around the market and even activities like talking and sneezing from people in the market probably increases the bacterial load.

Table 1: Variation in of bacterial population ($Log_{10}CFUg^{-1}$) from different tissues of the sampled *C. gariepinus.*

Fish Parts	Bacterial Populations				
	THB Log_{10}	TSC Log_{10}	TPC Log_{10}	TVC Log_{10}	TCC Log_{10}
SKIN	8.04±0.30[a]	5.79±0.40[a]	3.73±1.98[a]	5.78±0.80[a]	8.14±0.33[a]
GILLS	8.10±0.27[a]	5.62±0.50[a]	2.88±2.28[a]	5.59±0.63[a]	8.09±0.28[a]
INTESTINE	8.14±0.18[a]	4.92±1.17[a]	2.84±2.08[a]	4.93±2.11[a]	7.96±0.37 [a]

*****means with the same superscript along the columns are not significantly different ($p \geq 0.05$)
Key: THB=Total Heterotrohic Bacteria, TSC=Total Staphylococcal Count, TPC= Total Pseudomonads Count, Total Vibriod Count, TCC=Total Coliform Count.

Although all the *C. gariepinus* sampled in this research appeared to be healthy, they were heavily infected with various bacteria species and they showed no difference ($p \geq 0.05$). Bacterial infestation is encouraged by certain predisposing factors which include the fact that fishes live in microorganism-rich environment and are constantly exposed to attack or are vulnerable to pathogenic and opportunistic organisms.[5] Table 1 generally showed that the total heterotrophic bacteria (THB) and total coliform counts (TCC) were higher in all the samples ranging between $8.04\pm0.30 log_{10}CFUg^{-1}$(Skin) to $8.14\pm0.18 log_{10}CFUg^{-1}$(intestine) and 7.96 ± 0.37 $log_{10}CFUg^{-1}$(intestine) to $8.14\pm0.33 log_{10}CFUg^{-1}$(Skin). Total Staphylococcal counts (TSC) and total Vibriod count (TVC) had lower counts ranging from $4.92\pm1.17 Log_{10}CFUg^{-1}$(intestine) to $5.79\pm0.40 Log_{10}CFUg^{-1}$(skin) and $4.93\pm2.1 Log_{10}CFUg^{-1}$(intestine) respectively and total Pseudomonads counts (TPC) had the least count between $2.84\pm2.08 Log_{10}CFUg^{-1}$(intestine) to $3.73\pm1.98 Log_{10}CFUg^{-1}$(skin).

From the results of table 1 above, the high level of bacteria in the intestine could be due to the fact that the intestine is a natural habitat for most bacteria. This agrees with the results Sowunmi and Akani who recorded high levels of bacteria in the intestine of *C.gariepinus*[25,26] and the high level of bacteria on the skin could be attributed to the environmental factors which include the nature of water in which they are grown,[27] the mode of handling by possible infected sellers who may be carriers of these organisms thereby transferring to the fish, congestion of the fish in the pond which could lead to cross contamination. The result of bacterial population of 10^8 conforms to the finding of Egbere *et al*[28] who reported counts in the range of 10^6 – $10^8 CFUg^{-1}$. Nwankwo and Akani[27] reported $1.71\pm0.71\times10^8$ $CFUg^{-1.}$ However, the result of this research was low when compared to the finding of Adedeji[29] who reported counts in the range of 10^{12} - 10^{13} $CFUg^{-1}$.

Table 2: Susceptibility pattern of *Staphylococcus* spp obtained during the study period.

Antibiotics	Susceptibility Pattern		
	Susceptible (%)	Intermediate (%)	Resistant (%)
PEF(10µg)	0(0.0)	3(17.6)	14(82.4)
CN(10µg)	0(0.0)	4(23.5)	13(76.5)
APX(30µg)	0(0.0)	1(5.9)	16(94.1)
Z(20µg)	0(0.0)	0(0.0)	100(100)
R(25µg)	1(5.9)	3(17.6)	13(76.5)
CPX(10µg)	14(82.4)	2(11.8)	1(5.9)
SXT(30µg)	1(5.9)	2(11.8)	14(82.4)
S(30µg)	2(11.8)	8(47.1)	7(41.2)
E(10µg)	0(0.0)	4(23.5)	13(76.5)
OX(µg)	3(17.6)	5(29.4)	9(52.9)

Key: PEF=Pefloxacin, CN=Gentamycin, APX=Ampiclox, Z=Zinnacef, R=Rocephin, CPX=Ciprofloxacin, SXT= Septrin, S=Streptomycin, E=Erythromycin, OX=Oxacyline.

Result of the susceptibility pattern of the isolated *Staphylococcus* spp. against the tested antibiotics is presented in the table 2. Among the ten commercially sold antibiotics tested, the organism was most sensitive to Ciprofloxacin (82.4%). Complete resistance of all the *staphylococcu*s isolates was observed in Zinnacef with 100% resistance. Others antibiotics like Ampiclox (94.1%), Pefloxacin and Septrin (82.4%), Gentamycin, Rocephin and Erythromycin (76.5%) also showed some levels of resistance. This multidrug resistance of *Staphylococcus* agrees with the research of Agoba *et al*[30] who also reported high resistance of *Staphylococcus* isolates from selected fish farms in Ashanti region of Ghana to most of the tested antibiotics. Constant resistance of Staphylococcus to drugs could probably be attributed to the fact that they are believed to posses some genes that enable its resistance to drugs and depending on the type of drug, different genes encode for resistance.[31] From this research, *Staphylococcus* isolates had complete resistance to Zinnacef/Cefuroxime which is a 2nd generation cephalosporin antibiotic. Therefore, with the blasZ-plasmid and MecA-acquired from the

targets, *Staphylococcus* is able to alter the penicillin-binding protein (PBP) thereby conferring resistance against cephalosporin drugs.[31]

Susceptibility pattern of *Pseudomonas* spp. isolated from the sampled *C.gariepinus* is shown on table 3 below.

Table 3: Susceptibility pattern of *Pseudomonas* spp. obtained during the study period.

Antibiotics	Susceptibility Pattern		
	Susceptible (%)	Intermediate (%)	Resistant (%)
AU(30µg)	15(45.5)	7(21.2)	11(33.3)
CN(30 µg)	3(9.1)	17(51.5)	13(39.4)
PEF(30 µg)	11(33.3)	11(33.3)	11(33.3)
OFX(10 µg)	20(60.6)	7(21.2)	6(18.2)
S(30 µg)	6(18.2)	9(27.3)	18(54.5)
SXT(30 µg)	2(6.1)	5(15.2)	26(78.8)
CH(10 µg)	7(21.2)	2(6.1)	24(72.7)
SP(10 µg)	8(24.2)	11(33.3)	14(42.4)
CPX(10 µg)	29(87.9)	4(12.1)	0(0.0)
AM(30 µg)	12(36.4)	8(24.2)	13(39.4)

Key: AU=Augmentin, CN=Gentamycin, PEF=Perfloxacin, OFX=Tarivid, S=Streptomycin, SXT= Septrin, CH=Chloramphenicol, SP=Sparfloxacin, CPX=Ciprofloxacin, AM=Amoxicillin

The result of the susceptibility pattern of the *Pseudomonas* isolates from the sampled *C. gariepinus* is shown above. Among all the tested antibiotics, Ciprofloxacin (87.9%) had the highest susceptible effect on all the isolates. Tarivid/Ofloxacin (60.6%) also had high susceptible effect. The isolates showed to be resistant to septrin (78.8%), Chloramphenicol (72.7%) and Streptomycin (54.5%).

Table 4: Susceptibility pattern of *Vibrio* spp. obtained during the study period.

Antibiotics	Susceptibility Pattern		
	Susceptible (%)	Intermediate (%)	Resistance (%)
AU(30µg)	40(58.0)	17(24.6)	12(17.4)
CN(30 µg)	30(43.5)	23(33.3)	16(23.2)
PEF(30 µg)	32(46.4)	16(23.2)	21(30.4)
OFX(10 µg)	51(73.9)	9(13.0)	9(13.0)
S(30 µg)	16(23.2)	10(14.5)	43(62.3)
SXT(30 µg)	12(17.4)	4(5.8)	53(76.8)
CH(10 µg)	3(47.8)	14(20.3)	22(31.9)
SP(10 µg)	30(43.5)	25(36.2)	14(20.3)
CPX(10 µg)	61(88.4)	7(10.1)	1(1.4)
AM(30 µg)	33(47.8)	19(27.5)	17(24.6)

Key: AU=Augmentin, CN=Gentamycin, PEF=Perfloxacin, OFX=Tarivid, S=Streptomycin, SXT= Septrin, CH=Chloramphenicol, SP=Sparfloxacin, CPX=Ciprofloxacin, AM=Amoxicillin

The susceptibility of the *Vibrio* isolates follows same pattern with that of *Pseudomonas*. This could be because both are gram negative rods. Ciprofloxacin (88.4%) showed to have the highest susceptible effect followed by Tarivid (73.9%) which is similar to that of the *Pseudomonas* isolates (87.95 and 60.6%) respectively. Resistance is shown with septrin (76.8%) and Streptomycin (62.3%) which is similar to that of the *Pseudomonas* isolates (78.8 and 54.5%) respectively. The high resistance in septrin conforms to the finding by Nsofor *et al*[32] where septrin was recorded to be 78.9% in *V. parahaemolyticus* isolated from sea foods sold in mile1 and mile 3 markets in Port Harcourt. Septrin and streptomycin are commonly used antibiotics for the treatment of bacterial infections and therefore is usually abused and the organism may have developed resistance to them.

CONCLUSION AND RECOMMENDATION

The bacterial load observed in this study is of public health concern. Although these fishes appeared physically healthy, the organisms (*Escherichia coli*, *Bacillus* spp, *Pseudomonas* spp, *Vibrio* spp and *Staphylococcus* spp) isolated from them are potential health hazards. It was also shown that several pathogenic bacteria associated with *C. gariepinus* were resistance to most of the antibiotics tested. It is therefore recommended that ciprofloxacin should be used for the treatment of diseases of *Clarias gariepinus* due to these microorganisms by incorporating them in their feeds. Also, on consumption of the diseased fish, these drugs can be used by the individuals for treatment.

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