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Bacteriological Examination of Tilapia (*Oreochromis niloticus*) in Makoko Market in Lagos, Nigeria

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Abstract

Bacteriological examination was conducted on forty fresh tilapia (*Oreochromis niloticus*) selected randomly from a market in Lagos using standard microbiological methods. The fish were carefully weighed and measured. Of the 40 fish, 16 (40%) weighed between 51and100 g, 12 (30%) weighed between 201and 250 g, while 4 (10%) weighed 1-50 g, 101-150 and 201-250 g respectively. Twenty(50%) of the fish had lengths ranging from 11-15 cm, 12 (30%) within 21-25 cm and 8 (20%) within 16-20 cm length while none of the fish was less than 10 cm in length. A total of 188 isolates which belong to seven different bacterial species were recovered from the gills and intestines of the tilapia. *Escherichia coli* constituted 48 (25.5%), *Proteus* spp., 36 (19.2%), *Klebsiella* spp., 32 (17%), *Pseudomonas* spp., 20 (10.6%), *Enterobacter* spp., 18(9.6%), Coagulase negative Staphylococci (CONS) 18 (9.6%), while *Staphylococcus aureus* 16 (8.5%) was the least. The bacterial isolates were evenly distributed in the gills 92 (48.9%) and intestines, 96 (51.1%). Similar bacteria were isolated from the gills and intestines of the fish. The bacteria showed a high susceptibility to all the antibiotics tested, thus are not likely to pose a public health threat.

Keywords: Tilapia, Bacterial pathogens, Morphometrics, Antibiotic susceptibility.

Introduction

Tilapia is a group of fish having economic, commercial and nutritional importance.

Oreochromis niloticus is a species having tolerance to low water quality and disease, yet in recent years, its cultivation has been faced with problems related to bacterial infections (Huicab-Pech *et al.,* 2006). The success of Tilapia production is due to their rapid growth; they are easy to propagate, are tolerant to many environmental conditions, easy acceptance of natural foods and supplements greater resistance to disease. (FAO, 2014).

Fish has been one of the main foods of human for many centuries. The short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level beyond the reach of the low income group especially in Nigeria (Ezeri *et al,* 2001).

There is however, increased demand for fish because it is the cheapest source of animal protein. Fresh water fish like tilapia represents an important source of animal protein to human nutrition. The challenge however due to pathogenic organisms especially bacteria has limited its effective production and availability (Olugbojo and Ayoola, 2015).

According to Hudson (1990), disease occurrence in aquatic animal production is showing a significant impact in yields. The possible economic issues a fish farmer may suffer in event of disease occurrence are high mortality, stunted growth during and after disease outbreaks, cost of treatment or prevention, profit loss as well as loss of investors' confidence. In addition, damage to the wild population which may lead to both loss of resources and decreased biodiversity which often result in a shift in ecological balance (Olugboju and Ayoola, 2015).

The fresh water or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as microorganisms introduced from terrestrial animal and plant sources (Adam and Tobaias, 1999). Many shell fishes are used for food particles from large volume of waters and if the waters are contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be consecrated by the filter feeding activities of shell fish (Adam and ,1999; Olugbojo and Ayoola, 2015). Through careless handling of fish, the natural flora of the environment may be contaminated with microorganisms associated with man which include members of the family Enterobacteriaceae and *Staphylococcus aureus* that grow optimally between 30 and 37^{O}C (Johan and Carina, 2007).

Most important bacteria diseases includes aeromoniasis, edwardsiellosis pseudomonias, flavobacteriosis, vibriosis, micobacteriosis as well as streptococcosis which have wide host range, and geographical distribution as well as zoonotic potentials (Haenen, 201; Wamala *et al,* 2018). The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish diseases and infections are very common and thus constitute one of the most difficult health problems to deal with, as such, bacteria can enter the fish through gills or skin or it can stay on the surface of the body (Sheyin and Solomon, 2017). By monitoring the bacterial contents of fish organs, the quality of fish can be assured since this will affect the storage life and quality of the fishery products (Olugbojo and Ayoola, 2015). A good knowledge of fish disease agents is required to prevent and cure fish diseases or minimize their negative effects. Of all fish diseases, bacterial diseases are widespread and can be of serious concern in fish farming as they are responsible for heavy mortality in both wild and cultured fishes (Hudson, 1990).

There is paucity of information concerning bacterial diseases of tilapia and there antibacterial susceptibility especially in developing countries like Nigeria.

This study analysed the bacteriological quality of tilapia by isolating, identifying and characterizing bacteria present in tilapia; it also investigate the prevalence or occurrence of such bacteria and assessed the antimicrobial susceptibility pattern of such bacterial isolates. In addition, the weight and sizes of tilapia were also evaluated.

Materials and Methods

Sample collection

Forty (40) samples of Tilapia *(Oreochromis niloticus)* were randomly selected and purchased from Makoko Market in Lagos and transferred in sterile iced packed cooler immediately to the Department of Microbiology laboratory of the Lagos State University, Ojo for analysis.

Morphometric parameters

Morphometric parameters of the fish sample such as weight, total length and standard length were measured with the use of top loading balance (Mettler's Balance) for weight and graduated measuring ruler for length. Values were recorded in two decimal places (Alabi *et al,* 2008).

Preparation of stock culture

A section of the gills and intestine of each of the forty samples of fish were specially removed by means of sterile scalpels and a pair of scissors and razor blade and kept in sterile Petri dishes. Four grammes of each section were homogenized using mortar and pestle to obtain uniform distribution of cells through stock culture.

Bacteriological analysis

Serial dilutions of the original stock culture from gills and intestines were prepared. Each dilution was plated on the solid dried freshly prepared nutrient agar and spread using a sterile glass rod and incubated at 37^{o}C for 24 h after which the colonies that developed on the plate were counted. Those counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC) District colonies from each plate were then plated

by means of a sterile loop and sub-cultured onto a freshly poured nutrient agar medium, MacConkey agar, Manimitol salt agar, eosin methylene blue agar and blood agar contained in sterile plates. This was done with a view to obtaining pure growth and the plates were incubated at 37^0C for 24 h.

Characterisation of pure isolate was performed and this involves colonial characters, cell micro-morphology, motility test and biochemical tests to identify isolates to generic and species level according to Cheesbrough, (2000).

Antibiotic susceptibility testing of isolates

Isolates were selected for antimicrobial susceptibility testing according to Kirby-Bauer disc diffusion techniques on Mueller–Hinton agar using the following, antibiotic discs (Oxoid) in microgrammes: gentamycin (10µ), penincilin G(10µ), tetracycline (10µ), chloramphenicol (30µ), rifampicin (25µ), streptomycin (5µ), and cefotaxime (15µ). The zone of inhibition was interpreted according to CLSI, (2010).

Statistical analysis

MS-Excel, V.7 and SPSS version 27 were used to present data obtained in this study.

Results

The total bacterial counts in *O.niloticus* showed that counts varied from one fish part to another. The intestine showed a mean count of 2.5 x 10^5 cfug- while the gill revealed a mean count of 4.2 x 10^4 cfug.

During the bacteriological investigation of the fish, a total of 7 species were recovered from the intestine and gills. The recovered bacterial belong to six genera of which *E. coli* had the highest prevalence of 48 (25.5%), followed by *Proteus* spp. with 36 (19.2%), while the least recovered bacterium was *S. aureus* with16 (8.5%) (Figure 1).

Table 1: Count and mean count of total viable bacterial in Tilapia fish

Parameter	Total viable bacteria Minimum	Total viable bacteria Maximum	Total viable bacteria Mean
Intestine	2.10 x 10^5	8.2 x 10^5	2.5 x 10^5
Gills	1.2 x 10^4	1.0 x 10^5	4.2 x1 0^4

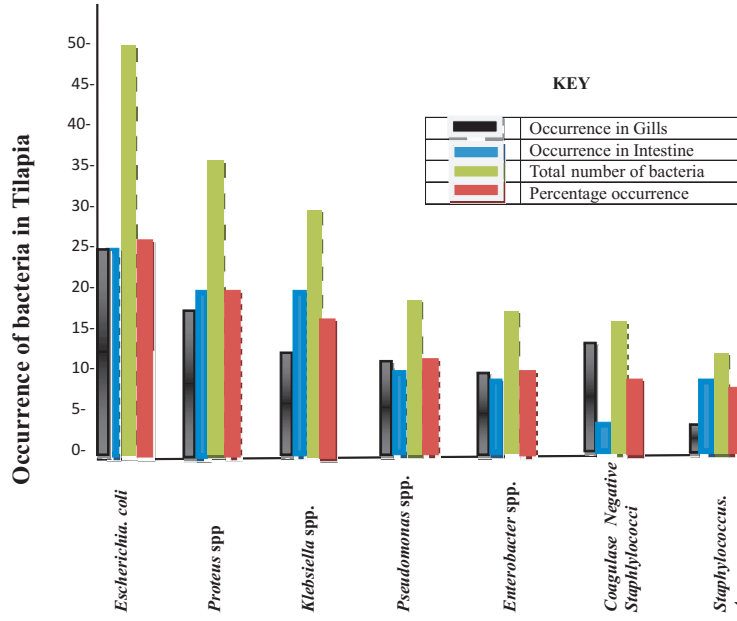

Figure 1: Distribution of Bacterial isolates from Tilapia

Table 2: Weight and length of Tilapia examined

WEIGHT (G)		TOTAL LENGTH (cm)		STANDARD LENGTH (cm)	
Range	**Frequency**	**Range**	**Frequency**	**Range**	**Frequency**
1-50	4	6-10	-	1-10	8
51-100	16	11-15	20	11-15	12
101-150	4	16-20	8	16-20	20
151-200	12	21-25	12	21-25	-
201-250	4	26-30	-	26-30	

The data in Table 2 revealed that most of bacterial isolates tested against various antimicrobial agents were susceptible to the antimicrobial agents evaluated.

Table 3: Antibiotic susceptibility profile (%)

Antibiotic/ Species	Ceftraxone	Streptomycin	Rifampicin	Ampicillin	Tetracycline	Pen G	Gentamycin
E . coli (40)	75	80	80	75	75	80	80
Proteus spp. *(35)*	93.3	9.71	85.7	91.4	91.4	85.7	85.7
Klebsiella spp. *(30)*	93.3	83.3	93.3	83.3	83.3	83.3	86.7
Pseudomonas spp. *(20)*	90	100	90	85	85	85	100
Enterobacter spp. *(18)*	88.9	94.4	94.4	88.9	88.9	8.33	100
CONS (16)	100	93.8	93.8	100	100	93.8	100
S. aureus(15)	100	93.3	86.7	86.7	100	100	93.3

A study of the weight of the fish revealed that 16(40%) weighed between 51and100 grammes and 12 (30%) weighed between 151 and 200 g while 20 (50%) were between 11-15 cm and 12 (30%) were between 21-25 cm in length (Table 2).

Discussion

Fish is one of the most important sources of human dietary animal protein worldwide especially in Nigeria and many other African countries. The Lagos Lagoon is exposed to many pollutants including untreated sewage, agriculture and industrial waste, which compromise the healthy state of the fisherman and the population inhabiting the contaminated areas (Moro *et al.,* 2016). Fish samples of Lagos lagoon were found to have very high content of bacterial pathogens as well as opportunistic pathogens.

Microorganism of human origin such as *E.coli, S. aureus*, *Salmonella* spp. and *Klebsiella* spp. have been found to proliferate in the gut and tissues of fish which render fish a carrier of disease causing bacteria (Udeze *et al.,* 2012). In this study, different types of bacteria of medical significance were isolated from the fish *(Oreochromis*, *niloticus*) caught from the Lagos Lagoon. Moro *et al.,* (2016) reported the recovery of thirteen enteric bacterial species from recirculatory aquaculture system in some fish farms in Lagos.

The results from this study showed that the bacterial loads vary between the gill and intestine. A high bacterial load in all samples were observed with gills having 4.2 x1 0^4 $cfug^{-1}$ and intestines 2.5x 10^5 $cfug^{-1}$. This may be attributed to the high ambient temperature in the lagoon where the fish was caught which is very close to optimum temperature for many mesophilie bacteria. The 5.5 x 10^9 cfug-^{1} intestinal bacterial load reported in Ferades (1989) is comparable to the result from this study. Bacterial load in the fish might increase with increase in temperature. The gills had a lower bacterial population compared to the intestine. The number of bacteria associated with gills is usually maintained at low level in order to keep the bacterial number low, and therefore afford the fish some degree of protection against bacterial invasion by the microflora of the gill (Ezeri *et al*., 2001; Lewis *et al,* 2002).

According to ICSMF (1986), any fish that has more than x10^6 bacterial count in a gramme is not suitable for human consumption, but because the gill and gut are always removed and discarded, there is a tendency for safety. It is however advisable that good and effective processing precautions be employed such as washing scraping of scales,

removal of gill and gut as well as cooking (Olugbojo and Ayoola, 2015). These will reduce the microbial load, thus prevent contamination of the flesh and muscle, hence safer for consumption. It is noteworthy that the microbial loads from the *O. nilotcus* fall within acceptable limit.

On the basis of percentage frequency, the Staphylococci (*S.aueus* and coagulase negative Staphylococci) which were the only Gram positive cocci were the least recovered. It is noteworthy that the remaining five bacterial species were members of the family Enterobacteriaceae. While the Staphylococci often originate from the fish handlers, the enteric bacteria such as *E.coli, Peroteus* spp., *Klebsiella* spp, *Enterobacter* spp and *Pseudomonas* spp. are likely to be of faecal origin. This finding agreed with Okpokwasili and Ogbulie (1993) that bacterial flora associated with Nigeria water culture include the genera, *Lactobacillus*, *Staphylococcus,* E*scherichia*, *Proteus*, *Enterococcus* and others, which can be traced to faecal contamination. The high prevalence of these bacteria which are indicator agents of pollution confirms that the lagoon was polluted .*Escherichia. coli* can cause urinary tract infection, bloody diarrhoea, severe anaemia and kidney failure which often result in death or further complications (Cabral, 2010). The bacteria isolated in this study can cause high mortality of the fish with attendant loss to the fishermen as such fish will definitely not be attractive to buyers (Moro *et al.,* 2016 ; Sheyin and Solomon, 2017).

The occurrence of *E. coli, Proteus* spp., *Klebsiella* spp., and *Pseudomonas* spp., in the fish may result from indiscriminate deposition of human and animal excreta as well as other environmental wastes into the lagoon that harbours fish or through washing of land surfaces into water bodies and through leaching during raining season (Cabral, 2010). These bacterial isolates can as well be traced to free roaming animals especially dogs and birds which contribute to faecal contamination of surface water (Green *et al,* 2012; Mauffret *et al*., 2012). It was observed that the Tilapia spices studied showed variation in length and weight. The absence of fish below 11cm and those above 25cm total length may be associated with selectivity. However, variation in weight could be due to several reasons which include age, fish size, season, feeding, sex, fishing activities and environmental changes. A similar finding was reported by King (1988) on the allometric growth pattern for Tilapia species. The weight and size of the tilapia showed that both morphometric parameters are directly proportional to each other.

The results of this study revealed that all the bacterial isolates from *O. niloticus* fish were susceptible to all the antibiotics tested. The bacterial isolates appear not to have been exposed to the antibiotics as against those produced through aquaculture which have the likely tendency to be treated and fed with feeds into which antibiotics have been incorporated. The bacteria recovered from this study are therefore not likely to pose a source of public health hazard.

Conclusion

A high distribution of bacterial species especially enteric bacteria and the staphylococci in the *O. niloticus* was found in this study. The bacterial isolates were susceptible to most of the antibiotics tested, thus appeared not to present a public health threat to consumers. There is, however, the need for fish mongers to take their personal hygiene seriously to avoid contamination of such fishes with bacterial pathogens which inadvertently may pose a health hazard to the unsuspecting consumers.

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