Antibiotic Sensitivity Pattern of Bacteria Isolated From Some Selected Fishes Sold In Bodija Market, Ibadan, Nigeria

ABSTRACT

Fish constitutes the cheapest source of animal protein in the world. The nutritional qualities and the shelf ability of fish can be affected due to microbial contamination after harvesting. Hence, this study is designed to investigate the microbiological analysis of fish and the antibiotic sensitivity pattern of the isolated bacteria from some selected fish sold in Bodija Market in Ibadan, Nigeria. Three different fish samples: Catfish (*Clarias gariepinus*), Sabalo fish *(Prochilodus lineatus*) and Tadpole fish (*Raniceps raninus*) were bought from Bodija market in an ice packed bag and then transported to Microbiology laboratory for further analyses. Microbial analysis were carried out using serial dilution and pour plate methods. The antibiotic sensitivity pattern on the bacteria isolates were carried out using commercial antibiotics. .*E. coli, Pseudomonas aureginosa*, *Staphylococcus aureus*, *Serratia* spp, *Klebsiella* spp, *Salmonella* spp, *Shigella* spp were isolated. Gram negative bacteria were more susceptible to most of the antibiotics used than gram positive bacteria. *E. coli* showed the highest susceptibility to all the antibiotics with varied zone of inhibitions. *Salmonella* spp show the highest susceptible to septrin with the least zone of inhibition to pefloxacin. *Shigella* spp had the least zone of inhibition to gentamycin, rocephin, septrin and erythromycin when compared to other bacterial isolates. *Serratia* spp exhibited highest zone of inhibition to gentamycin. Fish contamination can be averted by employing good hygienic conditions and preservation methods to avoid spoilage. The antibiotics sensitivity showed that all the bacteria isolated were more susceptible to antibiotics thereby developing a resistant strain.

Keywords: Antibiotic sensitivity, Zone of inhibition Selected fishes, Bodija market.

INTRODUCTION

Fish constitutes the cheapest source of animal protein in Africa. It is one of the main food components of humans for many centuries and still constitutes an important part of the diet of many homes (Abbas *et al.*, 2009). The advantage of fish as a food resulted from its easy digestibility and high nutritional value. Fishes are naturally found in water or artificially bred either for individual consumption or for commercial purposes. The earth surface is covered by 70% of water. The fresh water and salt water serve as natural reservoir for different type of fishes (Abbas, 2002). The quality of fish as well as its potential keeping time deteriorates rapidly leading to losses with regards to acceptable quality due to the growth of microorganisms or non-microbial causing lipid oxidation. Africa is endowed and constitute a rich source of numerous species of fresh fishes which include *Clarias* spp*, Bagrus* spp, Tilapia and others (Ahmed and Amos, 2007; Akinyele *et al*., 2013). The smoking of fish from smouldering wood helps in fish preservation giving the product a desirable taste and odour, longer shelf life through its antibacterial and oxidative

effect, lowering of pH, impartation of desirable colouration, acceleraton of the drying process and antagonising spoilage agents (Akinola, 2006). Spoilage of food products can be due to chemical, enzymatic or microbial activities (Ajayi *et al*., 2018). Chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fish products every year (Ahmed and Amos, 2007), and one-fourth of the world's food supplies through microbial activity alone (Adekola *et al*., 2006). Around 4-5 million tons of trawled and shrimp fish are lost every year due to enzymatic and microbial spoilage because of improper on-site storage (Archer, 2002). Spoilage of fish result in changes in its components by forming a new compounds which are responsible for the changes in odour, flavour and texture of the fish meat (Venugopal, 2002). Higher energy demanding freeze-storage preservation can be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage (Watkins *et al*., 2008). Combination of different preservation methods such as smoking and refrigeration retard the growth of spoilage microorganisms (Reij and Den Aantrekker, 2003). Compositional changes during fish spoilage result in lipid oxidation and protein degradation as well as the loss of other valuable molecules (Oni *et al*., 2018). In order to develop optimum preservation techniques for these value added products in active forms, deep understanding of the mechanism responsible for their degradation is essential **(**Hirsch *et al*., 1999). Bacteriological quality is of importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. Microbial hazards causing infections and poor health are closely related to food safety concerning the animal proteins derived from marketed food fish, fishery products and meat products (Adekoya *et al*., 2006). This creates a burning question for all consumers with a high risk commodity with regard to pathogenic bacteria contaminations which is alarming to food safety challenge. Food borne disease results from the ingestion of bacteria and the toxins produced by microorganisms present in the marketed (Kirby *et al*., 2003). However, this study identify pathogenic microorganisms associated with three different species fish commonly sold in Bodija market, Ibadan and also to know the antibiotic sensitivity pattern of the microorganism isolated from the fish species.

Materials and methods

Sample collection

Fish samples: Catfish (*Clarias gariepinus*), Sabalo fish*(Prochilodus lineatus*) and Tadpole fish (*Raniceps raninus*) were bought from Bodija Market, Oyo State from five different sellers between January and March. The samples were collected in an ice-packed bag and the immediately transported to the Microbiology laboratory for further microbiological analyses.

Isolation, enumeration and identification of bacterial isolates

Standard pour plate method was used for the isolation of microorganisms associated with the fish samples as described by Fawole and Oso (2004). About 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The samples were crushed into small pieces in a sterile mortar and homogenized in 10 ml sterile distilled water; 1 ml aliquot was measured and dispensed into test tube containing 9ml sterile distilled water and

serially diluted up to 10^6 dilution factor. Zero point one milliliter (0.1ml) of the diluent was aseptically pipetted and dispensed into sterile Petri dishes. The inoculated plates were pour plated using already sterilized molten agar medium (Nutrient agar, *Salmonella Shigella* agar and Mannitol salt agar). The plates were incubated at 37^{o}C for 24 hours. After incubation, the colonies were enumerated and recorded. Pure culture of the bacterial isolates were obtained by continuous streak on the appropriate medium. The bacterial isolates were identified based on their morphological and biochemical characteristics (Holt *et al*., 1994).

Antibiotics sensitivity test

Antibiotic sensitivity of the bacterial isolates was tested according to CLSI (2009) by disc diffusion method with an inoculums and agar dilution method. The interpretive categories were defined according to the zone diameter of inhibition. The broth culture of the bacterial isolates were prepared. Sterilized Muller Hinton agar was poured into sterile Petri dish allowed to gel, and was seeded with 16-24 hour old broth culture by swabbing using sterile glass spreader. The commercial antibiotics sensitivity disc were gently placed on the plate and labeled. The plates were inoculated at 37^{o}C for 24 hours and then examined for zones of inhibition. The antibacterial susceptibility pattern was measured by the zones of inhibition, examined and interpreted accordingly (Tiamiyu *et al*., 2015). The antibiotics used were PEF- Pefloxacin, GEN-Gentamycin, APX-Ampliclox, Z-Zinnacef, AM-Amoxacillin, R-Rocephin, CPX- Ciprofloxacin, SXT- Septrin and E-Erythromycin.

RESULTS

Total bacterial counts

Table 1 shows the total bacterial counts from the fish samples. The highest bacterial loads 2.0 were obtained from Sabalo fish *(Prochilodus lineatus*) while the Catfish (*Clarias gariepinus*) and Tadpole fish (*Raniceps raninus*) showed no significant different in their least counts 1.0. The bacteria isolated were *E. coli, Pseudomona auroginosa*, *Staphylococcus aureus*, *Serratia* spp, *Klebsiella* spp, *Salmonella* spp, *Shigella* spp (Table 2). *Proteus* spp and *Serratia* spp were found to be most occurring bacteria followed by *Esherichia* coli and *Salmonella* spp while *Staphylococus* spp and *Shigella* spp had no occurrence.

Morphological and biochemical characterization of bacterial isolates

Table 3 and 4 show the morphological and biochemical characterization of the bacterial isolates. The bacteria isolated were *E. coli*, *Pseudomonas aureginosa*, *Staphylococcus aureus*, *Serratia* spp, *Klebsiella* spp, *Salmonella* spp, *Shigella* spp.

Antibiotic sensitivity test of the bacterial isolates

Figure 1 shows the antibiotics sensitivity test of the bacterial isolates. All the bacteria isolated were susceptible to all the antibiotics used *E. coli* showed the highest susceptibility to all the antibiotics with varied zone of inhibitions. *Salmonella* spp show the highest susceptiblity to SXT with the least zone of inhibition to pefloxacin. *E. coli* had the highest zone of inhibition to gentamycin when compared to other isolates. *Shigella* spp had the least zone of inhibition to gentamycin, rocephin, septrin and erythromycin when compared to other bacterial isolates. *Serratia* spp exhibited highest zone of inhibition to gentamycin.

Table 1: Total bacterial counts from the fish samples

Sample	**Total bacterial counts (cfu/g) x 10^5**
Catfish (*Clariasgariepinus*)	1.0
Sabalo fish *(Prochilodus lineatus*)	2.0
Tadpole fish (*Raniceps raninus*)	1.0

Table 2: Occurrence and percentage occurrence of bacteria isolated from fish sample

Isolate	Samples			% Occurrence
	Catfish (*Clariasgariepinus*)	Sabalo fish *(Prochiloduslineatus*)	Tadpole fish (*Ranicepsraninus*)	
Proteus murabilis	-	+	+	33
Escherichia coli	-	-	+	17
Serratia spp	+	+	-	33
Salmonella spp	-	-	+	17
Staphylococus spp	-	-	-	0
Shigella spp	-	-	-	0

Table 3: Morphological Characterization of the bacterial isolates

Isolate code	Elevation	Edges	Optical characteristic	Colour	Surface	Cell arrangement
Cf 1	Raised	Entire	Opaque	Pink	Smooth	Chain
Cf 2	Raised	Entire	Opaque	Cream	Smooth	Cluster
Sab1	Raised	Entire	Translucent	Dark green	Smooth	Cluster
Sab 2	Flat	Entire	Opaque	Cream	Smooth	Cluster
Tad 1	Flat	Entire	Opaque	Cream	Smooth	Cluster
Tad 2	Entire	Entire	Opaque	Yellow	Smooth	Cluster

Table 4: Biochemical Characteristics of the bacterial isolates

Isolate code	Indole	Urease	Motility	Catalase	Voges-Proskauer	Maltose	Sucrose	Glucose	Lactose	Gram	Reaction	Probable Organism
Cf 1	+	+	+	+	-	A	A	A	A	+	Rod	*Proteus vulgaris*
CF2	+	-	+	-	-	AG	AG	AG	AG	-	bacilli	*Escherichia .coli*
Sab 1	-	+	-	-	+	A	AG	AG	AG	-	Cocci	*Serratia* spp
Sab 2	-	-	+	+	-	A	NA	AG	AG	-	Rod	*Salmonella* spp
Tad 1	-	-	-	+	-	A	A	A	AG	+	cocci	*Staphyloccus aureus*
Tad 2	-	-	-	-	-	NA	NA	NA	NA	+	Rod	*Shigella* spp

Keys : + = positive, - = negative, AG= Acid and Gas production, NA= NO Acid, A=Acid

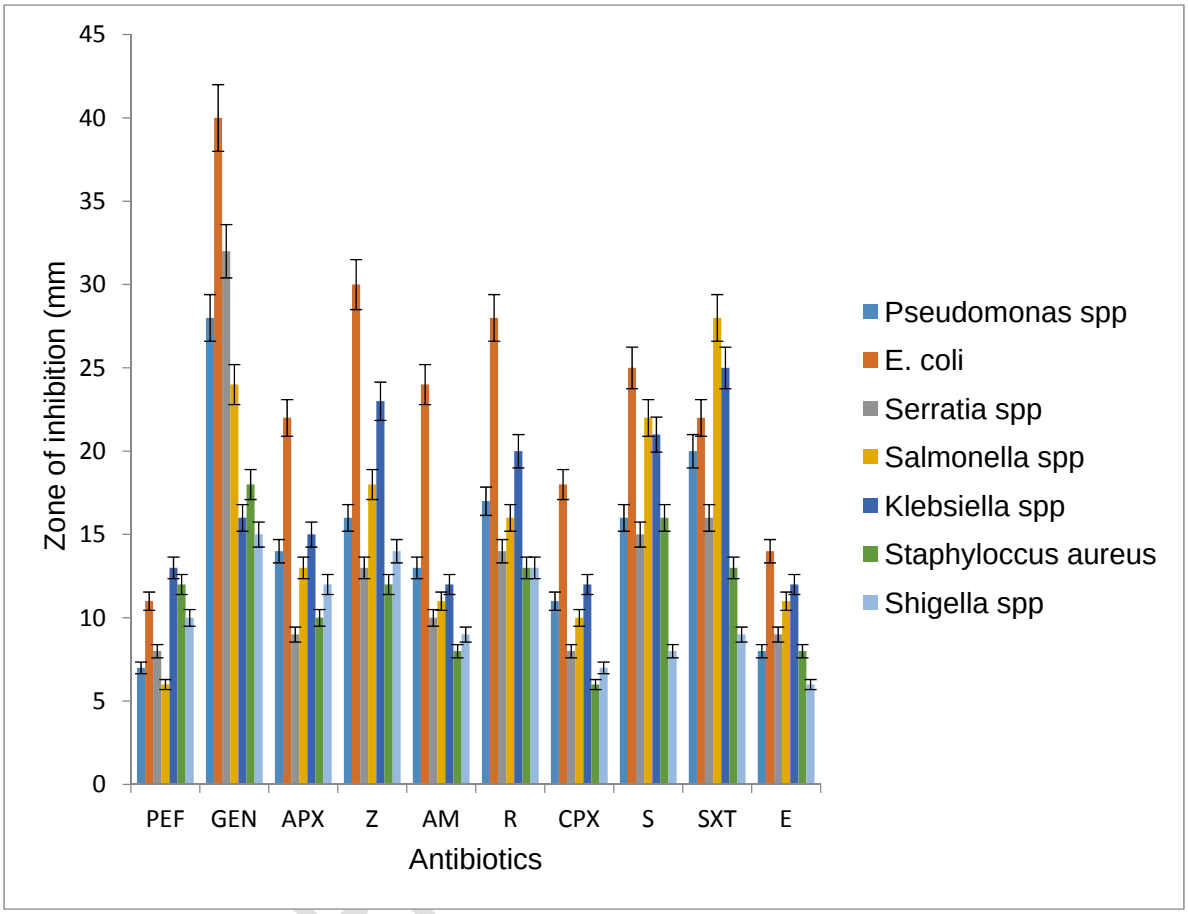

Figure 1: Antibiotic sensitivity test on the bacterial isolates

Key: PEF- Pefloxacin, GEN-Gentamycin, APX- Ampliclox, Z-Zinnacef, AM-Amoxacillin, R-Rocephin, CPX-Ciprofloxacin, SXT- Septrin, E-Erythromycin S- Septromycin.

DISCUSSION

The growth and survival of fishes in a water depend on the physicochemical conditions surrounding the water environment. The presence or accumulation of hazardous waste materialin the water bodies might affect the survival and quality of fishes. Different microorganisms are present in nature likewise those found in the water body can be natural microflora of fish. The growth of these microorganisms when considered in commercially sold fishes might render it unacceptable. This could pose health concern by ensuring fishes are free of contaminant mostly the pathogenic microorganisms. The occurrence of this microorganisms might arise possibly contamination during sales

and unhygienic handling of fish products, organic waste used for feeding of the fishes and their ability to survive in different habitats. Isolation of *E.coli* and *Salmonella* sppfrom marketed pawpaw fruit has been reported (Charo and Oirere, 2000). The presence of *Staphyloccus aureus* may lead to contamination of food and eventually affects the health of the consumers (Ayoola, 2007). Olukitibi *et al*. (2017) reported the occurrence of *Staphylococcus aureus, Escherichia coli* and *Samonella* spp from ponmo sold in Ogbese market. The result obtained from this study agrees with the findings of Akinola (2006) who reported isolation of *Staphylococcus aureus* from smoked fish.

Assessment of the antibiotic resistance among bacteria against antimicrobial agents is important for update on the bacterial antibiotic resistance patterns. It is part of a surveillance system aiming at monitoring emerging antibiotic resistant bacteria and their widespread. Isolation of antibiotic resistant bacteria from fish and aquaculture environment indicates the health risk associated with the aquaculture. The antibiotic sensitivity test help to ascertain their level of susceptibility or resistance of microorganisms to commonly used antibiotics. The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment. The high susceptibility level of *E.coli* to gentamycin when compared to other antibiotics had been reported by Kondro (2000). The resistant of microorganisms to some antibiotics might be due to resistance gene possess by them thereby causing cell lysis.Rompré *et al*. (2002) reported susceptibility of most pathogenic microorganisms to gentamicin and Zinnacef. Low frequency of antibiotic resistant bacteria may indicate the less activity associated with the contamination of antibiotics in the area (Yamaguchi *et al*., 2003). The use of antibiotics clearly show its effectiveness in control the growth of pathogenic microorganisms, inhibiting protein synthesis which prevent their permeability through their peptidoglycan layer (Spanggard *et al*., 2003). The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1%. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria.

Conclusion

High microbial load encountered in this study could account for the pathogenic microorganisms isolated and this can be controlled through proper hygienic and good maintenance of fish and fish products. The use of antibiotics inhibit the growth of spoilage and pathogenic microorganisms. Therefore, awareness of antibiotic resistance threat should be instilled in the community regardless of age as precaution and prevention step against dissemination of antibiotic resistant bacteria.

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