



## Bacteriological Evaluation of Deteriorated Frozen Fish Sold in Minna Metropolis, Niger State, Nigeria

<sup>1\*</sup>Baba, J., <sup>1</sup>Jibril, F.L., <sup>1</sup>Yakubu, Y.H., Muhammad, I.L., <sup>2</sup>Abdul-Rahman, A.A., <sup>1</sup>Dauda, D. and <sup>1</sup>Mabekeje O.O.

<sup>1</sup>Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Nigeria

<sup>2</sup>Department of Microbiology, Federal University Lokoja, Nigeria

### ABSTRACT

This study was aimed at analyzing the bacteriological deterioration of frozen fish sold in Minna metropolis. The fish samples were collected from four different markets within the metropolis. The bacterial load was determined using the pour plate method. Isolation and Identification of the various isolates were based on Gram staining technique and biochemical tests. Samples collected from Bosso market had the highest bacterial count of  $1.07 \times 10^6$ cfu/mg and Chanchaga market had the lowest Bacterial count of  $0.62 \times 10^7$ cfu/mg. Identification of bacteria showed the presence of *Staphylococcus aureus* 5(20%), *Streptococcus* sp 6(24%), *Pseudomonas aeruginosa* 4(16%), *Klebsiella* sp 5(20%) and *Bacillus* sp 5(20%). The antibiotics susceptibility test indicated that *S. aureus* was 100% susceptible to most of the antibiotics used in the study. Other Bacterial isolates also showed high susceptibility to the antibiotics, except *Streptococcus* sp that resisted Rifampicin, Amoxil, and Gentamicin by 50% each. *Bacillus* sp isolates also exhibited resistance to Erythromycin, Amoxil and Gentamicin by 50% each. It is evident from the study that proper hygienic measures must be taken concerning the handling of iced fish sold in the various markets in order to reduce bacterial contamination and subsequent infection to the barest minimum.

**Keywords:** Antibiotics, Bacterial, Identification, Isolation, Susceptibility

### INTRODUCTION

Fish is known for its high nutritional quality, relatively low fat content, saturated fat, cholesterol and high polyunsaturated fatty acids, protein and minerals, such as calcium, phosphorus, sodium, potassium and magnesium (Hanyel, 2008; Adewolu and Adeoti, 2010). Fish and fish products play a significant role in the diets of the populations of West African countries and constitute more than 60% of the total protein intake. In Nigeria, fish is eaten fresh, preserved or processed (Adebayo *et al.*, 2008).

Due to the crucial role played by fish production in meeting the protein demand, information on the parasites of fish becomes particular important, as they are known to significantly affect yield in fisheries (Hundson *et al.*, 2005). Despite all the benefits, there is a considerable risk of microbial contamination in this activity and consequently, in the finished product which represents critical concerns to public health questions (Ababouch, 2006). This occurs because large variety of pathogenic bacteria can

be introduced into different stages of the processing without proper control, affecting directly the sensorial quality, safety and the shelf life of fishery product (Kolodziejska *et al.*, 2002).

The natural habitat of fish is extremely prone to pollution that may be from domestic, industrial and agricultural discharges. The presence of bacteria in fish could be due to microbiological pollution of the lake, including storm-water that contain in deposits from wild life, agriculture, urban, forestry and rural settlement which is a potential health hazard to human (Bhagwat 2003; Popoff and Minor, 2007). The microbiological safety of sea food has been a source of concern to consumers, industries and regulatory agencies all over the world. The rapid and accurate identification of bacterial pathogens is important; both for quality

Received 11 June, 2021

Accepted 11 July, 2021

Address Correspondence to:

[babajohn200133@yahoo.co.uk](mailto:babajohn200133@yahoo.co.uk)

assurance and to trace bacterial pathogens within the food supply (Germini *et al.*, 2009).

Microbial activity starts in the different organs of fish after death, and the quality gradually deteriorates. The rate of deterioration or bacterial activity depends directly on storage and processing conditions of fish (Zambuchini *et al.*, 2008). Also the availability of vital nutrients in fish depends to a large extent on the methods of storage such as salting, roasting, drying, and freezing. A good storage condition can stop microbial activity and spoilage (Aaraas *et al.*, 2004).

Fishery products have been recognized as a major carrier of food-borne pathogens (Kama and Tejada, 2002; Upadhy and Lugg, 2010). Most fish related food borne illness are traced to *Staphylococcus spp*, *Escherichia spp*, *Vibrio*, *Clostridium perferenges*, *Streptococcus sp*, *Aeromonas sp*, *Salmonella sp*, *Shigella sp*, *E.coli* and *Yerisinia sp* (Ishood and Drake 2002; Yagoub and Ahmed 2009). Most human infections are acquired through consumption of under cooked food or contaminated water and food of animal origin (Lynch *et al.*, 2006; Majowicz and Nho, 2010). Bacterial infection of fish and fish products may influence human health by inducing disease and cause abdominal pain, acute gastroenteritis, bloody/mucoid diarrhea, nausea, vomiting and fever upon ingestion of contaminated fish products (Han *et al.*, 2001; Toranzo, 2005; Schmidt and Abdallah, 2006).

Frozen fish of different types are of great demands by the Nigerian consumers as it is a relatively cheaper source of animal protein. However the protein content decreases with increasing duration of frozen storage with fresh samples not frozen having higher protein content. Product dehydration, rancidity, drip loss and product bleaching are some of the disadvantages that have an overall effect on the quality of frozen food. In spite of some disadvantages associated with frozen storage, freezing is accepted as the effective way of preserving fish (Yagoub and Ahmed 2009).

Majority of the frozen fish marketed in Minna, Niger state are sold in open markets and therefore exposed to dust and contaminated air, encouraging the growth of microorganisms which might facilitate increased transmission and prevalence of pathogens. Most times the frozen fish are defrozen before they are bought

by customers, this encourages bacterial multiplication. Moreover poor personal hygiene, such as neglecting to wash hands after handling contaminated materials encourage contamination of frozen fish with pathogenic organisms. This study provides data on the bacteriological evaluation of deteriorated frozen fish sold in Minna metropolis, Niger state of Nigeria.

## METHODOLOGY

### Sample Collection

The study was conducted in Minna metropolis, Niger State. A total of ten samples were collected from each of the four different markets within Minna metropolis (Kure ultra-modern market, Bosso market Chanchaga market and Gwari market) at two weeks interval for two months (June-July, 2015). The samples were packed in a polythene bag and taken to the laboratory for analysis.

### Bacterial Colony Count

Preparation of serial dilution was done using the method of described by Cheesbrough (2006). The bodies of the fishes were scraped and swab stick was used to swab the fish body and inserted into the first test tube containing 9ml of distilled water to serve as the stock. Five other test tubes also containing 9ml of distilled water were arranged serially in the test tube rack. One (1) ml of the stock was taken using a pipette to the first test tube and from the first test tube to the second test tube, up to the fifth tube, i.e.  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  respectively. One (1) ml was taken from  $10^{-4}$  and  $10^{-5}$  factor into a sterilized petri dish, after which nutrient agar was added, the solidified ager plates were incubated at temperature of 37°C for 24hrs.

### Isolation of Bacteria from Frozen Fish Sample

Sub-cultured colonies were then streaked into Blood and MacConkey agar plates and incubated at 37°C for 24hrs for further isolation and identification.

### Characterization and Identification of the Isolates

All isolates were sub-cultured to obtain a pure culture and the pure isolates were Gram-stained to identify the isolates based on the method described by Cheesbrough (2006). The various

biochemical tests were performed for further identification of the isolates. The cellular morphology of the bacteria isolates were examined by studying their reaction to Gram stain and different biochemical tests such as catalase, coagulase, oxidase, indole, methyl red, voges-proskauer, citrate utilization, sugar fermentation etc.

### Antibiotic Susceptibility Testing

This was carried out by preparing a sterile Mueller-Hinton agar and was standardized as instructed by the manufacturer. The medium was dispensed in to petri dishes and allowed to solidify. The inocula were also prepared in test tubes in respect to 0.5 McFarland's standard. The test isolates were inoculated on the Mueller-Hinton agar using a sterile swab stick, after

which an antibiotic disc was placed onto the inoculated agar plates using a sterile forceps. The plates were incubated at 37°C for 24 hours. The plates were read and the zones of inhibition were measured to the nearest mm (Chesbrough, 2006).

### RESULTS

Table 1: Shows the average viable count of bacteria from the markets in Minna metropolis. It is evident from table 1 that the bacterial count across the market using dilutions  $10^{-4}$  and  $10^{-5}$  decreases with the increase in dilution factor. However, Bosso market had the highest bacterial count of  $1.07 \times 10^6$  at the dilution factor  $10^{-5}$ , while Chanchaga market had the lowest bacterial count of  $0.62 \times 10^7$  at the dilution factor  $10^{-5}$ .

Table 1: Average viable count of bacteria from the markets in Minna metropolis.

Markets	Dilution Factors	No. of Colonies	Population (cfu/ml)
Kure market	$10^{-4}$	103	$1.03 \times 10^6$
	$10^{-5}$	84	$0.84 \times 10^7$
Bosso market	$10^{-4}$	107	$1.07 \times 10^6$
	$10^{-5}$	92	$0.92 \times 10^7$
Chanchaga market	$10^{-4}$	80	$0.80 \times 10^6$
	$10^{-5}$	62	$0.62 \times 10^7$
Gwari market	$10^{-4}$	95	$0.95 \times 10^6$
	$10^{-5}$	90	$0.90 \times 10^7$

Table 2: Percentage incidence of the bacterial isolates from the markets in Minna metropolis

Isolates	No. of Isolates	Frequency of Occurrence (%)
<i>Staphylococcus aureus</i>	5	20
<i>Streptococcus</i> sp.	6	24
<i>Pseudomonas aeruginosa</i>	4	16
<i>Klebsiella</i> sp.	5	20
<i>Bacillus</i> sp.	5	20
	25	100

Table 3: Percentage susceptibility of bacterial isolates to antibiotics

Antibiotics	<i>S. aureus</i> n=5	<i>Streptococcus</i> sp n = 6	<i>P. aeruginosa</i> n = 4	<i>Klebsiella</i> sp n = 5	<i>Bacillus</i> sp n = 5
Streptomycin	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Ciprofloxacin	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Levofloxacin	100 (0)	80 (20)	50 (50)	100 (0)	75 (25)
Norfloxacin	100 (0)	80 (20)	65 (35)	100 (0)	80 (20)
Ampiclox	100 (0)	75 (25)	70 (30)	100 (0)	75 (25)
Chloramphenicol	90 (10)	100 (0)	100 (0)	100 (0)	100 (0)
Erythromycin	100 (0)	100 (0)	70 (30)	70 (30)	50 (50)
Rifampicin	100 (0)	50 (50)	60 (40)	85 (15)	55 (45)
Amoxil	100 (0)	50 (50)	50 (50)	100 (0)	50 (50)
Gentamicin	100 (0)	50 (50)	70 (30)	90 (10)	50 (50)

From the table 2 below, the percentage occurrence of the isolates indicated that *Streptococcus* sp is predominant with (24%) occurrence, while the least occurrence of the isolates was *Pseudomonas aeruginosa* (16%). *Staphylococcus aureus*, *Klebsiella* sp and *Bacillus* sp had equal occurrences of 20% each.

The reaction of the isolates to the different antibiotics is as shown in table 3 below. The percentage susceptibility of the isolates to the antibiotics indicated that *Staphylococcus aureus* was 100% susceptible to nearly all the antibiotics used in the study. Other bacterial isolates also showed considerable susceptibility to the antibiotics, except *Streptococcus* sp that resisted Rifampicin (50%), Amoxil (50%) and Gentamicin (50%). *Bacillus* sp exhibited resistance to Erythromycin (50%), Amoxil (50%) and Gentamicin (50%).

## DISCUSSION

The results obtained from this research indicates that frozen fish samples collected from Bosso market had the highest bacteria count of  $1.07 \times 10^6$ cfu/ml, while Chanchaga market had the least of bacteria count of  $0.62 \times 10^7$ cfu/ml. This could be due to insanitary conditions of the environment of vendors which leads to the contamination. According to Kam *et al.* (2001), the presence of these bacteria indicates poor sanitation within the environment and also during the handling and processing of the frozen fish. The results of this study also revealed that *Staphylococcus aureus*, *Streptococcus* sp, *Pseudomonas aeruginosa*, *Klebsiella* sp and *Bacillus* sp were the common pathogenic bacteria found associated with frozen fish in Minna metropolis. *Staphylococcus aureus* was isolated in fish samples. The presence of *Staphylococcus aureus* was due to the contamination of the fish by man through handling and processing. Its main habitat is humans and animals, and found mostly in nose, skin and throat of healthy individuals (Clucas and Ward, 2004). The genus *Staphylococcus* is made up of 36 Species which contain strains that are pathogenic, saprophytic or used as starter cultures for the food industry (Irlinger, 2007). According to Hencastre *et al.* (2007), the relevance of this species is related to its high capacity of colonization, because it can inhabit several environments such as soil, water and animal skin. *Pseudomonas aeruginosa* was also isolated from the frozen fish sample. This

finding is supported by Tripathy *et al.* (2007) that reported the bacteria not to be a cause of food borne illness, but are close to food deterioration. Tripathy *et al.* (2007) also reported *Pseudomonas* species to be associated with fish, and have been isolated from skin, gills and intestine. To further support the results from this study, *Pseudomonas aeruginosa* have been considered opportunistic pathogenic species (Altinok *et al.*, 2006). The findings of Moshood *et al.* (2012) corroborates the findings in this study, since common bacteria such as *Staphylococcus aureus*, and *Bacillus* sp were all isolated from frozen fish samples. The presence of these bacteria might be due to increase in moisture content of the product during storage. In recent study conducted by Tiamiyu *et al.* (2011), *Staphylococcus aureus*, *Bacillus* sp and *Streptococcus* sp were isolated from skin of *Clarias gariepinus* which also supports the outcome of this study. However, the outcome of this study does not agree with the findings of Efumtoye *et al.* (2012). The occurrence of *Streptococcus* sp and *Staphylococcus aureus* is in concurrence with Turker and Usta (2008). The isolation of *klebsiella* sp from this fish indicated faecal and environmental pollution and this support the findings of Yaqoub and Ahmed (2004) and Najjah *et al.* (2008). The occurrence of these bacteria in food (fish and fish products) may influence human health by inducing diseases infections and cause abdominal pain, acute gastroenteritis, blood/mucoid diarrhea, nausea, vomiting and fever (Akinjogunla *et al.*, 2009). According to Kamat *et al.* (2002), the presence of *Pseudomonas* sp in fish could be due to microbiological pollution of lake, including runoff and storm-water that contain deposits from wildlife, agriculture, urban, forestry and rural settlements which is a potential health hazard to human. According to Allshouse *et al.* (2004), *Salmonella* is the most common contaminant of fish and fishery products which does not conform with the results of this study. Huss *et al.* (2001) reported that the presence of these organisms could be due to contamination during multistep handling processes from harvest areas to the fish market resulting in deterioration of the quality of fish. The result of the antimicrobial susceptibility indicated that majority of the isolates were susceptible to nearly all the antibiotics used. *Pseudomonas aeruginosa*, *Bacillus* sp and *Streptococcus* sp were resistance to Amoxil, *Bacillus* sp and *Streptococcus* sp were resistance to gentamicin,

while *Streptococcus* sp, *Klebsiella* sp, *Pseudomonas aeruginosa* and *Bacillus* sp were susceptible to streptomycin and ciprofloxacin *Staphylococcus aureus* was susceptible to all the antibiotics. According to Adebayo and Janda (2006) *Streptococcus* sp (70%), *Staphylococcus aureus* (90%) and *Klebsiella* sp (80%) were susceptible to Gentamicin, while *Bacillus* sp (80%) and *Streptococcus* sp (80%) were susceptible to Norfloxacin which agrees with the findings of this study. There is a growing concern that excessive use of antibiotics in aquaculture is increasing the prevalence of antibiotic-resistant bacteria in aquatic food products (Cabello, 2006). Antibiotics resistance of some bacteria isolated from fish such as *Bacillus* sp, *Pseudomonas* sp and *Streptococcus* sp have been recorded by Mc Phearson et al. (2002) and this agrees with the results of this study.

### Conclusion

This study revealed that frozen fish sold within Minna metropolis could be a source of food borne bacteria pathogens, and this could lead to outbreak of food poisoning. The use of antibiotics in aquaculture, improvement in handling and processing of fish would help to minimize the prevalence of pathogenic bacteria in frozen fish.

### Acknowledgement

We wish to thank the Laboratory personnel who were very helpful in the course of the sample analysis, in the persons of, Mallam Hamza Aliyu and Mrs. Muyideen. A. Profound gratitude goes to Prof. I. B. Usman and Mallam Isah Chado for their useful suggestions regarding the sampling technique.

### REFERENCES

- Aaraas, N. M., Kaferstein, K. K., Molins, R. A., and Orban, E. (2004). Nutritional Quality of *Percafluviatillis*. *Food Chemistry*, 14, 255-264.
- Ababouch, L. (2006). Assuring Fish Safety and Quality in International Fish Trade. *Marine Pollution Bulletin*, 53 (12), 561-568.
- Adebayo, K. and Janda, J. M. (2008). Fisheries Statistics. *Fish Journal*, 4 (3), 121-125.
- Adewolu, B. and Adeoti, A. A. (2010). Fish as Diet for Man. *Fisheries*, 13, 116 – 125.
- Akinjogunla, O. K., Eghafona, N. O., and Ekoi, O. H. (2009). Diarrhoeagenic *Escherichia coli*. *Bacterial*, 1, 034 – 038.
- Altimok, I., Kayis, S., and Capkine, E. (2006). *Pseudomonas putida* Infection in Rainbow Trout. *Aquaculture*, 261 (3), 850 – 855.
- Allshouse, A. E., Buzby, D., and Harvey, D. (2004). Seafood Safety and Trade. *Agriculture*, 7, 782-789.
- Baftopadhyay, Y. (2000). Fish Spoilage. *Science*, 2 (3), 1651-1660.
- Bhagwat, M. (2003). Effects of Bacterial Diseases on Fish. *Biological Research*, 1, 106-118.
- Boscolo, W. R. (2001). Desempenho caracteristicas de Carcaca de Machos Revertidos de Tilapias do Nilo (*Oreochromis niloticus*), Nas fases iniciais de crescimento. *Revista Brasileira de Zootecnia*, 30 (5), 1391 – 1396.
- Burt, E., Orban, E., and Annes, E. (1992). Fisheries and Aquaculture. *Marine Pollution*, 53, 599-605.
- Cabello, F. C. (2006). Heavy Use of Prophylactic Antibiotics in Agriculture. *Environmental Microbiology*, 8, 1137 – 1144.
- Cheesebrough, M. (2006). Microbiological District Laboratory Practice in Tropical Countries. 2<sup>nd</sup> Edition. Cambridge Press, United Kingdom, 189.
- Clucas, M. O. and Ward, H. (2004). Antimicrobial Resistance in Bacteria Isolated From Aquaculture Sources. *Microbiology*, 100, 1103 – 1113.
- Daskalov, H. (2006). The Importance of *Aeromonas hydrophila* in Food Safety. *Food Control*, 17 (6), 474 – 483.
- Efumtoye, A. H., Barry, A., and Ayers, L. (2012). *Vibrio* Species Associated with Fishery Product. *Aquaculture*, 25: 633 – 637.

- Germini, D. E., Tatini, M., and Wagner, D. E. (2009). Incidence of Salmonella and Other Pathogenic Bacteria in Fish and Seafood. *Food Microbiology*, 63, 579-592.
- Hanyel, S. (2008). Characterization of Some Bacteria Isolated From Freshwater Culture Systems. *Microbiological Research*, 16, 391-396.
- Han, M. J., Yadav, N. S., and Moss, M. O. (2001). Fish Processing. *Science*, 13, 43-54.
- Hencaster, L., Armand, F., and Brish, H. (2007). The Ecology of Gram Positive Bacteria. *Microbiology*, 58, 762 – 772.
- Hundson, C. O., Ezeronye, O. U., and Chika, C. O. (2005). Parasites of Fishes. *Food Microbiology*, 31, 283 – 299.
- Huss, H. H., Jorgensen, L. Y., and Vogel, B. F. (2001). Control options for *Listeria monocytogens* in Seafood. *Food Microbiology*, 62, 267-274.
- Irlinger, M. S. (2007). Industrial Microbes. *Microbiology*, 102, 1167 – 1176.
- Ishood, B. L. and Drake, Y. S. (2002). Fish Pathogens. *Science*, 3, 256 – 259.
- Jalleawar, P. K. (2007). Genotypic Characterization of *Listeria* Species Isolated From Fresh Water Fish. *International journal of Food Microbiology*, 114 (1), 120-123.
- Kaferstein, K. K., Destro, M. T., and Fandos, E. (2000). Incidence of Enteric Bacteria in Fish and Fishery Products. *Food Microbiology*, 62, 191-196.
- Kama, F. L. and Tejada, M. (2002). Post-harvest Fisheries Development. *Food Science and Technology*, 4 (3), 121-125.
- Kamat, A. S., Bandekar, R. M., and Karami, S. (2002). Microbiological Quality of Some Major Fishery Products. *Microbiology*, 22, 51-61.
- Kam, K. M., Leung, T. M., and Saw, T. A. (2001). Outbreak of *Vibrio cholera* Related to Contaminated Fish Tank Water. *Public Health*, 109, 389-395.
- Kolodzieska, H. L., Hoh, J. G., and Reilly, A. (2002). Prevention and Control of Hazards in Seafood. *Food Control*, 11, 149-156.
- Lynch, E. L., Ipki, K., and Jonah, T. (2006). Survey of Specific Fish Pathogens. *Aquatic Science*, 21, 57-63.
- Majowicz, T. U and Nho, R. (2010). Parasites and Disease of Fish. *Aquatic Science*, 92, 111-119.
- Mc Phearson, R. M., Depaola, A., and Motes, M. L. (2002). Antibiotics Resistance in Gram-Negative Bacteria from Aquaculture Ponds. *Aquaculture*, 99, 203 – 211.
- Moshood, C. L., Lu, J. L., and Walt, P. C. (2012). Detection of Pathogenic Bacteria in Fishery Product. *Science*, 38, 207 – 209.
- Najiah, M. H., Rahl, L., Hamdan, W., and Wendy, M. (2008). Coliform Bacteria and *Salmonella* Species from Oyster. *Hydrobiology*, 3, 78-83.
- Olayemi, G. U., Offem, B. O., and Meyer, R. (2007). Bacterial Infection in Fish Farm. *Science*, 2, 153-161.
- Ophlen, R. (2009). Fisheries. *Biology*, 13, 66-71
- Popoff, J. K. and Minor, M. (2007). Microbiological Pollution of Lake. *Microbiology*, 20, 489-495
- Schmidt, H. Z. and Abdallah, M. E (2006). Incidence of Salmonella in Fish and Seafood. *Food microbiology*, 3, 579-592.
- Tejada, S. S., West, D., and Roth, E. (2007). Prevalence of Enteric Bacteria in Fishery Product. *Food Control*, 18, 1565-1568.
- Tiamiyu, A. K., Edwards, M. L., and Cooper, I. (2011). Isolation of Bacteria from *Clarias gariepinus*. *Science*, 35, 295 – 304.
- Toranzo, E.A. (2005). Bacterial food Spoilage. *Science*, 6:56-66.
- Tripathy, J. L., Dalsgard, I., and Brums, M. (2007). Isolation of *Pseudomonas* Species from Fish Product. *Aquaculture*, 53, 983 – 991.

- Turker, A. U. and Usta, C. (2008). Biological Screening of Some Turkish Medicinal Plant Extracts for Microbial and Toxicity Activities. *Production Research*, 22, 136-146.
- Upadhyaya Y. M. S. and Lugg, S. (2010). Prevalence of Enterotoxigenic *Staphylococcus aureus* in Fishery Product. *Marine*, 10 (12), 599-605.
- Yaqoub, S. O. and Ahmed, T. M. (2004). Pathogenic Microbes in Fresh Water Sample. *Husbandry*, 43, 32-37.
- Zambuchini, A., Embarek, P. K. and Huss, B. (2008). Critical Control Point in Ensuring the Safety of Raw Food. *Food Control*, 12, 347-356.