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Isolation and Identification of Bacteria from Ready-to-Eat Fish Sold in Makurdi Metropolis

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Abstract:
Fish is considered one of the sources of proteins, vitamins, and minerals as well as a necessary nutrient for dietary supplementation in both infants and adults. However, fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease. This research study was carried out to isolate and identify the bacterial contaminant of ready-to-eat roasted fish in Makurdi, Benue State. Twenty-five (25) samples were collected in clean polythene bags, 5 samples each from five different locations comprising High Level, Wadata, Wurukum, North Bank, and Kanshio. The samples were appropriately labelled and were taken to the laboratory for bacteriological analysis. The bacterial loads of the samples were determined using the Pour plate method of inoculation. The colonies obtained were sub-cultured repeatedly to obtain pure cultures. The isolates were identified using morphological and biochemical tests. The organisms isolated were Salmonella typhi, Escherichia coli, Shigella spp., and Staphylococcus aureus. The total viable counts ranged between $2.53x10^7$ to $5.5x10^7$cfu/g. The overall occurrence of bacteria isolated from the study area was 76.00%. Salmonella typhi (30.3%) had the highest occurrence while Shigella spp. (18.9%) had the least. With respect to locations, the North bank (28.3%) had the highest occurrence while the High level (15.1%) had the least. The implication is that consumers of this contaminated fish product are at high risk of getting infected with food-borne associated infections. Therefore, it is recommended that sanitary conditions under which ready-to-eat fish are handled, processed, and stored should be improved upon to reflect standard or good hygienic practices.

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INTRODUCTION

Fish is regarded as a source of proteins, vitamins, and minerals as well as a necessary ingredient that should be added to the diets of both infants and adults (Hadyait *et al.,* 2018; Shamsan *et al.,* 2019). Fish is a vital source of food for people and contributes about 60% of the world's supply of protein. 60% of the developing countries derive 30% of their annual protein from fish (Abisoye *et al*., 2011). It is the most important source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population. In Africa, fish supplies 17% of protein and it is one of the cheapest sources of protein. The advantage of fish as food is as a result of its easy digestibility and high nutritional value. Fish should be viewed not only as food but also as a ready source of income in the smallholder farming sector (Smith and Yoshida, 2010; Shamsan and Al-Jobory, 2018). Fish production in earth dams or ponds can revive the once abandoned lands and make them productive. Small-scale fish production also improves the livelihoods of the communal people and reduces the number of people who always depend on the government for economic assistance.

However, fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Yaqub *et al.,* 2017; Hefnawy *et al.,* 2019; Iqbal*et al.,* 2019). The microbiological diversity of fresh fish muscle depends on the fishing grounds and environmental factors around it. It has been suggested that the type of microorganisms that are found associated with a particular fish depends on its habitat. The bacterial pathogens associated with fish have been classified as indigenous and non-indigenous (Austin, 2011). The non-indigenous contaminate the fish or the habit one way or the other and examples include *Escherichia coli, Clostridium botulinum, Shigella dysenteriae, Staphylococcus aureus, Listeria monocytogens,* and *Salmonella*. The indigenous bacterial pathogens are found naturally living in the fish habitat for example *Vibrio* species and *Aeromonas* species. The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, and overstocking, which allow opportunistic bacterial infections to prevail (Austin, 2011). Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacterium, Streptococcus* spp*., Vibrio* spp*., Aeromonas* spp*., Salmonella* spp., and others.

The transmission of these pathogens to people can be through improperly cooked food or the handling of fish. The water quality parameters for fish farming impact fish life (Iqbal and Ashraf, 2020). There have been great economic losses reported due to food-borne illnesses such as dysentery and diarrhea resulting from the consumption of contaminated fish and such can be a problem to the immune-compromised, children and elderly people. Antibiotic-resistant bacterial infections have become a threat, in particular in developing countries (Iqbal and Ashraf, 2018; Ashraf *et al.,*2020).

The microbial association with fish compromises safety and the quality for human consumption; particularly critical when the microorganisms are opportunistic and/or pathogenic in nature (Mhango *et al*., 2010). The aim of the study was to isolate and identify the bacterial contaminant of ready-to-eat roasted fish in Makurdi metropolis, Benue State.

MATERIALS AND METHODS

Study area

Makurdi is the capital of Benue State; its commercial status was further enhanced when the Railway Bridge was completed and opened in 1932. Makurdi lies between latitude 7^{o} 43' 50" N and longitude 8^{o} 32' 10" E. It shares boundaries with Guma Local Government North East, Gwer to the South, Gwer-West to the west, and Doma Local Government Area of Nasarawa State to the North-West. The town is divided into two major blocks by River Benue, hence, the

North and South bank. Makurdi comprises eleven council wards and is inhabited predominantly by the indigenes of Makurdi; Tiv and Jukun people but Makurdi being the State capital, has become a cosmopolitan city with people of different tribes as Idoma, Igede, Agatu, Etulo, Igbo, Hausa and Igala with an estimated population of 500,797 with a landmass of 16km radius. The town has two universities which are, the Federal University of Agriculture and Benue State University (Britannica, T. Editors of Encyclopaedia, 2019).

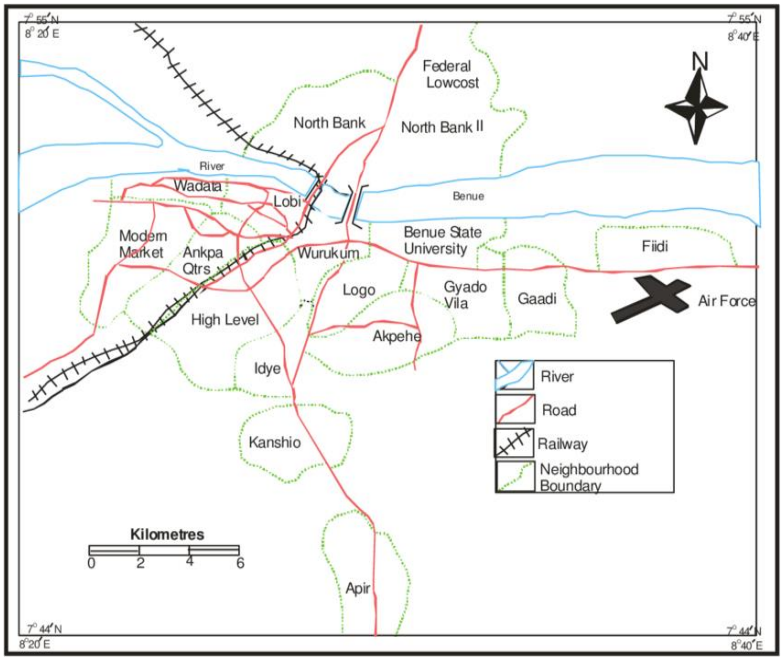

Fig. 1. Map of Makurdi Showing Study Area.

Source: Benue State Ministry of Lands, Survey and Solid Minerals (2015).

Collection of samples

Twenty-five (25) samples were collected in clean polythene bags, 5 samples each from five different locations comprising High Level, Wadata, Wurukum, North Bank, and Kanshio. The samples were appropriately labeled and were taken to Charis Rhema Research and Diagnostic Laboratories for bacteriological analysis.

Preparation of media

The media used in this work were Xylose-Lysine Deoxycholate agar, Mannitol Salt agar, Nutrient agar, Eosin Methylene Blue agar, Peptone Water, Sulphide Indole Motility Media, Simmon's Citrate agar, Triple Sugar Iron agar, and Urea Agar Base. They were all prepared according to their various manufacturers' instructions. The exact quantity of the dehydrated media was weighed and dissolved in the appropriate volume of sterile distilled water. The suspension was heated to boil and was sterilized using an autoclave at 121^{o}C for 15 minutes.

Determination of bacterial loads

The bacterial loads of the samples were determined using the Pour plate method of inoculation (Ochei and Kolhakter, 2008; Iqbal *et al.,* 2015**)**. The samples were serially diluted where 9ml of sterile distilled water was

measured into ten test tubes and labeled 10^{-1} to 10^{-10}. The samples were macerated and 1g was weighed and dissolved in 9ml of sterile distilled water and was properly mixed to have a homogenized mixture (stock solution). 1ml was taken from the stock sample and was transferred into the first tube labeled 10^{-1}, this was mixed properly and 1ml was taken out of the same tube (10^{-1}) and was transferred into the tube labeled 10^{-2}. This was done serially down to the last tube labeled 10^{-10} and 1ml was discarded from the last tube. 0.1ml was taken from tubes 10^{-7} and was aseptically inoculated into a petri dish and nutrient agar medium was poured into the petri dish containing the 0.1ml of the sample. The contents of the plate were mixed together by rotating the plates gently on a flat bench surface to ensure an even distribution of the sample in the medium. The plates were aseptically left undisturbed for some minutes for the medium to solidify and were inverted and incubated at 37^{o}C for 24 hrs. The colonies obtained after 24 hours of incubation, were counted using a colony counter and recorded in colony forming unit per milliliter (CFU/g).

Isolation of bacteria

The samples were inoculated into Trypticase Soy broth and incubated for 24 h. The broth cultures were sub-cultured onto EMBA, MSA, XLDA, and MA medium and were incubated for 24 hrs. The colonies obtained were further sub-cultured repeatedly to obtain pure cultures. The growth characteristics of the isolates on each growth medium were observed and recorded as described previously (Ochei and Kolhakter, 2008; Yunus *et al.,* 2016; Saleem*et al.,* 2018; Mohammad *et al.,* 2021).

Morphological/cultural identification

Gram staining

Gram staining was carried out according to the method described by Ochei and Kolhakter (2008). The colony of each isolate was emulsified using a wire loop, in a drop of normal saline on a clean microscope slide (smear). It was air-dried and was heat-fixed by passing the slide across a flame three times. The slide was placed on the staining rack was flooded with crystal violet for 1 minute and was rinsed with clean water. It was then covered with lugol's iodine (mordant) for 1 minute and rinsed with clean water. It was rapidly decolorized with acetone and was rinsed immediately with clean water. It was lastly stained with safranin (secondary dye) for 1 minute. The back of the slide was wiped with tissue and was allowed to air dry; the slide was mounted on the microscope stage and a drop of immersion oil was added. The specimen was brought into focus using a 100x objective with the iris diaphragm completely opened to increase the amount of light that entered the objective. Gram reaction was determined on the basis of the color of the dye retained; Gram-positive organisms retained the color of the primary dye (crystal violet) and appeared purple while Gram-negative organisms loosed the color of the primary dye after decolorization and picked up the color of the secondary dye (safranin) and appear red in color.

Motility test

The motility of the isolates was determined using sulfide motility indole media as described by Manhondo et al. (2010). The colony of the 24hrs isolate was picked using a straight inoculation needle; the medium was stabbed through the center to a depth of about 3 inches in the tube. The needle was removed from the medium along the same line it entered the medium. The tubes were incubated at 37°C and were examined daily for up to 7 days. The tubes were observed for a diffuse zone of growth flaring out from the line of inoculation which is indicative of motility motility-positive result; the inability of the organism to diffuse into the medium from the line of inoculation is indicative of motility-negative result.

Biochemical test

The isolates were further identified using various biochemical tests/characteristics as described by (Cheesbrough, 2006; Abdelhamid *et al*., 2006; Iqbal *et al.,* 2016).

Sugar fermentation test

The media (Triple Sugar Iron Agar) was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and incubated at 37^{o}C for 24hrs. The tube was observed for lactose, sucrose, and glucose fermentation on the basis of color change as a result of acid production in the medium due to the fermentation of the sugars by the test isolate.

Indole test

The test organism was inoculated in a test tube containing 5ml of sterile peptone water Cheesbrough, (2006) and incubated at 37^{o}C for 48 hours 0.5ml of Kovac's reagent was added and shaken gently and was examined for red color formation on the surface layer within 10mins. The formations of red color on the surface layer indicated an indole-positive result.

Coagulase test

The slide method as described by Cheesbrough, (2006) was used to carry out the test. A drop of distilled water was placed on a slide and colonies of the test organism were emulsified to make a thick suspension, and a loopful of plasma was added to the suspension and mixed gently and was observed for agglutination within 10 seconds. Agglutination within 10 seconds indicates coagulase coagulase-positive result.

Catalase test

A drop of hydrogen peroxide was placed on a microscope slide and the isolate was picked using a wire loop and was emulsified in the drop of the hydrogen peroxide and was observed for immediate bubble formation with indicated catalase positive result.

Citrate utilization test

The media was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the organism and incubated at 37^{o}C for 24hrs. A change in color from green to bright blue is indicative of a citrate-positive result.

Urease test

The media (urea agar base) was prepared in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and was incubated at 37^{o}C for 24hrs. The tube was observed for color change. A change in color from yellow to bright pink indicates urease positive result.

Statistical analysis

Percentage Occurrence of the bacterial isolates was calculated, and Chi-square was used to determine the relationship between bacterial isolates and sample location. P-values of less than 0.05 ($P<0.05$) were considered statistically significant.

RESULTS

Table 1 shows the total bacteria counts. The bacterial load ranged between $2.53 \times 10^7 – 5.51 \times 10^7$ cfu/ml. The occurrence rate varies significantly among the isolate [$X^2 = 14.712$, df = 4, P=0.000 (<0.05) between the different locations studied.

The cultural characteristics of the bacteria isolates are presented in Table 2 while Table 3 shows the biochemical characteristics of the isolates. The identified bacteria were: *Salmonella typhi, Staphylococcus aureus, Shigella* spp., and *E. coli.*

Table 4 shows the overall occurrence of the bacterial isolates with a statistically significant difference (<0.05) between the locations.

Table 1. Total Bacterial plate count with respect to the different locations.

Locations	Total plate count in CFU/ml (n=25)	Mean viable count
Wurukum	4.14×10^7	82.5
Wadata	2.53×10^7	50.6
Kanshio	5.51×10^7	110.2
North bank	5.17×10^7	103.4
High level	3.79×10^7	75.8
Total	21.14×10^7	84.56

$\chi 2$= 14.712; P= 0.000

Table 2. Cultural/Morphological Characteristics of Bacterial from Fish Isolates.

Code	EMBA	MSA	MA	XLDA	Presumptive isolates
A	No growth	yellow	No growth	No growth	*Staphylococcus aureus*
B	Greenish metallic sheen	No growth	Smooth pink	yellow	*Escherichia coli*
C	Colourless	No growth	Colourless	Red	*Shigella species*
D	Colourless	No growth	Colourless	Red with black center	*Salmonella typhi*

Key: EMBA = Eosin methylene blue agar; MSA= Manitol salt agar; MA= MacConkay agar; XLDA = Xylose lysine deoxycholate agar

Table 3. Biochemical Characteristics of the Isolates

Code	GR	Mot	Ind	Coa	Cat	Cit	Ur	Lac	Suc	Glu	Gas	H_2S	Slope	Butt	Isolates
A	-	+	+			-	-	+	+	+	+	-	A	A	*E. coli*
B	-	+	-			-	-	-	-	+	-	+	K	A	*S. typhi*
C	-	-	+			+	+	-	-	+	-	-	K	A	*Shigella* sp.
D	+	-	-	+	+	+	+	+	+	+	-	-	A	A	*S. aureus*

Key: Lac = lactose, Glu = glucose, Suc = sucrose, Ur = urea, Coa = coagulase, Cit = citrate, Mot = motility, Ind = indole, H_2S = hydrogen sulfide, A = acid reaction, K = alkaline reaction, - =negative, + = positive Gr = Gram reaction.

Table 4. The overall occurrence of Bacterial isolates with Respect to Location.

Location	No. Examined	No. of Positive samples (%)
Kanshio	5	4(80.00)
High level	5	2(40.00)
North bank	5	5(100.00)
Wurukun	5	5(100.00)
Wadata	5	3(60.00)
Total	25	19(76.00)

$\chi 2$=9.549, df = 4, p=0.000(<0.05)

Figure 2 shows the occurrence of the bacterial isolates. *Salmonella typhi* had the highest occurrence of 17(30.3%) followed by *E. coli* 14(25.0%), *Staphylococcus aureus* 12(19.7%), and the least *Shigella* spp. 10(15.8%). The occurrence rate varies significantly among the isolates [χ^2 = 21.767, df = 3, P=0.007 (<0.05)]. Table 5 shows the prevalence of the bacteria isolates with respect to location. The highest rate was observed in North Bank 15(28.3%), followed by Wurukun 11(20.7%), Wadata 10(18.8%), Kanshio 9(17.1%), and the least was observed in High Level with the occurrence rate of 8(15.1%). There is a significant difference between the isolates and location [χ^2=19.854, df = 3, p=0.042(<0.05).

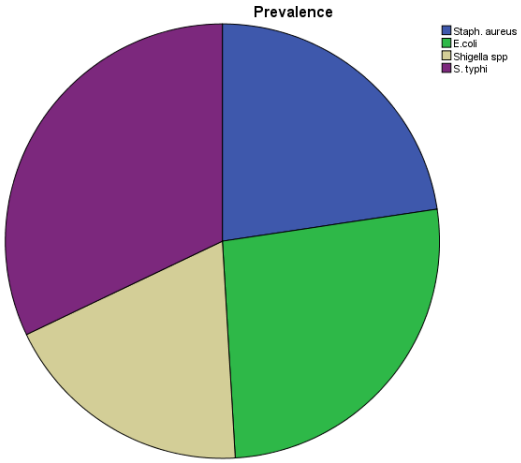

Fig.2. Occurrence of Bacteria Isolated from Ready-to-Eat Fish.

χ^2=16.259, df = 3, p=0.000(<0.05)

Table 5. Occurrence of the Isolates with Respect to Location.

Location	High level	Kanshio	North bank	Wurukun	Wadata
Staphylococcus aureus	1	3	5	2	1
Escherichia coli	3	2	2	4	3
Shigella species	0	1	4	3	2
Salmonella typhi	4	3	4	2	4
Total (%)	8(15.1)	9(17.0)	15(28.3)	11(20.8)	10(18.9)

χ2=19.854, df = 3, p=0.042(<0.05), (n=53

DISCUSSION

Ready-to-eat fish sold in Makurdi metropolis were analyzed for bacterial contamination. The overall occurrence of bacteria isolated from this study was 76.00%. This is quite high considering the fact that microorganisms are ubiquitous, this agrees with the findings of Eze et al. (2020). This high occurrence suggests the fact that these ready-to-eat fish sold in Makurdi Metropolis are highly contaminated with bacteria. The viable counts also suggest that the bacteria load in these ready-to-eat fish is highly significant. These pose a great risk to the consumers who usually eat them because the infective dose of these bacteria can be dangerous to humans when consumed, often leading to food poisoning. Another fact is that the viable counts of bacteria from this study are more than the accepted standard of (<10^4cfu/ml) as recommended by the World Health Organization (2006). The high rates of isolation suggest a significant public health concern. The implication is that consumers of these ready-to-eat fish are at high risk of getting infected with food-borne associated infections. This corroborates with the findings of Mhango et al. (2010) and Abisoye et al. (2011). The high number of *E. coli* 14(25.0%) is an indication of fecal contamination which could be attributed to the poor sanitary condition and unhygienic practices by the vendors in the study area. This study is similar to a study carried out by Abisoye (2011), who reported the presence of pathogenic organisms like *Salmonella typhi* and *E. coli* suggesting organisms that are transmitted via the fecal-oral route (Petronillah *et al.*, 2014; Christopher *et al*., 2009).

CONCLUSION

Bacteriological analysis of ready-to-eat roasted fish was carried out in this study. The occurrence of bacterial contaminants was found to be 76.0%. The organisms isolated were *Salmonella typhi, Escherichia coli, Shigella* spp., and *Staphylococcus aureus. S. typhi* had the highest rate of isolation. Ready-to-eat roasted fish from the North Bank were the most contaminated. The high rate of isolation recorded in this study calls for concern. This is because roasted fish which is contaminated with fecal material before or during harvest may cause outbreaks of intestinal infectious diseases such as typhoid fever.

RECOMMENDATIONS

Sanitary conditions under which fish are handled, processed, and stored should be improved upon to reflect standard or good hygienic practices.

Ready-to-eat fish should be displayed or sold in clean show glasses or properly packaged.

Fish handlers should be educated on proper hand washing during fish processing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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