Bacterial assessment of smoke-dried fishes sold at three landing market sites in Anambra State, Nigeria

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Abstract

The study focused on the bacteriological profile of four different smoke-dried fishes *Clarias gariepinus* (CG), *Oreochromis niloticus* (ON), *Malapterurus electricus* (ME), and *Citharinus citharus* (CC) sold at three landing market sites (Otuocha, Ose, and Ogbakuba) in Anambra State, Nigeria. Bacteria isolates from fish samples were examined and identified using microscopic, morphological, and biochemical characteristics. The results revealed the presence of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella flexneri* in all the fish samples from the three markets. The highest number of bacteria (1471 cfu/g), was found at the Ose fish landing market followed by Ogbakuba (1353 cfu/g), while the lowest number was found in Otuocha (957 cfu/g). Some of the isolated bacteria, *Staphylococcus aureus* and *Salmonella* sp are known to cause food borne diseases and are of public health concern. Therefore, regular disinfection and cleaning as well as regular inspection by health officers are advocated to improve the hygiene of the markets. However, it is recommended that consumers should cautiously subject the fish to additional processing such as adequate cooking in order to eliminate these bacteria and to avoid food poisoning.

Keywords: Bacteria, *Enterococus faecalis*, *Clarias gariepinus*, landing site, public health

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Introduction

Fish is one of the most significant sources of protein in the average Nigerian diet, hence, the most consumed in the country due to its distinct texture, and flavour (FAO 2016). Fish is a nutrient-dense food that contains high levels of vitamins, proteins, minerals, little or no saturated fat and low amount of carbohydrate (Edeh *et al* 2021). Being a source of vitamins and minerals, fish contains alternative vital nutrients needed to enhance the diet of adults and infants (Omoruyi and Ebhodaghe 2017; Alao *et al* 2017). It has a high acceptance due to the presence of the nature of its connective tissues making it easy to digest (Bogard *et al* 2017). Fish consumption has been shown in studies to reduce cardiovascular diseases, high blood pressure, cholesterol, Alzheimer's disease, and a variety of cancers (Belton *et al* 2018). In many Nigerian communities, smoked-dried fish products are popular (Omoruyi and Ebhodaghe 2017). Fish demand is increasing because of the health benefits and due to increase in human population (Mchazime and Kapute 2018). Fish is regarded as a delicacy by the majority of people, regardless of their religion, age and educational backgrounds (FAO 2018).

Fish is a perishable product with high moisture content, it is susceptible to deterioration as soon as it is collected, necessitating the need for preservation and processing (Ayeloja 2020). Common methods of preserving and processing fish are refrigeration, drying, or smoke-drying and canning (FAO, IFAD, UNICEF, WFP, and WHO 2020).

Smoke-drying of fish is a common and affordable method of fish preservation in Nigeria and some West Africa countries. Unfortunately, smoke-dried fish could be contaminated with different contaminants including bacteria and other pathogens, which could be of public health interest (Abiala *et al* 2020).

The various types of degradation and food poisoning caused by microorganisms can be avoided to a large extent by preventing microbial proliferation. According to Pilet and Leroi (2011), 10-20% of food-borne diseases are caused by fish and fish products. Smoked-dried fish products have been reported to harbour pathogenic

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bacteria such *Staphylococcus aureus*, *Salmonella* spp., pathotypes of *Escherichia coli*, and Listeria monocytogenes (Ayeloja *et al* 2018; Likongwe *et al* 2018; Udochukwu *et al* 2016).

During post-smoking handling, fish could easily be contaminated. Therefore, thermosensitive bacteria, such as Enterobacteriaceae could be used as markers of hygienic conditions and contamination of food with faces of either animal or human origin (Anihouvi *et al* 2019).

Some bacteria are not directly harmful to humans but through their activities, harmful secondary metabolites are produced. For instance, histidine decarboxylase activity in *Klebsiella pneumoniae* is known to enable the bacterium to produce histamine in fish products, which causes a variety of health issues in humans (Udochukwu *et al* 2016). This study was undertaken to evaluate the level of bacterial contamination of smoke-dried fish in three important markets in Anambra State to provide essential information that will guide proper fish handling and policy in order to avert adverse public health emergencies.

Materials and methods

Study area

Fish samples for this study were collected from Otuocha, Ose and Ogbakuba in Anambra East and Ogbaru Local Government Areas (LGAs) in Anambra State, Nigeria. Two important rivers, River Niger and River Anambra pass through these LGAs and thus sustain thriving fish markets in the area. The study sites lie in the tropical rain forest zone of Nigeria located in the southeastern region of the country. Anambra State is bounded by Delta State to the west, Imo State to the south, Enugu State to the east and Kogi State to the north (Anambra State of Nigeria, 2022). The sites experience two distinct seasons; rainy and dry seasons. The temperature of the sites between June and December, is usually 27-30°C, but between January and April, it rises to 32-34°C, with March having the greatest temperature of 36-38°C.

Sample collection

Smoked-dried freshwater fish samples were collected from February to April, 2021. A total of one hundred and twenty (120) fish samples, which included; 30 *Clarias gariepinus* (CG), 30 *Oreochromis niloticus* (ON), 30 *Citharinus citharus* (CC), and 30 *Malapterurus electricus* (ME) were collected from the markets. Fish samples within two to seven days of processing were randomly collected weekly from the fish processors at each site. The fish samples were collected in an aseptic manner using hand gloves and placed in well-sealed labelled Ziploc bags to avoid further contamination. The fish samples were transported at ambient temperature to the Nnamdi Azikiwe University Microbiology Laboratory for bacterial analysis.

Microbiological analysis

One gram (1g) of fish sample were weighed aseptically and placed in 10ml of sterile peptone water for bacteria isolation. The sample was thoroughly mixed to homogenize it. Peptone water was used as the diluent in a 10-fold serial dilution of each of the samples. The culture plates were incubated, 0.1ml of appropriate dilutions (10^{-2}) of the material were pour plated in sterile plates of Nutrient agar (NA) EMB, MacConkey agar, and Salmonella-Shigella agar. The culture plates were cultured at 37°C aerobically for 24-48 hours. All experiments were performed in triplicate. The number of developing colonies on the nutrient agar was counted to determine the overall viable population. Sub-culturing the bacteria into different nutrient agar plates yielded several discrete colonies, which were then identified using Balows *et al* (1991) approach.

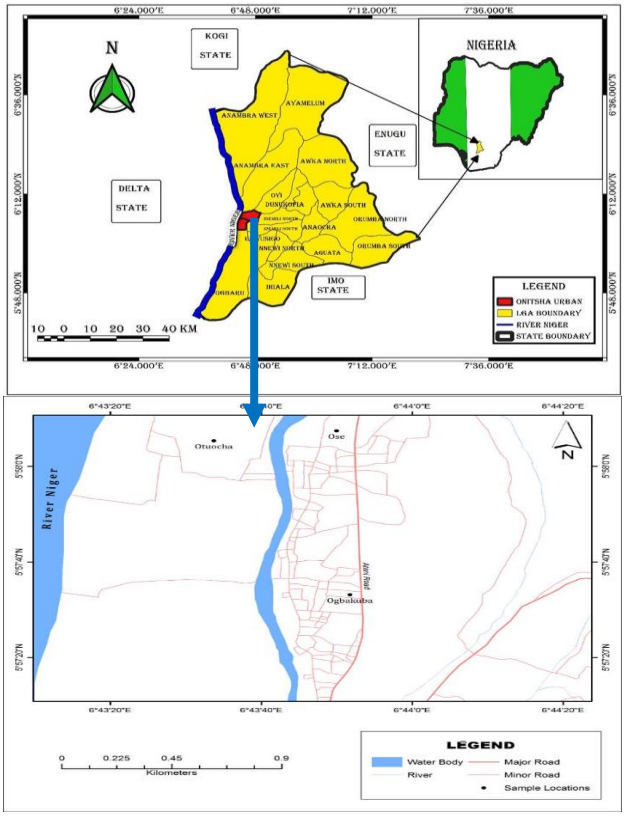

Figure 1. Map of the study sites with insert of map of Anambra State and Nigeria

Characterization and identification of bacteria

The bacterial isolates were identified by observing colony morphological traits that can be observed with the naked eye, such as shape changes, odour, and a raised surface on the agar. Biochemical assays such as gram staining reaction, catalase, citrate, sugar fermentation, coagulase, motility, oxidase, urease, indole, methyl red, and Voges-Proskauer tests were used to characterize and identified the isolates from the various fish samples collected from the selected sites, as described by Olutiola *et al* (2000).

Statistical analysis

The difference between mean bacterial load of the different fishes and sites were compared using ANOVA followed by Duncan multiple post hoc for multiple comparisons. Statistical analyses were performed using SPSS statistical software version 16.0.

Results

Twenty-one (21) different species of bacteria, which include *Enterococcus faecalis, Escherichia coli, Salmonella enterica, Shigella flexneri, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* were isolated from fishes collected from the three landing sites (Table 1). The number of isolates from the fish samples varied by location (Table 1). *Citharinus Citharus* (CC) samples had the highest mean number of isolated bacteria, with 442 cfu/g, 382 cfu/g, and 270 cfu/g from Ogbakuba, Ose and Otuocha, respectively. The number of bacteria isolates from *Citharinus Citharus* (CC) varied significantly ($p<0.05$) between the different landing sites. *Clarias gariepinus* (CG) had the lowest mean number of isolated bacteria, 218 cfu/g from Otuocha while *Orechromis niloticus* (ON) had the lowest mean number of bacteria at the Ogbakuba fish landing site (232 cfu/g).

Three thousand, five hundred and sixty-eight (3568 cfu/g) bacteria were identified from the fish samples collected at the different sites (Table 2). *Pseudomonas aeruginosa* (22.8%), *Enterobacter aerogene* (10.20%) and *Escherichia coli* (14.71%) were the dominant bacteria at Otucha, Ose and Ogbakuba markets, respectively. The highest percentage of bacteria contaminants were isolated from fish samples from Ose site (41%), while the lowest number of bacteria isolates was found at Otuocha site (26%) (Figure 2)

Table 1: Mean frequency of bacteria isolated from fishes from Otucha, Ose and Ogbakuba Markets

	Sites											
	Otuocha				**Ose**				**Ogbakuba**			
Bacteria Isolates	CG	ON	ME	CC	CG	ON	ME	CC	CG	ON	ME	CC
Aeromonas salmonicida	-	-	-	-	-	-	-	-	-	-	60	-
Bacillus licheniformis	-	-	-	-	-	-	-	45	-	-	-	42
Bacillus pumilus	-	-	-	-	-	-	40	-	-		-	
Bacillus subtilis	-	-	-	-	-	26	28	-	-	25	-	30
Citrobacter freundii	-	-	61	52	-	-	-	-	-	-	42	86
Enterobacter aerogene	-	-	40	37	45	42	63	-	38	-	78	-
Enterobacter cloacae	-	-	-	-	-	-	52	-	-	-	-	-
Enterococcus faecalis	40	-	-	-	-	52	-	59	-	47	-	41
Escherichia coli	50	56	-	-	58	-	54	-	60	-	78	61
Klebsiella planticola	-	-	-	-	-	-	-	42	-	-	-	-
Klebsiella pneumoniae	-	32	-	-	-	-	-	61		44	46	47
Micrococcus halobius	-	-	-	-	36	34	-	-	42	-	-	-
Micrococcus luteus	28	-	-	-	-	48	-	-	-	-	-	-
Micrococcus roseus	-	-	-	49	-	-	-	-	-	-		-
Proteus mirabilis	-	-	-	-	-	47	49	-	-	58	-	-
Proteus vulgaris	-	25	-	24	44	47	-	-	50	-	-	51
Pseudomonas aeruginosa	58	50	50	54	-	-	-	67		-	-	-
Salmonella enterica	-	38	34	-	42	38	41	-	55	58	-	-
Salmonella typhimurium	-	-	-	34	38	-	34	56	60	-	-	-
Shigella flexneri	-	20	25	20	-	-	85	52		-	30	74
Staphylococcus aureus	42	38	-	-	46	-	-	-	50	-	-	-
Total Occurrence (cfu/g)	**218**	**259**	**210**	**270**	**309**	**334**	**446**	**382**	**355**	**232**	**334**	**442**

- Not seen; CG-*Clairas gariepinus*, ON- *Orechromis niloticus*, CC-*Citharinus Citharus*, ME- *Malapterurus electricus*

Discussion

In this study, several bacteria species were isolated from smoke-dried fish from different markets. Some of the isolates such as *Psudomonas* spp and *Aeromonas* spp have been reported as specific spoilage organisms (SSO). This group of bacteria contributes to the spoilage of smoked fish, depending on storage and temperature (Ayeloja *et al* 2018). *Pseudomonas aeruginosa* was the dominant bacterium in Otuocha market, the dominant presence of this species in fish sold in the market suggests that fish from the market could have short shelf life and need additional processing and proper storage to prevent spoilage and consequent reduction in acceptability, palatability and nutritional quality.

Smoke drying is the most affordable method of fish preservation and well smoked fish can last for several months without spoilage if stored properly (Omoruyi and Ebhodaghe 2017). Consumption of smoked fish is very common in Nigeria and several West African countries as it is used to prepare many exquisite dishes. Previous reports have shown that bacterial contamination of smoked fish is possible (Udochukwu *et al* 2016; Ayeloja *et al* 2018; Likongwe *et al* 2018; Anie *et al* 2019).

Bacteria isolated from fish samples sold in Ose revealed that *Malapterurus electricus* (ON) had the highest number of isolates such as *Shigella flexneri, Enterobacter aerogene, Escherichia coli, Enterococcus faecalis* and others, while *Clarias gariepinus* (CG) had the least number of bacteria, which was predominated by *Staphylococcus aureus* and *Escherichia coli.* This finding is consistent with the findings of Anie *et al* (2019) that *Staphylococcus aureus* was the most isolated bacteria in smoked-dried fish samples from Delta State, Nigeria,

Table 2: Frequency and percentage distribution of bacteria isolates from the fish landing sites

	Sites					
	Otuocha		Ose		Ogbakuba	
Organism	**No**	**%**	**No**	**%**	**No**	**%**
Aeromonas salmonicida	-	-	-	-	60	4.43
Bacillus licheniformis	-	-	45	3.06	42	3.1
Bacillus pumilus	-	-	40	2.72	-	-
Bacillus subtilis	-	-	54	3.67	55	4.07
Citrobacter freundii	113	11.81	-	-	128	9.46
Enterobacter aerogene	77	8.28	150	10.2	116	8.57
Enterobacter cloacae	-	-	52	3.54	-	-
Enterococcus faecalis	40	4.3	111	7.55	88	6.5
Escherichia coli	106	11.4	112	7.61	199	14.7
Klebsiella planticola	-	-	42	2.86	-	-
Klebsiella pneumoniae	32	3.44	61	4.15	137	10.1
Micrococcus halobius	-	-	70	4.76	42	3.1
Micrococcus luteus	28	2.93	48	-	-	-
Micrococcus roseus	49	5.27	-	-	-	-
Proteus mirabilis	-	-	96	6.53	58	4.29
Proteus vulgaris	49	5.12	91	6.19	101	7.46
Pseudomonas aeruginosa	212	22.8	67	4.55	-	-
Salmonella enterica	72	7.74	121	8.23	113	8.35
Salmonella typhimurium	34	3.66	128	8.7	60	4.43
Shigella flexneri	65	6.99	137	9.31	104	7.69
Staphylococcus aureus	80	8.6	46	3.13	50	3.7
Total (cfu/g)	**957**		**1471**		**1353**	

- Not seen

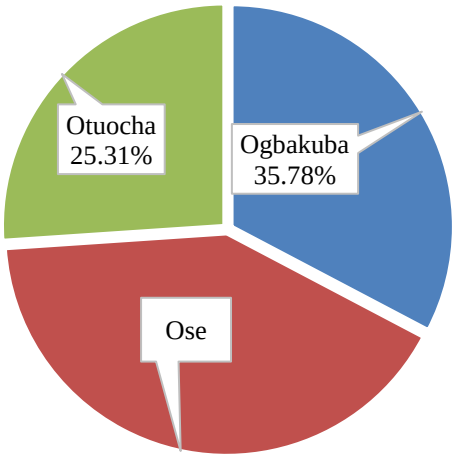

Figure 2. Percentage contamination of fish samples from the landing sites

accounting for 25%, while *Aeromonas* spp, *Micrococcus* spp, *Shigella* spp, *and Klebsiella* spp each accounted for 5% of bacterial load. Because *Staphylococcus aureus* is widely found on the hands, skin, clothing, utensils, air, and other surfaces, the presence of *Staphylococcus aureus* in the fish samples indicates that contamination may have come from the vendor's hands during processing (Kadariya *et al* 2014). Furthermore, *Staphylococcus aureus* produces enterotoxin, which has been reported as one of the major causes of food borne diseases (Kadariya *et al* 2014). This could lead hospitalization and loss in human productivity. The symptoms of the disease include nausea, vomiting, abdominal cramps, with or without diarrhoea (Kadariya *et al* 2014).

Enterobacteriaceae such as *E. coli*, *Klebsiella*, *Enterobacter*, *Salmonella*, *Shigella* and *Proetus* spp are indicators of poor hygiene and food contamination after cooking (Health Protection Agency 2009). These bacteria were isolated from the fish collected from the different markets, which shows that fish from the markets were contaminated with faecal microparticles from animals and/or humans during post-smoking handling. This finding is consistent with those of Ezemba *et al* (2017) and Ineyougha *et al* (2015), who isolated numerous comparable bacteria genera of public health concern from processed fish samples in different Nigerian markets. In this study, the highest number of bacteria was found at the Ose fishing site while the lowest number of bacteria isolates was found at the Otuocha location. This variation in bacterial load could be attributed to the processing hygiene of the fish handlers across the various sites, as also observed by Ibemenuga *et al* (2017), Ibanga *et al* (2019), Ikechukwu *et al* (2022) and Ineyougha *et al* (2015) in different sites in Nigeria. The findings of this study have revealed that high bacteria occurrence was linked to all smoke-dried fishes in Ogbakuba, Ose and Otuocha fish landing sites in Anambra State and strict caution should be employed to further process the fish before consumption.

Conclusion

Our study has shown that bacterial contamination of smoked fish is high and it is mostly attributed to post smoking handling and poor hygiene practices in the different markets. Some of the contaminating bacteria could cause early spoilage of fish, which could lead to further contamination and loss of nutritional value. In additional, consumers of contaminated fishes could be exposed to pathogenic bacteria. Therefore, regular monitoring of these fish markets is advocated, health officers should visit these markets regularly to ensure that standard hygiene level is maintained always for public safety.

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