Isolation and Identification of Bacteria Associated with Commercially Hawked Ready-To-Eat Fried Fish Sold in Jos Metropolis

ABSTRACT

Aims: This study aimed to isolate and identify bacteria associated with commercially hawked fried fish sold in Jos metropolis in Plateau State,Nigeria.
Methodology: A total of 20 fried fish Samples were obtained through random sampling of fish from vendors and collected in a sterile polyethylene bag and packaged in a sterile foil paper and then it was transported to the laboratory.The media used such as Nutrient agar (NA), Blood agar and MacConkey agar were prepared following the manufacturer's specification. For microbial isolation, 5mL of each sample was dissolved into 50mL sterile water inside different beakers. Then, 1mL of the dissolved sample was aseptically transferred into 9mL sterile distilled water and serially diluted up to the appropriate dilutions (i.e., 10^{-1} to 10^{-6} dilutions). From the dilutions 10^{-3}, 0.1mL was aseptically transferred into sterile Petri dishes and their spread plated with already sterilized molten media. The Petri plates were incubated at 37^{o}C for 24hrs for bacteria. After incubation, the cultural characteristics, colonial morphology were observed on the plates. Pure colonies were obtained by sub-culturing on a fresh growth media and stored at a refrigeration temperature of 0-4^{o}C. Microbial isolates were identified by biochemical and phenotypic characterization.
Results: A total of 35 bacteria species were isolated from 20 commercially hawked fried fish samples sold within Jos metropolis of Plateau State, Nigeria. The laboratory analysis revealed a significant microbial contamination. Pathogenic bacteria Isolated includes: *Staphylococcus aureus*, *Klebsiella aerogenes*, *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa* and *streptococcus spp.,* while non-pathogenic or opportunistic isolates includes: *Bacillus coagulans, Bacillus circulans, Citrobacter spp., Coagulase-negative staphylococci* and *Rahnellainusitata.* Total bacterial counts (TBC) reached alarming levels, such as 3.6 x 10^6 CFU/g in sample ABTe. Notably, *Klebsiella aerogenes* was the most frequently isolated organism, accounting for 28.57% of all isolates, indicating widespread contamination in the local aquatic environment.
Conclusion: The presence of pathogenic bacteria underscores the need for improved food safety measures among vendors. Comparative analysis with prior studies shows consistent contamination levels, reinforcing the necessity for stringent hygiene practices.

Keywords: *Isolation, Identification, Total bacterial counts (TBC), Microbial examination*

1. INTRODUCTION

In many countries of the world, fish is eaten and considered a rich source of protein (Gufe et al., 2019). With over 16% of the animal consumed worldwide coming from it, it is the most important source of protein. To accommodate demand, fish is frequently hawked commercially in markets and streets, making it readily available to consumers (ElGarhy et al., 2023). However, the handling and storage techniques of commercially hawked fish in Nigeria are frequently insufficient, providing a favourable habitat for bacterial growth (Schijns et al., 2021). Fish are frequently exposed to contaminants including dust, dirt, and other environmental pollutants, which can promote the growth of dangerous bacteria (Meador et al., 2018). Furthermore, fish dealers' lack of sufficient storage facilities, limited refrigeration, and poor hygiene standards increase the danger of infection (Owoeye et al., 2023). The World Health Organisation (WHO) estimates that foodborne infections impact around 1.5 billion people globally, resulting in 3 million deaths per year (Shrivastava et al., 2016). Foodborne illnesses are a major public health concern in Nigeria, with gastrointestinal infections being particularly common in children and the elderly (Olusegun et al., 2018). Fish is a common carrier of foodborne infections, including bacteria like Salmonella, Vibrio, and Escherichia coli. These bacteria can cause a variety of ailments, from mild gastroenteritis to potentially fatal infections like septicaemia and meningitis (Wang & Park, 2022). Nigeria's tropical climate, weak food safety regulations, and limited access to proper healthcare all contribute to an increased risk of foodborne

infections related with fish eating (Saeed et al., 2021). Despite the importance of fish as a food source and the potential health hazards connected with its intake, there has been little investigation into the bacterial contamination found in commercially hawked fish in Nigeria (Chukwu, 2013).The presence and kinds of pathogenic microorganisms in fish and fish products are well established, based on epidemiological data from the CDC, FDA, and the United States Department of Agriculture (USDA), as well as survey research described in the scientific literature (Barrett et al., 2017). A few reviews have summarised the occurrence of these pathogens in seafoods, with Salmonella spp., L. monocytogenes, Vibrio spp., Yersinia spp., C. botulinum, S. aureus, and Aeromonas spp. being the most commonly reported pathogens. Salmonella has been the primary bacterial cause of fish-related epidemics. Despite its low occurrence in fish outbreaks, Listeria monocytogenes is the most prevalent bacterial cause of fish and fish product recalls.

Furthermore, L. monocytogenes's ubiquity in the processing environment, as well as its capacity to multiply at low temperatures, make it a big problem for ready-to-eat fish products. Aside from the aforementioned pathogens, which are listed as bacterial pathogens of greatest concern in the Fish and Fishery Products Hazards and Controls guidance (Costley-Jessie, 2019), Aeromonas spp. have attracted attention as an emerging human pathogen associated with seafood products (Costley-Jessie, 2019). Aeromonas hydrophila, which is extensively dispersed in aquatic habitats, can not only cause infections in fish and humans, but it can also raise histamine levels, presenting a chemical risk to human health (Vergis et al., 2021). Antibiotic-resistant bacteria (ARB) have recently emerged as a significant concern for not only aquaculture, but the entire food business (Pepi and Focardi, 2021). AMR has emerged as both a global public health problem and an environmental pollution concern (Limbu, 2023). The plasticity of bacterial communities allows for the rapid spread of ARGs to other microbial communities via horizontal gene transfer methods such as conjugation, transformation, and transduction. Mobile genetic elements (MGEs) are DNA segments that may travel inside a genome and across bacteria, such as plasmids, transposons, and bacteriophages; these MGEs have been found to play an important role in horizontal gene transfer and ARG spread (Zhao et al., 2021).Plasmid-mediated conjugation is the most prevalent way for bacteria to horizontally acquire resistance genes (Zhao et al., 2021). Once the ARGs are transferred to a bacterium, resistance to antibiotics can be developed by destroying or modifying the antibiotic itself, developing an active efflux system to pump the antibiotic out, and/or reducing membrane permeability to decrease influx, modifying antibiotic receptors, altering metabolic pathways, and other biological mechanisms (Yuan et al., 2023).

This study intends to fill a knowledge vacuum by isolating and identifying bacteria found in commercially hawked fish in Nigeria, establishing their prevalence, and assessing the potential health consequences of consuming contaminated fish.

2.MATERIALS AND METHODS

2.1 Sample Collection

A total of 20 pieces of ready-to-eat fried fish (5 pieces from each of the study locations) were aseptically collected from each location, namely Farin-Gada Market (Labelled FGa-FGe), Abattoir Market (Labelled ABTa-ABTe), Building Market (Labelled BDa-BDe) and Bukuru Market (Labelled BKa-BKe), all located in Jos North and Jos South Local Government areas of Plateau State, Nigeria. To prevent contamination, samples were collected in sterile aluminium foil paper and polyethene bags and immediately transported to the National Veterinary Research Institute's (NVRI) Vom microbiological laboratory for analysis.

2.2 Media preparation and microbial analysis

The media used such as Nutrient agar (NA), Blood agar and MacConkey agar were prepared following the manufacturer's specification. For microbial isolation, 5mL of each sample was dissolved into 50mL sterile water inside different beakers. Then, 1mL of the dissolved sample was aseptically transferred into 9mL sterile distilled water and serially diluted up to the appropriate dilutions (i.e., 10^{-1} to 10^{-6} dilutions). From the dilutions 10^{-3}, 0.1mL was aseptically transferred into sterile Petri dishes and their spread plated with already sterilized molten media. The Petri plates were incubated at 37^{o}C for 24hrs for bacteria. After incubation, the cultural characteristics, colonial morphology were observed on the plates. Pure colonies

were obtained by sub-culturing on a fresh growth media and stored at a refrigeration temperature of 0-4^{o}C. Microbial isolates were identified by biochemical and phenotypic characterization.

2.3 Microbiological analysis of fish samples

2.3.1 Determination of the Total Number of Viable Cells

One gram of each fish sample was crushed in a sterile laboratory mortar with a pestle. The crushed samples were weighed and aseptically transferred into a test tube containing 9ml of sterile distilled water. The mixture was well shaken, followed by a 5-fold dilution in different test tubes. 1ml each of dilution factors 10^3 was pipetted and plated onto MacConkey agar (MCA) using the spread plate method. It was then incubated at 37°C for 24 hours. The total number of viable bacteria on each agar plate was obtained by counting the visible and distinct colonies using an electronic digital colony counter to determine the total number of viable bacteria and the total number of coliform bacteria. Individual colonies were picked after morphological observation, and purified by re- streaking on nutrient agar plates, and stored on nutrient agar slants at 4°C for further biochemical characterization and identification (Adesoji et al., 2019).

2.4 Identification and characterization of bacteria isolates

The pure colonies were examined macroscopically for the colony morphology and gram staining was carried out to determine its microscopy. The characterization of the microbial isolates was done using standard biochemical tests. The biochemical test carried out includes Catalase, Indole, methyl red, Urease, Oxidase and sugar fermentation test (Adesoji et al., 2019).

3.RESULTS AND DISCUSSION

3.1 RESULTS

A total of 35 bacterial species were isolated from 20 commercially hawked fried ready-to-eat fish samples sold in Jos, Plateau State, Nigeria. Table 1 displays the viable Bacteria counts from commercially hawked fish samples, while Table 2 presents the biochemical features of the bacteria isolates. Table 3 lists the bacteria species isolated from each fish sample analysed, whereas Table 4 shows the number of occurrences and frequency distribution of the bacterial species in the fish samples analysed.

Table 1. Viable bacteria counts from commercially hawked fried fish samples sold within Jos South and Jos North

S/N	SAMPLE ID	RAW COUNT (10^{-3}) TBC	TBC (CFU/g)	RAW COUNT (10^{-3}) TCC	TCC (CFU/g)
1	FGa	260	2.6×10^6	0	0
2	FGb	147	1.47×10^6	6	6×10^4
3	FGc	278	2.78×10^6	0	0
4	FGd	204	2.04×10^6	0	0
5	FGe	298	2.98×10^6	0	0

6	BDa	32	3.2×10^5	14	1.4×10^5
7	BDb	15	1.5×10^5	0	0
8	BDc	34	3.4×10^5	0	0
9	BDd	35	3.5×10^5	0	0
10	BDe	16	1.6×10^5	0	0
11	ABTa	19	1.9×10^5	17	1.7×10^5
12	ABTb	208	2.08×10^6	9	9×10^4
13	ABTc	280	2.8×10^6	147	1.47×10^6
14	ABTd	346	3.46×10^6	25	2.5×10^5
15	ABTe	360	3.6×10^6	0	0
16	BKa	14	1.4×10^5	4	4×10^4
17	BKb	170	1.7×10^6	138	1.38×10^6
18	BKc	315	3.15×10^6	218	2.18×10^6
19	BKd	122	1.22×10^6	12	1.2×10^5
20	BKe	262	2.62×10^6	147	1.47×10^6

Key: TBC = Total bacterial count, TCC = Total coliform count, FG = Faringada, BD = Building materials, ABT = Abattoir, BK = Bukuru market(s)

Table 2. Biochemical characterization of isolates from commercially hawked fried fish samples

Biochemical tests	**Bacteria isolate**				
	K. aerogenes	CoNS	*Bacillus*spp	*Streptococcus* spp	*S. aureus*
Gram stain	GNR	GPC	GPR	GPC	GPC
Catalase	+	+	+	-	+
Citrate	+	-	-	NA	-
Coagulase		+	NA	-	+
Gelatin hydrolysis	-	-	-	-	-

Hemolysis	-	-	+	α	-
TSIA	AAG	NA	NR		NA
H2S	-	NA	-		NA
Indole	-	-	-		-
Motility	-	-	+		-
Oxidase	-	-	-		-
Urease	+	+	+		+
Adonitol	+		-		
Arabinose	+		+		
Cellobiose	+	-	+		-
Dulcitol	+		-		
Glucose	+		+		
Inositol	+		+		
Lactose	+	-	+		-
Maltose	+	+	+		+
Mannitol	+	+	+		+
Mannose	+	+	+		+
Melibiose	+		-		
Raffinose	+	-	-		-
Rhamnose	+		+		
Salicin	+	-	-		-
Sorbitol	+		+		
Sucrose	+	+	-		+
Trehalose	+	+	+		+
Xylose	+	-	+		-

Key: GNR = Gram negative rod, GPC = Gram positive cocci, GPR = Gram positive rod, NA = Not applicable, NR = Non reactive, + = Positive reaction, - = Negative reaction, AAG = Acid-Acid-Gas, α = Alpha hemolysis

Table 3. Bacteria species isolated from commercially hawked fried fish samples

S/N	Sample ID	Bacterial isolate
1	FGa	Coagulase-negative Staphylococci (CoNS)
2	FGb	*Bacillus coagulans, Klebsiella aerogenes, Rahnellainusitata*
3	FGc	*Staphylococcus aureus,* CoNS
4	FGd	*Bacillus spp., Staphylococcus aureus*
5	FGe	*Bacillus circulans, Staphylococcus aureus, CoNS*
6	BDa	*Klebsiella aerogenes, Streptococcus spp.*, CoNS

7	BDb	*CoNS*
8	BDc	*CoNS, Bacillus spp.*
9	BDd	*Staphylococcus aureus*
10	BDe	*Staphylococcus aureus*
11	ABTa	*Streptococcus spp.*, *Citrobacterspp.*
12	ABTb	*Klebsiella aerogenes,* CoNS
13	ABTc	*Klebsiella aerogenes*
14	ABTd	*Streptococcus spp.*, *Klebsiella aerogenes*
15	ABTe	*CoNS, Bacillus spp.*
16	BKa	*Klebsiella aerogenes*
17	BKb	*Klebsiella aerogenes*
18	BKc	*Klebsiella aerogenes*
19	BKd	*Klebsiella aerogenes, CoNS*
20	BKe	*Klebsiella aerogenes, Bacillus spp.*

KEY: FG = Faringada, BD = Building materials, ABT = Abattoir, BK = Bukuru , spp., = Specie, coNS = Coagulase-negative Staphylococci

Table 4. Percentage of occurrence of bacteria species isolated from commercially hawked fried fish samples.

Organism	Number of occurrence	Percentage of occurrence (%)
Staphylococcus aureus	5	14.3
Bacillus coagulans	1	2.85
Bacillus spp.	4	11.42
Citrobacterspp.	1	2.85
Bacillus circulans	1	2.85
Klebsiella aerogenes	10	28.57
Rahnellainusitata	1	2.85
Streptococcus spp.	3	8.6
Coagulase-negative Staphylococci (CoNS)	9	25.71
Total	**35**	**100**

Key: spp, = Species

3.2 DISCUSSION

The findings of this study provide useful information on the microbiological quality of commercially hawked fish samples sold within Jos metropolis. Bacteria isolated in this study include *Staphylococcus aureus*, *Bacillus coagulans, Bacillus circulans, Bacillus spp., Citrobacter spp., Klebsiella aerogenes, Klebsiella aerogenes, Rahnellainusitata, Streptococcus spp.*, and *Coagulase-negative Staphylococci* (CoNS). The total bacterial counts (TBC) and total coliform counts (TCC) indicate significant microbial contamination in the fish samples, with some samples showing alarmingly high levels of viable bacteria. For instance, sample ABTe had a TBC of 3.6 x 10^6 CFU/g, which raises concerns regarding the safety of these fish products for consumption. The presence of high bacterial loads can be attributed to various factors, including improper handling, inadequate refrigeration, and exposure to contaminated water sources during harvesting and processing. The isolation of pathogenic bacteria such as *Staphylococcus aureus* and *Klebsiella aerogenes* from the fish samples highlights the potential health risks associated with consuming these products. *Staphylococcus aureus* is known for its ability to cause food poisoning through the production of enterotoxins, while *Klebsiella aerogenes* can lead to gastrointestinal infections and other serious health issues. Additionally, the presence of coagulase-negative staphylococci (CoNS) in multiple samples indicates possible contamination from skin flora or environmental sources, further complicating the safety profile of these fish which is agreement with the findings of Chukwu et al (2013).

The percentage occurrence analysis revealed that *Klebsiella aerogene*s was the most frequently isolated organism, accounting for 28.57% of all isolates. These findings raised concerns as it suggests that this pathogen might be prevalent in the local aquatic environment or introduced through poor handling practices. Coagulase-negative staphylococci also represent a significant portion of the isolates (25.71%), indicating that while they are typically less pathogenic than their coagulase-positive counterparts, their presence can still signify contamination. In comparison with previous studies conducted in similar settings, the levels of microbial contamination observed in this study are in agreement with the findings of (Olusegun et al., 2018 and Owoeye et al., 2023) who had TBC of 3.72 x 10^6 CFU/g and 4.24 x 10^6 CFU/g respectively and also isolated same bacteria pathogens among others. It also agrees with the work done by (Harris et al., 2021) which highlight the risks associated with street-vended food products.On the contrary, the bulk of the values recorded in this study are greater than the Total Aerobic Plate Count range of 103 to 104 reported by Oranusi and Nubi (2016) in their study on microbiological safety evaluation of RTE hawked fish marketed along the Lagos-Shagamu Motorway. This might be due to the origins of the raw ingredients utilised, as well as differences in the hygienic and sanitarypractices adopted by the vending locations. Other studies (Matereke et al., 2020; Ali et al., 2022) have found comparable amounts of bacterial contamination in fish and seafood products, reinforcing the importance of strict hygiene standards during handling and preparation.

4.CONCLUSION

In conclusion, this investigation highlights the considerable microbial contamination found in commercially hawked fried ready-to-eat fried fish samples in Jos Metropolis. The high total bacterial count and the isolation of dangerous bacteria like *Staphylococcus aureus* and *Klebsiella aerogenes* create major public health concerns about food safety. These findings highlight the critical need for improved food safety regulations and practices among vendors in order to reduce microbial contamination and protect consumer health. Public awareness programs emphasising proper food handling techniques are critical for educating both vendors and customers about the risks connected with tainted food products. Further research is needed to investigate intervention options that could improve food safety in street-vended fish products, ensuring that they do not endanger public health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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