

Isolation and Identification of *Staphylococcus aureus* from Fresh Fish (*Clarias gariepinus*) Sold at Evening Markets in Yenagoa, Bayelsa State, Nigeria.

Daworiye, P.S and Abere, T.F.

Department of Biology,

Isaac Jasper Boro College of education, Sagbama,

Bayelsa State, Nigeria.

Corresponding author: stephendaworiye@gmail.com

Abstract

Fish provides a major source of income and food for man. Preparation and handling of fish by sellers, buyers and consumers can expose fish to infection by *Staphylococcus aureus*, an opportunistic pathogen. Samples obtained from market sellers were prepared and cultured on nutrient agar and mannitol salt agar and subjected to biochemical tests and molecular identification. Total bacterial count was within the range of 4.0×10^8 to 1.03×10^8 CFU/g. The differences in bacterial growth observed on nutrient agar and mannitol salt agar was significant at $p(0.05)$, $r = 0.529$. The biochemical tests of isolated organisms showed that 2 of 10 isolated organisms are Gram positive, Coagulase positive and Catalase positive *Staphylococcus aureus*. Molecular identification using PCR assay showed a match with *Staphylococcus aureus* strain IJA899. The presence of this bacterium is of serious public health significance. A high degree of hygienic practices is recommended for both buyers and sellers to prevent potential cases of Staphylococcal food poisoning. Enforcement of standard hygienic practices is important to minimize food safety risks. The study provides critical baseline of risks associated with evening markets and indicate areas requiring sanitary interventions.

Introduction

Fish serves not just as a source of diet but major source of protein for people all over the world including the riverine communities in the Niger Delta region of Southern Nigeria where it is an excellent and highly recommended source of protein (Olubiyet *al.*, 2022). The quality of raw

fish (fresh or iced) at markets varies widely. Fish is a cheap source of protein with numerous health benefits due to a rich composition of essential nutrients (Singh *et al.*, 2025). Fish plays a significant role in diet, providing over 60% of animal-sourced food. This represents a crucial source of micro-nutrients. The quality of fish mainly depends on the time between harvesting and processing. At the time of capture, fresh fish contain on their bodies a wide variety of microorganisms (microflora). Increasing fish production contributes to a sustainable economy (Noreen *et al.*, 2025). During handling, natural flora may be contaminated with organisms from anthropogenic sources.

Catfish (*Clarias gariepinus*) is appreciated in most parts of Nigeria due to ease of production, exceptional taste and flavour (Alfa *et al.*, 2014; Olubiyet *et al.*, 2022). Fishery products can also act as sources of various food borne diseases due to the presence of contaminating pathogenic microorganisms. Bacteria found on fish are influenced by season and harvesting conditions. The proportion of the initial bacterial population can change after harvesting depending on the ability to adapt to new environment. The quality of fish and its products is of major concern to consumers given the unhygienic conditions where they are harvested and sold (Tusayiet *et al.*, 2021).

Rearing fish in high population densities within aquaculture plants result in susceptibility to bacterial pathogens that can cause disease outbreaks (Irrshathet *et al.*, 2023).

Spoilage bacteria are predominant in newly caught fish. Pathogenic bacteria could be present in various organs (skin, gills, gut). Pathogenic bacteria in fish may be indigenous (*Clostridium botulinum*, *Listeria monocytogenes*, *Aeromonas* spp, *Schewanella* spp and *Vibrio* spp) or non-indigenous (*Salmonella* spp, *Escherichia coli* and *Staphylococcus aureus*) (Alagoa *et al.*, 2018; Salama and Chennaoui, 2024). The microbial composition is influenced by pollution levels and temperature. Occurrence of pathogenic bacteria on fish is a consequence of process contamination or mishandling. The presence of indigenous pathogenic bacteria may not represent hazard itself because they occur at very low levels. Problems can arise when handling, processing or storage conditions provide a favourable environment for the growth of pathogenic bacteria. Sanitary conditions during processing of food products reflect on the bacteriological content of the finished product (Bedane *et al.*, 2022).

At the point of harvesting, contamination may occur through equipment used and may result in spoilage. Catfish (*Clarias gariepinus*) as one of the exceptionally perishable foods often face a lot of criteria such as freshness, also seen as the most important attribute of catfish quality. Fish and fish products are generally regarded as high-risk commodity in respect to pathogen contents, natural toxins and other possible contaminants (Chintagariet *al.*, 2017). Food borne infections are a major cause of illnesses and death worldwide, resulting in about 420,000 deaths annually. Food borne diseases most often result from the injection of bacterial toxins and possible microorganisms present in food, especially in fresh fishes (Bintsis, 2017).

Methodology

The experimental approach was adopted for the study. Standard microbiological techniques were used to determine bacteriological content of fresh fish sold at evening markets in Yenagoo (4°55'N, 6°16'E), Bayelsa State, Nigeria. Samples were obtained from Swali Market, Opolo Market and Tombia Road Market.

Sample collection and preparation

Samples were collected randomly from 6 sellers in each of the selected markets. The samples were labeled, packed in polythene bags and transported in ice to the laboratory for preparation and analysis. Fish samples were pounded using mortar and pestle and 10g of the ground sample weighed into 90ml of sterile distilled water (1:10 w/v) (Oku and Amakoromo, 2013). This was then homogenized using a Stomacher blender and 1ml of the homogenate was measured using a sterile pipette and diluted into 9ml of sterile distilled water (10^{-1}). Serial dilutions were repeated further to obtain dilutions of 10^{-6} used for the analysis.

Using a sterile pipette, 0.1ml aliquot of diluent was poured onto Nutrient agar and Mannitol Salt agar in duplicates (Popovic *et al.*, 2010). Standardization of *Staphylococcus aureus* was done using 0.5 MacFarland standard (Nix *et al.*, 2020). With the aid of sterile glass rod, this was then spread evenly on the media surface. Prepared plates were incubated at 37°C for 24-48 hrs.

Isolation and characterization

Plates were observed for bacterial growth and recorded. Observed colonies were transferred to agar slants with the aid of wireloop and flame sterilization. Slants were incubated at 37°C for 24 hrs for further identification. Biochemical identification of bacteria involved the use of Gram staining, Coagulase test and catalase test.

Total bacterial count was obtained using the formula:

$$\text{CFU/g} = \frac{\text{No of Colonies (CFU)}}{\text{Volume Plated (mL)} \times \text{Dilution Factor}}$$

Molecular identification of bacteria was by PCR based multiplex assay (Lee and Ringdale, 2008) using target genes that code for bacterial subunits (16s). Quantification was done using Nanodrop 1000 Spectrophotometer while the Multiplex PCR was used for amplification. Big Dye Terminator kit was used for sequencing and similar sequences downloaded from the National Centre for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool Nucleotide (BLATN) (van der Mee-Marquet, 2004; Daworiye *et al.*, 2019). Evolutionary history was inferred using the Neighbour-Joining method in MEGA 6.0 (Saitou and Nei, 1987) and evolutionary distance computed using the Jukes-Cantor method (Erickson, 2010).

Results

After 24 hrs of incubation, plates were observed for bacterial growth and the results presented in Table 1. The table shows bacterial counts obtained from inoculation on Nutrient Agar and Mannitol Salt Agar. On Nutrients Agar, the counts from Swali Market were in the range of 1.6×10^8 – 4.3×10^8 CFU/g. At Opolo Market the range was 1.3×10^8 – 3.1×10^8 CFU/G while at Tombia Rd Market, the counts ranged from 5.9×10^8 – 1.03×10^9 CFU/g. The counts on Mannitol Salt Agar Showed a range of 5.0×10^7 – 1.3×10^8 for Swali Market, 4.0×10^7 – 8.0×10^7 for Opolo market and 1.4×10^8 – 2.9×10^8 for Tombia Rd Market.

The colonial descriptions of the isolated bacterial species are presented in Table 2. Ten (10) bacterial species were identified from the colonial descriptions out of which 2 showed biochemical characteristics of *Staphylococcus aureus* (Gram positive cocci, Coagulase positive, catalase positive). Cultures were standardized using 0.5 MacFaland standard with a colony count of about 1.5×10^8 CFU/ml. Uploading the DNA sequence of extracted and amplified 16s DNA

for the 2 samples to the NCBI database produced a match. All samples had a 100% match with *Staphylococcus aureus* strain.

Discussion

The results indicated that fresh fish (*Clarias gariepinus*) and its products obtained from evening markets within Yenagoa metropolis had significant incidence of the versatile bacteria *Staphylococcus aureus*. Presence of the bacteria in the selected samples is of public health significance since the opportunistic parasite is able to cause a variety of diseases ranging from skin infections to food borne diseases.

High counts of the bacteria in samples from Tombia Rd Market is indicative of the socioeconomic activities within the vicinity. Contamination may be due to contact with anthropogenic sources. Exposure of fish to the surrounding environment from morning to the evening when they are sold will result in significant exposure to the bacteria (Oh *et al.*, 2007). Non-hygienic practices by buyers, sellers and passers-by could also result exposure to the bacteria a normal flora inhabiting the skin and nasal passage. Recent evidence has shown that *Staphylococcus aureus* develops antibiotic resistance (van der Mee-Marquet *et al.*, 2004; Nix *et al.*, 2020). This results in the presence of toxigenic strains of the bacteria in food with serious public health consequences.

The presence of the bacteria in the samples may not indicate immediate health risk since fish and fish products are cooked before consumption. Occurrence of *Staphylococcus aureus* in fish and fish products was also reported by Narmanno *et al.* (2005) and Oh *et al.* (2007). However, the presence of Coagulase positive strains of the bacteria calls for caution. There are several cases of Staphylococcal food poisoning in cooked and preserved food. Food safety begins with production and continues to handling and consumption.

The results obtained were significant at $p(0.05)$ using the Pearson correlation coefficient. Better hygienic practices should be adopted by sellers. Consumers are advised to properly clean and cook fish before consumption. The use of specially designed tables and sheds with nets are recommended to prevent unnecessary exposure of fish and fish products to various species of bacteria.

The study showed African catfish sold at evening markets had significant bacterial load with *Staphylococcus aureus* present at the various markets. As high as 1.03×10^9 CFU/g. Confirmed *Staphylococcus aureus* has the ability to produce heat-stable enterotoxins. In a high traffic hub like markets, this poses severe public health risks for staphylococcal food poisoning. Contributing to knowledge, the study provides high-genetic confirmation of *Staphylococcus aureus* strain IJ899. It also establishes a critical baseline in respect to risks associated with evening markets and points at areas requiring urgent sanitary interventions.

Sample ID		Nutrient Agar		Mannitol Salt Agar	
		No. of Colonies	CFU/g	No. of Colonies	CFU/g
Swali Market	A1	38	3.8 x 10 ⁸	12	1.2 x 10 ⁸
	A2	43	4.3 x 10 ⁸	13	1.3 x 10 ⁸
	A3	22	2.2 x 10 ⁸	5	5.0 x 10 ⁷
	A4	41	4.1 x 10 ⁸	11	1.1 x 10 ⁸
	A5	16	1.6 x 10 ⁸	8	8.0 x 10 ⁷
	A6	33	3.3 x 10 ⁸	6	6.0 x 10 ⁷
Opolo Market	B1	13	1.3 x 10 ⁸	6	6.0 x 10 ⁷
	B2	23	2.3 x 10 ⁸	8	8.0 x 10 ⁷
	B3	19	1.9 x 10 ⁸	4	4.0 x 10 ⁷
	B4	28	2.8 x 10 ⁸	4	4.0 x 10 ⁷
	B5	31	3.1 x 10 ⁸	5	5.0 x 10 ⁷
	B6	16	1.6 x 10 ⁸	7	7.0 x 10 ⁷
Tombia Rd Market	C1	62	6.2 x 10 ⁸	29	2.9 x 10 ⁸
	C2	103	1.03 x 10 ⁹	15	1.5 x 10 ⁸
	C3	91	9.1 x 10 ⁸	16	1.6 x 10 ⁶
	C4	71	7.1 x 10 ⁸	18	1.8 x 10 ⁸
	C5	66	6.6 x 10 ⁸	21	2.1 x 10 ⁸
	C6	59	5.9 x 10 ⁸	14	1.4 x 10 ⁸

Table 1. Colony counts from various samples.

Isolate	Colonial Morphology	Gram's Reaction	Coagulase Test	Catalase Test	Probable Identity
DA 1	Golden-yellow, pigmented, smooth	+	+	+	<i>Staphylococcus aureus</i>
DA 2	White, opaque, pin-point, moist surface	+	-	-	<i>Streptococcus</i> spp
DA 3	Creamy, dull, flat, irregular shape	+	-	+	<i>Bacillus</i> spp
DA 4	Yellow, opaque, shining, convex	+	+	+	<i>Staphylococcus aureus</i>
DA 5	Yellow, pigmented, convex, smooth	+	-	+	<i>Micrococcus</i> spp

DA 6	Creamy, circular, smooth, moist	+	-	-	<i>Enterobacterspp</i>
DA 7	Brown, mucoid, smooth, raised	-	-	+	<i>Shewanella putrifaciens</i>
DA 8	White, circular, smooth, convex	+	+	-	<i>Erysipelothrix rhusopathiae</i>
DA 9	Colourless, Small, smooth, translucent	-	-	+	<i>Shigellaspp</i>
DA 10	White, tiny, smooth, opaque	+	-	-	<i>Enterobacterspp</i>

Table 2. Colonial description and Biochemical reactions of isolated bacteria.

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