

Microbial and parasitic analysis of fresh -water catfish from Patigi and Kaima local government areas of Kwara State, Nigeria

Kayode Abel Yusuf, Ekedegwa Benedict Adole *, Folajinmi Ayodeji Olawuni and Olusegun Babatunde Aina

Department of Perishable Crops Research, Nigerian Stored Products Research Institute (NSPRI) Ilorin, Kwara State, Nigeria.

Magna Scientia Advanced Research and Reviews, 2026, 17(01), 001-008

Publication history: Received on 20 March 2026; revised on 26 April 2026; accepted on 28 April 2026

Article DOI: <https://doi.org/10.30574/msarr.2026.17.1.0033>

Abstract

Contamination of Agricultural commodities, food and food products by microbial pathogens poses significant challenges to human health leading to illnesses, diseases and sometimes if not well managed can lead to death. Fresh water catfish possess substantial proteins and is acceptable by all with little or no religion and cultural bias. It is a major source of quality animal protein and omega3 fatty acid for catfish lovers especially the aged population as alternative for red meat. However, some concerns have emerged regarding the quality of fish in the market due to microbial contamination. Patigi and Kaima in Kwara state were identified as prominent areas where fishing activities are pronounced. This study isolated, identified and enumerated different pathogenic and enteric coliform bacteria from fresh water catfish samples from the study areas through microbiological techniques using differential and selective media for isolation and identification of the organism. Wet preparation for parasite was carried out, *Ichthyophylus mulifilis* and *Ichthyophylus lioteri* of the class protozoan recorded the highest number (10.8% and 8.1%) found basically in the fins and gills of the catfish. Pour plate method, differential and selective media of MSA, EMB and PDA identified *Staphylococcus sp.* (37.3×10^5 cfu/ml), *Eschereria coli* (22.0×10^5 cfu/ml) and *Aspergillus flavus* (22.1×10^5 cfu/ml) as the pathogenic organism isolated from the fresh water catfish samples from the study areas. Results of this study suggest that fresh water catfish from the study areas poses a significant health threat to catfish lovers due to high microbial contamination through human water contact activities.

Keywords: Catfish; Parasites; Bacteria; Fungi

1. Introduction

Fish represents a significant source of protein and is widely accepted across diverse populations with minimal religious and cultural restrictions, giving it a distinct advantage over red meat as an alternative animal protein source. However, it is a highly perishable commodity distributed globally [1]. For centuries, fish has constituted a major component of human nutrition and continues to be a vital part of diets in most nations [2]. Its consumption is increasing due to its high biological value, which includes efficient protein retention, low cholesterol content, and the presence of essential amino acids [3]. Consequently, fish has become an increasingly crucial source of balanced protein, vitamins, minerals, polyunsaturated fatty acids, and other elements necessary for maintaining human health [4]. Within Africa, Nigeria leads other nations such as Egypt, Ghana, Kenya, Zambia, Cameroon, South Africa, and Uganda in fish production, with an annual output exceeding 1.1 million metric tons of species including tilapia, catfish, white leg shrimp, carp, and salmon [5]. However, the limited supply of animal protein, coupled with a rapidly growing population, has increased its cost beyond the reach of many low income groups [6].

* Corresponding author: Kayode Abel Yusuf

The Federal Department of Fisheries reported in 2018 that among all farmed fish species globally, the African catfish is preeminent in terms of nutritive value and constitutes a significant component of the human diet. Nigeria is the leading producer of catfish and the highest aquaculture producer of this species in Africa [7]. The country contributes more than 67% of total global production, followed by Uganda, Cuba, Sudan, Hungary, the Netherlands, Benin, and Brazil [8]. The preference for catfish is attributed to several favourable characteristics, including its ability to tolerate a wide range of environmental conditions, its suitability for high stocking densities in culture systems, and its rapid growth rate. It adapts well to artificial feeding, exhibits high fecundity, and commands a favourable market price [9]. This species is also preferred in the "point and kill" trade, where live fish are kept until it is needed for preparation. The African catfish (*Clarias gariepinus*) has been widely reported as a suitable species for aquaculture in Nigeria and other African nations [9].

Its production is of considerable importance to the Nigerian economy and the aquaculture industry, serving as a source of income, reducing unemployment rates, and increasing the Gross Domestic Product (GDP) [10]. Although Nigeria is a major oil producer, the aquaculture sector accounted for over 60% of the national GDP [11]. Furthermore, in 2022, catfish production alone contributed approximately 5.48% to the agricultural GDP, while agriculture as a whole contributed 20.24% to the national economy [10]. Thus, catfish cultivation is recognized as a major strategy for boosting fish production and moving the country toward self-sufficiency [11]. Also, a recent strategic initiative, FISH4ACP, was launched in Abuja on June 8, 2023, by the Food and Agricultural Organization (FAO) in partnership with the Federal Ministry of Agriculture and Rural Development (FMARD). This initiative aims to reinforce Nigeria's catfish value chain to achieve resilient, efficient, and environmentally sustainable production [12].

However, the consumption of fish poses potential health risks due to microbial contamination. Contamination often originates from domestic waste, agricultural runoff, industrial effluents, the use of animal manure in fish farming, and contaminated feed [13]. These contaminants can render fish unfit for human consumption [14], as fish harbor large numbers of microorganisms in their alimentary tract, gills, and skin. These organisms may cause disease in the host or act as reservoirs for transmission to humans [15].

Thus, this study aimed at sampling Edu Local Government Area of Kwara state (Patigi and Kaima) where human contact, industrial and fishing activities contribute to an increased chemical residue, microbial and parasitic pathogens which could be harmful to human health when consumed. These contaminations result in postharvest losses of African catfish (*Clarias gariepinus*), leading to reduced profits for fish farmers, scarcity and unavailability for consumption, and negative effects on the economy that need to be studied. This study revealed the status and level of contamination of freshwater catfish in the study areas.

2. Material and methods

2.1. Sample collection

This study was carried out at the Nigerian Stored Product Research Institute (NSPRI), Km 3 Asa dam Area, Ilorin Kwara State, Nigeria.

Forty (40) live Fresh water catfish samples of different sizes were harvested each from the river in Patigi and Kaima in Kwara state, Nigeria through fishermen and were transported with the NSPRI fish boxes to the food science laboratory of the Nigerian Stored Products Research Institute for further processing, treatment and analysis. Standard culture, selective and differential media for microbial isolation (Nutrient agar (N/A), Eosin methylene blue (EMB), Manitol salt agar (MSA) and Potato dextrose agar (PDA)) and all other reagents of analytical and food grades were provided by the Nigerian Stored Products Research Institute, Ilorin and Mich Nigerian Ltd.

2.2. Parasitological Analysis

Fresh water catfish samples were examined for parasitic organisms, using wet preparation techniques for both endo- and ecto-parasites. Particular attention was directed towards specific anatomical locations, including the gills, skin, fins, and the walls of the intestine and stomach [16].

2.3. Examination of the Ecto-parasite

An external examination of each fish specimen for parasites was performed according to the technique described by [16]. The skin, gills, tail, and fins were initially scrutinized for the presence of ecto-parasites using a hand lens. Subsequently, the aforementioned external parts were carefully washed into a Petri dish containing a 0.9% saline solution. Several drops of this resultant mixed solution were collected using a dropper, placed onto a clean glass slide,

and covered with a cover slip. The prepared slides were then observed under a light microscope. For a more detailed examination, the gills of each fish were dissected out using a sterile dissecting kit. Each gill arch was placed into a Petri dish containing 10 ml of normal saline. After a brief immersion, the gill was removed, placed on a glass slide, and covered with 1-2 drops of saline solution for observation under a binocular microscope.

2.4. Examination of the Endo-parasite

The abdominal cavity of each fish was opened to expose the internal organs. The stomach and intestine were carefully excised and longitudinally cut open. The contents of the gut lumen were washed into separate Petri dishes containing a saline solution. The inner lining of the gut wall was gently scraped with a scalpel, and the scrapings were added to the saline solution in the Petri dish. Subsequently, 1-2 drops of this preparation were transferred to a glass slide, covered with a cover slide, and meticulously observed under a light binocular microscope for the presence of endo-parasites. The motility of any parasites present, manifesting as a wriggling movement within the saline solution, facilitated their detection under microscopic observation. Preliminary identification of the recovered parasites was achieved by comparing their morphological characteristics with pictorial guides on fish parasites [14]. Definitive identification of specimens to the species level was conducted following the established taxonomic keys and methods outlined by [16].

2.5. Preparation of stock culture for the enumeration of Bacterial and Fungal pathogens

Sections of the gills, gut, and intestine from each fresh catfish sample were aseptically excised using a sterile scalpel and pair of scissors and placed into sterile Petri dishes. A 4-gram portion of these combined tissue sections were triturated using a sterile mortar and pestle. Homogenization was performed to achieve a uniform cellular distribution by dissolving the ground tissue in 40 ml of sterile distilled water. The resultant homogenous mixture served as the stock culture for the subsequent enumeration, isolation, and identification of bacteria and fungi [17]



Figure 1 Trituration of fish samples

2.6. Bacteria and Fungi enumeration

One ml aliquot from a 10^{-6} dilution of each homogenized sample was inoculated onto specific culture medium using the pour-plate technique. The media employed were Nutrient Agar (for total viable bacterial count), Eosin Methylene Blue Agar (for coliforms, notably *Escherichia coli*), Salmonella-Shigella Agar (for *Salmonella sp.* and *Shigella sp.*), Mannitol Salt Agar (for *Staphylococcus sp.*), and Potato Dextrose Agar supplemented with 0.1% streptomycin (for fungal enumeration). All plates were prepared in triplicate. Bacterial cultures were incubated under aerobic conditions at 37°C for 24-48 hours, while fungal cultures were incubated at ambient room temperature (approximately 25-28°C) for 3-5 days. Following incubation, the number of colonies on each plate was enumerated using a colony counter (Reichert, USA), and the results were expressed as colony forming units per gram of sample homogenous mixture (CFU/g) [17].

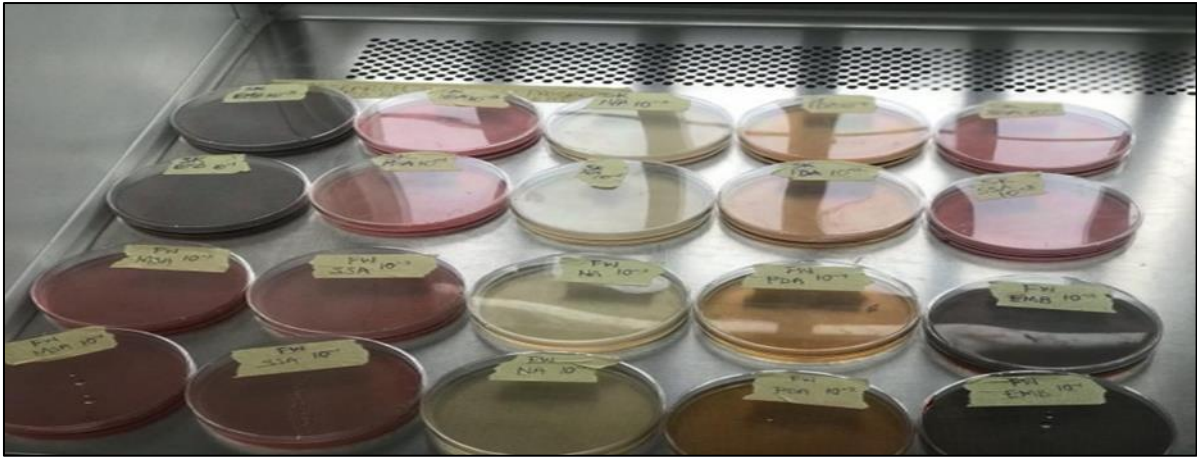


Figure 2 Inoculated plate with bacterial isolates

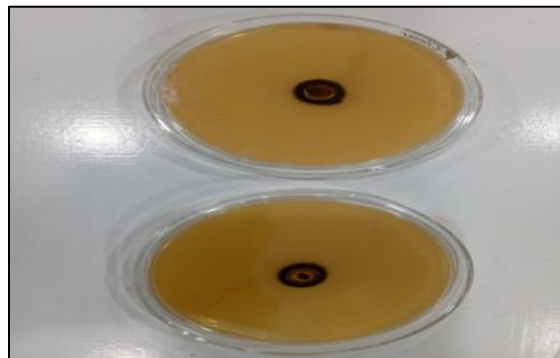


Figure 3 Invitro-antimicrobial activities of oils showing zone of inhibition

2.7. Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and tested for significant difference among treatments by New Duncan's Multiple Range F-Test (DMRT) at ($p < 0.05$) using SPSS software packages version 20.0.0 (IBM Statistics).

3. Results and discussion

3.1. Isolation and Identification of Parasites from the fresh water catfish samples

Forty (40) fresh water catfish were sampled from Kwara state out of which thirty-seven (37) representing 92.5% were infested with different species of parasites. Five (5) out of the forty catfish were within 0-10cm in length, eleven (11) were between 11-20cm, twenty (20) were between 21-30cm and four (4) were between 31-40cm. Five (5) of the catfish had weight ranges from 50-100g, seven (7) had weights ranging between 101-150g, eighteen (18) had weight ranges from 150-200g and ten (10) had 201-250g. Parasitic Trematodes recorded the highest number of parasites with 21.6% for *Opisthorchis sp* found precisely on the skin and the gills of the catfish samples and 18.9% of *Bagrus bayad* found inhabiting the skin, fins and the gills. *Ichthyophylius multifilis* and *Ichthyophylius lioteri* of the class protozoans recorded the second highest numbers, isolated and identified from the fresh water catfish samples with 10.8% and 8.1% respectively and they are found inhabiting the gills and the fins of the catfish samples. *Camallanus sp*, *Capillaria sp*. and *Ceanohabiditis sp*. of the class Nematoda had the least numbers with 5.4%, 2.7% and 2.7% respectively and they were isolated from the intestinal parts of the fresh water catfish. The skin of the catfish samples recorded 40.5% of total parasites followed by the gills with 27.0% while the intestine and the fins had the least number of parasitic counts with 16.2% as shown in table below.

Table 1 Examination and Identification of Parasites from Fresh water Catfish in Kwara State.

STATE	LENGTH (cm)	NO. EXAMINED	WEIGHT (g)	NO. EXAMINED	PARASITE	CLASS	NO. OF FISH INFECTED (%)	Infected part of <i>Clarias gariepinus</i>			
								GILLS (%)	FINS (%)	SKIN (%)	INTESTINE (%)
KWARA STATE (Patigi, and Kaima)	0-10	5(12.5)	50-100	5 (12.5)	<i>Ichthyophylus mulifilis</i>	Protozoa	3(8.1)	2(5.4)	-	1(2.7)	-
	11-20	11(27.5)	101-150	7 (17.5)	<i>Ichthyophylus lioteri</i>	Protozoa	4(10.8)	2(5.4)	-	2(5.4)	-
	21-30	20(50.0)	151-200	18(45.0)	<i>Camallanus spp.</i>	Nematoda	2(5.4)	-	-	-	2(5.4)
	31-40	4 (10.0)	201-250	10 (25.0)	<i>Capillaria spp.</i>	Nematoda	1(2.7)	-	-	-	1(2.7)
					<i>Bagrus bayad</i>	Trematoda	7(18.9)	2(5.4)	1(2.7)	4(10.8)	-
					<i>Diphillobothrium spp.</i>	Cestoda	5(13.5)	1(2.7)	1(2.7)	3(8.1)	-
					<i>Rhabdochona congolensis</i>	Nematoda	3(8.1)	-	-	-	3(8.1)
					<i>Opisthorchis spp.</i>	Trematoda	8(21.6)	3(8.1)	-	5(13.5)	-
					<i>Spirometra erinaceieropica</i>	Cestoda	3(8.1)	-	3(8.1)	-	-
					<i>Ceanohabditis elegan</i>	Nematoda	1(2.7)	-	1(2.7)	-	-
		40(100.0)		40(100.0)			37(92.5%)	10(27.0)	6(16.2)	15(40.5)	6(16.2)

A myriad of parasites were isolated from *Clarias gariepinus* in this study with the class Protozoans and the Nematoda being the dominant parasitic organism isolated from the samples in the study areas. *Ichthyophylus sp.*, *Camallanus sp.*, *Rhaddochona sp.*, *Bagrus bayad.* *Diphilobothrium sp.*, *Eimeria sp.*, and *Epistylis sp.* were all isolated. The findings of this report agree with the report of [18] in Jammu, India, who isolated the following class of parasites from catfish samples in his study. Also, [19] isolated *Camallanus spp.*, *Ichthyophylus sp.* and *Diphilobothrium sp.* in catfish samples in his study. Different parts of the catfish from the study areas are infected with parasites, with the skin and the gills recording the highest number of parasites with 15(40.5%) and 10(27.0%). The finding of these reports disagrees with the report of [20], where intestinal parasites were the dominant specie in his study. The reports of fresh water catfish parasitic infestation and contamination in this study may be due to different unhygienic and unethical bad industrial, human and animal water contact behavioral approach and activities to water bodies for daily chores like washing, bathing, defecating, urinating, swimming [21]; [22] which may facilitate and encourage parasitic habitation or deposition on leaves and debris in the water bodies which may be eating up by the fish, such parasites may affects the physiological activities of the fish and may eventually leads to death and may also not be harmful to the fish but when such fish are consumed by humans may lead to food poisoning. The findings of this study agree with the reports of [23] who reported high prevalence of protozoans and nematoda parasites from fresh water catfish samples in his study in Niger state and he concluded that it was due to unethical and bad industrial activities to water bodies in the region. Report of [24] also agrees with the findings of this study with isolated Nematodes, Trematodes and protozoans from fresh catfish samples in the Northern part of the country.

3.2. Isolation and Identification of Bacteria and fungi from the fresh water catfish samples

Microbial enumeration and identification of fresh water catfish from Patigi and Kaima Local Government Areas of Kwara state through selective and differential medial.

Fresh water catfish samples from both study areas (Patigi and Kaima) recorded a total general/ hetero-trophic bacteria count of 54×10^5 cfu/ml ± 4.9 .

Manitol salt agar (MSA) recorded a total bacterial count of 37.3×10^5 cfu/ml ± 17.5 which are suspected to be *Staphylococcus sp.* Eosin methylene (EMB) also recorded a total bacterial count of *Eschereria coli* (22.0×10^5 cfu/ml ± 3.4) while Potato dextrose agar had 21.3×10^5 cfu/ml ± 4.06 and the pathogens are suspected and confirmed to be *Aspergillus flavus* due to its morphological appearance such as hyphae pattern, colour and shape. Salmonella-shigella agar (SSA) had no visible bacterial growth and there was no *Salmonella sp* or *Shigella sp* counts from the fresh water catfish samples from the above study area.

Bacteria and fungi count from the catfish samples therefore had microbial contamination that is above the permissible level of consumption of (1.0×10^5 cfu/g) according to the International Commission on Microbiological Specification for Food, 2020 [4] and the microbial guideline for ready to eat food which is less than 10^5 [4]. However, all the bacteria and fungi pathogens isolated from the fresh water catfish in this study have been reported to cause food poisoning in human and severe illness when consumed in high amount such as Listeriosis, Diarrhea, Bacillary dysentery, intestinal obstruction, urinary tracts infection and Typhoid fever [25].

Table 2 Microbial enumeration of fresh water catfish samples

Study Area	Sample	Differential and Selective media				
		N/A (cfu/ml)	MSA (cfu/ml)	EMB (cfu/ml)	SSA (cfu/ml)	PDA (sfu/ml)
Patigi and Kaima	Fresh water catfish	54.67 $\pm$ 4.91 ^b	37.33 $\pm$ 17.57 ^a	22.00 $\pm$ 3.46 ^b	0.00 $\pm$ 0.00	21.33 $\pm$ 4.06 ^b

Result shows mean $\pm$ Standard error (SE) of triplicate readings. Means in the same column with unshared superscript are significantly different ($p < 0.05$) Key: N/A=Nutrient agar, MSA=Manitol salt agar, EMB=Eosin Methylene Blue, SSA=Selective Saline agar, PDA= Potato dextrose agar

According to [26], *E.coli*, *Staphylococcus sp* and *Aspergillus sp* are microbes of public health importance, they are thermally not stable and their presence are indicative of enteric microorganism with bacillary dysentery and diarrhea when consumed.

However, the findings and the report of this study pose major health threat to catfish lovers in Patigi and Kaima area of Kwara state [14];[19]. As it was reported by [27] the entero-toxins produced by *Staphylococcus sp.* could cause gastroenteritis and inflammation of some body organs after consumption of the infested fish. Also, *E. coli* have been

implicated in causing gastroenteric diseases such as diarrhea, dysentery and vomiting, while *Aspergillus flavus* have been implicated in causing cancer [27].

4. Conclusion

Based on the findings of this study, freshwater catfish from Patigi and Kaima Local Government Areas of Kwara State contain microbial pathogens that pose significant risks to human health. Consumers are therefore advised to ensure proper handling and cooking to avoid food poisoning. Government agencies and non-governmental organizations should intensify health education efforts to discourage unsafe practices in water bodies and promote hygiene. Furthermore, effective control measures should be implemented to reduce microbial contamination and postharvest losses.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest throughout all the stages of this work.

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