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INCIDENCE OF INDICATOR ORGANISMS, OPPORTUNISTIC AND PATHOGENIC BACTERIA IN FRESH CATFISH (*Clarias gariepinus*) SOLD IN DAMATURU, YOBE STATE.

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Abstract: *Catfish is a highly nutritious and easily digestible food, yet it is susceptible to microbial contamination, particularly in open markets. This study assessed the microbiological quality of street-vended catfish in Damaturu, Yobe State, from May to August 2024. Samples from randomly selected vendors were analyzed for microbial load, indicator organisms, and pathogenic bacteria using conventional microbiological methods. Coliforms were found in 88% of samples, however, no sample exceeded acceptable limits ($1.0x10^2$ CFU/g). While Escherichia coli was absent, other coliforms such as Citrobacter and Klebsiella species, alongside Salmonella species, were isolated. Unsanitary water ($2.4x10^1$ CFU/ml) used during gutting contributed to higher contamination levels in gutted fish ($2.3x10^1$ CFU/g). Common isolates included Staphylococcus, Salmonella paratyphi (26-47%), Vibrio cholerae, and Klebsiella pneumoniae. The presence of enteric bacteria highlights cross-contamination risks, posing health hazards, particularly to immunocompromised individuals. Enforcement of vendor registration and sanitation standards is essential to ensure fish safety.*

Keywords: *Catfish, Street Vendors, Microbial Contamination, Coliforms, incidence.*

INTRODUCTION

The advantage of fish as food is as a result of its easy digestibility and high nutritional value. Fish are a crucial source of food for people and are the most important source of high-quality protein. Fish provide approximately 16% of the animal protein consumed by the world's population. However fish are susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease.

Fish is an excellent source of high quality protein; however, it is susceptible to microbial spoilage. Fish carries high microbial load on the surface of the skin, in the intestine and in the gills.

Food security and poverty reduction have been central to the world development agenda but the principal themes have changed with the growing population, and changes in the world economy, technology and state of the environment.

Clarias gariepinus, also known as the African mud catfish, exists in the wild but it is also cultivated in ponds, cages, and pens and is of great commercial importance. This is an omnivorous fish with a preference for a planktonic diet (Khedkar, 2003).

The catfish (*Clarias garienpinus*) is a remarkable fish species in Nigeria where it is the leading aquatic crop. It has the credentials of fast growth, resistance to disease and handling stress. It has air breathing structure and can, therefore, tolerate very low oxygen levels in any aquatic environment. Nigeria is presently one of the largest producer of catfish (FAO, 2005).

Pathogenic and potentially pathogenic bacteria associated with fish and shellfish include *Mycobacterium, Streptococcus spp., Vibrio spp., Aeromonas spp., Salmonella spp.* and others (Petronillah *et al*., 2013). However the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Adebayo *et al.,* 2012).

Foodborne illnesses such as dysentery and diarrhea resulting from the consumption of contaminated fish can result in economic losses. Microbial associations with fish compromise the safety and quality of human consumption; this is particularly critical when microorganisms are opportunistic and/or pathogenic in nature (Mhango *et al.*, 2010 & Novotny *et al.,* 2004).

Bacteria found on African catfish majorly belong to genera of Pseudomonas, Staphylococcus, Flavobacterium, Vibrio, Micrococcus, Bacillus and Aeromonas. Some exist as microflora in pond water and large water bodies. These bacteria can be found on the gills, skin, and fin or in the intestinal tracts of fish under normal conditions but due to environmental stress they may produce epizootics diseases while some are also pathogenic organisms (Afolabi *et al.*, 2020). The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to prevail. Other studies have also demonstrated the presence of indicator micro-organisms of faecal pollution, opportunistic and pathogenic bacteria to humans in fish samples (Petronillah *et al.*, 2013).

Preservation of fish by smoking is carried out after catching and may be eaten without further cooking. From the processing centers to the market centers, smoked fishes are often contaminated with bacteria among other microorganisms (Moshood *et al.*, 2012).

Smoked fish is highly desirable because of its enhanced flavour and texture, in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations (Sowumi, 2007). Smoking demonstrated a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content. Smoking dehydrates and makes the fish muscle to be tough which provides a longer shelf life, lowers the pH and thereby making it less susceptible to spoilage (Akinwumi, 2014).

The microorganisms connected with smoked fish are typically those of the normal flora of the fish and that of human body, *Mucor spp* even the fungi that are airborne and that of the soil. These organisms including *Bacillus brevis* are normal bacteria present in fish (Cheikyula & Awobode, 2019). *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Salmonella spp*, *Micrococcus luteus*, *Bacillus subtillis*, *Escherichia coli, Proteus spp*, *Enterobacter aerogenes* are usually the main bacteria that are isolated often associated with smoked fish. These microorganisms may have found their way into the fish through hand to hand contact. Apart from *Bacillus brevis*, the other bacteria are a subset of the normal flora found on the human skin. (Akinwumi & Adegbehingbe, 2015).

METHODOLOGY

Description of the Study Area

Damaturu is a Local Government in Yobe state. The state is located within latitude 11^{o} North and longitude 13.5^{o} East with a total land area of 47,153 square kilometers. It shares common boundaries with Borno state to the east and southeast, Jigawa state to the northwest, Bauchi and Gombe states to the southwest. It also shares an international border with the Republic of Niger. This boundary stretches over 323km to the north of the State. The population of the State according to the National Head Count conducted in 2006 is about 2.6 million.

Sample Collection

Forty (40) catfish samples from street vendors were collected and transported to the Federal Polytechnich Damaturu, Yobe State, for further analysis. The samples were transported within 2 hours from sampling areas to the laboratory in individual sampling bags and carried in cooler boxes filled with ice. The samples were analyzed at the Science Lab., Technology Laboratory. All samples were aseptically deboned and

blended at 12,000 rpm for 2 minutes and stored at -20°C until analysis (Da Silva *et al.,* 2002). Frozen samples were thawed at room temperature for 10 – 15 minutes and prepared as described by Cunnif, (1999).

Culture

Total Bacterial Count (TBC)

The blended fish samples (25g) were aseptically homogenized with 225ml of 0.1% sterile peptone water in a sterile stomacher bag. The 1:10 dilution in peptone water was further serially diluted. Aliquots (1ml) were pour-plated with sterile molten nutrient agar. The duplicated plates were incubated at 10°C for 7 days for psychrophilic/psychrotrophic and 25°C for 48 hours for mesophilic counts. All plates with colony forming units between 30 and 300 were recorded [Trytinopoulou *et al.,* 2002; Baixas-Nogueras, 2005].

Total and Fecal coliforms

Fish samples (25g) were homogenized with 225ml of 0.1% sterile peptone water and blended at 12,000 rpm for 2 minutes. Serial dilutions were made with 0.1% sterile peptone water. Presumptive test for total coliforms was carried out using a 3-tube multiple fermentation most probable number (MPN) technique. Aliquots (1ml of each dilution) for three consecutive dilutions were added to lauryl tryptose broth containing test tubes. The test tubes were incubated at 35°C for 24- 48 hours and examined for growth and gas production. Total coliforms were confirmed by transferring a loopful of broth of positive gas production tubes into 2% brilliant green broth. These were incubated at 35°C for 48 hours (FDA, 2002). Another loopful of LTB inoculum was transferred to previously prepared EC broth for confirmatory purposes and incubated at 44.5°C for 48h (FDA, 2002).

Isolation and identification of other opportunistic bacteria:

For isolation of *Vibrio, Pseudomonas* and other Gram-negative bacteria, the blended fish samples (25g) were added to 225ml of alkaline peptone water (APW) with 1% NaCl at pH 8.6 for enrichment. This was incubated at 37°C for 8 and 24 hours. Thiosulphate citrate bile salts sucrose agar plates were streaked with a loopful of enrichment broth after 8 hours and another after 24 hours. This was done to allow for the growth of *Vibrio* species, which might be overtaken by the overgrowth of enteric bacteria after long hours of incubation. The plates were incubated at 37°C for 24 hours. Small green and yellow colonies from plates inoculated after 8 hours of incubation of enrichment broth were presumed *Vibrio* isolates. Typical yellow, green, blue-green and black colonies which appeared after 24 hours of incubation were purified and isolates grown on TSI agar slants and subjected salt tolerance test and Gram stain. The isolated colonies were further identified by API 20E system [FDA, 2002; Corry *et al.,* 2008].

RESULT

Table 1: Microbial population distribution in fish incubated at Mesophilic range of temperature

	% of samples with coliforms and population ranges in CFU/g				
Sample	**10^2-10^3**	**10^3-10^4**	**10^4-10^5**	**10^5-10^6**	**>10^6**
SL-A	13	11	29	44	16
SL-B	05	07	12	17	08
SL-C	00	27	36	19	03
SL-D	19	53	38	17	00

Key: CFU = Coliform Forming Unit

Table 2: Occurrence of different bacteria on fresh catfish from Damaturu

	Prevalence (%)				
Bacterial species	***SL-A (n=10)***	***SL-B (n=10)***	***SL-C (n=10)***	***SL-D (n=10)***	***TOTAL (n=40)***
Proteus spp.	1	2	2	1	6 (12)
Vibrio cholerae	3	4	3	6	11 (36)
Staphylococcus spp.	1	1	0	0	2 (5)
Eschericia coli	0	0	0	0	0 (0)
Salmonella spp.	0	2	0	0	2 (5)
Klebsiella spp.	1	1	0	0	1 (2)
Citrobacter spp.	5	3	6	6	18 (40)

Values in parentheses indicate the % prevalence.

Table 3: The prevalence of pathogens in fish sold in Damaturu (%)

Isolates	**Catfish Sample (**%)	**Gutting water (**%)
Aeromonas hydrophila	-	06
Citrobacter braakii	-	10
Citrobacter freundii	10	-
Enterobacter sakazakii	-	05
Enterobacter cloacae	-	-
Klebsiella pneuminiae	18	12
Proteus vulgaris	10	02
Proteus mirabilis	-	10
Salmonella species	22	24
Staphylococcus species	17	17
Vibrio cholera	23	14

- Signifies absence of the bacteria.

Statistical Analysis

Statistical analysis was performed using the statistical package for social science, SPSS v21 for Windows. Value of $p<0.05$ was used to indicate significant deviation from the total samples analyzed.

DISCUSSION

The bacterial population in food indicates the general quality of the food and the degree of spoilage it might have undergone. The occurrence of total bacterial counts in many of the samples investigated having though having <105 CFU/g raises concern about the hygienic status of the point of sale environment. More concern was the occurrence of mesophilic bacteria though within acceptable limits. The specification for bacterial counts of fish which is <105 CFU/g is considered acceptable (Andrew, 1992). The moderate bacterial counts in the fresh catfish suggested poor handling during handling process by vendors. The bacterial counts especially in the catfish supports the argument that the water in use during degutting becomes highly contaminated with mesophilic bacteria. The moderate bacterial counts of whole catfish compared to the gutted one from street vendors, suggest to what extent reusing water can

contribute to the microbial load of the street vended fish. According to Reij *et al.* (2001) poor hygiene and unsanitary handling of food are the causes of contamination of food.

Most of the catfish samples had mesophilic bacteria in it. Their presence could have been from the evisceration of the catfish on the streets. The slow air drying of the catfish in unsanitary environments attracted flies and insects which might also have contributed to the higher bacterial counts. While no *E. coli* was isolated from all the fish investigated, the very presence of coliforms above the acceptable limit in fish, marketed on the streets presents a risk to the consumers. This is because coliforms grow rapidly at ambient temperatures and spoil the fish in a short period of time (Galdreich and Clarke, 1966).

The occurrence of *Salmonella* on street vended whole tilapia, gutted catfish and tilapia and not from frozen supermarket fish suggests a temperature effect.

CONCLUSION

This study has revealed a moderate level of bacterial contamination of Catfish sold in Damaturu by street vendors. The lack of knowledge of sanitary handling of food and poor processing conditions on the streets contributed to the bacteria levels in the fish. The gutting of fish by street vendors in a washing basin introduced more pathogens to the fish. These circumstances, therefore, represent a potential health risk to the fish consuming individuals in the society if left unmonitored. However, the microbial load still fall within the recommended microbial limit for ready to-eat foods indicating that the fish sold in different location of Damaturu is fit for human consumption. The microbial load on different fish samples are not significantly different ($p > 0.01$) from each other. Rigorous regulations and monitoring activities coupled with food safety training of handlers (fishermen and traders) and consumers on various aspects of proper hygiene.

RECOMMENDATION

It is therefore recommended that Catfish stores should be well screened and ventilated so as to avoid contamination of smoked fish products. There is also need for more researches on antibiotic susceptibility surveillance in aquatic environments where the fresh fish are obtained.

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