MICROBIAL PROFILE OF SMOKED FISH SOLD IN THE NYANKPALA AND TAMALE MARKETS OF NORTHERN GHANA

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ABSTRACT

Smoked fish and fish products are widely consumed in Ghana because they are comparatively affordable. However, studies in other parts of Africa have reported microbial contamination of processed fish and fish products. It is for this reason that this study was undertaken to assess the microbial quality of smoked fish vended in the Nyankpala and Tamale markets. Fish samples were collected from vendors from Nyankpala and Tamale Markets and analysed using standard procedures and methods. Out of the 66 samples, 22.76% had Shigella spp. whilst 77.36% were free of Shigella spp. The highest count of 4.31 × 10^5 cfu/ml Shigella spp. isolates was from Tamale Central Market samples and lowest count of 1.00 × 10^3 cfu/ml was from the Tamale Vodafone Office Area Market. Out of the 66 samples, only 13.63% of the samples had Salmonella spp. and 86.37% were free of it. Salmonella spp. showed the highest count of 1.00 × 10^5 cfu/ml and lowest count of 1.00 × 10^3 cfu/ml of samples from Tamale Central Market. Out of the 66 samples, 18.20% had E. coli whilst 81.80% were free from it. The highest count of 2.74 × 10^5 cfu/ml E. coli isolates were recorded from samples from the Tamale Central Market and lowest count of 1.00 × 10^3 cfu/ml from Lamashegu Market. Shigella spp., Salmonella spp. and E. coli were the microbes obtained from the smoked catfish. Shigella spp., Salmonella spp. and Escherichia coli could be attributable to vendors' unhygienic practices and unsanitary ambient circumstances. It is therefore recommended that the processors and vendors should employ best practices in processing and handling fish and fish products to minimise the level of contamination.

Keywords: *Microbial contamination, markets, Northern Ghana, smoked fish, vendors*

INTRODUCTION

Fish is a vital source of nutritional protein and micronutrients including iron, iodine, zinc, calcium, vitamin A, and vitamin B for many populations in rural areas, especially in the low-income districts of Ghana (Aheto *et al*., 2017). Fish is a significant source of non-animal sources of protein in Ghana since locals consume 75% of the country's annual fish harvest (MOFA, 2017). Frequent fish imports into the nation are therefore required to meet the high demand of this quality protein in our diets (FACP, 2016). Fish is the most perishable food that loses freshness rapidly, if preservation is delayed after harvesting owing to autolysis and microbiological deterioration (Dehghani *et al*., 2018). Fresh fish

preservation in tropical areas is therefore a key challenge in resource-limited communities due to inadequate infrastructure and unfavorable environmental and climatic factors (Anihouvi *et al*., 2012).

The smoked fish industry is vital in Ghana for employment creation, revenue generation, food and nutrition security, and export revenues, all of which promote the country's financial sustainability (Asiedu *et al.,* 2018). According to earlier report, 95% of Ghanaian fish processed for consumption is smoked, 3% is salted, and the remaining fish is dried, fried, or fermented (SMFP, 2015). Processed fish species that are smoked in Ghana includes Catfish (*Clarias* spp.), herring (*Sardinella aurita*), mackerel (*Scomber* spp.), anchovy (*Anchoa guineensis*), tuna (*Thunnus albacores*) *Sphyraena* spp., *Caranx* spp. and *Tilapia* spp. (Asiedu *et al*., 2018).

Fish is often consumed fresh, but it is also commonly smoked, dried, or even in powder form (Aheto *et al*., 2017). The socio-economic well-being of the communities participating in the artisanal smoking industry is undoubtedly impacted by contamination as *Salmonella* spp., *Staphylococcus aureus, Shigella* spp. and *Escherichia coli* have been connected to fish. These fish are sold in the markets on strewn tables after being smoked with wood placed on filthy carpets or floors.

Microbial contamination can lead to infection and disease; environmental pressures can destabilise prospective pathogens and their hosts (Iqbal *et al.,* 2012). When these bacteria are consumed, they have a wide range of effects on the human health system, from minor illnesses to severe illnesses and even death. Fish is consumed by a large proportion of the population and fewer studies focused on the hygiene and safety of both domestic and frozen fish value chains. The objective of the study was therefore to determine the *Shigella* spp*., Salmonella* spp. and *Escherichia coli* of smoked fish vended in Nyankpala and Tamale Markets of Northern Ghana.

MATERIALS AND METHODS

Study Area

The study was carried out at the University for Development Studies, Nyankpala Campus, Nyankpala Township of the Tolon District and Tamale Township markets located in the Tamale Metropolis of Northern Region. Selected markets in the Tamale Township includes Lamashegu Market, Aboabo Market and Tamale Central Market. The laboratory procedures and analysis were carried out in the Spanish laboratory complex of the University for Development Studies.

Sampling of Smoked Fish

At various selling points, samples were obtained from vendors depending on the types that are available. Samples were collected from various smoked fish sale sites in Nyankpala Township and the University for Development Studies Nyankpala Campus. Smoked fish samples were also collected from vendors at various selling joints in Tamale Markets such as Aboabo Market, Lamashegu Market, Central Market, and other vendors in the Vodafone GH corporate area in Tamale Metropolis. Herring, catfish, salmon, frankfish (Aba knifefish, odo), snakehead fish, whitefish, redfish, tilapia, and mudfish were among the smoked fish gathered. These fish were packaged in a sterile black polythene bag and placed inside an ice-filled ice box before being transferred to the Spanish laboratory complex and preserved in a refrigerator. A total of 66 specimens of smoked fish were collected.

Media Preparation

Media used for the study includes; *Salmonella-Shigella* (S-S) agar, MacConkey agar, Simmons citrate agar, Triple Sugar Iron (TSI) agar, DNase agar and Buffered Peptone Water-BPW (all from Oxoid, Basingstoke, UK). The S-S agar and MacConkey agar were used for detection, enumeration and isolation; Citrate and DNase agar were for biochemical testing, and BPW which is a liquid medium was also used for sample processing. All the media used were prepared following the manufacturer's instructions. Required

grams of powdered medium each was weighed and dissolved in sterile distilled water, brought to boil with frequent agitation for complete particle dissolution. It was then sterilised by autoclaving at 121°C for a 15 mins at 1.5 p.s.i. All prepared media were allowed to cool before use. Slants of citrate and TSI agar in sterile test tubes were made.

Preparation of Buffered Peptone Water (BPW) OXOID

20 g of buffered peptone powder was measured and added 1 L of distilled water. Each sample required 90 mL of prepared buffered peptone water. Hence, ten (10) g of each sample required 90 mL of prepared buffered peptone water, therefore 66 samples by 90 mL (66 × 90 mL = 5940 mL = 6 L). It indicates that 120 g of BPW was added to 6 L of distilled water to get our peptone water for all the samples. It was sterilized by autoclaving at 121°C for a 15 min.

Preparation of Saline Solution (0.9% w/v)

A saline solution was prepared for serial dilution by weighing 22.50 g of NaCl which was then dissolved in 2.5 L of distilled water. The test tubes were sterilised by autoclaving for 15 min at 121°C. The serial dilution of 10-folds with levels of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were used where each test tube required 9 mL of the homogenised saline solution. One (1) mL of homogenised sample was then pipetted into each test tube containing 9 mL of saline solution. This technique was carried out for a total of 132 petri plates.

Determination of *Shigella* spp., *Salmonella* spp. and *Escherichia coli*

Ten (10) g of the smoked fish was homogenised with 90 mL of the prepared peptone. The pour plate method was utilised, which involved carefully pouring the prepared S-S and MacConkey media into sterile petri dishes already containing the required volume (100 µL) of diluted samples from respective test tubes, and allowing it to solidify. The prepared S-S and MacConkey media, which had been allowed to cool to around 50 °C, was carefully poured into the sterile petri plates containing the dispensed diluted samples and gently shaken to mix uniformly. This was repeated for each of the 66 petri plates with diluted samples. It was allowed to solidify before being incubated at 37 °C and 45.5 °C for 18 - 24 hours to allow *Shigella* spp. and *Salmonella* spp., and *E. coli* to proliferate, respectively.

Biochemical Test

The biochemical test conducted on the isolates from the smoked fish samples confirms the presence of *Shigella* spp., *Salmonella* spp. and *Escherichia coli.* A biochemical test was performed using citrate test, triple sugar iron test, dnase test and indole test on the different microbes for their identification base on how they react. The Table 1 indicates the results of the biochemical test.

Table 1: Biochemical test on the isolates from the smoked fish

Sample	Microbe	Citrate Test	Indole Test	Dnase	Triple Sugar Iron Test					
					Sucrose	Lactose	Glucose	Dextrose	H□S	Gas production
C10a	*Shigella* spp.	+	+	_	_	_	_	+	_	_
L6	*Shigella* spp.	_	_	+	+	+	+	+	_	+
C2	*Salmonella* spp.	+	_	_	+	+	+	+	+	+
	Salmonella spp.	+	_	_	+	+	+	+	+	+
C1	*E.coli*	+	_-	_-	+	+	+	+	_-	+
L1c	*E.coli*	_	_	_	+	+	+	+	_	+

RESULTS AND DISCUSSION

Shigella spp.

The mean figures of the total microbial count for *Shigella* spp., *Salmonella* spp. and *Escherichia coli* samples of the smoked fish are indicated in Table 2. Out of the 66 sample, only 22.76% of them contain *Shigella* spp. loaded and 77.36% were free of *Shigella* spp. *Shigella* spp. with the highest total count of ($4.31 \times 10abc^5$ cfu/ml) was found in the sample (C10 - Tilapia) vended from the Tamale Central Market whilst the lowest count of (1.00×10^3 cfu/ml) was found in sample (Vm7-Catfish) vended in the Vodafone Office Market Centre (Table 2). Most of *Shigella* spp. counts of smoked fish were lower than recommended limit of 10^5 cfu/g (ICMSF, 1986) except samples from L6, C10b, C10c, Cl2 and Cl3. Poor handling conditions among smoked fish vendors might account for this high numbers. It is recommended by the ICMSF that a maximum bacterial count of 10^5 cfu/ml for good quality products and 10^5 cfu/ml for products of relatively decent quality (Nwachukwu and Madubuko, 2013).

Shigella spp. had the highest count. This is primarily because there was probably improper handling procedures and the exposure of fish products to filthy surroundings. This microorganism is well-known for producing the infection shigellosis, which causes diarrhoea, fever and stomach cramps (Gaurav *et al*., 2013).

Table 2: Microbial count (cfu/ml) of the smoked fish samples

Site	*Parameter*		
	***Shigella* spp. (cfu/ml)**	***Salmonella* spp. (cfu/ml)**	***E. coli* (cfu/ml)**
Vm7	1.00×10^3	0	0
Vm7	1.00×10^3	0	0
Vm7	1.00×10^3	0	0
L6	7.50×10^5	0	6.00×10^3
L6	1.50×10^5	0	2.00×10^5
L6	8.25×10^5	0	1.60×10^5
C2	2.00×10^3	1.00×10^3	6.00×10^3
C2	2.00×10^3	1.00×10^3	6.00×10^3
C2	2.00×10^3	1.00×10^3	6.00×10^3
C10a	2.47×10^5	1.00×10^3	0
C10b	3.69×10^5	1.00×10^3	0
C10c	4.31×10^5	1.00×10^3	0
C1	4.80×10^5	5.00×10^3	2.21×10^5
C2	1.80×10^5	1.00 x 10□	2.74×10^5
C3	1.38×10^5	1.00 x 10□	2.17×10^5
L1c	0	0	1.00×10^3
L1c	0	0	1.00×10^3
L1c	0	0	1.00×10^3

***Salmonella* spp.**

Salmonella spp. was also observed with the highest total count of (1.00 × 10□ cfu/ml) from sample (C1-Salmon) vended from the Tamale Central Market and the lowest count of (1.00 × 10^3 cfu/ml) from sample (C2 and C10a thus frankfish (aba knifefish) and tilapia) vended from the Tamale central market (Table 2). Out of the 66 smoked fish samples, *Salmonella* spp. isolates of 13.63% was recorded whilst 86.37% of the total samples were free of *Salmonella* spp. *Salmonella* spp. count of 13.63% may perhaps be attributable to improper handling procedures and faecal contamination from water sources. *Salmonella* spp. are likely to be present in fresh or processed fish collected in faecal-polluted water bodies and either maintained in unclean conditions or consumed raw or barely cooked (Norhana *et al*., 2010). According to Aberoumand (2010), the majority of food poisoning outbreaks are linked to the intake of unprocessed or poorly heat-treated fish, as well as cross-contamination through processing. Approximately 12% of gastrointestinal infections related with fish intake are caused by bacteria such as ***Salmonella***. *Salmonella* can cause diseases such as fever, diarrhoea and stomach cramps.

Escherichia coli

Escherichia coli was also observed to have the highest total count of (2.74 × 10^5 cfu/ml) from sample (C1-Salmon) sold from the Tamale central market and the lowest count of (1.00 × 10^3 cfu/ml) from sample (L1c- Salmon) sold from Lamashegu market (Table 2). Out of the 66 smoked fish samples cultured, 18.20% were found to contain *Escherichia coli* and 81.80% were free of *Escherichia coli. Escherichia coli* count was also low in the smoked fish. The high presence of *E. coli* levels is more than the acceptable limit of 2 cfu/g (ICMSF, 1986) hence the likelihood of faecal pollution from the smoked fish handlers, water and air. *E. coli* is the utmost preferred coliform indicator for mishandling and contamination of fish (FAO, 1992). The primary route by which harmful strains of bacteria cause disease is through faecal and oral transfer. Because cells can live outside the host body for a short period, they are ideal diagnostic organisms for testing environmental samples for faecal contamination (Thompson, 2007). It is also associated with poor handling practices and faecal contamination and is known to cause gastroenteritis in humans, and fever among others. Polluted fish consumption can lead to gastroenteritis showed commonly by diarrhoea (Lopes da Silva *et al*., 2010) and its severity depends on strain ingested.

Similar studies found pathogenic bacteria like *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli* in smoked fish (Ayeloja *et al*., 2018; Likongwe *et al*., 2018). *S. aureus*, *E. coli,* fungi and other mesophilic aerobic bacteria are also found in soil, untreated water, animals, and humans (FAO, 1992; Tegule, 2011). The activeness of biological (bacteria, virus, and parasite) and the chemical (biotoxins) dangers in raw fish raises serious safety issues. These hazards exist in pre-harvest fish and, as a result, are difficult to control. However, there are several techniques for preventing harmful germ development throughout the processed fish supply and preservation.

Total bacteria viable counts were higher in fresh and lower in smoked catfish (Salaudeen and Osibona, 2018). Improper smoking and unhygienic handling of the smoked fish might lead to variable microbial loads in the fish (Abolagba and Iyeru, 1998; Daniel *et al*., 2013). Smoked fish samples may have high water content which would promote microbial growth (Eyo, 2001). According to Pilet and Leroi (2011), 10% to 20% of foodborne illnesses are caused by fish and fish products. Additionally, pathogenic bacteria like *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli* have been found in smoked fish (Ayeloja *et al*., 2018; Likongwe *et al*., 2018). *S. aureus*, *E. coli,* fungi and other mesophilic aerobic bacteria are found in soil, untreated water, animals and humans (FAO, 1992; Tegule, 2011).

Fish samples roasted on charcoal / wood barbeque were displayed for sale in markets on filthy

Some of the smoked fish sample from the various sites

(a)

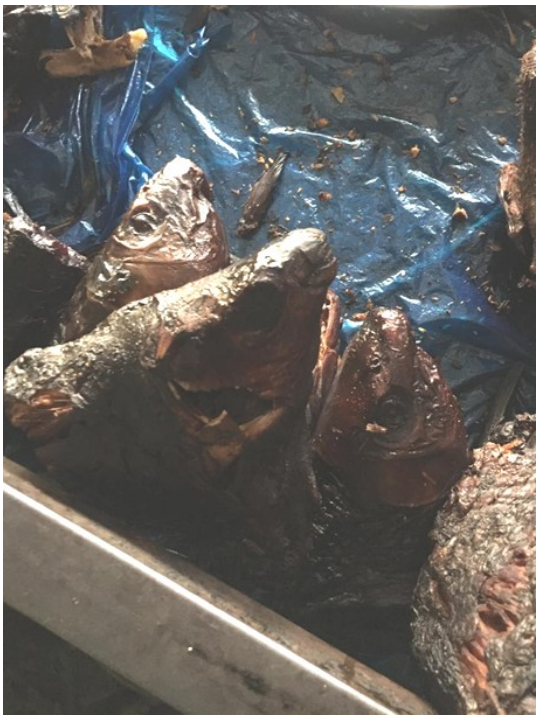
(b)

(c)

(d)

(e)

(f)



(g)

(h)

(i)

floors / mats / trays / open containers or messy tables (Plates a to i). Processed fish can be contaminated with germs in nature, through processing, and also if not handled properly after post-processing under sterile conditions.

CONCLUSION

Shigella spp., *Salmonella* spp. and *Escherichia coli* were found in the smoked fish. Generally, the levels of the microbial load of some bacteria exceeded the recommended microbial limit for ready-to-eat foods, indicating that the smoked fish sold in Markets are unsafe for human consumption. The harmful bacteria discovered in the smoked fish samples have serious implications for global health. Proper hygiene conditions can lessen the risks associated with contamination or the survival of biological intoxicants during processing. Smoking at sufficiently high temperatures can control microbial growth and contamination in fish, whilst the heat provided may not be adequate to kill all microbial pollutants. Smoked fish must be preserved carefully because improper storage and handling of the goods has been shown to expose dried fish to microbial illness. It is highly encouraged that the processed fish be dried at a temperature that will reduce moisture content suitable for microbial growth. Environmental cleanliness and good food processing and handling skills, particularly among smoked fish traders is encouraged.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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