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Prevalence of *Salmonella* organisms in fresh and smoke-dried fish within parts of Kaduna metropolis, Kaduna State, Nigeria

DO Esonu*, BV Maikai & AJ Oghumu

Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna State Nigeria

*Correspondence: Tel.: +234 8061604710; E-mail: esonu25@gmail.com

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Abstract

Aquatic environments are the major reservoirs of *Salmonella* and fishery products have been recognized as major carrier of food-borne pathogens. To determine the prevalence of *Salmonella* in fish, 112 fresh and smoke-dried fish samples were pre-enriched and enriched with buffered peptone water and selenite broth respectively, before plating on Desoxycholate Citrate Agar. Of the 112 samples, 75% (84/112) were contaminated with non-lactose fermenters. Upon subjecting them to biochemical tests, 13.1% (11/84) of the *Salmonella* suspects showed reactions consistent with that of *Salmonella* species, *Proteus* 65.5% (55/84) and *Citrobacter* 21.4% (18/84) species. The total prevalence of *Salmonella* out of the 112 fish sampled was 9.8% (11/112). *Salmonella* species was slightly higher in the fiber tank farm 12.9% (95% CI: 5.134-28.852) than in the earthen pond 12.1% (95% CI: 4.816-27.326) but this association was not significant (Fishers exact test=1.0). Prevalence was higher in fresh fish 12.5% (95% CI: 6.472-22.775) compared to smoke-dried fish 6.25% (95% CI: 2.148-16.835), though this association was not statistically significant (Fishers exact test= 0.347). Among the dried fish, the prevalence was higher 9.5% (95% CI: 0.017-0.289) in the weight range 30-49.9g compared to the smallest weight range of 10-29.9g with prevalence of 4.0% (95% CI: 0.002-0.195). For the fresh fish, the prevalence was higher 14.04% (95% CI: 7.287-25.324) in shorter length fish of 20-39cm than the lengthier ones of 40-49cm with 0.0% (95% CI: 0.000-35.433) prevalence. This study has demonstrated the presence of *Salmonella* species in fresh and smoke-dried fish in parts of Kaduna metropolis, Kaduna State, Nigeria. This is of public health significance and poses a potential risk especially among immunocompromised consumers.

Keywords: Biochemical test, Earthen pond, Fresh fish, Fiber tank, Prevalence, Salmonella, Smoke-dried fish

Introduction

Salmonella species are the leading causes of acute gastroenteritis in several countries (Soltan *et al*., 2009). Salmonellosis is the most common food-borne disease in both developing and developed countries, although incidence rates vary between humans and animals. *Salmonella* species are important sources of contamination of the environment and the food chain (Ponce *et al*., 2008). *Salmonella* serovars are zoonotic, leading food borne pathogens, responsible for outbreaks of both human and animal diseases and

have important health significance worldwide. There are several transmission routes for salmonellosis, but the majority of human infection are derived from consumption of contaminated foods (Mehemet *et al*., 2003) such as insufficiently cooked meat, fish or improperly pasteurized milk and milk products (Jackson *et al*., 2007). *Salmonella* infections can lead to numerous clinical conditions, such as enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by non-typhoid microorganisms (Bailey & Maurer, 2005).

Fish is a very important source of animal protein in the diets of man. They constitute about 60% of the total protein intake in adults especially in the rural areas (Akise *et al*., 2013). In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adebayo-Tayo *et al*., 2008). Fish that is sold to the general public is often subjected to several unhygienic processes. Water used in washing the fish can also serve as source of contamination. If the fish is not properly washed, they can serve as vehicles for transmission of pathogenic organisms (Bailey & Maurer, 2005). Therefore, there is need to evaluate the safety of fish sold for human consumption because consumption of either fresh or smoked fish has been associated with gastroenteritis which indicates lack of proper hygiene practices during fish handling and preservation. This can constitute a food safety hazard. It is therefore pertinent to determine the prevalence of this organism in fresh and smoked fish.

Bacterial culture, isolation and biochemical characterization identification method has been used for the laboratory diagnosis of *Salmonella* organisms since it has been proven to be reliable. These cultural methods for the detection of *Salmonella* are also relatively easy to perform, cheap and readily available hence the use in this research study.

Materials and Methods

Study area

This study was conducted in Kawo and Ungwan Shanu districts of Igabi and Kaduna North Local Government Areas of Kaduna State respectively. The geographical coordinates of Kawo are 10° 34' 44'' North, 7° 26' 56'' East while that of Ungwan Shanu are 10° 31' 47'' North and 7° 21' 41'' East (Google map, 2014).

Sample collection

A total of 112 fish samples comprising of 64 fresh and 48 smoke-dried fishes, were randomly collected between July and September, 2014 using systematic random sampling method. The fresh fish samples were collected from fish farms located in Ungwan Shanu district. Every 3rd fish picked from the net was used for the study. The farms are made up of earthen ponds and fiber tanks while the smoke-dried fish were bought from Kawo market in Kawo district.The fish samples were purchased exactly the way they were sold to other consumers. The samples were packed in sterile polythene bags and transported to the bacterial zoonosis laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. The fish samples were kept in the refrigerator not longer than twelve (12) hours if they were not immediately processed. Before the processing of each sample, the total weight of each fish was determined and in addition, total length of the fresh fish was also determined.

Laboratory procedures

From each sample collected, 10 grams (comprising the intestine, gills and parts of the skin) were weighed out using weighing balance and cut into smaller pieces after which it was placed in a large sterile white polythene and 90mls of buffered peptone water was added to it. The samples were then homogenized in the 90mls peptone water for 5 minutes using a laboratory blender (Stomacher L-B 400). This gives a dilution factor of 1:10. The homogenized samples were then incubated at 37°C for 24hours. From the clear middle layer (below the upper fat layer) of the homogenate, 1ml was pipetted and inoculated into 5mls of selenite broth. This was incubated at 37°C for 24 hours.

Using a sterile Pasteur's loop, a loop full of incubated selenite broth was streaked on Desoxycholate Citrate Agar to ensure the growth of isolated colonies. The plates were labeled, incubated at 37°C for 24 hours. The colonial morphology on the plate was then appraised and those colonies that were colorless with black centers indicating non-lactose fermenters, were stored on Nutrient Agar slants in bijour bottles and incubated at 37°C for 24hours and kept in the refrigerator at 4°C pending biochemical characterization.

The purity of the isolates stored on Nutrient Agar slants was ascertained by plating on Desoxycholate Citrate Agar before stabbing and streaking on the Triple Sugar Iron (TSI) and this was incubated at 37°C for 24 hours. Typical *Salmonella* suspects give alkaline and acids reaction on the slant and butt of the TSI respectively, with or without hydrogen sulphide gas production. Isolates were also streaked on prepared urea and citrate agar slants. These were also

incubated at 37°C for 24 hours. *Salmonella* suspects maintained the original color of urea and change the green color of citrate to blue, indicating urea negative and citrate positive respectively.

Furthermore, Sulfide Indole Motility (SIM) agar was also inoculated by stabbing to test for motility, sulphur and indole production. Presence of cloud around stab-line after incubating for 24 hours at 37°C indicates motile organism, also black color formation indicates production of hydrogen sulphide. About 0.5ml of Kovac's reagent was added to SIM tubes and shaken. A pinkish color indicates indole production. Lastly, methyl red voges proskeur (MR-VP) was also inoculated and incubated at 37°C for 24 hours and 2 drops of methyl red was added, pinkish coloration indicates methyl red positive.

Data analysis

Data was analyzed using Statistical Package for Social Science version 20.0. Fisher's exact test was used to test for association between prevalence of *Salmonella* in fresh and smoke-dried fish and factors such as weight, length, preservation and management practices of fish. $P \leq 0.05$ was considered significant.

Results

On processing the one hundred and twelve (112) samples collected, 75% (84/112) yielded non-lactose fermenting colonies upon plating on Desoxycholate Citrate Agar and were considered *Salmonella* suspects. The suspects were then subjected to biochemical tests and 13.1% (11/84) of them showed reactions consistent with that of *Salmonella* species while the remaining 86.9% (73/84) that were not *Salmonella,* showed reactions typical for *Proteus* 65.5% (55/84) and *Citrobacter* 21.4% (18/84) (Table 1).

The total prevalence of *Salmonella* out of the 112 fish sampled was 9.8% (11/112). Information on management practices obtained from each of the farms showed that fiber tank farm has a better management practice than that of the earthen pond in terms of hygienic practices. Though *Salmonella* spp. was slightly higher in the fiber tank farm 12.9% (95% CI: 5.134-28.852) than in the earthen pond 12.1% (95% CI: 4.816-27.326) but there was no significant association (Fisher's exact test = 1.0) between *Salmonella* spp. and management practice. The isolation of *Salmonella* with respect to the preservation method of the fish showed that fresh fish was higher 12.5% (95% CI: 6.472-22.775) compared to smoke-dried fish 6.25% (95% CI: 2.148-16.835), though there was no significant association (Fisher's exact test = 0.347) between *Salmonella* spp. and preservation method. Among the dried fish, the prevalence was higher 9.5% (95% CI: 0.017-0.289) in the weight range of 30 – 49.9g compared to the smallest weight range of 10 – 29.9g with 4.0% (95% CI: 0.002-0.195) prevalence. No *Salmonella* was seen in the bigger 50 – 69.9g fish. This is in contrast to the fresh fish category where all the *Salmonella* positive fresh fish 14.0% (95% CI: 7.287-25.324) were isolated in the smallest weight range of 150-499g. No *Salmonella* was isolated in the 500-799g and 800-1099g weight ranges. It was observed that the prevalence was higher 14.04% (95% CI: 7.287-25.324) in shorter length fish of 20-39cm than the lengthier ones of 40-49cm 0.0% (95% CI: 0.000-35.433) (Table 2).

Discussion

The result of this study shows the presence of *Salmonella, Proteus* and *Citrobacter* in fresh and smoke-dried fish obtained from Igabi and Kaduna North Local Government Areas of Kaduna State. The prevalence of *Salmonella* 9.8% (11/112) that was gotten out of the total number of fish sampled is of public health significance as no *Salmonella* is expected to be present in food meant for consumption. Though, Agu *et al*. (2013) reported a higher prevalence of 20% in Benin, Nigeria while Raufu *et al*. (2014) reported a prevalence of 11.5% in Catfish reared in Maiduguri, sub-Saharah, Nigeria. The isolation of *Salmonella* from fish in this study may either be introduced through handling post-harvest, or contamination via the water used. Also, environmental contamination may have contributed to the isolation of other organisms such as *Proteus* and *Citrobacter*. Likewise, materials used for the preparation or the holding time may have contributed to the contamination (Uzeh *et al*., 2006; Ogbonna *et al*., 2012).

The slightly higher rate in the fiber tank than the earthen pond may be attributed to contamination from other sources such as feed or utensils used other than those from water or environment probably because there were no strict biosecurity measures

Table 1: Prevalence of bacterial isolates in fresh and smoke-dried fish in parts of Kaduna metropolis, Kaduna State.

Pathogens	Number positive (%)
Salmonella spp.	11 (13.1)
Proteus spp.	55 (65.5)
Citrobacter spp.	18(21.4)
Total	84 (75.0)

Table 2: Prevalence of *Salmonella* spp. in fresh and smoke-dried fish in parts of Kaduna metropolis, Kaduna state.

Factors			Number examined	Number positive	Fisher's exact test
Preservation Method	Preservation	Fresh fish	64	8 (12.5)[a]	0.347
		Smoke-dried fish	48	3 (6.25)[b]	
Management practice	Fresh fish	Fiber tank	31	4 (12.9)[c]	1.0
		Earthen pond	33	4 (12.1)[d]	
Weight (g)	Smoke-dried fish	10-29.9	25	1 (4.0)[e]	
		30-49.9	21	2 (9.5)[f]	
		50-69.9	2	0 (0.0)[g]	
	Fresh fish	150-499	57	8 (14.0)[h]	
		500-799	6	0 (0.0)[i]	
		800-1099	1	0 (0.0)[j]	
Length (cm)	Fresh fish	20-39	57	8 (14.04)[k]	
		40-49	7	0 (0.0)[l]	
Total			112	11 (9.8)[m]	

95% CI: a= 6.472-22.775; b= 2.148-16.835; c=5.134-28.852; d= 4.816-27.326; e= 0.002-0.195; f= 0.017-0.289; g= 0.000-0.822; h= 7.287-25.324; i= 0.000-39.033; j= 0.000-94.871; l= 7.287-25.324; m= 0.000-35.433

employed in the farm. The higher contamination in fresh fish compared to the smoke-dried ones may be attributed to prolonged heat treatment the dried fish was subjected to during the process of preservation which must have helped in reducing the risk of *Salmonella* contamination of the smoke-dried fish.

In this study, bigger fish were observed to have lower or no *Salmonella* as compared to smaller ones. This may be because microbial contamination may cover more of the entire surface area of a smaller organism faster than a larger organism and since it was not the entire fish that was processed, one can likely miss out some contaminated part more easily in a bigger fish than in a smaller one. This also applies in the case of lengthier fish as compared to smaller ones. This could also be due to the sampling method as more small and shorter length fish were sampled. This finding seems to be in contrast to the report of Emere & Egbe (2006) and Omeji *et al*. (2011) on parasite eggs and oocysts in fish whereby it was more in bigger and lengthier ones compared to smaller ones.

This study has demonstrated the presence of *Salmonella* in fish. Though the prevalence was relatively low, it is still of public health significance. We therefore recommend proper cooking of fish at all times before consumption and improvements in the detection and investigation of foodborne illnesses so that possible source of infection can be traced to ensure proper control measures.

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Conflicts of Interest

The authors declare no conflict of interest.

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