NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., March 2020 Vol 41 (1): 32 – 40.
https://dx.doi.org/10.4314/nvj.v41i1.5
ORIGINAL ARTICLE

Occurrence of *Salmonella* and *Shigella* on Dried Crayfish (*Procambarus Clarkia*) Sold in Zaria and Kaduna Central Market, Kaduna State, Nigeria

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SUMMARY

Dried crayfish is a rich source of protein and an important crustacean consumed all over the world.To determine the occurrence of Salmonella and Shigella in dried crayfish and crayfish sellers practices which may predispose the food item to microbial contamination, dried crayfish were sampled from 100 stalls in Samaru (n=20), Sabo (n=30) and Central market (n=50) markets of Kaduna state. Dried crayfish samples were each homogenized, analyzed for total coliform plate count, then pre-enriched and enriched in peptone water and Rappaport-Vassiliadis broth respectively, which was further cultured on Salmonella Shigella Agar (SSA). Non-lactose fermenting colonies were subjected to biochemical and Sugar tests. The overall mean coliform count was 14.95log$_{10}$cfu/100ml. Salmonella and Shigella species were isolated from 12 (35.3%) and 5 (14.3%) samples respectively. Locational distribution of dried crayfish showed, Sabon gari (13.3%) and Central market Kaduna (6%) having the highest occurrence of Salmonella and Shigella suspect species respectively. Questionnaire survey administered to 100 of the crayfish sellers revealed that most of them 71 (74%) eat uncooked dried crayfish, 39% (44/100) of them use their bare hands unwashed while packaging the crayfish at point of sale. The presence of Salmonella and Shigella in crayfish from these locations is of public health significance. Therefore, public enlightenment on hygiene, sanitation and proper storage and packaging of crayfish to prevent foodborne disease outbreak such as Salmonellosis and Shigellosis in Nigeria is highly advocated.

Key words: Crayfish, Salmonella, Shigella, food contamination and hygiene

INTRODUCTION

Coliforms bacteria are commonly used indicator of sanitary quality of food and water. Coliform can be found in the aquatic environment, in soil and in vegetation (Doyle and Erickson, 2006). Coliforms are aerobic or facultative anaerobic gram negative and non-spore forming bacilli or rods that are able to

ferment lactose with gas production. Coliforms are broad class of bacteria found in the environment, including the faeces of man and other warm-blooded animals (Fresno, 2009).

Salmonella is a rod-shaped gram negative bacterium of the family *Enterobacteriaceae*. Two species of *Salmonella* are recognized which are; *Salmonella bongori* and *Salmonella enterica*. It is further divided into six sub-species and over 2500 serovars (CDC, 2008). *Salmonella* is further divided into two categories; typhoidal and non-typhoidal. Non typhoidal *Salmonella* is the most common form and it is carried by both humans and animal e.g. *Salmonella enteritidis* and *Salmonella daviana*. Typhoidal *Salmonella* which causes typhoid fever is caused by *Salmonella typhi* which is carried by humans. Symptoms of the infection include diarrhoea, abdominal cramps, fever and vomiting (Yabuuchi *et al.*, 2002).

Salmonella and *Shigella* are closely related bacteria which are Gram-negative, facultative anaerobic, non-spore-forming, non-motile and rod-shaped (Yabuuchi *et al.*, 2002).The causative agent of human shigellosis, *Shigella* causes disease in primates, but not in other mammals. It is only naturally found in humans and gorillas (Pond, 2005). *Shigella* is one of the leading bacterial causes of diarrhea worldwide, as of 2005 the WHO reported that *Shigella* causes about 165 million cases of severe dysentery, with a million resulting in death each year, mostly among children in the developing world (World Health Organization, 2005).

Crayfish (*Procambarus clarkii*) is a crustacean that forms greater proportion of shellfish, abundant in the fresh waters of Delta region of Nigeria. Crayfish is classified as animal polypeptide consisting about 36 – 45% protein (Ibironke *et al.*, 2014). The protein is relatively cheaper than other animal protein and possesses high nutritional value (Abou-Zaid and Mohammed, 2014). Crayfish is used to a large extent in local food preparation in Nigeria. Reports showed that crayfish has found use in complementary food formulations (Ibironke *et al.*, 2014).

The implication of poor post-harvest handling of crayfish has also been reported (Kumolu-Johnson *et al*, 2010). *Salmonella* and *shigella* remains the major food safety concern in ready-to-eat food such as crayfish which can be transmitted through contaminated uncooked or faecal-oral infection of man in the absence of adequate hygienic practices. Food borne infections and illness is a global health problem resulting in morbidity and mortality (Adak *et al.*, 2005). In many situations, the hands are major vehicles that contaminate crayfish during the process of packaging the dried crayfish by wholesalers and retailers in the local market. Lack of personal hygiene among food handlers is one of the most commonly reported practices contributing to food-borne illness and poor hand surface hygiene is also a "significant contributory factor" (Cogan *et al*., 2002).

Contamination of crayfish by bacteria organisms such as *Salmonella* and *Shigella* upon consumption can pose a major health risk, hence the need for proper handling, preservation, processing, packaging, and transportation of crayfish to ensure its safety and wholesomeness in consumption. Therefore, the aim of this research is to determine the coliform count, *Salmonella* and *Shigella* contamination of dried crayfish and the practices among those selling it in Samaru, Sabo and Kaduna Central markets, Nigeria.

MATERIALS AND METHODS

Study design

This was a cross-sectional study in which three markets within Kaduna state were selected, namely Sabo (30), Samaru (20) and Kaduna central markets (50), giving a total sample size of 100.

Questionnaire Administration

Structured close ended questionnaires (100) were administered to crayfish sellers (employing oral interview) within the selected markets to determine the relationship between knowledge and practices of dried crayfish sellers in relation to its handling and sale within the study area.

Sample collection

A total of one hundred samples were collected from the retailers at the point of sale. The smoked dried crayfish were bought from sellers who consented to the questionnaire survey. Each sample were packaged in small polythene, properly labelled with an identity number and then transported to the Laboratory.

Laboratory procedures

Pre-enrichment, enrichment and total coliform count of samples

From each sample collected, 10 grams was weighed and placed in a large sterile Stomacher bag 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 1:9. The suspensions were further serially diluted 10-fold down to 10^{-5}. From the clear middle layer (below the upper fat layer) of the homogenate, lml was pipetted and inoculated into 9mls of Rappaport-Vassiliadis broth. This was incubated at 37' C for 24 hours.

Another 0.1ml each of the serially diluted samples of the homogenate was inoculated and spread on the surface of the freshly prepared MacConkey agar with a glass rod. It was incubated at 37°C for 24 hours for enumeration of total coliform plate count,

Selective plating

Using a sterile Pasteur loop, a loopful of each broth inoculum with identifiable growth on the Rappaport broth was streaked on Salmonella-Shigella agar (SSA) to ensure the growth of isolated colonies as described by American Public Health Association (1992). The plates were labeled, incubated at 37°C for 24 hours. Non-lactose fermenting colonies which appear as small to moderate sized button-like colonies or colourless colonies with a black center was picked and subcultured unto nutrient Agar slant. This will further be incubated at 37°C for 24hours and stored in the refrigerator at 4°C pending biochemical characterization.

Biochemical tests

The isolates stored on the nutrient agar slants were placed on Salmonella-Shigella agar before the stabbing of Triple Sugar Iron (TSI) agar and inoculation of TSI. This was than incubated at 37^0C for 24 hours. Typically suspected *Salmonella* gives an alkaline and acid reaction on the slant and butt of the TSI with or without H_2S and gas production while *Shigella* gives an alkaline and acid reaction on the slant and butt of the TSI respectively. Isolates were also streaked on prepared urease agar slants, incubated at 37^0C for 24 hours and typical *Salmonella* and *Shigella* suspects showed no change in colour in the urease agar slants.

Sulphur Indole Motility (SIM) agar was also inoculated by stabbing to test for motility, H_2S and indole production. Presence of

cloudiness around the stab line after 24 hours of incubation at 37^0C indicates the organism is motile. Following the addition of 2 drops of Kovac's reagent to the SIM tubes, a pinkish-red colouration at the upper meniscus indicated the organism was positive for indole, a cream-brown colouration indicated the organism was negative for indole. Blackening of the stab line as well as the surroundings of the SIM agar indicated H_2S production.

Sugar Fermentation Test

Further confirmation was carried out using sugar fermentation test following the recommendation of Cox and Williams (1979). A 1% solution, each of lactose, sucrose, mannitol, dulcital, arabinose and maltose, to which 1% androne peptone was added to each, was prepared and autoclaved at 115^0C for 20 minutes. The *Salmonella* and *Shigella* isolate was inoculated into each test tube containing the sugars and then incubated at 37^0C for 24-28 hours. Colour change from light cream to pinkish-red indicated fermentation.

Data analysis

Data obtained were analyzed using SPSS version 20. The mean total coliform counts were expressed in $\log_{10}$. The total number of positive expressed in percentages. Results obtained were presented using tables. The result from the structured questionnaires was obtained using multi-linear regression. P-values less than or equal to 0.05 were considered statistically significant.

RESULTS

From the total coliform count of the 100 samples of dried crayfish purchased from Samaru, Sabon-gari and Central market Kaduna, the overall mean coliform count was $14.95\log_{10}$cfu/100ml and a Standard Deviation= $29.23\log_{10}$cfu/100ml (Table 1). Out of the 100 samples processed, 12(35.3%) were positive for *Salmonella*. *Salmonella* was isolated from 2(10%) samples from Samaru, 4(13.3%) from Sabo and 6(12%) from Kaduna Central markets (Table 2). Also from the 100 samples processed, 5(14.3%) were positive for *Shigella*. *Shigella* was isolated from Samaru (5%), Sabo (3.3%) and Kaduna Central market (6%) (Table 3).

TABLE 1: Total coliform count of dried crayfish sampled in Samaru, Sabo and Central market Kaduna

Location	Number examined	Range of Log_{10} of TCC (cfu /100ml) $x10^6$	Mean Log_{10} of TCC (cfu /100ml) $x10^6$	Standard Deviation Log_{10} of TCC (cfu /100ml) $x10^6$
Samaru	20	0.00-111.0	8.30	24.69
Sabo	30	0.00-62.0	9.30	14.00
Central Market	50	0.00-292.00	27.24	49.00
Total	100		44.84	87.69

Overall mean$\log_{10}$ Total Coliform Count =$14.95\log_{10}$cfu/100ml and the Standard Deviation= $29.23\log_{10}$cfu/100ml

TABLE 2: Location and Distribution of *Salmonella* Isolated from Dried Crayfish Sellers Sold in Samaru, Sabo and Central Market Kaduna, Kaduna State.

Location	Number examined	Number Positive (%)
Samaru	20	2(10%)
Sabo	30	4(13.3%)
Central Market	50	6(12%)
Total	100	12(35.3%)

TABLE 3: Location and Distribution of *Shigella* Isolated from Dried Crayfish Sellers Sold in Samaru, Sabo and Central Market Kaduna, Kaduna State.

Location	Number examined	Number Positive (%)
Samaru	20	1(5%)
Sabo	30	1(3.3%)
Central Market	50	3(6%)
Total	100	5(14.3%)

The result as shown in Table 4 indicate the degree to which the under listed variables are contributory to transmission of *Salmonella* and *Shigella*, thus the respondent indicated sourcing the dried crayfish mostly from the wholesalers which implies a high level of contracting *Salmonella* and *Shigella* as this variable has a level of significance of 0.031 which is lower than the level of confidence of 0.05 as shown in Table 5. The variables show a regression value of 0.512 which signifies to the modelling of Table VI as such been the determinant of *Salmonella* and *Shigella*.

DISCUSSION

The values from the study on the total coliform count and the standard deviation established the presence of bacterial contamination of dried crayfish. The presence of coliform in the crayfish samples in this study was inferred from colony morphology in the selective and differential media used. A lower mean bacterial count (cfu/ml) on crayfish samples A ($2.5x10^4$) and B ($2.1x10^4$) was reported by Ugwu (2019) on crayfish samples sold at Ogbete main market in Enugu metropolis Nigeria. The higher total coliform counts obtained from the present study, implies low standard of personal and environmental hygiene.

The study has also demonstrated various percentages of *Salmonella* and *Shigella* suspects in Samaru, Sabo and Central Market Kaduna. Though this study reported a higher percentage of *Salmonella* than that reported by El-Kholie *et al*. (2012) who worked on crayfish purchased from fishermen along the River Nile in Egypt. Also no *Salmonella* and *Shigella* was reported by Orogu *et al*. 2018 and Ugwu (2019) who carried out a bacteriological analysis of dried crayfish sold in Ozoro market, Delta State and Ogbete main market, Enugu State respectively. The incidence of *Salmonella* and *Shigella* in the samples may be attributed to external

TABLE 4: Demography of Respondents in the study of the Knowledge and practices of Dried Crayfish sellers in Samaru, Sabo and Kaduna central market

VARIABLES	SAMARU	SABON GARI	CENTRAL MARKET	TOTAL
Age (yrs) - <20	3(15%)	3(10%)	1(2%)	7(7%)
- 20 – 39	12(60%)	12(40%)	28(56%)	52(52%)
- 40-59	4(20%)	15(50%)	18(36%)	37(37%)
- ≥60	1(5%)	0(0)	3(6%)	4(4%)
Gender - Male	4(20%)	10(33%)	22(44%)	36(36%)
- Female	16(80%)	20(67%)	28(56%)	64(64%)
Duration of Selling (yrs)				
- **<1**	2(10%)	2(6.67%)	4(8%)	8(8%)
- **1-5**	7(35%)	1(3.33%)	9(18%)	17(17%)
- **6-7**	7(35%)	12(40%)	31(62%)	50(50%)
- **11-15**	2(10%)	15(50%)	6(12%)	23(23%)
- **>16**	2(10%)	0(0)	0(0)	2(2%)
Eating of Uncooked crayfish				
- **yes**	13(65%)	30(100%)	28(56%)	71(71%)
- **No**	7(35%)	0(0)	22(44%)	29(29%)
Tasting Dried Crayfish				
- Yes	9(45%)	10(33%)	19(38%)	38(38%)
- No	11(55%)	20(67%)	31(62%)	62(62%)
Source of buying the Crayfish				
- Source	1(5%)	6(20%)	16(32%)	23(23%)
- Whole seller	13(65%)	24(80%)	29(58%)	66(66%)
- retailer	6(30%)	0(0)	5(10%)	11(11%)
packaging				
- source	3(15%)	3(10%)	4(8%)	10(10%)
- whole seller	5(25%)	15(50%)	22(44%)	42(42%)
- retailer	2(10%)	12(40%)	4(8%)	18(18%)
- self	10(50%)	0(0)	20(40%)	30(30%)
Measuring				
- Bare hands	6(30%)	8(27%)	30(60%)	44(44%)
- Measuring cup	13(65%)	21(70%)	7(14%)	41(41%)
- Hand covered with polythene bag	1(5%)	1(3%)	13(26%)	15(15%)
Symptoms associated with consumption of uncooked dried crayfish				
- Vomiting	2(10%)	1(3%)	1(2%)	4(4%)
- Diarrhoea	0(0%)	0(0%)	0(0%)	0(0%)
- Fever	0(0%)	0(0%)	0(0%)	0(0%)
- Stomach ache	0(0%)	0(0%)	0(0%)	0(0%)
- None	18(90%)	29(97%)	49(98%)	96(96%)

TABLE 5: Multi-linear Regression of the knowledge and practices of Crayfish sellers in Samaru, Sabo and Kaduna central market

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	Beta	Std. Error	Beta		
(Constant)	1.614	.148		10.912	.000
Processing of dried crayfish	.026	.027	.097	.959	.340
Eating of uncooked dried crayfish	.001	.023	.003	.029	.977
Tasting dried crayfish before buying	.039	.021	.188	1.822	.072
Source of buying the crayfish	-.045	.020	-.256	-2.189	.031
Packaging of the dried crayfish	.004	.010	.041	.387	.699
Supplies of dried crayfish	-.037	.027	-.162	-1.367	.175
Storage of dried crayfish	-.017	.020	-.086	-.869	.387
Measuring and package of dried crayfish	.016	.015	.114	1.083	.282
Other uses of dried crayfish	.166	.039	.408	4.219	.000
Consumption of uncooked dried crayfish	.010	.014	.072	.728	.469

TABLE 6: Model Summary of Multi-linear Regression of the knowledge and practices of Crayfish sellers in Samaru, Sabo and Kaduna central market

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.512[a]	.262	.178	.091

contamination through poor handling in the course of harvesting, processing, storage and display at the point of sale.

The result of this study shows that dried crayfish handled and packaged for human consumption could be a source of bacterial infection to man as this was done with the bare hands unwashed as observed during sampling. Majority of the respondents packaged/stored their crayfish in big cellophane bags, raffia bags, or basins while others in cement paper bag or old newspapers. This implies that some of the crayfish sellers still use old methods of storing/packaging of crayfish which may be detrimental to the product and human health. Iwuchukwu *et al*. (2017) revealed that the use of plastic and laminated packaging bags to store crayfish is the most reliable method of packaging. This is because they are designed to prevent dehydration and oxygen penetration which invariably controls deterioration.

The sellers also revealed that majority of the crayfish were processed by smoking, while just a few are processed by washing in salt water or sun drying. Smoking is more commonly used as it gives crayfish a desirable taste and increases the consumer's desire or demand for it. According to Agwumba (2009), smoking of crayfish is the alternative method used when sun-drying is impossible because of the frequent rains during the rainy season

The consumption/tasting of uncooked dried crayfish as indicated by the respondents from the questionnaire depicts that, this could serve as the dominate factors for *Salmonella* and *Shigella* infection especially when the product is poorly processed.

CONCLUSION

The study has established the presence of *Salmonella* and *Shigella* in dried crayfish sold in Samaru, Sabo and Central market Kaduna. The level of total coliform counts from dried crayfish samples were high. This study has also revealed that there is poor packaging of dried crayfish, eating/tasting of uncooked dried crayfish, poor storage and unhygienic handling of dried crayfish. Proper/strict hygienic practices is therefore recommended in handling, packaging and storage of dried crayfish and dried crayfish should be properly cooked before consumption to prevent transmission of food-borne diseases to man

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