Original Research Article

Evaluation of Bacteriological Quality of Dried Cray Fish (*Astacus leptodactylus*) Sold at Ogbete Main Market in Enugu Metropolis, Nigeria

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A B S T R A C T

Cray fish (*Astacus leptodactylus*) is one of the foods used as a source of protein for both the high- and low- income earners, hence harbor pathogenic bacteria of public health significance. This study is aimed at evaluating the bacteriological quality of dried crayfish (*Astacus leptodactylus*) sold at Ogbete main market in Enugu metropolis, Nigeria. The two kinds of dry crayfish (*Astacus leptodactylus*) samples were screened bacteriologically using ten-fold dilution method. A total of sixty(60) dried crayfish samples (30 each for both sample) were collected from different sellers at Ogbete main market, Enugu. Two kinds of crayfish (Orini and Ishekeri) sold in the market were labelled as sample A and sample B. *Staphylococcus aureus, Escherichia coli*, *Bacillus* sp and *Klebsiella* sp were isolated. The mean bacterial load of these organisms ranged from 2.2×10^4 to 2.5×10^4 cfu/ml for both fish types. The prevalence rates are as follows: *Staphylococcus aureus* 30% and 26.67%, *Escherichia coli* 20% and 20%, *Bacillus* sp 13.33% and 16.67% and *Klebsiella* sp10% and 13.33% for both samples respectively. This result suggested that the samples were contaminated with bacteria which have been reported to be pathogenic to humans and animals, hence adequate hygiene must be observed during the collection, processing, transportation and sale of the edible food.

Introduction

Cray fish (*Astacus leptodactylus*) is one of the foods used as a source of protein for both the high- and low- income earners. It is a freshwater crustacean resembling small lobsters. Some species are found in brooks and streams where there is running fresh water, while others thrive in swamps, ditches and paddy fields. Fish is known as a low fat, high protein food and usually contains useful fatty acids such as Omega-3 fatty acids which are important for optimal brain and nervous system development (Orogu *et al.,* 2018). Fish constitutes more than 60% of the protein intake in adults especially in rural areas of

Nigeria (Adeleye, 1992). Crayfish contains a good amount of vitamin A, C,B6, thiamine, riboflavin (Willard, 2006).It is also rich in sodium, potassium and phosphorous with good amount of iron, zinc, copper and manganese(Orogu *et al.,* 2018). Crayfish is preserved from bacterial deterioration by drying, smoking, freezing, chilling and banning, but drying is the commonest way used to prolong the shell life of the products by reducing the water content thereby protecting the products (Orogu *et al.,* 2018). The species are both contaminated through water pollution, during processing and packaging. The contamination of dried crayfish increases when they are expose to flies, dust and handling with dirty hands in the market.

Crayfish species are contaminated by several microorganisms. These microorganisms are resistant to some antibiotics which can be a major threat to public health(Adenalke *et al.,* 2016).These organisms are mainly enteric bacteria that can colonise gut as resistant commensals or cause gastroenteritis (Adenalke *et al.,* 2016). Some of these organisms are *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Salmonella sp*.

The aim of this study is to determine the bacteriological quality of dried crayfish of two different types sold at Ogbete main market, Enugu in Enugu State, Nigeria, with the view of highlighting the implications of consuming raw foods.

Materials and Methods

Sample Collection

A total of sixty(60) dried crayfish samples (30 each for both sample) were collected from different sellers at Ogbete main market, Enugu. Two kinds of crayfish (Orini and Ishekeri) sold in the market were labeled as sample A and sample B. Their names are based on the towns where they are harvested. The samples were packed aseptically into two sterile containers and were taken to the Applied Microbiology laboratory at Enugu State University of Science and Technology, Enugu State for microbial identification.

Sample Preparation

A total of 5g each of the sample A and sample B were weighed into a test tube containing 20ml of sterile distilled water. This was swirled evenly in order to homogenize the mixture and this was labelled as the stock. A ten-fold dilution of the homogenates was made with sterile normal saline as diluents. This was carried out as described by Cheesebrough (2002). A total of 9 ml of the normal saline was measured into eight test tubes. 1 ml of the stock was collected using a pipette and serially diluted into the first tube and from the first tube to the second test tube up to the eighth test tube respectively (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}). Approximately 0.1 ml was taken from dilution10^{-3} and 10^{-4}into different sterile agar plates in duplicates. All plates were incubated at 37°C for 24 h. After incubation, the bacterial colonies on the agar plates were counted and representative colonies were transferred onto nutrient agar slants for biochemical identification.

Calculations of Colony Forming Unit

The method described by Collins *et al.,* (1989) for estimating bacterial counts was used to enumerate the total viable counts of the isolates. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was for 0.1 ml of the original sample. The mean colony count on the positive growth plates were used to estimate the total count.

Characterization and Identification of the Isolates

All the organisms isolated were sub-cultured in nutrient agar plates to obtain a pure culture. Gram stain and biochemical tests were carried out to identify the organisms Cheesebrough (2002). The biochemical tests included catalase, coagulase, oxidase, indoleand sugar fermentation tests like lactose, sucrose, mannitol and glucose.

Results and Discussion

Determination of Bacterial load for two Crayfish types

Two types of crayfish labelled as sample A and sample B were examined and their bacterial loads were determined. The result is shown in Table 1.

Determination of the Morphology andBiochemical Characteristics of the Bacterial Isolates

Based on Gram stain and biochemical reactions, the isolates were identified as shown in Table 2.

Determination of the Prevalence Rate of Bacteriaon the CrayfishSamples

A total of 60 samples of dried crayfish comprising of 30 samples each for both A and B respectively were examined. A total of four (4) different organisms were isolated. *Staphylococcus aureus* was found to have the highest prevalence rate followed by *Escherichia coli, Bacillus sp and Klebsiella*sp as shown in Table 3.

Dry crayfish (*Astacus leptodactylus*) is one of the main protein rich foods. The greatest challenge in this food sample is its contamination by bacteria. This is as a result of exposing it to flies, dust and poor handling by both the sellers and consumers. This present study revealed the bacterial load of the sample with values ranging from 2.1x 10^4cfu/ml to 2.5x 10^4cfu/ml for both samples (Table 1). This study is in agreement with the work done by Kasozi *et al.,* (2016) which shows microbial load of 2.4x 10^4cfu/ml to 7.2 x 10^4cfu/ml and Israel *et al.,* (2016) who reported the microbial load of crayfish from 4.2x 10^2cfu/g to 4.4x 10^2cfu/g. This study shows a bacterial growth of 73.33% and non-bacterial growth of 26.67 % for sample A while 76.67% for bacterial growth and 23.33% for non-bacterial growth for sample B.

This shows that 45(75%) samples collected were contaminated. This could be as a result of the fact that during handling of crayfish, the natural flora of crayfish environment will be contaminated with organisms associated with man, such as *Staphylococcus aureus* which was isolated in this study. This study revealed the prevalence rate of bacteria as follows: *Staphylococcus aureus* 30% and 26.67%, *Bacillus* species 13.33% and 16.67%, *Escherichia coli* 20% and 20% and *Klebsiella* species 10% and 13.33% respectively for both samples (Table 3). This study is in agreement with the findings of Israel *et al.,* (2016) who found the presence of bacteria like *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Also this study correlates with the findings of Elsherief *et al.,* (2014) who found 12% and 8% of *Escherichia coli* from both retailed fish and fish farm respectively, the work of Yagoub(2009)who isolated 13% of *Escherichia coli* from raw fish and the findings of Yahyazadeh *et al.,* (2016) who isolated 17.14% of *Staphylococcus aureus* from crayfish and the work done by Kasozi *et al.,* (2016) who isolated *Bacillus*sp, *Staphylococcus aureus* and *Escherichia coli* with the values ranging from 11.11% to 22.22%. This study revealed 10% and 13.33% of *Klebsiella* species from both samples.

Table.1 Themean bacterial count (cfu/ml) of the crayfish samples

Types of Crayfish	Average count (cfu/ml)
Sample A	2.5×10^4
Sample B	2.1×10^4

Table.2 Morphological and Biochemical Characteristics of the Bacterial Isolates from crayfish samples

Isolate colony microscopy description	Gramstain	Catalase	Coagulase	Oxidase	Indole	Sugar fermentation Lactose	Sucrose	Mannitol	Glucose	Organisms found
1. Small smooth cocci in yellow colonies butches with glistering surface on Mannitol salt agar.	+	+	+	-	- +	+	+	+	+	*Staphylococcus* sp
2. Raised pinkish Short colonies on MacConkey agar.	-	-	-	+	+ +	+	+	+	+	*Escherichia coli*
3. Pale colonies Short rods on MacConkey agar in chains.	+	+	-	-	- -	+	+	+	+	*Bacillus* sp
4. Colonies appeared larger, mucoid and red with diffusing red pigment on MacConkey agar.	Short rods -	-	-	-	+	+ +	+			*Klebsiella* sp

Table.3 Prevalence rate of bacteria on the crayfish samples

Types of Crayfish	Number of Sample	Bacterial Growth	Non Bacterial Growth	*Bacillus*	*Staphylococcus aureus*	*Escherichia coli*	*klebsiella* sp
Sample A	30	22(73.33%)	8(26.67%)	4 (13.33%)	9 (30%)	6 (20%)	3 (10%)
Sample B	30	23(76.67%)	7(23.33%)	5 (16.67%)	8 (26.67%)	6 (20%)	4 (13.33%)

This is not in line with the study of Elsherief *et al.,* (2014) who had 2% of *Klebsiella* species from retailed fish. Isolation of these organisms from these samples are due to storage conditions (Sheen *et al.,* 2012) and as well as exposing the food samples to dirty environment, improper handling and poor hygiene by both the sellers and consumers. The higher prevalence (20%) of *Escherichia coli* in this study may suggest higher contamination with faecal matter. The increase of food poisoning in our environment is worrisome. It is therefore, imperative that public awareness should be disseminated to both sellers and buyers informing them the impeding danger of poor handling of edible foods.

From this study, contamination of dried crayfish could be due to improper handling, improper storage and dirty environment in which they are sold.

Recommendations

It is therefore recommended that:

Crayfish should be properly preserved and handled to avoid been contaminated with pathogenic organisms.

Crayfish should be covered to avoid flies on them.

Crayfish should be displayed in a hygienic environment.

Crayfish should be cooked properly before consumption.

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