Original Research Article

Bacteriological Assessment of Smoked-Dried White Shrimp (*Nematopalaemonhastatus*Aurivillius, 1898) Sold in Calabar, Nigeria

ABSTRACT

Aim: Enumeration of bacterial counts is an important index of assessing the safety and quality of food products. This study aimed to investigate the occurrence of pathogenic bacteria in crayfish samples obtained from popular market serving consumers in Calabar.

Study design: This study was a cross sectional study conducted between April 2017 and April 2018.

Methodology: One hundred and twenty (120) samples of smoked dried *Nematopalaemonhastatus* (white shrimp) were analyzed for the presence of bacterial pathogens. Bacterial loads and identification of isolated organisms was determined using standard microbiological methods.

Results: The results showed that 66.7% of the analyzed shrimps had aerobic bacterial counts exceeding the upper permissible limit (<$1.0x10^{6}$Cfu/g) and 56.7% had unsatisfactory (>20Cfu/g) *Vibrio* counts. The study revealed the presence of different bacteria genera namely *Klebsiella, Salmonella, Pseudomonas, Serratia, Vibrio, Citrobacter, Proteus, Aeromonas, Streptococcus, Escherichia, Coagulase-negative Staphylococci* (CoNS), *Enterobacter* and *Bacillus*. Predominant organism was *Salmonella* sp (26.7%), followed by *Vibrio*sp (21.7%) while the least isolated organisms were *Bacillus*sp and Coagulase-Negative Staphylococci (1.7%) each. The occurrence of high counts of pathogens in seafood may cause food poisoning; especially in individuals who consume this seafood raw, or lightly or insufficiently cooked.

Conclusion: Government should constitute surveillance committee to educate the inhabitant of coastal areas about the health implication of direct contamination of coastal water with fecal matter.

Keywords: Assessment, bacteria, white shrimp, seafood, foodborne infections.

1. INTRODUCTION

White shrimp (*Nematopalaemonhastatus*, Aurivillius, 1898) is one of the most important commercially valuable seafood consume not only in Nigeria but in sub-Saharan Africa and other region in the world. Because of its nutritional benefits, they are consumed daily in soups and other recipe among coastal and inland dwellers (Omobepade*et al*., 2018). Seafood is an important source of animal protein, omega-3 fatty acids, essential amino acids, essential micronutrients, vitamin and minerals (Amaglianie*t al*., 2012; Venugopal &Gopakumar, 2017). Nutritionally, consumption of seafood is associated with potential health benefits, including neurologic development during gestation and infancy and reduced risk of heart disease (Iwamoto *et al*., 2010). Carbohydrate content in sea foods is known to be very small and as such recommended for weight lost (Elegbede&Fashina-Bombata, 2013; Venugopal &Gopakumar, 2017). In Nigeria, white shrimp are often sold and consumed in dried form, however, most coastal dwellers consumed it fresh (Omobepade*et al*., 2018).

Seafood constituteover 40% of the animal protein consumed by an average Nigerian compared to meat and it is relatively less expensive. This accounts for the mass preference for fish products (Nrior*et al.,* 2016). However, along with the nutrients and benefits derived from seafood consumption

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come the potential risks of eating contaminated seafood. Consumption of raw or undercooked seafood is recognized as a health risk to consumers. Sea foodsare prone to microbial contamination, especially filter feeders such as shrimps, which concentrate these pathogens in their filtration systems (Bakr *et al*., 2011; Popovic *et al.* 2010). Infectious agents involved in seafood contamination consist of bacteria, viruses and parasites, which can cause illnesses ranging from mild gastroenteritis to life-threatening diseases. Some of these pathogens are naturally present in the aquatic environment, while others can be introduced through animal or human fecal shedding and sewage pollution (Iwamoto *et al*., 2010). Additionally, seafood may become contaminated during handling, processing, or preparation. Microbiological quality of shell fishes has shown that they habour many pathogenic microorganisms (Nrior*et al.,* 2016) that have been implicated in outbreak of food borne diseases in many part of the world including Nigeria, where it is highly consumed (Udoh *et al.*, 2017). These illnesses include Typhoid fever, Cholera, diarrhea and other digestive disorders (Rashed *et al*., 2017). Seafood provides a good medium to the proliferation of known medically important pathogens including *Escherichia coli*, (Costa, 2013)*Salmonella* species (Amaglianiet *al*., 2012) *Vibrio* species, (Bakr *et al*., 2011) *Pseudomonas* species etc. (Hariharan &Amadi, 2016; Nrior*et al.,* 2016). Adequate cooking kills most pathogens; however, unlike other foods, such as meat and poultry, that are usually fully cooked, seafood is often consumed raw or with minimal cooking. Clearly, the information available suggests that the bacteria associated with shrimp (*N. hastatus*), includes potential human pathogens (Ajibare*et al*., 2021; Orji *et al*., 2016). In light of these facts, the main aim of this study was to investigate the occurrence of pathogenic bacteria in crayfish samples obtained from popular market serving consumers in Calabar.

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2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Calabar, a city in Cross River State, in Southern Nigeria. The city lies between Longitudes 4^{o}57'0"N and 8^{o}19'30"E and Latitudes 4^{o}95'0N and 8^{o}32'0^{o}E. Calabar is divided administratively into Calabar Municipal and Calabar South LGAs. The city is mostly populated with Efik speaking people. The city has an area of 406 square kilometers and had a population of 371, 022 at the 2006 census. The study was conducted in two major markets (Watt market and Marian market) and two beaches (Esuk-Nsidung beach and ObufaEsuk beach). These sites were selected because they are the most visited market in Calabar metropolis (Etang*et al*., 2017).

2.2 Sampling Method

This study was a cross sectional study conducted between April 2017 and April 2018. A total of 120 white shrimps (Fig. 1) samples were purchased from 2 major market (i.e. Marian market and Watt market) and 2 beaches (i.e. Esuk-Nsidung beach and ObufaEsuk beach) in Calabar metropolis. Each sample was collected in a sterile polythene bag to avoid further contamination and then labeled appropriately by indicating the site of collection, time and date. These were then conveyed to microbiology laboratory for analysis. Shrimps (10g) samples were homogenized using sterile laboratory mortar. About 1.0g of the homogenate was mixed with 10mL of alkaline peptone water and incubated at 37^oC for about 6-7 hours as enrichment medium. The suspension of homogenized tissue was serially diluted 10-fold using normal saline then 0.5mL of the appropriate dilution was spread over a freshly prepared Thiosulfate Citrate Bile-Salt Sucrose (TCBS) Agar plate and MacConkey Agar plate, using a sterile bent spreader. The plates were incubated at 37^0C for 18-24 hours. After incubation, the incubated plates were observed for characteristic colonies. Each colony, distinguished mainly by colour and size, was counted and results were also recorded. Then discrete representatives of each colony type were randomly selected for purification, characterization and identification using conventional biochemical tests.

FIG. 1: Picture showing white shrimp (*N. hastatus*) (Source, Watt market)

2.3 Data Analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 and results presented in percentages. The contamination rate of the samples was interpreted using the guideline given by Center of Food Safety (2014).

3. RESULTS AND DISCUSSION

The results presented in Table 1 indicate that all the analyzed *N. hastatus* were contaminated with various degree of bacterial growth. Enumeration of bacterial count is an important index of assessing the safety and quality of food products. In line with Center of Food Safety guideline (2014), acceptable upper limit of aerobic bacterial count in shellfish is <1.0 x 10^6Cfu/g. The results showed that 33.3% (40/120) of the examined shrimps had bacterial count under the permissive limit, 56.7% (68/120) are at borderline (10^6-≤10^7Cfu/g) while 10.0% (12/120) of samples are unsatisfactory (>10^7Cfu/g) for consumption. Of the 12 unsatisfactory samples, 8 (26.7%) were from Nsidung beach and 4 (13.3%) from ObufaEsuk beach. Previous findings showed high microbial load in different species of shrimps.

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Ajibare*et al*.(2021) reported total heterotrophic bacterial counts (THBC) of 1.076 to 1.125 x 10^2Cfu/g from white shrimp (*N. hastatus*) in Ondo state, Nigeria. The high bacterial load observed in this study corroborated Orji *et al*. (2016) in Ebonyi state, Nigeria, who reported mean aerobic counts of 1.87±0.543 x 10^7Cfu/g from fresh shrimps (*Palaemon serratus*). Similarly, another study conducted by Amin *et al*. (2021) in Egypt recorded mean aerobic counts of 4.62 x 10^4 ± 1.7 x10^3Cfu/g. The results of the current study also agreed with Talukder*et al*. (2019) in Bangladesh who recorded total viable counts between log 2.92 ± 0.22 Cfu/g and log 3.41 ± 0.23 Cfu/g from Rupsha and Mongla respectively. In this study, 56.7% (68/120) of the analyzed samples had *Vibrio* counts higher than the upper acceptable limit (Center of Food Safety, 2014). This is comparable to other study. Talukder*et al*. (2019) reported *Vibrio* count ranged from log 2.06 to 2.11 Cfu/g from shrimp (*Penaeus monodon*). Nwosu *et al*. (2020) found heavy *Vibrio* counts in market smoked dried shrimps (*Litopenaeusvannamei*) compared to home smoke dried rinsed shrimps. The elevated aerobic bacterial counts recorded in this study may be attributed to pollution of coastal waters with domestic and industrial wastes (Nwosu *et al*., 2020). Unsanitary or unhygienic status of handlers and the untidy environment where these fish products were sold may have contributed to increased bacterial load (Adebayo-Tayo *et al*., 2012). Fresh shrimps are often washed with unclean sea water at the seashore before drying, this processing method may be the reasons for elevated microbial load observed in the current study (Nwosu *et al*., 2020). Dried crayfish are often sold in an opened space; hence, inappropriate storage and exposure to flies may have contributed to unacceptable contamination rate observed in this study (Gram &Dalgaard, 2002). The survival of any microbial group, within a particular ecological niche, is greatly dependent on environmental parameters, such as temperature, pH, aeration, availability of organic nutrients etc. Hence, abundance of plethora of microbial groups in the aquatic environment have a significant effect in the high contaminated rate (Nwachukwu *et al*., 2013).

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Microorganisms by nature are adapted to survive in diverse ecosystem. However, anthropogenic activities increase their diversity and numbers in any ecological system (Khatri &Tyage, 2014). Thirteen different bacterial genera were recovered from *N. hastatus* (Fig. 2). The bacteria isolated include species of *Klebsiella, Salmonella, Pseudomonas, Serratia, Vibrio, Citrobacter, Proteus, Aeromonas, Streptococcus, Escherichia, Coagulase-negative Staphylococci* (CoNS)*, Enterobacter* and *Bacillus*. These organisms were previously reported in seafood (Nrior*et al.,* 2016; Ajibare*et al*., 2021; Orji *et al*., 2016). The occurrence of these bacterial species is of public health concern because majority are known pathogen implicated in gastrointestinal disorder such as diarrhea, dysentery, typhoid fever, cholera among others (Ajibare*et al*., 2021).

TABLE 1: Contamination rate of dried crayfish obtained in Calabar

Location	No of samples	Aerobic Bacterial Counts			*Vibrio*Counts		
		Satisfactory	Borderline	Unsatisfactor	Satisfactor	Borderline	Unsatisfactory
		<1.0x10^6Cfu/g	10^6-≤10^7Cfu/g	>10^7Cfu/g	<20Cfu/g	20 - ≤10^3Cfu/g	>10^3Cfu/g
Watt market	30	10(33.3)	20(66.7)	0(0.0)	16(53.3)	8(26.7)	6(20.0)

Marian market	30	20(66.7)	10(33.3)	0(0.0)	12(40.0)	6(20.0)	12(40.0)
Nsi-dung beach	30	8(26.7)	14(46.7)	8(26.7)	6(20.0)	20(66.7)	4(13.3)
ObufaEsuk beach	30	2(6.7)	24(80.0)	4(13.3)	18(60.0)	10(33.3)	2(6.7)
Total	**120**	**40(33.3)**	**68(56.7)**	**12(10.0)**	**52(43.3)**	**44(36.7)**	**24(20.0)**

This is in agreement with Hariharan and Amadi (2016) who reported the prevalence of pathogenic bacteria in shellfish. This study indicates high occurrence of *Salmonella*spp (26.7%) and *Vibrio*spp (21.7%) in the analyzed samples which implies that their consumption should be of public health importance. Similar observation was made by Bakr *et al*., (2011) in their study, detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria, Egypt. The highest prevalence of *Salmonella*spp in crayfish is somewhat similar to Bakr *et al.* (2011). The potential source of *Salmonella* contamination in crayfish is likely due to poor water quality, surface water run-off and fecal contamination from wild animal or livestock (Moussa *et al*., 2013).

The occurrence of *Escherichia coli* in seafood is considered a sanitary case and may represent a risk to the consumers if related to pathogenic strains, especially diarrheagenic *Escherichia coli*. However, the presence of non-pathogenic *Escherichia coli* in seafood should also alert to public health, since this bacterium is recognized as an indicator of fecal contamination, possibly indicating the presence of other enteric pathogens (Costa, 2013).

The genus *Pseudomonas* is the most important in the order *Pseudomonales*. *Pseudomonas* species frequently cause nosocomial infections especially in immune compromised patients. These infections are complicated and life threatening (CDC, 2015). *Citrobacter, Proteus, Serratia, Klebsiella* and *Enterobacter* are mostly members of the *Enterobacteriaceae* family which are mainly fecal contaminants that could be picked from unhygienic sources. *Citrobacter* species are rarely the source of illness, except for infection of the urinary tract. The disease caused by *Staphylococcus aureus* is intoxication and common symptoms may appear with 2-4 hours of consumption of contaminated of food include nausea, but in severe cases dehydration can lead to shock and collapse (Nrior*et al*., 2016). *Aeromonas*spp is ubiquitous in fresh water environment, and their presence is in consonance with Markey *et al.* (2013). The presence of CoNS implies contamination from human during handling (Orji *et al*., 2016; Talukder*et al*., 2019). The differences in the prevalence of isolated organisms between this and earlier studies could be explained when factors such as differences in sample size, storage condition, hygiene, environmental and geographical variation are considered.

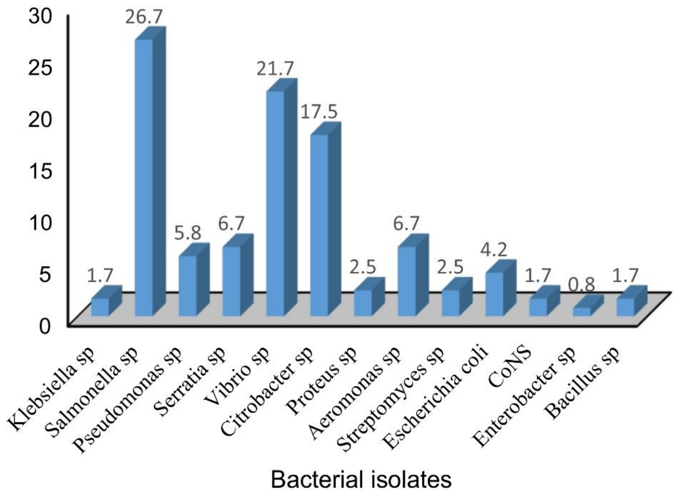

FIG 2: Prevalence rate of the isolated bacteria

4. CONCLUSION

The maximum upper limit for acceptable aerobic bacterial and *Vibrio* counts in crayfish is <1.0x10^{6}Cfu/g and <20Cfu/g respectively as given by Center of Food Safety guidelines. As observed in this study, 66.7% of the analyzed crayfish had aerobic bacterial counts exceeding the upper permissible limit. Similarly, 53.3% of the examined shrimps had unsatisfactory *Vibrio* counts. This may be due to human handling, improper hygienic conditions and fecal contamination of the coastal water. The finding of potential human pathogens in crayfish obtained from markets in Calabar underscores its nutritional values. In conclusion, the occurrence of high counts of pathogens in marine food may cause food poisoning; especially in individuals who consume this seafood raw, or lightly or insufficiently cooked. Government should constitute surveillance committee to educate the inhabitant of coastal areas about the health implication of direct contamination of coastal water with fecal matter. Before sewage disposal into the sea, it should be treated otherwise it may influence the organic load directly and bacterial load indirectly.

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