Bacteriological Assessment of Smoked-Dried White Shrimp (*Nematopalaemon hastatus* 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 white shrimp samples obtained from major markets 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 *Nematopalaemon hastatus* (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* spp (26.7%), followed by *Vibrio* spp (21.7%) while the least isolated organisms were *Bacillus* spp 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: Hence, seafood should be processed and packaged under standard hygienic conditions to reduce the risk of microbial contamination. In addition, public health awareness campaign targeted at consumers and vendors should be optimized with frequent monitoring by regulatory agencies.

Keywords: Bacteriological Assessment, Smoked White Shrimps, Calabar, Nigeria.

1. INTRODUCTION

White shrimp (*Nematopalaemon hastatus*, Aurivillius, 1898) is one of the most important commercially valuable seafood consumed not only in Nigeria but also in sub-Saharan Africa and other regions of the world. Due to 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 (Amagliani *et 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 seafood is known to be very little and as such recommended for weight loss (Elegbede & Fashina-Bombata, 2013; Venugopal & Gopakumar, 2017). In Nigeria, white shrimps are often sold and consumed in dried form, however, most coastal dwellers consume it fresh (Omobepade *et al*., 2018).

Seafood constitutes over 40% of the animal protein consumed in Nigeria compared to meat and it is relatively less expensive. This accounts for the mass preference for fish products (Nrior *et al.,* 2016). Despites the nutrients and benefits derived from seafood, consumption of contaminated seafood is accompanied with potential health risks (Bakr *et al*., 2011). Seafood are 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 include bacteria, fungi, viruses and parasites, which can cause illnesses ranging from mild gastroenteritis to life-threatening diseases (Ajibare *et al*., 2021). Additionally, 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. Furthermore, microbiological evaluation of shellfish has shown that they habour many pathogenic microorganisms that have been implicated in outbreak of foodborne diseases in many part of the world including Nigeria, where it is highly consumed (Udoh *et al.*, 2017). These illnesses include Typhoid fever, Cholera, diarrhoea and other digestive disorders (Nrior *et al.,* 2016; Rashed *et al*., 2017).

Also, seafood provides a good medium for the proliferation of known medically important pathogens including *Escherichia coli*, (Costa, 2013) *Salmonella* species (Amagliani *et al*., 2012) *Vibrio* species, (Bakr *et al*., 2011) *Pseudomonas* species etc. (Hariharan & Amadi, 2016; Nrior *et al.,* 2016). Although adequate cooking has been shown to inactivate most pathogenic contaminants in meat and poultry products, seafood is sometime consumed raw or with minimal cooking especially among coastal dwellers. Findings from previous studies have reported bacterial contaminants in white shrimps (Ajibare *et al*., 2021; Orji *et al*., 2016). However, there are paucity of related studies investigating the contamination rate of white shrimps in Calabar. Consequently, this study seeks to analyze the occurrence of pathogenic bacteria in white shrimp (*N. hastatus*) samples obtained from major markets serving consumers in Calabar.

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 Obufa Esuk 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 markets (i.e. Esuk-Nsidung beach market and Obufa Esuk beach market) in Calabar metropolis. Each sample was collected into a sterile polythene bag and sealed to avoid further contamination. All samples obtained were labeled appropriately by indicating the location, time and date of collection. The samples were subsequently conveyed for further evaluation and microbiological analysis. Ten (10 g) of each shrimp sample was homogenized using sterile laboratory mortar. About 1.0 g of the homogenate was mixed with 10 mL of alkaline peptone water and incubated at 37 ^{o}C for about 6-7 hours. The suspension was serially diluted 10-fold using normal saline, and 0.5 mL of the appropriate dilution was spread over freshly prepared Thiosulfate Citrate Bile-Salt Sucrose (TCBS) Agar plate and MacConkey Agar plate, using a sterile spreader. The plates were incubated at 37 ^{0}C for 18-24 hours. Following incubation, the plates were observed for characteristic colonies. Discrete representatives of each unique colony type were selected for purification, characterization and identification using conventional microbiological and biochemical tests.

FIG. 1: White shrimp (*N. hastatus*) (Source: Emmanuel E. Bassey, 2023, Watt market)

2.3 Data Analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) software version 20 and results presented as percentage. Samples contamination rate was interpreted using the guideline provided by Center of Food Safety (2014).

3. RESULTS AND DISCUSSION

Enumeration of bacterial count is an important index of assessing the safety and quality of food products. Results obtained revealed that all analyzed *N. hastatus* samples were contaminated with various degree of bacterial growth (Table 1). According to the Center of Food Safety guideline (2014), acceptable upper limit of aerobic bacterial count in shellfish is <1.0 x 10^{6} Cfu/g. The present study showed that 33.3% (40/120) of the examined shrimps had bacterial count under the permissive limit, 56.7% (68/120) were at borderline (10^{6}-≤10^{7}Cfu/g) while 10.0% (12/120) of samples were unsatisfactory (>10^{7}Cfu/g) for consumption. Of the 12 samples that were unsatisfactory, 8 (26.7%)

were obtained from Nsidung beach market while 4 (13.3%) were from Obufa Esuk beach market. Previous studies have shown that high microbial loads can be obtained from different species of shrimps. Thus, Ajibare *et al*. (2021) reported total heterotrophic bacterial counts (THBC) of 1.076 to 1.125 x 10^2 Cfu/g from white shrimp (*N. hastatus*) in Ondo state, Nigeria. Also, the high bacterial load observed in this study corroborated with Orji *et al*. (2016) in Ebonyi state, Nigeria, who reported mean aerobic counts of 1.87±0.543 x 10^7 Cfu/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 x10^3 Cfu/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 shrimps (*Penaeus monodon*) in 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 aligns with finding by Talukder *et al*. (2019) who reported *Vibrio* count ranging from log 2.06 to 2.11 Cfu/g in shrimps (*P. monodon*) and Nwosu *et al*. (2020) who reported heavy *Vibrio* counts in smoked dried shrimps (*Litopenaeus vannamei*) sold in markets compared to home-smoked dried rinsed shrimps.

The elevated aerobic bacterial counts recorded in this study could be attributed to factors such as the use of polluted water at the shores, poor handling, unhygienic environment where these fish products are sold, inappropriate storage and exposure to insect vectors (Adebayo-Tayo *et al*., 2012; Gram & Dalgaard, 2002; Nwosu *et al*., 2020). Inadequate basic amenities such as potable water in major fishing communities in Nigeria remain a major challenge (Nwachukwu *et al*., 2013; Nwosu *et al*., 2020). This has encouraged the use of polluted sea water in washing shrimps which inadvertently predisposes shrimps to further microbial contamination and may have contributed significantly to the observed contamination rate.

From this study, 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*. 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). These organisms have been previously reported as major contaminants of seafood (Nrior *et al.,* 2016; Ajibare *et al*., 2021; Orji *et al*., 2016). This also is in agreement with Hariharan and Amadi (2016) who reported the prevalence of pathogenic bacteria in shellfish.

TABLE 1: Contamination rate of dried crayfish obtained in Calabar

Location	**No of samples**	**Aerobic Bacterial Counts**			***Vibrio* Counts**		
		Satisfactory	**Borderline**	**Unsatisfactory**	**Satisfactory**	**Borderline**	**Unsatisfactory**
		$<1.0x10^6$Cfu/g	**10^6-$\leq 10^7$Cfu/g**	**$>10^7$Cfu/g**	**<20Cfu/g**	**20 - $\leq 10^3$Cfu/g**	**$>10^3$Cfu/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)
Obufa Esuk 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 study indicates high occurrence of *Salmonella* spp (26.7%) and *Vibrio* spp (21.7%) in the analyzed samples which is of public health importance. Similar observation on the prevalence of *Salmonella* spp was reported by Bakr *et al*. (2011) in Alexandria, Egypt. The potential source of *Salmonella* contamination in *N. hastatus* 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 health risk to consumers if related to pathogenic strains, especially diarrheagenic *Escherichia coli*. However, the presence of non-pathogenic *Escherichia coli* in seafood is a concern 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 presence of *Pseudomonas* species in the analyzed samples is worrisome. *Pseudomonas* species are implicated in Pseudomonal food poisoning. 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 and they are implicated in gastrointestinal disorder etc. (Ajibare *et al*., 2021). The disease caused by *Staphylococcus aureus* is foodborne intoxication and common symptoms 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 survival of any microbial group, within a particular ecological niche, is greatly dependent on environmental parameters and anthropogenic activities (Khatri & Tyage, 2014). Hence, abundance of plethora of microbial groups in the aquatic environment have a significant effect in the high contaminated rate (Nwachukwu *et al*., 2013). 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.

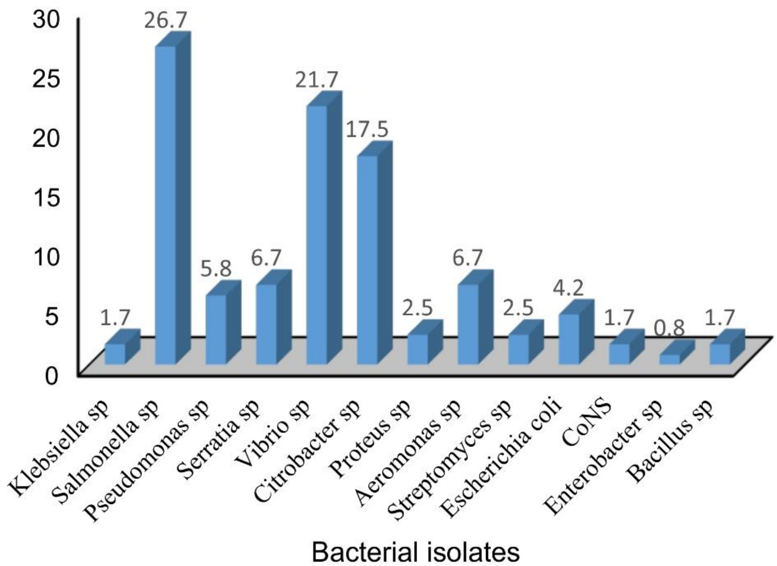

FIG 2: Prevalence rate of the isolated bacteria

4. CONCLUSION

White shrimps, like any other seafood has the potential of causing foodborne illnesses from bacterial, fungal, viral and parasitic pathogens under certain conditions. Hence, it is encouraged that the conditions of processing and packaging of seafood be done under standard hygienic measures to reduce the risk of microbial contamination. In addition, public health awareness campaign targeted at consumers and vendors should be optimized with frequent monitoring by regulatory agencies.

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