Bacteriological Quality of Fresh Prawn (*Macrobracium rosenbergii*) Sold in Port Harcourt.

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Abstract
Bacteriological quality of fresh prawn collected from different local markets was assessed using standard microbiological techniques. Nutrient and MacConkey agar were used as growth media for isolation of heterotrophic and coliform bacteria. Biochemical tests were performed to characterize and identify bacterial isolates. Isolates identified using ABIS Online(and frequency of occurrence) were Staphylococcus spp (21%),Pseudomonas spp (19%), Micrococcus spp (19%), Escherichia coli (26%), Klebsiella spp, Vibrio spp (10%), Lactobacillus spp (5%), and Aeromonas spp. Escherichia coli has the highest colony count of bacteria of 26% followed by Staphylococcus spp of 21% colony count. The total heterotrophic bacteria count range from $7.0x10^{-7}$ to $9.1 x 10^{9}$cfu/g, enteric bacteria ranged from $2.0x 10^{7}$ to $5.7x10^{9}$cfu/g. With respect to parts of fresh prawn analyzed, the appendage region of samples collected from Mile One Market (sample 2) had the highest colony count ($9.1x10^{9}$cfu/g), followed by the body region of samples collected from Mile One Market (sample 2) ($8.0x10^{9}$cfu/g). These values were significantly different from the count ranges of corresponding body parts of prawn collected from Creek Road and Mile Two Markets ($p>0.05$). Antimicrobial susceptibility test using common antibiotics as treatment on isolates revealed 80% of the isolates tested were susceptible to Gentamycin, Amoxicillin, Norfloxacine, Ceftriaxone, Levofloxacin, Erythromycin and Chloramphenicol, and only 20% were susceptible to Rifampicin, Ampiclox and Ciprofloxacin. Consequently the high bacteria load of these samples poses a public health risk and of great concern to consumers due to the inherent pathogenic potentials of these organisms. Contamination of fresh prawn due to poor sanitary standard of local markets, unhygienic practices of vendors or human and animal waste introduced into harvest sites worsen the situation. It is therefore recommended that improved sanitary practices during harvesting, storing and handling of fresh prawn be encouraged and maintained.

Key Words: *fresh prawn, bacteriological quality, antimicrobial susceptibility, culture techniques.*

Introduction

Prawn (*Macrobracium rosenbergii*) belong to crustaceans usually found in aquatic environment[1].Prawn is added to form a nutrient-rich diet which is widely eaten around the world. It provides the world's best prime source of high quality protein. Consumption of prawns provides some health benefits such as promotion of strong bones and teeth, improvement of immune function and reduction of heart disease. Because of its high nutritional value, nutritionist recommend consumption of prawn, this is because it is a vital protein source, with high low level of unsaturated fatty acids, which reduces the risk of cardiovascular disease. Vitamins, minerals are essential nutrients derived from prawn consumption also contribute to children's growth and development.[2]

Food safety is a complex issue where sea foods are generally regarded as high risk commodity due to the presence of pathogens, toxins and contaminants found in them[3].World health organization and Agricultural Organization of the United Nations postulated that food-borne infections are the most wide-spread health problems in the contemporary world, and an important cause of reduced productivity [4]. Several bacteria are associated with fresh prawn, some of which are pathogenic and poses serious health hazard if consumed raw or undercooked. Bacteriological quality of prawn has been reported as good for consumption when properly prepared[5]. However, some likely pathogenic bacteria associated with fresh prawn include species of the following genera: *Mycobacterium, Vibrio, Salmonella, Shigella,* and *Escherichia coli, Listeria monocytogenes, etc.*[6].

In the southern part of Nigeria, especially Port Harcourt, there is high demand for prawn due to its economic and nutritional value. Considering the health safety of consumers and economical sustenance, it is important to maintain the bacteriological quality of fresh prawn at consumable levels. The mode of contamination of fresh prawn include feeding, harvesting, storage and handling processes[7].

Prawns which constitute healthy diet for humans poses health threats when consumed raw or unhygienically prepared. Fresh prawn has been reported to be associated with seafood-borne diseases, which is caused by pathogenic bacteria present in it[8].

The study was aimed at ascertaining the bacteriological quality of fresh prawn sold in local markets in Port Harcourt. The objectives were to carry out microbiological analysis of the prawn, to observe the distribution of bacteria with respect to body parts, and to conduct antibacterial susceptibility tests.

Materials and Methods

Sample Collection

Fresh prawn samples were collected from three different local markets in Port Harcourt (Mile 3, Mile I, and Creek Road markets respectively). Samples were collected aseptically early in the morning using sterile polythene bags containing ice cubes and transported to the laboratory for analysis.

Microbiological Analysis

Microbiological analysis was carried out using Nutrient, MacConkey, and Salmonella Shigella (SS) Agars.

Nutrient Agar is a medium that supports the growth of non-fastidious microorganism. It is composes of 0.5% peptone, 0.3% beef extract, 1.5% agar, 0.5% sodium chloride, distilled water and a neutral pH. Nutrient agar was used in enumerating the total viable bacterial count in fresh prawn samples. Following the manufacturers specification, 28g of nutrient agar was dissolved in 1litre of distilled water and heated to dissolve completely. The media was sterilized in the autoclave for 15minutes at 121^{o}C, cooled to 45^{o}C and 20ml was dispensed into each sterile petri dish and allowed to solidify [9].

MacConkey Agar is a selective medium used selectively to isolate enteric bacteria from other gram negative bacteria based on their ability to ferment lactose. MacConkey agar used was prepared according to the manufacturer's specification by dissolving 47g of MacConkey agar in 1litre of distilled water and properly homogenized using a Bunsen burner. The media was then sterilized in the autoclave for 15minutes at 121^{o}C and allowed to cool at 45^{o}C then 20ml of the media was dispensed into each sterile petri dish and allowed to solidify[9].

SS agar is a selective and differential media used for the isolation of Salmonella species. It also differentiates between lactose and non-lactose fermenting bacteria. The media used was prepared using the manufacturer's specification by dissolving 62g of SS agar in 1000ml of distilled water and then heated with frequent agitation to dissolve. The medium was allowed to cool to 50^{o}C after which 20ml of the media was dispensed into sterile petri dishes and allowed to solidify[9].

Normal saline was prepared as described by Harigan and Mc-caine (1976). 85g of sodium chloride (NaCl) was weighed using a weighing balance and dissolved into 1litre of distilled water. A sterile 10ml pipette was used to transfer (ml of the saline into different test tubes and plugged with cotton wool to prevent contamination. Both test tubes and other contents were sterilized in an autoclave at 121^{o}C for 15 minutes[9].

Sterilization Glassware and Work Bench

Glass-wares such hockey glass rod (spreader) and pipettes were sterilized using the hot air oven at 160^{o}C for 1hour.

Test tubes and conical flasks containing media and physiological saline were sterilized in the autoclave at a temperature of 121^{o}C for 15minutes.The entire work bench was sterilized using 98% ethanol before and after work. The inoculating loop was sterilized through flaming before and after inoculation [10].

Preparation of Samples for Microbiological Analysis:

Sample Preparation

Each fresh prawn sample was dissected using a sterile dissecting blade into three parts (*i.e.* Head, Body and Appendages). These parts were homogenized separately using a sterile mortar and pestle.

Serial Dilution

1g of each homogenized sample part was added into 9ml of sterile normal saline contained in a test tube. This was shaken vigorously to form a stock solution of 10^{-1}concentration, and series of 10 fold serial dilutions were made.

Inoculation and Incubation

0.1ml (aliquot) from 10^{-6} dilutions was pipetted onto the surface of dried Nutrient agar, SS agar and MacConkey agar using a sterile 1ml pipette. The inoculum was spread evenly using a sterile glass spreader which was further sterilized by dipping into 98% ethanol and flamed with a Bunsen burner. The plates were then inverted and incubated at 37^{o}C for 24hours after the inoculation was completed [10].

Total Heterotrophic Bacterial Count

After 24 hours of incubation, visible bacterial colonies were counted and recorded. Morphological identification was facilitated by studying the colonial morphology under which the size, shape, margin and elevation of the colonies were considered.

Characterization and Identification of Bacterial Isolates

Biochemical tests were carried out to characterize the isolates and identification was done using ABS Online Microbial Identification Application[11].Core tests used include Gram reaction, Coagulase (to differentiate *Staphylococcus aureus* from other species of *Staphylococcus*); Catalase (helps to differentiate *Staphylococci* (catalase +) from *Streptococci* (catalase -)and also used to differentiate *Mycobacterium spp.*); Starch hydrolysis (This test was used to identify bacteria that produce amylase an enzyme that hydrolyses starch). Specific sugar fermentation

tests were carried out on glucose, maltose, manitol and lactose following the methods of Cheesebrough [12]. Starch hydrolysis test was also done to ascertain bacteria capable of hydrolysis starch. Others biochemical tests carried out were Oxidase, Motility, Indole, Methyl Red, Voges Proskauer (VP),

Antibiotics Susceptibility

Antibiotics susceptibility is the sensitivity of bacteria to antibiotics. Antibiotics susceptibility testing (AST) of bacteria was carried out to determine which antibiotics would be successful in treating a particular bacterial infection *in vivo*. But in this study, nutrient agar was used. Isolates were tested against common antibacterial drugs by disc diffusion assay on Nutrient Agar with antibiotic disc.

Results and Discussion

The results obtained from the bacteriological analysis of fresh prawns shows that bacteria were present in them. The samples analyzed were differentiated into regions of head, body and appendages. The estimation of the total heterotrophic bacteria count of the various samples regions are given in the tables below:

Bacterial Count (Total Colony Count)

Table 1: Total heterotrophic bacterial count of body parts of prawn

	Sample 1(Mile 3 Market)			Sample 2(Mile I Market)			Sample 3 (Creek Road market)		
	Head	**Body**	**Appendage**	**Head**	**Body**	**Appendage**	**Head**	**Body**	**Appendage**
Total	**1311**	**373**	**2754**	**1287**	**2411**	**367**	**402**	**223**	**576**
Mean	**437**	**124.3**	**918**	**429**	**803.7**	**122.3**	**134**	**74.3**	**192**
Cfu/g	**$4.37x10^9$**	**$1.24x10^9$**	**$9.18x10^9$**	**$4.29x10^9$**	**$8.03x10^9$**	**$1.22x10^9$**	**$1.34x10^9$**	**$7.4x10^8$**	**$1.92x10^9$**

There was a peculiar pattern of occurrence of bacterial loads with respect to body parts of fresh prawn and the markets sampled. Total heterotrophic bacteria occurred in all body parts irrespective of the market in question (Table 1).

Table.2: Total Coliform Bacterial Count

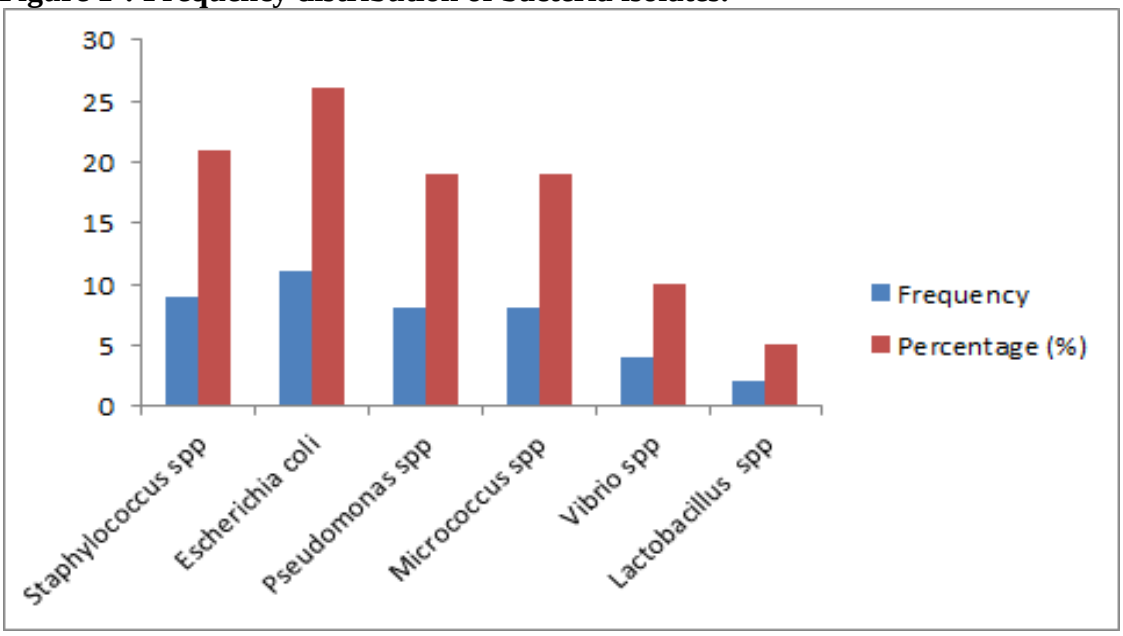
Samples 1(Mile 3 market)				Samples 2 (mile 1 market)			Samples 3 (Creek Road Market)		
	Head	**Body**	**Appendage**	**Head**	**Body**	**Appendage**	**Head**	**Body**	**Appendage**
Total	**-**	**-**	**1734**	**220**	**1861**	**15**	**9**	**-**	**20**
Mean	**-**		**578**	**73.3**	**620.3**	**5**	**2**	**-**	**6.7**
Cfu/g	**-**		**$5.78x10^9$**	**$7.3x10^8$**	**$6.20x10^9$**	**$5.0x10^7$**	**$2.0x10^7$**	**-**	**$7.0x10^7$**

On the contrary, total coliform counts scored 0% in the head and body parts of prawns obtained from Mile 3 Market. Body of fresh prawns from Creek Road market also scored 0% in coliform count (Table 2).

The study has shown that fresh prawn has high possibility of becoming contaminated with bacteria. Several bacterial species were isolated from the fresh prawn samples, which include *Staphylococcus spp, Pseudomonas aureginosa, Micrococcus spp, Escherichia coli, Klebsiella spp, Aeromonas spp. Vibro spp Lactobacillus spp.* There was no observable growth for *Salmonella spp.* and results obtained is presented on table 1 and 2. The total heterotrophic

bacterial count ranged from $7.0x10^{-7}$ to 9.1 x 10^{9}cfu/g. For enteric bacteria, from 2.0x 10^{7} to $5.7x10^{9}$cfu/g. Of all parts of fresh prawn samples analyzed, the appendages from Sample 2 (Mile I market) had the highest bacterial count ($9.1x10^{9}$cfu/g), followed by the body region from Sample 2(Mile I market) ($8.0x10^{9}$cfu/g). This shows that samples collected from Mile I market are more heavily contaminated compared to samples collected from Mile 2 and Creek Road market. This implies that the sanitary standard of these markets are very poor.

Figure 1`: Frequency distribution of bacteria isolates.



The occurrence of bacterial and their respective frequencies were *Staphylococcus spp.* (21%), *Escherichia coli* (26%), *Pseudomonas spp* (19%), *Micrococcus spp* (19%), *Vibrio spp* (10%) and *Lactobacillus spp.* (Figure 1). The result reveals that *Escherichia coli* has the highest percentage (26%) of occurrence, followed by *Staphylococcus spp (21%)*, this is due to high level of faecal and sewage contamination of prawn which may have occurred from harvest sites in water, unhygienic practices of vendors.

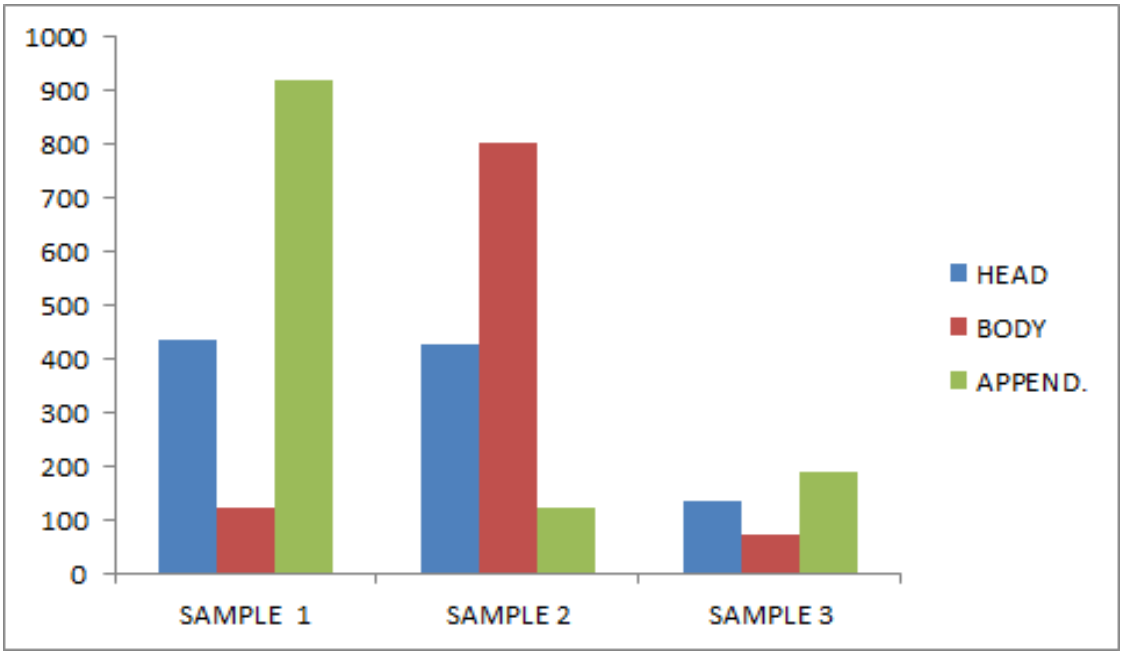


Figure 2: Bacteria distribution on body parts of fresh prawn.
Legend: Sample 1: Mile 3 Market;
Sample 2: Mile I market;
Sample 3: Creek Road market)

Figure 2 illustrates comparison of bacterial load of the different body parts of the samples analyzed. Comparing the head of the three samples analyzed, Sample 1 and Sample 2 has the highest bacterial colony count with no significant difference at p <0.05, for the body part; Sample 2 has the highest bacterial colony count followed by Sample 1. For the appendages, Sample 1 has the highest colony count followed by Sample 2 and Sample 3 but no significant difference. The difference in colony counts may be due to poor sanitary or unhygienic practices of vendors.

Table 3: Morphology and Biochemical Reactions of Bacteria Isolates

Iso	GR	Cat	Coa	Oxi	Mtl	Ur	SH	Vp	MR	Ind	Glu	Lac	Mal	Man	P.O.
A	GPC	+	-	+	+	-	+	-	-	-	AG	AG	AG	AG	*Staphylococcus arlettae*
B	GNR	+	-	+	+	-	+	-	+	-	AG	-	-	AG	*Pseudomonas aeruginosa*
C	GPC	+	-	+	-	-	-	-	+	+	AG	AG	AG	AG	*Macrococcus brunensis*
D	GPC	+	-	+	-	-	-	-	-	+	AG	A	AG	AG	*Macrococcus lamae*
E	GNR	-	-	+	+	-	-	-	+	+	AG	A	AG	AG	*Escherichia coli*
F	GNR	+	-	+	-	-	-	+	-	+	AG	AG	AG	AG	*Klebsiella Oxytoxa*
G	GPC	+	-	-	-	-	+	-	-	-	AG	-	AG	-	*Staphylococcus haemolyticus*
H	GPC	+	-	+	-	-	+	-	-	-	AG	-	AG	-	Staphylococcus Fleuretti
I	GNR	+	-	+	+	-	-	-	+	+	AG	AG	AG	AG	*Escherichia coli*
J	GPR	-	-	-	-	-	-	-	+	+	AG	-	AG	-	*Lactobacillus satsumensis*
K	GPC	-	-	+	-	-	+	-	+	+	AG	-	AG	A	*Macrococcus hajekii*
L	GPC	-	-	+	-	-	+	-	-	+	AG	AG	AG	AG	*Staphylococcus sciuri*
M	GNR	+	-	-	+	-	+	-	-	+	AG	AG	AG	AG	*Vibrio gazogenes*
N	GNR	+	-	+	-	-	+	-	+	-	AG	AG	AG	AG	*Aeromonas salmonicida*

Key: Iso: Isolate, Lac: Lactose, GPC: Gram Positive Cocci; GNR: Gram Negative Rod, GNC: Gram Negative Cocci, GPR; Gram Positive Rod, Mal: Maltose, Man Manitol, GR: Gram

Reaction, Cat: Catalase, Coa: Coagulase, Oxi: Oxidase, Mtl: Motility, Ur: Urease, SH; Starch Hydrolysis, Vp: Vouges Proskaeur, Glu: Glucose, P.O: Probable organism

Table 4: Antibiotics Susceptibility Test results

Bacteria	Sensitive
Staphylococcus spp	Levofloxacin(30mm),Erythromycin (28mm) Gentamycin (28mm),Ciprofloxacin (25mm) Rifampicin (34mm). Norfloxacin (23mm), Ceftriaxone (25mm)Ampicillin (28mm),
Escherichia coli	Levofloxacin(29mm),Gentamycin (23mm), Ceftriaxone (30mm), Chloramphenicol (21mm), Ampicilin (22mm),Ciprofloxacin (35mm), Norfloxacin (28mm), Amoxicillin (23mm)
Micrococcus spp	Gentamycin, Chloramphinicol Erythromycin, Lefloxacin

The bacterial species also exhibited resistance to some antimicrobials. *Staphylococcus* species to Chloramphenicol and Amoxicillin; *E. coli*to Rifampicin and Erythromycin, and *Micrococcus* species to Ampiclox, Rifampicin and ampicillin. Antibacterial susceptibility assays using *E. coli, Micrococcus spp. and S. aureus* showed that the isolates were sensitive to commonly used antibiotics, thus are recommended for prophylactic and therapeutic uses if fresh prawn consumers are infected with related organisms (Table 4).

Discussion

Bacteria such as *Staphylococcus spp., Pseudomonas spp., and Escherichia coli* were isolated from the head region; *Micrococcus spp, Escherichia coli, Klebsiella spp, Lactobacillus spp.* and *Staphylococcus spp* were isolated from the body region, and *Micrococcus spp., Staphylococcus spp., Vibrio spp.,* and *Aeromonas spp.* were isolated from the appendages.

The findings of this study reveals that fresh prawn samples sold in local market in Port Harcourt are heavily contaminated and several of the bacterial isolates are pathogenic in nature, as they are known to cause food borne infections. This urgently suggests the need for monitoring harvest sites, improving sanitary standards of local markets and encouraging hygienic practices of vendors, to ensure elimination of seafood borne infections, with needed cautions for the end-user.

Conclusion and Recommendations

The study has revealed several bacterial organisms associated with fresh prawns, with most of them being natural pathogens implicated in seafood borne infections and ailments. Irrespective of the health benefits derived from the consumption of seafood, such as fresh prawn, consuming raw or unhygienically prepared fresh prawn poses health threats, as fresh prawn harbors harmful bacteria which can cause food-borne diseases ranging from diarrhea to life threatening gastroenteritis.

Therefore, proper guideline for the improvement of the microbial quality of fresh prawns be strictly adhered to. In addition, fresh prawn should be properly cooked and items in recipe should be devoid of contaminating microorganisms in order to avert pathogens capable of causing food-borne diseases.

It is therefore recommended that:

- Sufficient handling and appropriate disposal of sewage should be carried out and maintained to avoid microbial contamination of harvest sites by pathogenic bacteria.
- Improved efforts should be undertaken to instruct all health professionals, food handlers and consumers concerning the microbiological risks associated with the consumption of raw or undercooked prawn.
- Careful washing and thorough cooking of fresh prawn prior to consumption should be undertaken to
- Get rid of microbial pathogens.
- Effective enforcement agency for prevention of harvesting or sales of fresh prawn from sewage-contaminated growing water should be developed and funded adequately.
- Appropriate antibiotics should be used or a physician be contacted in case of food poisoning.

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