



Full Length Research Paper

Microbial load of farm-reared *Macrobrachium vollenhovenii* (Herklots, 1857) and the health implications

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Cultured prawns are more prone to pathogenic aquatic microorganisms than the wild-captured prawns. Therefore, estimation of bacterial load in prawns has been variously used to ascertain the safety of the prawns for consumption. Samples of *Macrobrachium vollenhovenii* were analyzed for total viable bacterial count (TVC), total coliform count (TCC), total clostridia count (TCLC) and total *Salmonella-Shigella* count (TSS). Sampling was done weekly for four consecutive weeks. Water quality parameters were within recommended range for growth of prawns. Mean TVC of prawns from Badore was $2.10 \pm 0.28 \times 10^6$ while values for Badagry and Epe were $1.95 \pm 0.24 \times 10^6$ and $2.05 \pm 0.39 \times 10^6$ respectively. The TCC were $1.45 \pm 0.06 \times 10^3$, $1.40 \pm 0.19 \times 10^3$ and $1.68 \pm 0.19 \times 10^3$ for Badore, Badagry and Epe respectively. The TCLC were $0.65 \pm 0.72 \times 10^2$ (Badore), $0.57 \pm 0.61 \times 10^2$ (Badagry) and $0.43 \pm 0.67 \times 10^2$ (Epe), and corresponding TSS values were $1.42 \pm 0.28 \times 10^2$, $0.90 \pm 0.72 \times 10^2$ and $1.17 \pm 0.91 \times 10^2$. Mean TVC, TCC, TCLC and TSS were above ICMSF maximum acceptable limits. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp* and *Shigella sp* were isolated from all the samples. This result infers that the prawns are contaminated with pathogenic microorganisms and could serve as points of transmission of infection to consumers. Proper hygienic and sanitary conditions should be maintained.

Keywords: Farm-reared, Health Implications, *Macrobrachium vollenhovenii*, Microbial Load, Microorganisms, Water Quality

INTRODUCTION

Prawns are delicacies cherished by most people because the flesh of the prawn is a good source of protein. As a result of increased global demand in the past few decades, prawns have become one of the most important export products from many Asian and Latin American countries around the world. The implication of this increased demand is that aquaculture of prawns has to be employed by exporting countries in an attempt to meet the global demand. In fact, in 2014, aquaculture

accounted for 54% of global shrimp production (FAO, 2016). Aquaculture products have, however, been reported to be more prone to various pathogenic microorganisms which are present in the aquatic environment than those captured from the wild (Kamat *et al*, 2002). The contamination of shrimps with bacteria has been reported to be a reflection of several factors which include the harvesting environment, rearing water quality and post-process contamination (Solomon and Ibe, 2012).

Therefore, estimation of bacterial load in prawns has been variously used to ascertain the safety of the prawns for consumption (Okonko *et al.*, 2008; Abd-El-Aziz and Moharram, 2016; Yousuf *et al.*, 2008)

Aquaculture is generally regarded as a food-producing enterprise; however, while realizing this potential of aquaculture, there also exists potential health risks as a result of product contamination due to chemical and biological contaminants. Thus, consumption of aquaculture produce may sometimes cause disease due to infection or intoxication. Proper handling of fish between capture and delivery to the consumers is a crucial element in assuring quality of the final product. Standards of sanitation, method of handling and the time/temperature of holding fish are all significant factors to assure quality (Reilly and Kaferstein, 1999). Hence, caution must be exercised in view of the health risks associated with the aquaculture produce; as such an evaluation of quality and safety of the aquaculture produce must be given utmost priority.

In Nigeria, the most-farmed aquaculture species is the African mud catfish, *Clarias gariepinus* (Burchell, 1822). However, there are other fin/shellfish that have been reported to have great aquaculture potential; one of such is the African river prawn, *Macrobrachium vollenhovenii* (Herklots, 1857) (Jimoh *et al.*, 2013). It can be found in low salinity brackish water. This prawn is widely distributed in the country, attains relatively large size and commands high market demand/price. (Bello-Olusoji *et al.*, 2006; Jimoh *et al.*, 2012). Consequent upon the high price of this prawn in the Nigerian market, and in order to meet the market demand, partial farming of this prawn has been embarked upon. Thus, in view of the possible attendant health risks that could be associated with the farming of this prawn, this study was carried out to assess the microbial load of *Macrobrachium vollenhovenii* (Herklots, 1857) and the health implications on farm personnel and consumers of this aquaculture produce.

MATERIALS AND METHODS

Sources of Prawn Samples

Samples of *Macrobrachium vollenhovenii*, and pond water were obtained from three different freshwater shrimp farms located in Badore, Badagry and Epe areas in Lagos State, Nigeria. The ponds sampled in the three different locations each had an area of 0.95ha, 0.45ha and 0.64ha, and were stocked at 2,500, 1,000 and 800 post larvae respectively. All the ponds in the three sites were fertilized with cow dung. In Badore, there was periodic tidal water exchange by opening the inlet-cum-

outlet to small transition pond which was connected to a small canal.

Collection of Prawn and Water Samples

Samples of the prawns were collected weekly for four (4) weeks in all the selected farms. The prawns were transported on ice to the University of Lagos laboratory where microbial analysis was done. On the farm, water temperature, pH and dissolved oxygen were measured in-situ using the thermometer, digital pH meter and dissolved oxygen meter respectively. For other parameters, water sample was collected in clean bottle and taken to the laboratory where turbidity and total dissolved solids were measured using digital meter and electronic conductivity meter respectively while alkalinity was determined by the titration method.

Preparation of Samples for Microbial Analysis

The prawns were rinsed in sterile distilled water to remove any debris on the shell. Then, using sterile knife and forceps, 15g of sample flesh was cut out and blended for bacterial isolation. 10g of the blended sample and 90ml sterile physiological saline (0.85% w/v NaCl) were poured into conical flask to make a homogenized suspension and serial dilutions of the homogenate was prepared.

Enumeration of Bacteria

Inoculums from the prawn samples, under sterile conditions, were streaked on sterile culture media. The media used for the microbiological analysis were Nutrient Agar, NA (Difco, USA) for total viable bacterial count, MacConkey Agar, MA (Difco, USA) for total coliform count, Potato Dextrose Agar (PDA), Mannitol Salt Agar (MSA) for Gram-positive bacteria count and Deoxycholate Citrate Agar (DCA) for *Salmonella-Shigella* count. The inoculated plates were then incubated aerobically at 25°C for 24hrs. The total bacterial count for each sample was determined using the pour plate techniques and the counts expressed in colony forming unit per gram (cfu/g) of the sample.

Isolation and Identification of Bacteria

Isolated bacteria were characterized using both morphological culture characteristics and biochemical tests as described by Jolt *et al.* (1994). The Global Infectious Diseases and Epidemiology Network (GIDEON) software (Berger, 2001) was used to confirm the results obtained with the biochemical tests.

Statistical Analysis

Data generated from this study were analysed using Microsoft Excel 2007.

RESULTS

The physico-chemical parameters of water from the three prawn farms are presented in Table 1. Temperature ranged from 25.5 - 27.0 °C (mean = 26.0 ± 0.5 °C), 26.5 - 28.4 °C (mean = 27.4 ± 0.7 °C) and 26.5 - 28.0 °C (mean = 27.1 ± 0.7 °C) for Badore, Badagry and Epe respectively. The mean pH for Badore was 7.2 ± 0.3; Badagry was 7.4 ± 0.5 and Epe was 7.4 ± 0.7 while mean conductivity values were 20.0 ± 0.4 s·m⁻¹ (Badore), 15.9 ± 0.4 s·m⁻¹ (Badagry) and 22.0 ± 1.9 s·m⁻¹ (Epe); and the corresponding dissolved oxygen values were 3.0 ± 0.0 mg/l, 3.4 ± 0.1 mg/l and 3.3 ± 0.3 mg/l respectively. Water from Badore, Badagry and Epe had mean turbidity values of 10.8 ± 0.4 ppm, 3.0 ± 0.2 ppm and 10.7 ± 1.4 ppm respectively with corresponding total dissolved values of

21.6 ± 2.0 mg/l, 23.0 ± 2.3 mg/l and 17.5 ± 1.5 mg/l. The alkalinity values were 19.2 ± 1.0 ppm (Badore), 23.3 ± 0.5 ppm (Badagry) and 21.6 ± 1.7 ppm (Epe).

The results of the microbial load in farm-raised *M. vollehovenii* from the three farms are presented in Table 2. The mean total viable bacterial count of prawns recorded for Badore, Badagry and Epe were 2.10 ± 0.28 × 10⁶ cfu/g, 1.95 ± 0.24 × 10⁶ cfu/g and 2.05 ± 0.39 × 10⁶ cfu/g respectively and the corresponding total coliform count values were 1.45 ± 0.06 × 10³ cfu/g, 1.40 ± 0.19 × 10³ cfu/g and 1.68 ± 0.19 × 10³ cfu/g. Total clostridia count was 0.65 ± 0.72 × 10² cfu/g (Badore), 0.57 ± 0.61 × 10² cfu/g (Badagry) and 0.43 ± 0.67 × 10² cfu/g (Epe). The respective total *Salmonella-Shigella* counts for Badore, Badagry and Epe were 1.42 ± 0.28 × 10² cfu/g, 0.90 ± 0.72 × 10² cfu/g and 1.17 ± 0.91 × 10² cfu/g. Seven bacteria isolates were characterized from the three farms and these were *Bacillus sp*, *Clostridium sp*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp*, *Shigella sp*, *Micrococcus sp* and *Klebsiella* with *Micrococcus sp* isolated from prawn samples from the Badore farm only.

Table 1: Mean Water Quality Parameters of the Experimental Prawn Farms.

| Farm | Temp. (°C) | pH | Conductivity (s·m ⁻¹) | Turbidity (ppm) | DO (mg/l) | TDS (mg/l) | Alkalinity (ppm) |
|---------|------------|---------|-----------------------------------|-----------------|-----------|------------|------------------|
| Badore | 26.0±0.5 | 7.2±0.3 | 20.0±0.4 | 10.8±0.4 | 3.0±0.0 | 21.6±2.0 | 19.2±1.0 |
| Badagry | 27.4±0.7 | 7.4±0.5 | 15.9±0.4 | 3.0±0.2 | 3.4±0.1 | 23.0±2.3 | 23.3±0.5 |
| Epe | 27.1±0.7 | 7.4±0.7 | 22.0±1.9 | 10.7±1.4 | 3.3±0.3 | 17.5±1.5 | 21.6±1.7 |

DO = Dissolved Oxygen; TDS = Total Dissolved Solids

Table 2: Microbiological Assessment of *Macrobrachium vollehovenii* samples collected from the experimental prawn farms.

| Sample | TVC (x10 ⁶ cfu/g) | TCC (x10 ³ cfu/g) | TCLC (x10 ² cfu/g) | TSS (x10 ² cfu/g) | PMI |
|---------|------------------------------|------------------------------|-------------------------------|------------------------------|--|
| Badore | 2.10 ± 0.28 | 1.45 ± 0.06 | 0.65 ± 0.72 | 1.42 ± 0.28 | <i>Bacillus sp</i> <i>Clostridium sp</i> <i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Salmonella sp</i> <i>Shigella sp</i> <i>Micrococcus sp</i> <i>Klebsiella sp</i> |
| Badagry | 1.95 ± 0.24 | 1.40 ± 0.19 | 0.57 ± 0.61 | 0.90 ± 0.72 | <i>Bacillus sp</i> <i>Clostridium sp</i> <i>Staphylococcus aureus</i> <i>Salmonella sp</i> <i>Shigella sp</i> <i>Escherichia coli</i> <i>Klebsiella sp</i> |
| Epe | 2.05 ± 0.39 | 1.68 ± 0.19 | 0.43 ± 0.67 | 1.17 ± 0.91 | <i>Bacillus sp</i> <i>Clostridium sp</i> <i>Staphylococcus aureus</i> <i>Salmonella sp</i> <i>Shigella sp</i> <i>Escherichia coli</i> |

Key: TVC (Total Viable Count), TCC (Total Coliform Count), TCLC (Total Clostridia Count), TSS (Total *Salmonella-Shigella* Count) and PMI (Predominant Microorganisms Isolated)

DISCUSSION

Water quality and microbiology of the pond play a significant role in nutrient status of pond, well being of fish, potential fish yield and most importantly, quality and safety of fish raised from the pond (Reily and Kafastein, 1999). Thus, monitoring of fish ponds for microbial quality is necessary to obtain safe fish produce from the ponds. Several researchers have reported that there is a relationship between the microbial parameters and water quality parameters in the pond (Jun *et al.*, 2000; Surendraraj, *et al.*, 2001). All water parameter recorded in this study were within the recommended range for shrimp culture as reported by New (2002).

The total viable bacterial count (cfu/g) of *Macrobrachium vollehovenii* from Badore was $2.10 \pm 0.28 \times 10^6$, Badagry was $1.95 \pm 0.24 \times 10^6$ while Epe was $2.05 \pm 0.39 \times 10^6$. These values are above the ICMSF maximum acceptable limits of 10^6 cfu/g for prawns (ICMSF, 1978). This is in accord with the results of several authors who also reported high TVC values in shrimps (Ibe, 2008; Okonko *et al.*, 2008). When bacterial load is 10^6 cfu/g or higher in food, those foods are considered as spoiled (Conseulo *et al.*, 1983); however, high bacterial load does not imply that the prawn is unfit for human consumption since only a portion of the total flora contributes to spoilage (Huss *et al.*, 2007). The implication of these higher bacterial counts is the possibility of shellfish-borne diseases and the eventual transmission of these diseases to consumers. Cultured prawns have been reported to have higher bacterial load than the wild-caught prawns (Jayasree, *et al.*, 1999; Ogbondeminu and Okaeme, 1986), and in the case of this study, this may be due to bird droppings and the organic manure that was used to fertilize the ponds.

In this study, the total coliform count (TCC) varied from $1.45 \pm 0.06 \times 10^3$ cfu/g (Badore) to $1.40 \pm 0.19 \times 10^3$ cfu/g (Badagry) and $1.68 \pm 0.19 \times 10^3$ cfu/g (Epe). These values are above the ICMSF maximum acceptable limits of 10^2 cfu/g for prawns (ICMSF, 1986) as was also reported by Yousuf *et al.* (2008), Solomon and Ibe (2012), Okonko *et al.* (2008). Coliforms are used as indicator microorganisms to serve as a measure of faecal contamination (Nayem *et al.* 2011; Clarence *et al.* 2009; Okonko *et al.*, 2008), and most reports have verified the general absence of coliform bacteria on the natural flora of wild-caught shrimps (Miget, 1991). Hence, the isolation of *Escherichia coli* and other coliform bacteria in this study is an indication that there was faecal pollution in the sample areas, which might have been as a result of the use of poultry wastes for fertilizing the pond or seepage from septic tanks around the pond areas. The presence of coliforms could also have resulted from unsanitary conditions during harvesting and handling of the prawns (Abd-El-Aziz and Moharram, 2016; Clarence *et al.*, 2009).

Thus, indicating poor hygienic conditions of farm personnel.

Mean TSS values of $1.42 \pm 0.28 \times 10^2$ cfu/g, $0.90 \pm 0.72 \times 10^2$ cfu/g and $1.17 \pm 0.91 \times 10^2$ cfu/g were recorded for Badore, Badagry and Epe respectively. In this study, *Salmonella* and *Shigella* were recorded in all the samples. The *Salmonella-Shigella* (SS) count exceeded the ICMSF recommendation as was also reported by Kamat *et al.*, (2002), Nayem *et al.*, (2011) and Okonko *et al.*, (2008). The prevalence of *Salmonella* and *Shigella* species are low in raw wild-caught crustaceans; however, the incidence of *Salmonellae*, *Enterobacteriaceae* and *Listeria* species are high in farm-raised shrimps (Huss, 1995), which was also observed in the study area. The high level of diverse microbial contamination in rearing waters and cultured aquatic animals is due to the excess deposition of unconsumed feed at the base of the ponds, the use of animal and human manure and agricultural wastes leaching into the water supply (Moriarty, 1997). This probably explains why the incidence of *Salmonella*, *Shigella*, *Listeria* and *Vibrio* species are more common in rearing pond waters and cultured seafood (Huss, 1995). This may account for the presence of these organisms in the prawn as animal manure was used for fertilization of ponds in the three locations.

The microorganisms associated with cultured prawns reflect the microbial population of pond water, pond surrounding environment, harvesting and handling procedures. In this study, the predominant microorganisms isolated were *Bacillus sp*, *Clostridium sp*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella sp*, *Shigella sp*, *Micrococcus sp* and *Klebsiella sp*. and this is similar to the microorganisms reported by Okonko *et al.* (2008) and Bankole *et al.* (2004). Some of these isolated bacteria are pathogenic and, consequently, of public health significance. *Escherichia coli* are naturally-occurring bacteria in the intestinal tract of warm-blooded animals. Most are not pathogenic, but some strains are pathogenic, and these pathogenic strains are transmitted to prawns through sewage pollution of the coastal environment. *E. coli* is generally spread through contaminated food and water. Symptoms of *E. coli* infection in food include diarrhea, abdominal cramping, nausea and vomiting. *Salmonella* is a bacterium which causes typhoid fever and transmitted through contaminated food and water. The disease has been reported as a major public health problem in developing countries due to poor sanitary conditions and inadequate potable water (Ibekwe *et al.*, 2008). *Staphylococcus aureus* is also a pathogenic bacterium of public health significance that causes intoxication, and humans have been reported as the major source of *Staphylococcus* transmission to prawns through improper handling (Omemu and Bankole, 2005). Minute amounts of the toxin

produced by *S. aureus* causes illness, and on rare occasions, it is fatal. The presence of these pathogenic microorganisms in *Macrobrachium vollenhovenii* imply a serious public health concern and prawn producers as well as farm personnel should be educated on the importance of providing hygienic environment, practicing good personal hygiene and strict adherence to good management practice.

CONCLUSION AND RECOMMENDATIONS

Prawn farms used for this study sourced water from surrounding natural water bodies, which are most times contaminated with microorganisms through human activities. This contamination also affects the cultured prawns that depend on these sources as water source, which might, in turn, affect those handling the prawns. Also, consumption of these contaminated prawns is hazardous to man. Results from this study showed that prawn samples from all the studied locations were contaminated with *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Shigella* raising serious public health concerns that might arise from consumption of these prawns. Therefore, it is necessary to embrace measures that will reduce the bacterial load of prawns. Hence, it is important that Government makes Laws which will stop or reduce human activities that could contaminate natural water bodies. It is also necessary to follow the code of safe practice concerning prawn handling, harvesting and storage. Proper hygienic condition should be maintained at every stage of production and materials used for production must be maintained in good and hygienic condition. Borehole should be used to supply water to prawn farms, located far away from septic tanks and deep enough to avoid contamination. Also, consumers should be educated on the possible health hazards of raw/undercooked prawns.

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