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Assessment of Microbial Loads, Species Characterization and Composition in Prawn (*Macrobrachium vollenhovenii*) Fillets from Major Wetlands in Akwa Ibom State, Nigeria

***EFFIONG, MU; ADEYEMI, AV**

Department of Animal and Environmental Biology, University of Uyo, Uyo, Nigeria.

**Corresponding Author Email: mmanduueffiong@uniuyo.edu.ng; ORCID ID: https:orcid.org/0000-0003-0087-2450*
*Co-Author Email: **adeyemiadekunle.vincent@gmail.com***

ABSTRACT: The objective of this paper is to assess the assessment of microbial loads, species characterization and composition in prawn (*Macrobrachium vollenhovenii*) fillets from major wetlands (Nwaniba, Ibaka, Ibeno and Itu) in Akwa Ibom State, Nigeria. The microbial loads, species characterization and composition in prawn fillets were determined using standard microbiological procedures. Results from the study revealed total heterotrophic bacterial counts ranging from 2.10 x 104cfu/g in samples from Ibeno to 7.30 x 104cfu/g from Itu samples. Samples from Itu also recorded the highest values (3.5 x 104cfu/g) of total heterotrophic fungal counts. A total of eight bacterial (*Staphilococcus aureus, S. albus, Enterobacter aerogenes, Bacillus cereus, Escherichia coli, Micrococcus luteus, Arthrobacter freundii* and *Salmonella ecterica*) and six fungal (*Candida tropicalis, Aspergillus niger, A. flavus, A. terreus, Mucor mucedo*, and *Rhizopus sp*) species were isolated. The bacterial species, *Micrococcus luteus* and *Arthrobacter freundii* had 100% frequency of occurrence while in the fungal group it was *Candida tropicalis.* The presence of these pathogenic organisms in prawn samples from these wetland areas may infer possible threat to the health of prawn consumers especially when the products are undercooked or poorly processed before consumption.

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The increasing outbreaks of seafood poisoning around the world has highlighted the importance for microbial control in the fishery industry. Studies have revealed that microbiological risk assessment had become an emerging tool for the evaluation of the safety of food and water supplies (Effiong and Christopher, 2020). Prawns had been reported to harbor pathogens capable of causing seafood-borne diseases (Iwamoto *et al.*, 2010). Some of these pathogenic organisms (*Vibrio* sp., *Salmonella* sp., *Streptococcus* sp. and *Staphylococcus* sp.) had been reported to be responsible for various health problems in humans (Lipp and Rose, 2011). Despite the health and nutritional benefits of prawns it is highly perishable and can habour a large number of bacteria in the gut from water, sediment and feed (Shakila *et al.*, 2006). Thus, prawns deteriorate due to improper handling, and further processing may not bring back its original fresh quality. Several bacteria species are associated in the spoilage of fresh prawn, some of which are pathogenic and pose serious health hazard. Contamination may be due to poor hygienic condition including inappropriate processing, preservation and storage condition (Eze *et al*., 2011). The animal can also be exposed to a range of hazards from the water, feeding, storage, harvesting and through handling processes. The hazard agents may involve bacteria, viruses, parasites, natural toxins, and chemical contaminants (Effiong and Isaac, 2019). The African river prawn (*Macrobrachium vollenhovenii*) is a large,

**Corresponding Author Email: mmanduueffiong@uniuyo.edu.ng*

commercially important prawn species from the family Palaemonidae. It is a catadromous species that could move from freshwater to brackish area for spawning purposes. There had been tremendous development in shrimp and prawn culture globally (Rahman *et al*., 2002; Alam, 2007) and constitute an important part of the artisanal fishery production in some parts of Nigeria especially Akwa Ibom State (Abdolnabi *et al.,* 2015). In 2014, aquaculture accounted for 54% of global shrimp production (FAO, 2016). The study of Zabbey (2007) stated that prawns are highly relished and among the leading priced seafood on the global markets. It 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. The consumption of prawn is highly recommended because of its vital protein source, with light level of unsaturated fatty acids, which reduces the risk of cardiovascular disease in consumers (Disegha and Onuegbu, 2018). Prawns are among the most commonly consumed seafood by the people of Akwa Ibom State. Studies by FDA (2007) identified *Salmonella* spp., *Clostridium botulinum*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Yersinia pseudotuberculosis*, *Vibrio cholerae*, *Clostridium perfringens*, *Bacillus cereus*, *Aeromonas* spp., *Plesiomonas shigelloides*, *Shigella* spp., *Streptococcus* spp. as some pathogens of prawn that have been implicated in food borne outbreaks at some time with *Shigella sonnei* being the leading cause of shigellosis from food. The microbiological protection of seafood products could thus be achieved by ensuring the absence of these pathogenic microorganisms and by all means preventing their increase in the aquatic habitat (Edema and Omemu, 2004). Therefore, the objective of this paper is to assess the assessment of microbial loads, species characterization and composition in prawn (*Macrobrachium vollenhovenii*) fillets from major wetlands (Nwaniba, Ibaka, Ibeno and Itu) in Akwa Ibom State, Nigeria

MATERIALS AND METHODS

Collection of Experimental Fish: The prawn samples were procured from four major wetlands (Nwaniba, Ibaka, Ibeno and Itu) in Akwa Ibom State, Nigeria. A total of 100 prawn samples were collected (25 samples from each site) and transported to the laboratory of the Department of Microbiology, University of Uyo, for detailed examination and analysis.

Sample Preparation and Microbiological Analysis: The collected samples were prepared separately and made into fine particles using a manual blender. The method of Prescott (2005) was adopted and used for the microbial analysis. A 10g sample was homogenized with 90ml of sterile water and the homogenate was allowed to settle for 10 minutes. This formed the stock solution. Thereafter, 5 ml of the supernatant was transferred to make a 10-fold serial dilution. A1ml of the diluted sample was inoculated using pour plate technique and a 0.5 % Nutrient agar medium was poured at 40°C on the plates. The sample and the medium were then mixed and allowed to set before incubating at 25°C for 48 hours. Colonies were sub-cultured to get pure cultures. These were further screened for the presence of indicator organisms. Microbial assay of prawn fillets was carried out to determine bacterial load, identification and frequency of occurrence using the method described by (Cheesbrough, 2006). Plates with colonies ranging between 50 – 200 were selected for determination of total bacterial count and isolation of individual bacterial groups. Total load of bacteria was estimated thus:

Total load of bacteria (cfu/ml) = C x D x 10 x V/W (Cheesbrough, 2006)

Where: C= Number of colonies found, D= Dilution factor, V= Volume of physiological saline, W=Weight of fish sample.

Characterization of Bacteria/Fungal Identification: The identification of fungal isolates was conducted by cultural and morphological characteristics such as: surface texture, topography and pigmentation according to the methods of Effiong and Isaac (2019). Microscopic identification was done by placing a drop of 5% potassium manganese (kMnO4) on a slide and a small portion of representative fungi mycelium was removed and teased onto the potassium manganese stain using a sterile needle. The slide was covered, mounted and viewed at 40x objective microscope. Photos of identified fungal isolates were taken and compared with a documented book of fungi by St-Germain and Summerbell (2003). Visual observation of morphological characteristics such as shape, size and colour of the bacterial colonies were done. Shape of the individual isolate was determined by Gram staining method with the young culture. The motility test was performed by hanging drop method. Biochemical tests such as catalase activity, oxidase, indole production, gelatin liquefaction and proteinase test were performed using bacterial isolates from fresh culture according to the methods of Effiong and Isaac (2019). The pure fungal isolates were identified using both cultural and morphological characteristic texture (glabrous, powdery, granular, fluffy, downy, cottony,)

surface topography (flat, raised, heaped, folded, domed, radial, grooved) surface pigmentation (white, creamy, yellow, brown, pink, grey, black) reverse pigmentation (yellow, none, brown, red, black). The procedure involved visual examination of isolates in culture (pigmentation, texture, reverse side, colony surface) and observation of stain preparation (nature of conidia, hyphae and spores) under microscope. Fungi plates were incubated at 28^0C for 5 – 7 days. After incubation, the plates were observed and visible colonies counted with the aid of a Quebec colony counter (Cheesbrough, 2006).

Data Analysis: Data collected for microbial counts were subjected to descriptive statistics. Differences in microbial species loads and diversity were analysed using one-way analysis of variance (ANOVA) at 95% probability level using SPSS version 20. The bacterial and fungal isolates were presented in tables.

RESULTS AND DISCUSSION

The results of microbial densities isolated from prawn samples are presented in Table 1. From the results, total hetrotrophic bacterial count (THBC) ranged from 2.70 to 3.10 cfu/g in Nwaniba landing site, 6.90 – 7.60cfu/g in Itu, 2.10 – 2.60cfu/g in Ibeno and 5.00 – 5.70cfu/g in Ibaka. The total heterotrophic fungal count (THFC) ranged from 1.90 – 2.50cfu/g in Nwaniba landing site, 3.00 – 3.50cfu/g in Itu, 1.30 – 1.60cfu/g in Ibeno and 1.70 – 2.00 in Ibaka. Total coliform count (TCC) was observed to range from 1.40 – 1.60cfu/g in Nwaniba landing site, 4.50 – 5.00cfu/g in Itu, 1.20 – 1.50cfu/g in Ibeno and 1.00 – 1.30 in Ibaka. The mean value of TCC from Nwaniba landing site was 1.50cfu/g, 4.80cfu/g in Itu, 1.33cfu/g in Ibeno and 1.13cfu/g in Ibaka. Faecal coliform count (FCC) was observed to range from 0.90 – 1.10cfu/g in Itu landing site and 5.80 – 6.10cfu/g in Ibaka. The mean value of FCC from Itu landing site was 1.00cfu/g while Ibaka was 5.97cfu/g. *Salmonella shigella* count was observed to range from 4.80 – 5.10cfu/g in Ibaka landing site and had a mean of 4.97cfu/g.

The results in Table 2 revealed the biochemical characterization of bacterial isolates. The results showed that all the bacterial isolate exhibited different reaction during biochemical characterization such that, *Bacillus cereus* under gram reaction was positive and had thick rod shape. In catalase and coagulase tests the organism showed positive and negative reactions respectively while under motility and starch hydrolysis tests it showed positive reactions. Citrate utilization test was negative while Urease and Methyl – red tests showed negative reactions. Voges Proskauer and Spore formation tests were positive while hydrogen sulphide production showed negative. Under maltose, fructose, sucrose and galactose tests the organism showed acid reaction while under glucose it showed acid and gas.

The results of microscopic characterization of fungal isolates are presented in Table 3. From the table, it was observed that *Candida tropicalis* had a creamy white colony with a pseudohyphae soma and septate hyphae. Its asexual spore was blastoconidia with a special reproductive structure, conidia. Its conidial head was rachate and its vesicle shape was globose with apotecium special vegetative structure. *Aspergillus niger* possessed a compact white or yellow basal dark colony with a filamentous soma and septate hyphae. Its asexual spore had globose conidia while its special vegetative structure was footcell. It had a smooth walled erect conidophores special reproductive structure, globose conidial head and a globose vesicle shape. *Aspergillus flavus* possessed a dense felt yellow – green colony with a filamentous soma and septate hyphae. It had sub-glubose vesicle shape, radiate conidial head, foot cell special vegetative structure, globosecobidia asexual spores. Its special reproductive structure was phialides borne directly on the vesicle sclerotia. *Mucor mucedo* had sporangiosphore asexual spore, symbolically branched sporangiophore special reproductive structure. It also had coenocytichypae, filamentous soma and creamish yellow colonies. Vesicle shape, conidial head and special vegetative structure are absent.

Table 1: Microbial densities of organisms isolated from prawn samples at 95% confidence interval for Mean.

Microbes	Nwaniba	Itu	Ibeno	Ibaka
THBC $x10^{-3}$cfu/g	2.87 ± 0.12	7.30 ± 0.21	2.37 ± 0.14	5.27 ± 0.22
THFC $x10^{-3}$cfu/g	2.17 ± 0.18	3.23 ± 0.15	1.47 ± 0.90	1.87 ± 0.90
TCC $x10^{-3}$cfu/g	1.50 ± 0.06	4.80 ± 0.15	1.33 ± 0.90	1.13 ± 0.90
FCC $x10^{-3}$cfu/g	-	1.00 ± 0.01	-	5.97 ± 0.90
SSC $x10^{-3}$cfu/g	-	-	-	4.97 ± 0.80
TVC $x10^{-3}$cfu/g	-	-	-	-
TSC $x10^{-3}$cfu/g	8.80 ± 0.25	1.03 ± 0.90	3.97 ± 0.80	7.00 ± 0.20

Where: THBC =Total heterotrophic bacteria count; THFC =Total heterotrophic fungal count; TCC =Total coliform count; FCC =Fecal coliform count; SSC – Salmonella shigella count; TVC – Total Vibrio count; TSC – Total Staphylococcal count; cfu/g – Colony forming unit per gram

Rhizopus species had tall sporangiophores in groups, black brown sporangia special reproductive structure. Its asexual spore was ovoid sporangiosphore and its stolons rhizoids special vegetative structure coenocytic hyphae, filamentous soma and white becoming grayish brown colony colour. Vessicle shape and conidial head were absent. *Aspergillus terreus* had a brownish colour becoming darker with age colony colour, filamentous soma, septate hyphae, footcell special vegetative structure, globose conidia asexual spore, and a long columnar conidial head. It also had hemispherical vesicle shape

Table 2. Biochemical Characterization of Bacterial Isolates

S/N	1	2	3	4	5	6	7	8
Gram reaction	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
Shape	cocci in cluster	rod	cocci in cluster	-ve	-ve	cocci in pairs	thick rod	Rod
CAT	+	+	+	+	+	+	+	+
COA	+	-	-	-	-	-	-	-
MOT	-	+	-	+	+	-	+	+
Starch	-	-	-	-	-	+	+	-
CIT	+	+	+	+	-	+	-	+
Urea	-	-	-	-	-	+	-	-
MR	-	+	-	-	+	+	-	+
VP	+	-	+	+	-	-	+	-
Spore	-	-	-	-	-	-	+	-
H_2S	-	-	-	-	-	-	-	+
HAE	B	-	-	-	-	-	-	-
GLU	A	AG	A	AG	AG	-	AG	A
Mal	A	AG	A	AG	AG	A	A	A
Lact	A	AG	-	AG	-	-	-	-
Fruct	AG	-	A	-	-	A	A	-
Suc	-	-	A	AG	-	-	A	-
Man	AG	A	-	AG	AG	A	-	AG
Gal	A	-	A	-	-	A	A	-
Possible organism	*Staphylococcus aureus*	*Atrobacter freundii*	*Staphylococcus albus*	*Enterobacter aerogenes*	*Escherichi a coli*	*Micrococcus luteus*	*Bacillus cereus*	*Salmonella enterica*

Where: -ve = negative, +ve = positive, CAT = Catalase test, COA = Coagulase test, MOT = Motility test, STARCH = Starch hydrolysis, CIT = Citrate utilization test, UREASE = Urease test, MR = Methyl – red test, VP = Voges Proskauer test, H_2S = Hydrogen sulphide production, GLU = Glucose, MAL = Maltose, LACT= Lactose, FRUCT = Fructose, SUC = Sucrose, MAN = Mannitol and GAL = Galactose.

Table 3. Macroscopic and Microscopic Characteristics of Fungal Isolates

Probable Organism	*Candida tropicalis*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus terreus*	*Mucor mucedo*	*Rhizopus* species
Vesicle shape	Globose	Globose	Subglobose	Hemispherical	-	-
Conidial head	Rachate	Globose	Radiate	Long columnar	-	-
Special reproductive structure	Conidia	Smooth walled erect conidiophores	Phialides borne directly on the vesicle, Sclerotia	Short conidiophores	Sympodically branched sporangiophore	Tall sporangiophores in groups, black brown sporangia
Asexual spore	Blastoconidia	Globose conidia	Globose conidia	Globose conidia	Sporangiospore	Ovoid sporangiosphores
Special vegetative structure	Apothecium	Footcell	Footcell	Footcell	-	Stolons rhizoids
Nature of hyphae	Septate	Septate	Septate	Septate	Coenocytic	Coenocytic
Type of Soma	Pseudohyphae	Filamentous	Filamentous	Filamentous	Filamentous	Filamentous
Colony colour	Creamy white colony	Compact white or yellow basal dark colony	Dense felt yellow – green colony	Brownish colony becoming darker with age	Creamish yellow colonies	White becoming greyish brown

The results of the occurrence of bacteria isolates are presented in Table 4. From the table, *Staphylococcus aureus* was present in Itu, Ibeno, Ibaka landing sites and absent in Nwaniba. Its percentage of occurrence was 75. *Enterobacter aerogenes* and *Bacillus cereus* were present in Nwaniba, Itu and Ibaka landing sites but was absent in Ibeno with percentage of occurrence of 75%. *Staphylococcus albus* was present in Itu and Ibeno landing sites and was absent in Nwaniba and Ibaka landing site. Its percentage of occurrence was 50. *Escherichia coli* was present in Itu and Ibaka landing sites and absent in Nwaniba and Ibeno. It had a 50% of occurrence. *Micrococcus luteus* and *Atrobacter freundii* were present in all the landing sites and had a 100% of occurrences. *Salmonella enterica* was present in Ibaka landing site and was absent in Nwaniba, Itu and Ibeno, with percentage of occurrence of 25.

The results of the occurrence of fungi isolates in prawn samples are presented in Table 5. From the table, *Candida tropicalis* was present in Nwaniba, Itu, Ibeno

and Ibaka landing sites. It had a percentage of 100. *Aspergillus niger* was present in Itu and Ibaka landing sites and was absent in Nwaniba and Ibeno landing site. It had 50% occurrence. *Aspergillus flavus* occurred in Nwaniba, Ibeno and Ibaka landing sites and was absent in Itu, with a 75% occurrence. *Mucor mucedo* was present in Nwaniba and Itu landing sites and absent in Ibeno and Ibaka. It had a percentage of occurrences of 50. *Rhizopus* sp. was present in Itu, Ibeno and Ibaka landing sites and was absent in Nwaniba. It had a percentage occurrence of 75. *Aspergillus terreus* was present in Nwaniba and Itu landing sites and absent in Ibeno and Ibaka, with a percentage of occurrence of 50.

Table 4. Percentage frequency of Occurrence of Bacterial Isolates in Prawn Samples

Bacteria isolates	Nwaniba	Itu	Ibeno	Ibaka	% prevalence of occurrence of organisms
Staphylococcus aureus	-	+	+	+	75
Enterobacter aerogenes	+	+	-	+	75
Bacillus cereus	+	+	-	+	75
Staphylococcus albus	-	+	+	-	50
Escherichia coli	-	+	-	+	50
Micrococcus luteus	+	+	+	+	100
Atrobacter freundi	+	+	+	+	100
Salmonella enteric	-	-	-	+	25

Table 5. Percentage Frequency of Occurrence of Fungal Isolates in Prawn Samples

Fungal isolates	Nwaniba	Itu	Ibeno	Ibaka	% prevalence of occurrence of the organisms
Candida tropicalis	+	+	+	+	100
Aspergillus niger	-	+	-	+	50
Aspergillus flavus	+	-	+	+	75
Mucor mucedo.	+	+	-	-	50
Rhizopus sp.	-	+	+	+	75
Aspergillus terreus	+	+	-	-	50

The African river prawn had been identified to be highly appreciated and among the leading priced seafood. Its consumption had been highly recommended because of its essential protein source, with high levels of unsaturated fatty acids (Disegha and Onuegbu, 2018). The results of the present study revealed that microorganisms present in prawns include both bacteria and fungi species including: The microbial organisms isolated from the samples were *Staphylococcus aureus, Enterobacter aerogenes, Bacillus cereus, Staphylococcus albus, Escherichia coli, Micrococcus* sp., *Atrobacter* sp., *Salmonella* sp., *Candida tropicalis, Aspergillus niger, Aspergillus flavus, Mucor mucedo, Rhizopus* sp., and *Aspergillus terreus.* Reports had shown that infection rates of these microbial agent on organisms are a reflection of the general infestation from the habitats, inappropriate processing, preservation and storage condition (FDA, 2007; Ella *et al.,* 2013; Bassey and Effiong, 2016; Effiong and Isaac, 2019; Effiong and Obot, 2020; Bubu-Davies *et al.,* 2023). The reports of the present study agreed with the work of Srinivasan *et al.* (2015) who recovered similar organisms from the gut of farmed giant freshwater prawn, *Macrobrachium rosenbergii*. The organism, *E. coli* had been reported to be an indicator of health risk from water contact with seafood (EPA, 2012; Effiong and Isaac, 2019; Effiong and Christopher, 2020). Its evidence could be as a result of faecal samples deposited into the water bodies. In a related study, Adelaja *et al.* (2013) reported that *E. coli* could cause diarrhea and kidney damage if consumed in infected fish products that is not properly processed and this could also pose a major treat of urinary infection in complicated community.

The presence of *Bacillus cereus, Micrococcus and Staphylococcus* observed in the prawn samples was consistent with other studies (Effiong and Isaac, 2019; Effiong and Obot, 2020). It could be suggested that the presence of these organisms in prawn samples from these wetlands may be as a result of poor sanitary condition and improper handling of the samples by the fisher folks at the landing sites. Thus, leading to the contamination of the products and eventual health risk to the final consumers. The presence of *Atrobacter freundii* and *Enterobacter aerogenes* isolated from the prawn samples in the present study were also reported in selected brands of some herbal products (Ella *et al.,* 2013) vended in Nigeria. The fungi species, *Aspergillus flavus* and *A. niger* observed in the studied prawn samples are of great health interest due to their mycotoxigenic potentials. *A. flavus* had been reported to have the ability to produce aflatoxins, which could deteriorate the liver and kidney in man resulting to death (Bassey and Effiong, 2016; Esenowo *et al.,* 2023). The *Aspergillus* species had been reported to

possess rapid growth in a freezing temperature and may subsequently develop in the stored food product (Mounir *et al.*, 2011). The species *Rhizopus* and *Mucour* isolated from prawn samples are also of adverse effect to human health. The various microbial isolates recorded in the present study were also identified in smoked *Trachurus trachurus* and *Scomber scombrus* by Agbabiaka and Agu (2019) and Effiong and Christopher (2020) respectively. These organisms had been reported to be food borne pathogens and had been implicated in health issues arising from consumption of uncooked or poorly processed seafood (Amusan *et al.*, 2010). The high occurrence of *E. coli* in Ibaka landing site could be as a result of high deposit of faecal sample in the water body, since *E. coli* is a faecal coliform bacteria. Furthermore, consumption of raw or under cooked seafood is a major risk because the products might habour these pathogens and this risk could be further increased by mishandling of food during processing as stated by Food and Environmental hygiene Department (FEHD, 2005). Thus, seafood products should be properly handled and processed to reduce the pathogen load to the barest minimum.

Conclusion: The consumption of bacteria and fungi infested prawns could be of great danger to the human health if not properly processed and cooked. The polluted habitat and poor handling of prawn products could majorly increase microbial density, and this could pose serious threat to the lives of consumers. It is advisable that proper handling and storage methods be employed in other to reduce the microbial density in prawns. Based on the findings of this study, the following recommendations are made to educate the public on the potential health implications associated with fungi and bacteria contaminated prawns. Proper handling and suitable disposal of sewage should be carried out and maintained to avoid microbial contamination of harvest sites by pathogenic bacteria. Educating all health professionals, food handlers and consumers concerning the microbiological risks involved in the consumption of raw or undercooked prawn. Fresh prawn prior to consumption should be careful washed thoroughly before cooking. Physician should be contacted in case of food poisoning.

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