MICROBIAL ASSEMBLAGE OF THE ANATOMICAL PARTS OF GERCACINID CRAB FROM A TROPICAL MANGROVE SWAMP

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ABSTRACT

The microbial assemblage and occurrence in the gut, flesh and shell of Cardisoma armatum, from the tropical mangrove swamp of Lagos Lagoon, Nigeria, was analysed using standard microbiological techniques. The crab gut had the highest total heterotrophic bacteria count and total coliform count with respective significant ($p<0.05$) values of 6.90 ± 0.16 x10^{4}CFU g^{-1} and 3.45 ± 0.12 x10^{2}CFU g^{-1}. Total feacal coliform of the crabs were 0.05 ± 0.00 x10^{2}CFU g^{-1}, 0.01 ± 0.00 x10^{2}Cfu g^{-1} and 0.01 ± 0.00 x10^{2}CFU g^{-1} in gut, flesh and shell, respectively. Highest total fungal count of 7.50 ± 0.04 x10^{3}CFU g^{-1} was recorded in the crab shell. The bacteria and fungi species isolated and their frequencies of occurrences in percentage were: Bacillus sp. (21.4 %), Citrobacter sp. (3.6 %) Enterobacter sp. (7.1 %), Escherichia coli (10.7 %), Klesiellia pneumonia (17.9 %), Providencia sp. (3.6 %), Serratia sp. (10.7 %), Staphylococcus aureus (10.7 %), S. epidermis (7.1 %), Vibrio sp. (7.1 %), Aspergillus flavus (8.6 %), Aspergillus fumigatu (5.7 %), Aspergillus niger (40.0 %), Fusarium sp. (14.3 %), Penicillium sp. (5.7 %) and Saccharomyces sp. (25.7 %). The isolation rates in the different anatomical sites of the crab follow the order: guts > flesh > shell and shell > flesh > guts for bacterial and fungi infestation respectively. The results of this study showed that the gercacinid crab, C. armatum in the tropical mangrove of Lagos harbours microorganisms including those that are pathogenic.

Keywords: Crab, Coliform, Fungi, Mangrove swamp, Microbes, Pathogen

INTRODUCTION

Food safety has recently become a catchphrase, where crustaceans have been implicated in food poisoning, cholera, salmonellosis, shigellosis and yersinia food infection (Uaboi-Egbenni *et al*., 2010). Survey on the microbiological quality of crustaceans such as crab shows that they harbor pathogenic organisms. This is because the water bodies from which the crabs are harvested are heavily polluted. The ability of crab to concentrate pollutants and potential human pathogens has led to the establishment of test procedures and standards aimed at assuring the safety of crab as a human food source (Givens *et al*., 2013). Indicator organisms, most frequently coliforms, have been used to determine the likelihood of contamination of crabs with fecal matter and hence with human pathogens (Ding *et al*., 2017; Parlapani *et al*., 2019). Failure to detect evidence of *Salmonella* and *Shigella* spp. is generally regarded as an indication of the safety of the crab with regard to pathogenic bacteria (Givens *et al*., 2013).

Crabs are usually infected with a wide range of microbes in aquatic ecosystems. The types of micro-organisms found associated with

crab depend on its habitats and it has long been recognized that aquatic microorganisms in general, have a strong affinity for surfaces (Dvoretsky, 2012). According to Kambiré *et al*. (2016) the main microbiological indicators consist of bacteria of the genera *Escherichia, Salmonella* and *Clostridium*, main pathogens such *Vibrio* and *Clostridium,* and secondary pathogens such as *Campylobacter*, *Staphylococcus* and *Aeromonas*, fungi and viruses. Fungi and their spores are ubiquitous in the environments. Some genera, such as *Aspergillus* spp., have been found to elaborate hazardous mycotoxins that are mutagenic, teratogenic, hepatotoxic, immunotoxic and nephrotoxic (Żukiewicz-Sobczak, 2015). Pathogenic microbes have been identified from different parts of various crustaceans and factors causing damage to crustaceans were also analyzed. Occurrence of pathogenic bacteria in gills, flesh and shell cavity of mud crab *Scylla serrate* from Malaysia was also reported (Najiah *et al*., 2010).

The mangrove land crab, *Cardisoma armatum* Herklots, 1851 (Decapoda: Gecarcinidae) is one of the most economically and ecologically valuable species in Nigeria (Lawal-Are *et al*., 2019). To understand the roles of microbial flora of the land crab, the first step to be taken is to investigate the composition of microbial groups in the anatomical parts of the host. Generally, the assessment of normal flora of some crabs have been studied in an attempt to define the bacterial group associated with the crabs so as to determine its microbiological quality for consumption (Uaboi-Egbenni *et al*., 2010; Akpaniteaku *et al*., 2019), nevertheless, dearth of information still exists on the microbiological assemblage of gecacinid crabs from mangrove swamps of Nigeria. Meanwhile, the microbiological quality is of importance to public health since it directly relate to crab spoilage and may cause food poisoning. It is therefore important to monitor the quality of harvested crabs to ensure that the crab products do not pose health risks to end users. Hence, this research therefore seeks to determine the bacteria and fungi assemblage associated to the gut, flesh and shell of *C. armatum* inhabiting the University of Lagos (Unilag) mangrove swamp in Nigeria.

MATERIALS AND METHODS

Study Area and Sample Collection: The sampled site, Unilag mangrove swamp lines the Abule-Eledu Creek, one of the several adjoining creeks of the Lagos Lagoon with latitude 6^{o} 26' - 6^{o} 37'N and longitude 3^{o} 23' - 4^{o} 20'E (Figure 1). It is a typical estuarine water zone with extensive mangrove but low transparency and alkaline, with a pH above 7 (Moruf and Lawal-Are, 2015). A total of 68 crabs were obtained using a hand-net during the hours of 18.00 and 21.00 on a weekly basis from October to December, 2021. The crabs were aseptically transported in ice chest box to the Ecotoxicological Laboratory located at the Department of Marine Sciences, University of Lagos for further processing and analysis.

Sample Preparation: Samples of *C. armatum* were measured with the aid of Sartorius top loading balance (Model 1106) to the nearest tenth of a gram. Specimens with weight range of 68.50 – 90.70 g were used for the study. The specimens were sacrificed and the incidental materials adhering to the shells were removed by washing the crab aseptically with sterile distilled water before opening the ventral surface with sterile scissors to expose the carapace, gut and flesh. Five (5) grams of each specimen were mixed with 225 mL of sterile 0.1 % peptone water in sterile beaker and thoroughly homogenized under aseptic conditions. Thereafter, the homogenized samples were serially diluted to 10^{6} as described by APHA (2005).

Enumeration and Isolation of Heterotrophic Bacteria, Fungi and Coliforms: Standard pour plate technique described by Collins *et al.* (2004) and Dubey and Maheshwari (2014) was employed for the analysis of crabs for total heterotrophic bacteria, coliforms, fecal coliforms and fungi in colony forming unit per gram (CFU g^{-1}). The prepared samples were analyzed immediately.

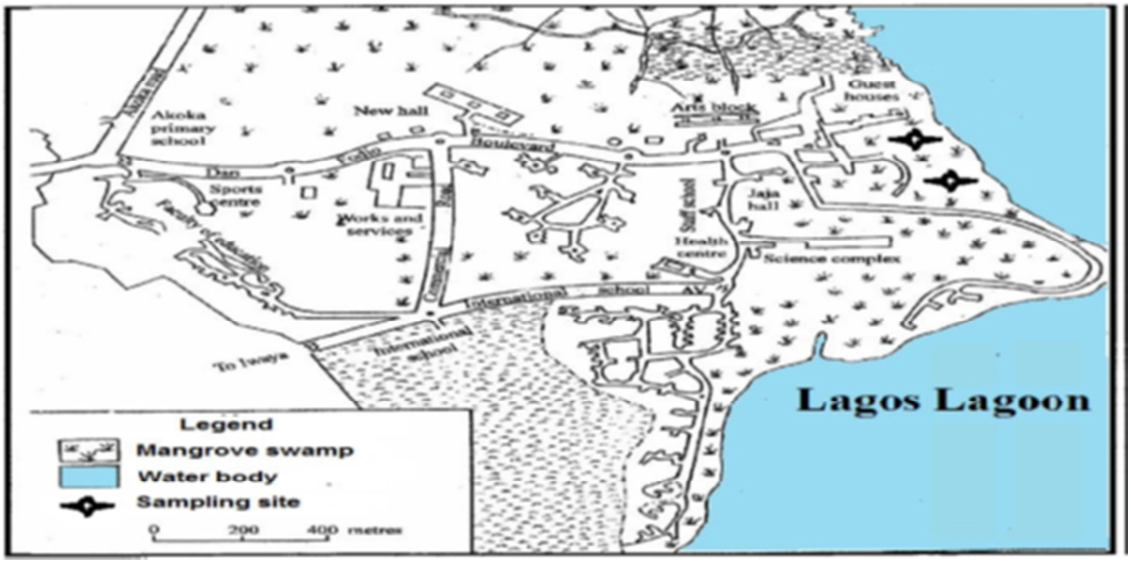

Figure 1: Map of University of Lagos Lagoon Front showing the mangrove swamp (Lawal-Are *et al.*, 2019)

One (1) gram of each of the sample were taken and diluted serially in 9 ml of sterile distilled water into five folds (10^{-1} to 10^{-5}). One hundred microliter (100 µl) of two different dilutions was inoculated into sterile Petri dishes in duplicates with the aid of micropipette fitted with sterile tips. Sterile molten nutrient agar, eosine methylene blue agar and potato dextrose agar (supplemented with 1 mg per ml of chloramphenicol to inhibit bacterial contaminants) were poured into the inoculated plates. They were swirled to ensure even distribution of the inoculum and left to solidify. The inoculated plates were then incubated aerobically at 37^{o}C for 24 – 48 hours (bacteria) and 28 ± 2^{o}C for 5 days (fungi). The developed colonies were counted in duplicates using colony counter. Average colonies of the dilutions that met up with the standard pour plate technique of 30 – 300 colonies were taken and multiplied by the corresponding dilution factor to give the total number of bacteria, coliforms, fecal coliforms and fungi population per gram of the analyzed samples.

Characterization of Isolates: Gram's staining and motility test were done following the method of Harrigan and McCance (1976). The method used for the determination of fecal contamination indicators is the method of fermentation in multiple tubes which is based on the seeding of a series of three tubes containing liquid media, then the determination of the number as described by Hamiroune *et al.* (2020). Biochemical tests were done according to methods described in Collins *et al.* (2004). Further identification of bacterial isolates into species was done according to the methods described in Bergey's Manual of Systemic Bacteriology (Krieg and Holt, 1984).

Statistical Analysis: Data were analyzed using Microsoft Excel (2010). Significant difference was set at $p<0.05$. Results are presented as means ± standard error (SE). Duncan Multiple Range Test (DMRT) was used to sort out the differences in the means.

RESULTS AND DISCUSSION

Microbial Load in the Anatomical Parts of Crab: Microbial load in the anatomical parts of *C. armatum* from tropical mangrove swamp is shown in Table 1. The crab gut had the highest total heterotrophic bacteria count (THBC) and total coliform count (TCC) with respective significant values ($p<0.05$) of 6.90 ± 0.16 x10^{4}CFU g^{-1} and 3.45 ± 0.12 x10^{2}CFU g^{-1}. Total feacal coliform (TFC) was obtained as 0.05 ± 0.00 x10^{2}CFU g^{-1}, 0.01 ± 0.00 x10^{2}Cfu g^{-1} and 0.01 ± 0.00 x10^{2}CFU g^{-1} in gut, flesh and shell of crabs, respectively.

Table 1: Microbial load (CFU g^{-1}) in *Cardisoma armatum* from tropical mangrove swamp

Indicators	Gut	Flesh	Shell
Total heterotrophic bacteria count ($x10^4$)	6.90 ± 0.16^b	2.72 ± 0.12^a	2.00 ± 0.21^a
Total coliform count ($x10^2$)	3.45 ± 0.12^b	1.84 ± 0.06^a	1.45 ± 0.21^a
Total feacal coliform ($x10^2$)	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Total fungal count ($x10^3$)	2.00 ± 0.06^a	7.30 ± 0.61^b	7.50 ± 0.04^b

$^{a, b}$ means with different superscript letter within a row are statistically different ($p<0.05$)

The higher bacterial counts in the crab gut samples may be attributable to natural feed such as trash fish, molluscs and farm waste that facilitate the entry of microbial pathogen into the digestive tract. This finding suggests that the crab gut is colonized by its own intestinal bacterial community. Chen *et al.* (2015) reported the colonization of bacterial community in the intestinal tract of Chinese mitten crab (*Eriocheir sinensis*) in Lake Tai, China. This observation in line with the findings of Zhang *et al*. (2016) who reported high population of bacteria in the gut of the crab *E. sinensis* when compared to the population of bacteria in its gills. Givens *et al.* (2013) and Sivasubramanian *et al*. (2017) also reported high bacterial loads in crab gut. Although, the total bacteria count of crab rarely indicate the quality of the crab, it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health conditions of consumers of such contaminated crab (Geetha *et al*., 2016).

In the present study, highest total fungal count (TFC) of 7.50 ± 0.04 $x10^3$CFU g^{-1} was recorded in the crab shell. The shell and flesh portrayed a similar trend in TFC, being significantly higher ($p<0.05$) than the 2.00 ± 0.06 $x10^3$CFU g^{-1} recorded in the crab gut. The level of mean TFC reported in this study was however lower than the 9.36 ± 2.20 $x10^3$CFU g^{-1} reported for *Galatea paradoxa* from Cross River (Udoh, *et al*., 2017). Furthermore, the total plate counts for both bacteria and fungi did not exceed the range of specified microbiological limits recommended for fish and fishery products by International Commission on Microbiological Specification for Foods (ICMSF). According to ICMSF (1998), microbial counts of crab meat below 10^5 CFU g^{-1} are considered good quality and counts between 10^5 and 10^6 CFU g^{-1} are considered marginally acceptable quality.

Cultural and Morphological Characterization of Fungi isolated from the Anatomical Parts of Crab: The cultural and morphological characterization of fungi isolated from the anatomical parts of *C. amatum* is shown in Table 2. The predominant fungi species isolated were *Aspergillus flavus, A. fumigatus, A. niger, Fusarium* sp*., Penicillium* sp*.* and *Saccharomyces* sp*.* The crab flesh had the highest diverse fungi isolates with *Aspergillus niger, A. flavus, A. fumigatus, Saccharomyces* sp*.* and *Penicillium* sp*.* identified. These fungi were similar to the genera reported for a marine crab (*Xenograpsus testudinatus*): *Aspergillus penicillioides, Aspergillus versicolor, Penicillium citrinum* and *Penicillium paxili* (Shaumi *et al*., 2021). The majority of these fungi belong to the Phylum: Ascomycota, with *Aspergillus* and *Penicillium* being the most speciose genera, and these are also two of the most speciose genera in the marine environment (Jones *et al*., 2015). *Aspergillus* spp. appears to be very common in all the anatomical parts of *C. armatum.* This corroborates the work of Xu *et al*. (2021) on the detection of *Aspergillus* spp. in the anatomical parts of Chinese mitten crab.

Morphological and Biochemical Characterization of Bacteria isolated from the Anatomical Parts of Crab: Eight genera consisting both gram-positive and gram-negative bacteria were isolated from the anatomical parts of *C. amatum* (Table 3). The isolates were identified as *Bacillus* sp*., Citrobacter* sp*., Enterobacter* sp*., Escherichia coli, Klesiellia pneumonia, Providencia* sp*., Serratia* sp*., Staphylococcus aureus, S. epidermis* and *Vibrio* sp. using their morphological and biochemical characteristics (catalase, oxidase, citrate, indole, mannitol, motility, spore and shape).

Table 2: Cultural and morphological characterization of fungi isolated from *Cardisoma armatum* inhabiting the University of Lagos mangrove swamp

Sample	Cultural character	Cellular Morphology	Probable identity
Gut	Filamentous mold with dark-pigment	Septate hyphae, conidiophore is enlarged at the tip forming vesicle and spores enclosed	*Aspergillus niger*
	Cream, raised, soft colonies with alcohol odour	Oval shaped with some budded	*Saccharomyces* sp.
Flesh	Filamentous mold with dark-pigment, gray-yellow and blue-green pigment	Septate hyphae, conidiophore is enlarged at the tip forming vesicle and spores enclosed	*Aspergillus niger, A. flavus, A. fumigatus*
	Cream raised, soft colonies with alcohol	Oval shaped, some budded	*Saccharomyces* sp.
	Filamentous mold with whitish colony	Fusiform, sickle-shaped and elongated	*Penicillium* sp.
Shell	Filamentous mold with dark pigment and white plane colony	Septate hyphae with sporangium filled spores	*Aspergillus niger, Fusarium* sp.
	Cream raised, soft colonies with alcohol odour	Oval shaped, some budded	*Saccharomyces* sp.

Table 3: Morphological and biochemical characterization of bacteria isolated from *Cardisoma amatum* collected from the University of Lagos mangrove swamp

Sample	Gram reaction	Catalase	Oxidase	Citrate	Indole	Mannitol	Motility	Spore	Shape	Probable organisms
Gut	-ve	+	+	-	+	-	+	+	Rod	*Bacillus* sp.
	-ve	+	-	+	-	+	-	-	Rod	*Klesiella pneumonia*
	-ve	+	-	-	+	+	+	-	Rod	*Escherichia coli*
	+ve	+	+	+	-	+	-	-	Cocci	*Staphylococcus aureus*
	-ve	+	_	+	-	+	+	-	Rod	*Enterobacter* sp.
	-ve	+	+	+	+	+	+	-	Rod	*Vibrio* sp.
	-ve	+	-	+	-	+	-	-	Rod	*Citrobacter* sp.
Shell	+ve	+	+	+	-	+	-	-	Cocci	*S. aureus*
	+ve	+	+	-	-	-		-	Cocci	*S. epidermis*
	-ve	+	+	-	+	-	+	+	Rod	*Bacillus* sp.
	-ve	+	-	-	+	+	+	-	Rod	*E. coli*
	-ve	+	-	+	-	-	-	-	Rod	*Klesiellia pneumonia*
	-ve	+	-	+	-	+	+	-	Rod	*Serratia* sp.
Flesh	-ve	+	+	-	+	+	+	-	Rod	*Vibrio* sp.
	-ve	+	-	+	-	+	+	-	Rod	*Serratia* sp.
	-ve	+	-	-	+	+	+	-	Rod	*E. coli*
	-ve	+	+	-	+	-	+	+	Rod	*Bacillus* sp.
	-ve	+	-	+	+	+	+	-	Rod	*Providencia* sp.
	+ve	+	-	+		+	-	-	Cocci	*S. aureus*
	-ve	+	-	+	-	-	-	-	Rod	*Klesiellia pneumonia*

- *= absent, + = present, -ve = negative, +ve = positive*

Table 4: Percentage occurrence of isolates from different anatomical parts of *Cardisoma armatum* inhabiting the University of Lagos mangrove swamp

Isolates	Crab part			Total (%)
Bacteria	Gut	Flesh	Shell	
***Bacillus* sp.**	3	2	1	6(21.4)
***Citrobacter* sp.**	1	0	0	1(3.6)
***Enterobacter* sp.**	1	1	0	2(7.1)
Escherichia coli	2	0	1	3(10.7)
Klesiellia pneumonia	2	2	1	5(17.9)
***Providencia* sp.**	0	0	1	1(3.6)
***Serratia* sp.**	1	1	1	3(10.7)
Staphylococcus aureus	1	1	1	3(10.7)
S. epidermis	0	2	0	2(7.1)
***Vibrio* sp.**	1	0	1	2(7.1)
Total	12	9	7	28(100.0)
Fungi				
Aspergillus flavus	0	3	0	3(8.6)
Aspergillus fumigatus	0	2	0	2(5.7)
Aspergillus niger	2	4	8	14(40.0)
***Fusarium* sp.**	0	0	5	5(14.3)
***Penicillium* sp.**	0	2	0	2(5.7)
***Saccharomyces* sp.**	1	3	5	9(25.7)
Total	3	14	18	35(100.0)

Bacillus sp. and *Klesiella pneumonia* occurred across the three crab anatomical parts. These species are implicated in causing a wide range of infectious diseases including abscesses, food borne infections, ear infections, respiratory and urinary infections (Afolabi *et al.*, 2020) and also some of these isolates are potential spoilage organisms of unprocessed crab.

In the present study, the detection of coliforms of feacal origin and *E. coli* gives relevant information regarding the food safety and sanitary conditions of crabs and the mangrove swamp respectively. Therefore, the presence of *E. coli* may be due to the presence of faecal pollution caused by human and other environmental wastes in the water bodies from which the *C. armatum* was obtained, similar observation was also made by Moruf (2022). *Vibrios* spp. are predominant in high salinity sites whereas *Pseudomonas* and *Aeromonas* spp. were common in low salinity sites (Joseph and Ravichandran, 2012).

Occurrence of Isolates in the Anatomical Parts of Crab: The percentage occurrence of isolates from the sample is presented in Table 4. In the bacterial group, *Bacilus* sp. had the highest frequency of occurrence (21.60 %), while *Citrobacter* sp. and *Providencia* sp. occurred the least (3.40 %). Bacterial specimens in guts of the crabs had the highest number of isolates (12), followed by the crab flesh (9) and lastly the shell (7). Among the fungal isolates were species of *Aspergillus*, a taxon including ubiquitous saprotrophic fungi that play an important role in recycling carbon and nitrogen on Earth (Fang and Latge, 2018) was found to have the highest relative abundance (54.30 %) among the fungal genera recorded in *C. armatum*. The dominant *Aspergillus* species was *A. niger* (40.00 %), which was isolated from all the anatomical parts of the crab, while *A. flavus* (8.60 %) and *A. fumigatus* (5.70 %) were only isolated from the crab flesh. Previous studies have demonstrated that *Aspergillus* species are essential components of crab fungal communities. For instance, *Aspergillus* has been described as the dominant genus retrieved from the crushed dilutions of the vent crab *X. testudinatus* (Pang *et al.,* 2019).

Conclusion: The results of this study showed that the gercacinid crab, *C. armatum* in the tropical mangrove of Lagos Lagoon harbours microorganisms including those that are pathogenic. These results contribute to filling the gap in our knowledge of microorganisms in the studied crab species and further research to study the function(s) of the dominant bacteria and fungi in each anatomical part may further provide understanding of the relationship between the symbiotic microbes and the host.

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