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Isolation of gut associated bacteria from *Callinectes amnicola* (Crustacea: Portunidae) collected from two interconnecting tropical lagoons in Nigeria

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Abstract

The occurrence and relative distribution of bacterial flora associated with the gut of the crab *Callinectes amnicola*, as well as sediment and water, from Badagry and Epe Lagoons, were investigated. The water samples from the Epe Lagoon had the highest total bacteria count of 7.5±1.2 x 10^7 cfug-^{1}, whereas the crab gut from the Badagry Lagoon had the lowest total bacteria count of 3.6±0.5 x 10^7 cfug-^{1}. The analyzed plate counts from the gut of *C. amnicola*, included the total heterotrophic bacteria, total coliform and total faecal coliform which were greater in the Epe Lagoon than in the Badagry Lagoon although these differences were not significant ($p>0.05$). The values recorded for the Epe Laggon were 4.5±0.2 x 10^7 cfug-^{1}, 3.4±0.1 x 10^5 cfug-^{1} and 1.5±0.3 x 10^5 cfug-^{1} for total heterotrophic bacteria, total coliform and total faecal coliform respectively. All the nine (9) identified isolates, with the exception of *Streptococcus sp*, were largely gram-negative bacteria, and included *Escherichia coli*, *Salmonella typhi*, *Shigella sp*., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus sp.*, *Aeromonas hydrophila* and *Vibrio cholera*. Except for *Shigella sp.*, which was absent in the Badagry Lagoon during the study period, the isolates found in this study were comparable in both locations. The most common isolates in the gut of *C. amnicola* obtained from the two interconnected lagoons were *A. hydrophila* and *E. coli*. This study is useful for authorities to develop coastal contamination monitoring strategies and quantify the human health risks connected with the consumption of aquatic crabs.

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1. Introduction

Coastal lagoons serve as nursery and feeding areas for many continental and marine species. In tropical lagoons, *Callinectes species* predominate, accounting for the majority of landed crabs. The Lagoon Crab (*Callinectes amnicola*), belonging to the Portunidae is a decapod crustacean of high commercial value in Nigeria (Moruf & Lawal-Are, 2017a). In human and animal diets, the species is commonly considered as a valuable source of protein and minerals (Chindah et al., 2000; Moruf *et al.*, 2019), as well as the most important food organism collected in West African coastal (inshore) fisheries and lagoons (Lawal-Are & Kusemiju, 2000; Moruf & Adekoya, 2020).

Food safety has recently become a catchphrase, thanks to rising consumer knowledge of ill effects of consuming substandard food and the role of independent media as ombudsmen (Geetha *et al.*, 2016). Crabs and shrimps have been implicated in *Vibrio parahaemolyticus* food poisoning, cholera, salmonellosis, shigellosis and Yersinia food infection (Uaboi-Egbenni *et al*., 2010). Furthermore, bacterial diseases are the second major cause of mortality in both wild and cultured crabs, while the major cause being viral infections. The actual role of these microorganisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other diseases

process (Akintola *et al.*, 2009). Opportunistic pathogens are organisms that usually do not cause disease unless the host's immune system is compromised.

It has been shown by research that the microfauna of aquatic animals is highly variable and reflects the individual animal's surroundings, particularly the food they eat while a majority of these bacterial flora are carried in the gastrointestinal tract (Akintola *et al.*, 2009). Previous work on *C. amnicola* focused on its distribution (Abowei & George, 2010), diet ecology (Moruf & Lawal-Are, 2017b) and reproductive biology (Lawal-Are, 2010). The significance of pathogenic bacteria in the *Callinectes sp* from the Lagos Lagoon has been investigated (Uaboi-Egbenni *et al*., 2010), but little is known about *C. amnicola* gut bacterial flora in the country. Therefore, this work would provide baseline data on the occurrence and distribution of gastro-intestinal bacteria in wild stocks of *C. amnicola* from two important lagoon systems namely the Badagry and Epe Lagoons, in the Southwestern part of Nigeria.

2. Materials and Methods

2.1 Study site

The two study sites, the Badagry Lagoon (6º25′ 415″ N and 2º60′ 43″ E) and the Epe Lagoon (6° 29′ 6.38″ N and 3° 35′ 4° 40″E) are located within Lagos State, South-West Nigeria (Figure 1). The two lagoons are part of a continuous system of lagoons and creeks along the coast of Nigeria. Six sampling stations were selected in each lagoon. Site selection was based on increasing anthropogenic effects, particularly from the discharge of domestic and solid waste.

2.2. Collection of Samples

The sampling was conducted between May and October 2019, where similar numbers of samples were collected from each station. A total of 120 fresh samples of *C. amnicola* collected live were transported to the laboratory and weighed sartorius top loading balance (Model 1106) to the nearest tenth of a gram. Specimens in the weight range of 178 – 226 g and 116 - 204 g, from Badagry Lagoon and Epe Lagoon respectively, were used for the study. The surface water temperature was measured by dipping the glass (bulb) of the thermometer (HT09 in Coimbatore, Tamil Nadu) into the water, 15 cm below the water surface and allowed to stand for 2 minutes before taking the reading between the hours of 16-8 am. The pH was measured in the field using an Oakton pH Meter (Model 35624-00, Cerritos, California). Six representative samples of bottom sediment (to a depth of 2 cm) were collected from each station using a Veen Grab (wt. 25 kg; height—20 cm, m) following the procedure of dos Santos *et al*. (2000).

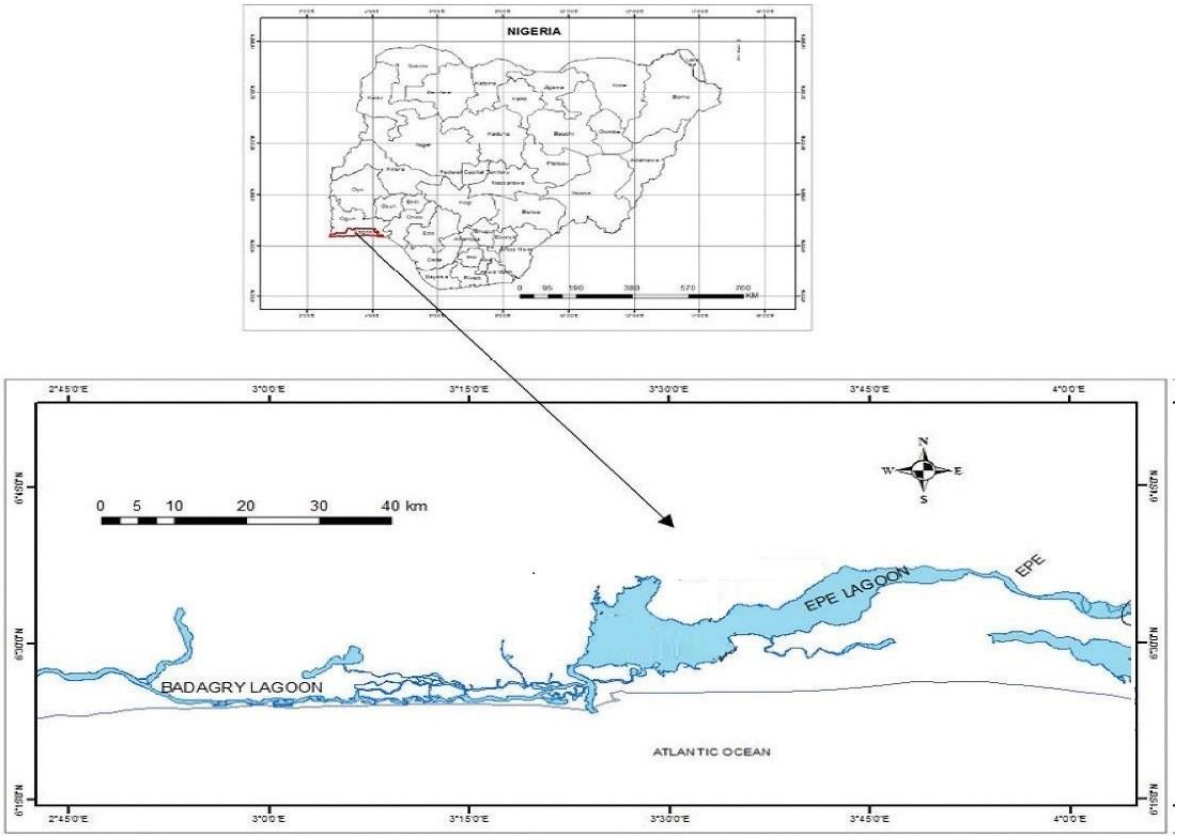

Fig 1. Map of Badagry and Epe Lagoons (adapted from Akinwunmi & Moruf, 2021)

2.3. Preparation of Samples

Twenty randomly chosen specimens from each lagoon were sacrificed and the number of incidental organisms was removed by washing their skin with 70% ethanol before opening the ventral surface with sterile scissors to expose the gut. Five grams of each crab gut sample was mixed with 225 mL of sterile (0.1%) peptone water in a sterile beaker and thoroughly homogenized under aseptic conditions. Thereafter, the homogenized samples were serially diluted to 10^6 as described by the American Public Health Association (2001).

2.4. Isolation and Characterization of Isolates

Gram's staining procedure and the method for ascertaining motility followed Harrigan & McCance (1976). The method for determining fecal contamination indicators is the fermentation in multiple tubes method, which is based on seeding a series of three tubes with liquid media, then determining the number of positive tubes by referring to table of three probability tubes set out in Mc Grady (Hamiroune *et al.,* 2020). The most probable number technique was employed in the microbial examination of water and sediment with different agar to identify each target organism using the pour plate method (Saxena *et al.,* 2015).

Biochemical tests were done according to tests and descriptions of Collins & Lyne (1984). Further identification of bacterial isolates into species was done according to tests and descriptions in the Bergey's manual of Systemic bacteriology (Krieg & Holt, 1984).

2.5. Statistical Analysis

Data were analyzed using the descriptive statistic SPSS (version 20). Differences in means were tested using t-test with the significance level being set at $P<0.05$.

3. Results

The water quality condition of the crab collection sites are given in Table 1. The highest mean temperature of 31.8±2.8 °C (range = 29-34.6 °C) was recorded in the Badagry Lagoon in May while the lowest mean temperature of 23.1±1.3 °C (range= 21.8-24.4 °C) was recorded in the Epe Lagoon in September. Similarly, the highest pH mean value of 7.9±0.5 (7.9-8.9) was recorded in the Badagry Lagoon in October while the lowest mean value of 6.1±0.7 (5.4-6.8) was recorded in the Epe Lagoon in May (Table 1).

Table 2 depicts the mean total bacteria counts in water, sediment and gut samples of *C. amnicola* from the Badagry and Epe lagoons. The highest total bacteria count of 7.5±1.2 x 10^7 cfug^{-1} was seen in water samples from Epe Lagoon while the least value of 3.6±0.5 x 10^7 cfug^{-1} was obtained in the crab gut from Badagry Lagoon. Significant difference ($P<0.05$) was only observed between the bacterial counts of the two lagoons (Badagry Lagoon, 5.7±0.5 x 10^7 cfug^{-1}; Epe Lagoon, 7.5±1.2 x 10^7 cfug^{-1}).

Table 1. Mean±standard error values of temperature and pH in two interconnecting lagoons, Nigeria

Month	Temperature (°C)		pH	
	Badagry Lagoon	Epe Lagoon	Badagry Lagoon	Epe Lagoon
May	31.8±2.8(29-34.6)	31.1±2.3(28.8-33.3)	7.6±0.3(7.3-7.9)	6.1±0.7(5.4-6.8)
June	26±3.3(22.7-29.3)	29.1±0.2(28.9-29.3)	7.2±0.7(6.5-7.8)	6.7±0.9(5.8-7.6)
July	24.6±1.1(23.5-25.7)	29.2±0.65(28.5-29.8)	6.8±0.9(5.9-7.6)	7.4±0.1(7.3-7.5)
August	25.45±3.5(22.0-28.9)	28.2±1.4(26.8-29.6)	6.9±0.1(6.8-7.0)	6.2±0.3(5.9-6.5)
September	28.6±0.7(27.9-29.3)	23.1±1.3(21.8-24.4)	6.9±0.3(6.6-7.1)	7.0±0.4(6.6-7.4)
October	26.2±0.3(25.9-26.5)	27.5±0.6(26.9-28.1)	7.9±0.5(7.9-8.9)	6.9±0.1(6.8-7.0)

Values are not significantly different between sites ($p>0.05$)

Table 2. Mean ± standard values of the total bacterial counts (cfug^{-1}) in water, sediment and the guts of *Callinectes amnicola* from two interconnecting tropical lagoons in Nigeria.

Component	Badagry Lagoon	Epe Lagoon	*p*-value (t-test)
Crab gut	3.6±0.5 x 10^7	4.5±0.2 x 10^7	0.4
Water	5.7±0.5 x 10^7	7.5±1.2 x 10^7	0.0*
Sediment	4.5±0.2 x 10^7	6.7±0.5 x 10^7	0.1

*: Significantly different at $p<0.05$

Bacterial loads in the guts of *Callinectes amnicola* from the Badagry and Epe lagoons are shown in Table 3. Morphological and biochemical characterization of cells found in the gut of *C. amnicola* from the two lagoons indicated that a total nine (9) bacterial species were present (Table 4). With the exception of *Streptococcus sp*, all the characterized cells were predominantly gram-negative bacteria.

Table 3. Mean± standard error values of bacterial loads (cfu g^{-1}) in the guts of *Callinectes amnicola* from two interconnecting tropical lagoons in Nigeria.

Plant Count	Badagry Lagoon	Epe Lagoon	*p*-value (t-test)
Total heterotrophic bacteria	$3.6\pm0.5 \times 10^7$	$4.5\pm0.2 \times 10^7$	0.1
Total coliform counts	$2.7\pm0.3 \times 10^5$	$3.4\pm0.1 \times 10^5$	0.2
Total fecal Coliform	$1.2\pm0.2 \times 10^5$	$1.5\pm0.3 \times 10^5$	0.5

Values are not significantly different between sites ($p>0.05$)

Table 4. Morphological and biochemical characteristics of bacterial isolates from the gut of *Callinectes amnicola* from Badgary and Epe lagoons in Nigeria.

Parameter	Probable organism								
	a	b	c	d	e	f	g	h	I
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Comma	Cocci
Gram reaction	-	-	-	-	-	-	-	-	+
Catalase test	+	+	-	-	+	+	+	+	-
Motility	+	+	-	+	+	+	+	+	-
Urease	-	-	-	+	-	-	-	-	-
Oxidase	-	-	-	-	+	+	-	+	-
Citrate test	-	-	-	+	+	-	-	+	+
indole	+	+	-	-	-	+	+	+	-
mannitol	+	+	-	-	+	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+	+	+	+
Lactose	+	-	-	-	-	+	+	+	+

a: *Escherichia coli*; b: *Salmonella typhi*; c: *Shigella sp.*; d: *Proteus vulgaris*;e: *Pseudomonas aeruginosa*; f: *Bacillus sp.*; g: *Aeromonas hydrophila*; h: *Vibrio cholerae*; i: *Streptococcus sp.*

The gram-negative bacteria species found commonly in the gut of *C. amnicola* were *Escherichia coli*, *Salmonella typhi*, *Shigella sp*., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus sp.*, *Aeromonas hydrophila* and *Vibrio cholera*. The relative distribution of isolates from Badagry Lagoon showed that *Aeromonas hydrophila* was the highest in the gut of *C. amnicola* in May, June, September and October, accounting for 28.75 % (n=23), 33.33 % (n=19), 25.81 % (n=16) and 27.78 % (n=20) respectively (Table 5). *Shigella sp.* was not recorded from crabs from the Badagry Lagoon.

In the Epe Lagoon, all the identified isolates were present in the gut of *C. amnicola* throughout the period of study (Table 6). The highest distribution of bacterial isolates (23.58 %) was found to be of *Escherichia coli* in August, followed by *A. hydrophila* representing 21.21% of the isolates in May. With the exception of May and June, *E. coli* was predominant throughout the months of the study.

4. Discussion

The water quality of the sites from where crabs were collected during the present study showed optimum conditions for this species. The mean temperature range was 25 - 32°C whereas the pH range was 7.5–8.5 which were similar to the conditions required for the production of portunid crabs (Ikhwanuddin, 2020).

Table 5. Monthly percentage distribution of isolates in the gut of *Callinectes amnicola* from the Badagry Lagoon

Bacteria	May		June		July		August		September		October	
	N	%	N	%	N	%	N	%	N	%	N	%
Escherichia coli	11	13.75	13	22.81	16	25.81	19	26.39	14	22.58	16	22.22
Salmonella typhi	15	18.75	7	12.28	15	24.19	9	12.50	14	22.58	10	13.89
Shigella sp.	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Proteus vulgaris	4	5.00	2	3.51	2	3.23	18	25.00	3	4.84	5	6.94
Pseudomonas aeruginosa	5	6.25	11	19.30	8	12.90	0	0.00	3	4.84	9	12.50
Bacillus sp.	0	0.00	2	3.51	5	8.06	4	5.56	1	1.61	5	6.94
Aeromonas hydrophila	23	28.75	19	33.33	13	20.97	15	20.83	16	25.81	20	27.78
Vibrio cholerae	18	22.50	0	0.00	0	0.00	5	6.94	7	11.29	5	6.94
Streptococcus feacalis	4	5.00	3	5.26	3	4.84	2	2.78	4	6.45	2	2.78

Table 6. Monthly percentages of isolates in the gut of *Callinectes amnicola* from the Epe Lagoon

Bacteria	May		June		July		August		September		October	
	N	%	N	%	N	%	N	%	N	%	N	%
Escherichia coli	14	10.61	17	14.91	24	18.90	29	23.58	23	17.16	19	16.10
Salmonella typhi	23	17.42	19	16.67	13	10.24	15	12.20	17	12.69	19	16.10
Shigella sp.	9	6.82	0	0.00	0	0.00	2	1.63	4	2.99	2	1.69
Proteus vulgaris	15	11.36	13	11.40	21	16.54	11	8.94	17	12.69	13	11.02
Pseudomonas aeruginosa	4	3.03	9	7.89	18	14.17	17	13.82	14	10.45	10	8.47
Bacillus sp.	9	6.82	11	9.65	12	9.45	15	12.20	11	8.21	13	11.02
Aeromonas hydrophila	28	21.21	23	20.18	19	14.96	14	11.38	23	17.16	19	16.10
Vibrio cholerae	23	17.42	19	16.67	13	10.24	15	12.20	17	12.69	19	16.10
Streptococcus feacalis	7	5.30	3	2.63	7	5.51	5	4.07	8	5.97	4	3.39

However, this condition is also known to be favourable for the proliferation of microorganisms as reported by Nayak (2010).

Even though shellfish, such as crabs are substantially nutritious and have become an increasingly significant source of inexpensive proteins and other essential nutrients, they harbour some pathogenic microorganisms which could be attributed to poor hygienic conditions of the water bodies from where they are obtained (Oranusi *et al*., 2018; Okon *et al*., 2020). Evidence indicates that the gastro- intestinal bacteria of aquatic organisms is highly variable and is a reflection of their environment (Nayak, 2010).

In the present study, the highest bacteria load was recorded in a water sample from the Epe Lagoon while the lowest was in the crab gut from the Badagry Lagoon. Significant difference was only observed in the bacterial counts in water samples from the two lagoons. Furthermore, the higher total heterotrophic bacteria, total coliform and total faecal coliform were recorded in the gut of *C. amnicola* from the Epe Lagoon. These high bacterial loads substantiated the results of Akintola *et al*. (2009), who reported a range of $5.7 \pm 0.3 \times 10^7$ cfu g^{-1} to $8.1 \pm 0.3 \times 10^7$ cfu g^{-1} total viable counts in the gut of shellfish from the Epe Lagoon.

Moreover, a total of nine bacterial species were identified from the gut of *C. amnicola*. With the exception of *Streptococcus sp*, all the characterized cells were predominantly gram-negative bacteria, which included *Escherichia coli*, *Salmonella typhi*, *Shigella sp.*, *Proteus vulgaris*;e, *Pseudomonas aeruginosa*, *Bacillus sp.*, *Aeromonas hydrophila* and *Vibrio cholera*. The isolates obtained in this study were similar for both locations except that *Shigella sp*. was absent in the Badagry Lagoon throughout the study period. *A. hydrophila* and *E. coli* were the most dominant bacterial isolates in the gut of *C. amnicola* collected from the two lagoons. These results are similar to those obtained by Uaboi-Egbenni *et al.* (2010), where *E. coli, Proteus vulgaris, Salmonella sp, Streptococcus agalactiae, Vibrio sp,* and *Staphylococcus aeureus* were prevalent in *Callinectes sp*. obtained from the Lagos Lagoon in Nigeria. Reports have shown that the occurrence of both *Salmonella typhi* and *Vibrio cholerae* in the aquatic organism is evidence of high human interactions associated within the two lagoons (Akintola *et al.*, 2009; Uaboi-Egbenni *et al*., 2010). In addition, the presence of coliform bacteria indicates previous contamination of the lagoon with raw sewage.

5. Conclusions

This study showed the presence of a consortium of pathogens in the gut of *Callinectes amnicola*, as well as sediment and water, from Badagry and Epe Lagoons in Nigeria. The high occurrence of these pathogenic bacterial species in the lagoons indicates high levels of untreated sewage and solid waste disposal into the lagoon environment and this calls for urgent intervention. This study is useful for authorities to develop coastal contamination monitoring strategies and for assessing human health risks connected with the consumption of aquatic crabs.

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