

## Survey of bacteria associated with abdominal flap of freshwater crab (*Potamon ebonyicum*) at Ebonyi River basin Nigeria

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### Abstract

Ebonyi River basin of south east Nigeria is home to the freshwater crab *Potamon ebonyicum*, which are consumed indiscriminately by inhabitants and tourists. Bulk of the crab meat is obtained by crabbing. A preliminary survey of pathogenic bacteria associated with vulnerable morphological part of the crab species was carried out in the basin. Bacteria were isolated from abdominal flaps of the male and female crab. A total of 53 bacterial isolates consisting 4 bacteria species such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Aeromonas hydrophila* were recorded. Percentage composition of each bacterial species was recorded in the reproductive features of the samples. *Pseudomonas aeruginosa* (72%) and *Staphylococcus aureus* (48%) were dominant among other bacterial species in the abdominal flaps of the male and female crab. Population of *E. coli* (4%) was significantly low and only recorded in the abdominal flap of the male crab. The results of the study showed clearly that the reproductive feature of freshwater crab was vulnerable to pathogenic microorganisms. The edible crabs from sampled site were contaminated with bacteria species which were considered by Centre for Food Safety as hazardous to human health. Investigation on sample from other area would enhance regulation on aquaculture, handling, processing and consumption of freshwater crab.

**Keywords:** Pathogenic bacteria, Reproductive feature, Freshwater crab, *Potamon ebonyicum*, Ebonyi River basin

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## Introduction

The ebonyi crab *Potamon ebonyicum* is found in large number at Ebonyi River basin, where some investigations on its aquaculture have been conducted (Akpaniteaku, 2013; Akpaniteaku, 2017). Decline in the population of the crab species (Akpaniteaku, 2015) suggested investigation on other aspects of biology including the impact of bioaccumulation of essential and non essential elements in the environment (Akpaniteaku and Okoye, 2018; Akpaniteaku and Udeozor, 2018). Some parasites could devastate mud crab population in the wild if there was no action by researchers. But they have attracted less attention because of lack of the description of their infection as compared to other commercial exploited crustaceans and aquaculture species (Ihwan *et al.*, 2015). Depleted wild stock could be protected against diseases of which bacteria are a leading agent. Bacteria and their components could be targeted to curb devastating effects of their quorum sensing infection (Atujona *et al.*, 2018). Many kinds of pathogens may be present in crabs living in various environments such as freshwater (Centre for Food Safety, 2015). Diverse array of bacterial species including several potential human pathogens could be isolated from edible crabs (Faghri *et al.*, 1984). The ability of freshwater crab to accumulate pollutants and human pathogens has necessitated investigations on safety of the crab species as human food source. And

potential approach to controlling bacterial diseases of crab, should be helpful and practicable (Wang, 2011) as findings would be used in solving problems of seed multiplication. It could also help in addressing the problem of population decline in the wild. The survey was therefore aimed at identifying various pathogenic bacteria that could contaminate *P. ebonyicum* through reproductive features, and compare them with safety standards for aquaculture and consumption.

## Materials and methods

The ebonyi crab *P. ebonyicum* used for the research were collected in the month of April 2019 from Ishieke community of Ebonyi State. The location is 12.9km west from Abakaliki the capital of the state on latitude 6° 15' 18" N, longitude 8° 05' 55" E with total area of 5,533km<sup>2</sup>. This investigation involved equal number of male and female crab (25 each) and a total of 50 crabs were used (Table 1). They were collected in plastic tub and transported to the microbiology laboratory of the University for Analysis.

Medium was prepared by dissolving nutrient agar (NA) in distilled water and autoclaved. The abdominal flap (AF) of the crab sample was swabbed using sterile cotton bud and streaked on to the NA medium separately, and one plate without sample maintained as control. The plates were incubated at 37°C for 24h for observation of bacteria colony.

**Table 1: Morphometrics of specimens of *Potamon ebonyicum* used for the investigation.**

Measurement	Male			Female		
	Min	Max	SD	Min	Max	SD
Weight (g)	19.04	60.20	1.55	18.97	50.09	10.97
Carapace length (cm)	1.50	4.40	0.89	3.00	4.80	0.38
Carapace width (cm)	4.50	6.50	0.52	5.00	6.50	0.47
Length of abdominal flap (cm)	2.00	3.50	0.38	3.00	4.80	0.61
Width of abdominal flap (cm)	1.50	2.00	0.26	2.00	3.50	0.48

Min = Minimum, Max = Maximum, SD = Standard Deviation, g = Gram, cm = Centimetre.

Test for confirmation of isolates was conducted with selective agar which included Centrimide Agar (CA), Mannitol salt Agar (MSA), Eosin Methylene Blue Agar (EMB) and MacConkey Agar (MA). The colonies were selected from each sample and streaked three times on to NA medium to receive pure culture. Phenotypic characteristics, gram staining and biochemical tests were determined for all isolates.

Regression analysis and Pearson's correlation coefficient were used to estimate sex variable, and determine relationship between size of male and females *P. ebonyicum* affected by various bacterial species. One-way analysis of variance (ANOVA) was used to identify variation at 5% significant limit ( $p > 0.05$ ).

## Results

Bacteria were isolated from the male and female *P. ebonyicum*. A total of 4 bacterial species were represented in 53 isolates from 50 samples of the ebonyi crab (Table 2). Highest number of isolates was recorded by *Pseudomonas aeruginosa* 29(58%), followed by *Staphylococcus aureus* 12(24%) and *Aeromonas hydrophila* 11(22%). The

least isolated bacterium was *Escherichia coli* 1(2%).

Percentage composition of each bacterial species was recorded in the AF of the male and female crab (Table 3). The dominant bacterial species isolated from the AF of the female was *Pseudomonas aeruginosa* with 72% compared to 44% in the AF of the male. The density of *Staphylococcus aureus* and *Aeromonas hydrophila* in the AF of the male and female were not significantly ( $p > 0.05$ ) different. The composition percentages were 48% and 44%, and 24% and 20% respectively. *Escherichia coli* were found only in the AF of the male, and in the lowest density (4%).

Regression analysis showed there was no discrimination in the sex of *P. ebonyicum* affected by the bacterial species (Table 4). The coefficient of relationship between the size of the male and the female crab contaminated with bacteria was negative ( $r = -0.00$  to  $r = 0.00$ ).

Morphological and biochemical characterization of cells found in the reproductive features of the ebonyi crab *P. ebonyicum* (Table 5) indicated that a total 4 bacterial species were identified.

**Table 2: Bacterial isolate from abdominal flap of specimens of freshwater crab *Potamon ebonyicum* at Ebonyi River basin**

Bacterial Species	Abdominal Flap		No. of crab	% of crab
	Male	Female		
<i>Pseudomonas aeruginosa</i>	+	+	29	58
<i>Escherichia coli</i>	+	-	1	2
<i>Staphylococcus aureus</i>	+	+	12	24
<i>Aeromonas hydrophila</i>	+	+	11	22

+ Presence of bacterial species, - Absence of bacterial species

**Table 3: Percentage bacterial isolate from abdominal flaps of male and female crab *Potamon ebonyicum* specimens at Ebonyi River basin**

Bacterial Species	Abdominal Flap	
	Male (%)	Female (%)
<i>Pseudomonas aeruginosa</i>	44	72
<i>Escherichia coli</i>	4	0
<i>Staphylococcus aureus</i>	24	48
<i>Aeromonas hydrophila</i>	20	44

**Table 4: Relationship between size (carapace width) of male and female *Potamon ebonyicum* contaminated with various bacterial species at Ebonyi River basin**

Bacterial Species	Variable (CW)	RE	CC
<i>Pseudomonas aeruginosa</i>	M & F	$y = 18422.62 + 3350.55x$	$r = -0.00$
<i>Escherichia coli</i>	M & F	-	-
<i>Staphylococcus aureus</i>	M & F	$y = 223.04 + 43.14x$	$r = -0.00$
<i>Aeromonas hydrophila</i>	M & F	$y = 4.55 + 0.17x$	$r = 0.00$

CW = Carapace Width, M = Male, F = Female, RE = Regression Equation, CC = Correlation Coefficient

**Table 5: Morphological and biochemical characteristics of bacterial species isolated from male and female crab *Potamon ebonyicum* specimens at Ebonyi River basin.**

Identification of Bacteria	<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Aeromonas hydrophila</i>	
	ME	FC	MC	FC	MC	FC	MC	FC
Gram staining shape	G <sup>-ve</sup>	G <sup>-ve</sup>	G <sup>-ve</sup>	-	G <sup>-ve</sup>	G <sup>-ve</sup>	G <sup>-ve</sup>	G <sup>-ve</sup>
Motility	M	M	M	-	NM	NM	M	M
Indole test	-	-	+	-	-	-	+	+
Citrate utilization test	+	+	-	-	+	+	-	-
Catalase test	+	+	+	-	+	+	+	+
MaCconkey Agar	-	-	-	-	-	-	Pink	Pink
Centrimide Agar	Blue	Blue	-	-	-	-	-	-
Mannitol Salt Agar	Green	Green	-	-	Golden Yellow	Golden Yellow	-	-
Ethyl Methline Blue Agar	-	-	Green	-	-	-	-	-
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All the characterized cells were predominant by gram-negative bacteria.

The bacteria species found common in the AF of both sex were *Pseudomonas*

*aeruginosa*, *Staphylococcus aureus* and *Aeromonas hydrophila*.

### Discussion

Shellfish affected by pathogen or sewage discharge is known to be a health hazard, and bacterial species associated with the tissue of crab indicates possible contamination, which may occur through contact with the source (Faghri *et al.*, 1984). Fecal by products from humans and cattle due to open grazing and defecation is significant, and can affect the quality of water and fish resources in the river basin (Pers. Obs.). General information about bacterial diseases of crab is available (Wang, 2011). The visible decline in the wild population as reported by Akpaniteaku (2014) could in part, emanate from the bacterial contamination with the reproductive features of the crab species. The presence of bacterial species in the *P. ebonyicum* as reported in the present study may have modified implementation of habitat regulation such as closed area, minimum size, and sex based restrictions recommended for conservation of the crab species (Akpaniteaku and Emmanuel, 2017). Brood stock parasite of mud crab *Scylla serrata* was discovered in two separate cases during the evaluation for mass aquaculture in Australia, which appeared to be responsible for 100% mortality (Kvingedal *et al.*, 2006). The contamination of abdominal flap of the female *P. ebonyicum* with pathogenic bacteria in the present study may also

pose potential threat to crab aquaculture in the basin. Akpaniteaku (2016) reported that experimental aquaculture potentials of *P. ebonyicum* depends on those that are genetically endowed with capacity to inhabit aquatic environment, as some of them would struggle to escape to adjacent area. Bacterial contamination of crab from the sampled area irrespective of size may seem to indicate that selective capture and consumption is irrelevant. Atujona *et al.* (2018) reported that immunity of the consumers determine pathogenic response by secretion of virulence factors that facilitate their proliferation. Perhaps failure to isolate fecal indicator bacterium (*E. coli*) from the abdominal flap of the female crab, and insignificant density in the male indicate that fecal by products were low in the area. Al-Sheraa *et al.* (2018) reported high presence of *E. coli* and *Pseudomonas aeruginosa* in mitten crab *Eriocheir sinensis* as indicator of fecal contamination in the bank of Khor Al-Zubair canal. Despite low density of *E. coli* in the present study, there may be possibility of fecal contamination due to high presence of *Pseudomonas aeruginosa*. Wang (2011) reported that new diseases associated with pathogens have appeared in aquaculture-exploited crab species. And according to Centre for Food Safety (2015) consumption of raw or uncooked crabs increases the risk of developing food borne diseases.

## Conclusion

The results of this study revealed that pathogenic bacteria were isolated from abdominal flaps of the crab species. The isolated bacterial species were predominately *Pseudomonas aeruginosa*, indicating that the sampled site was contaminated with waste from human activities. Experiment on freshwater crab aquaculture in earthen pond could be reconsidered for areas that are not prone to contamination. Raw consumption of appendage or whole crab should be prohibited by relevant authority. The need to have mitigation policy for various levels of wastes, and adverse effect of large scale animal rearing in the basin is advocated. Further study on other organisms at the higher trophic level than the crabs might reveal relatively the status of food chain in the basin.

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