

Original Research Article

Isolation and Identification of *Vibrio* Spp. from Catfish (*Arius heudelotii*) from Wild Environment in Rivers State, Nigeria

ABSTRACT

This study was aimed at the identification and isolation of *Vibrio* spp. associated with *Arius heudelotii*, a catfish from a wild environment. Twenty-four *Arius heudelotii* (Catfish) were purposively sampled from the New Calabar River Choba, in Obio- Akpor LGA in Rivers State, Nigeria. The *Vibrio* spp. associated with the gills, skins, and intestines of *Arius heudelotii* fish of economic value and water samples from the New Calabar River were isolated and identified. Ten presumptive *Vibrio* species (*V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus*, *V. mytili*, *V. angular*, *V. furnissi*, *V. diazotrophic*, *Allivibrio fisheri*, *V. xuii*, and *V. guzogene*) were isolated based on the morphological and biochemical test. A total of 96 *Vibrio* species were detected n=31 (32.4%), n=11 (11.5%), n=24 (25%), and n=30 (31.2%) for gills, skin, intestine and water samples, respectively. Among the vibrio species isolated, *V. Parahaemolyticus* had 18/96 (18.6%), was the most predominant, this was followed by *V. Vulnificus* 17/96 (17.7%), *V. Cholerae* 14/96 (14.6%), *V. alginolyticus* 11/96 (11.6%), *V. mimicus* 8/96 (8.3%), *V. furnissi* 7/96 (7.3%), *V. mytili* 6/96(6.3%), *V. angularum* 6/96 (6.3%), and *Allivibrio fisheri* 6/96 (6.3%) and *V. diazotrophic* was the least predominant 3/96 (3.1%). *Vibrio* organisms can be pathogenic. Therefore, it is important to investigate the presence of *Vibrio* spp. that are capable of causing sickness to humans and animals.

Keywords: *Vibrio*; *Arius heudelotii*, wild environment, catfish; bacteria

Introduction

Fish is an important source of high-quality protein for human consumption (1). Globally, the demand for fish and fish products has increased tremendously (2, 3). Fish is highly economical and highly nutritious (4). Fish contains important vitamins that are essential for good health, such as Vitamin K in fish, which is responsible for anti-haemorrhage factors, fish oil, and vitamin E reduces inflammation (5).

Various types of fish, *Arius heudelotii*, *Clarias gariepinus*, and others are readily available for human consumption. *Arius heudelotii* is also usually found in the brackish water, swampy, or paddy fields of wild environments and is consumed by individuals as food. *Arius heudelotii* has significant economic and nutritional values (6, 7). However, the production of this economic and nutritious food in Africa has been facing challenges for over a decade (8). In

Comment [r1]: Suggested to please maintain uniform font size for all words

Comment [r2]: suggested to write it as ""isolation and identification"

Comment [r3]: Suggested to please reframe this sentence
Twenty four were collected from the New.....Nigeria and were processed to sample their skin, gills and intestines.

Comment [r4]: suggested to please delete

Comment [r5]: suggested regular font instead of italics

Comment [r6]: suggested to please delete word "presumptive"

Comment [r7]: Many species have been wrongly spelled eg. *V. vulnificus* (correct is *V. vulnificus*) *V. parahaemolyticus* (correct *V. parahaemolyticus*). There are others also. Suggested to please check and write correctly.

Comment [r8]: incorporate " and presumptively identified" after isolated

Comment [r9]: suggested to replace detected by "isolated"

Comment [r10]: please incorporate word "samples" after water

Comment [r11]: Suggested to please correct the spellings of different *Vibrio* species. Also suggested to please avoid using both formats of numerical figures (eg 18/96 or 18.6%) i.e only percentages could convey the result.

Comment [r12]: consumption

Comment [r13]: Suggested to please delete

Comment [r14]: suggested to please replace Fish by "It"

Comment [r15]: V : please use small cap

Comment [r16]: Suggested to please delete "in fish"

Comment [r17]: Suggested to please replace factors by properties

Comment [r18]: suggested to please remove fish oil from here. If desired it can be used elsewhere in the text. Not required here as the sentence only talks about vitamins.

Comment [r19]: Suggested to rewrite as "known to reduce inflammation"

Comment [r20]: It would be better to add some more types of fish

Comment [r21]: Suggested to please incorporate a reframed sentence " However, *Arius heudelotii*, an economic and nutritious food in Africa has been facing challenges for over a decade (8).

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recent times, the species have been deteriorating in Africa, especially in Nigeria due to various unhealthy activities of man against the natural habitat. Another major problem affecting the growth of these species is pathogenic infections (9, 10). Over the years, the Nigerian aquaculture industry has suffered negligence and lack of adequate support to fish farmers until recently, due to the incessant fall in oil production and prices.

Studies have shown that pathogenic infections are caused by the use of contaminated water from wild environments and artificial feeds which have contributed to the high growth of several pathogens (11). In an attempt to curb the high rate of growth and advancement of infectious agents in cultured and wild environments, fish farmers used several antibiotics that have caused multidrug-resistant microorganisms in aquaculture (12). Aquaculture production has witnessed lower output as a result of contamination by these agents. They hinder development as well as the sustainability of the global fish sector (13).

Currently, the use of drug therapy strategy for the management of disease is no longer effective due to microorganism's resistance to drugs. The aquaculture industry has suffered greatly due to the rapid spread of the disease. Fish diseases are significant challenges nowadays that disrupt the stable supply of fish around the world, especially those caused by *Vibrio* spp., *Aeromonas* spp., and others. These microorganisms are opportunistic, gram-negative, rod-shaped, facultative anaerobic bacteria, oxidase-positive, motile or non-motile, and non-spore-forming, and are considered a major challenge for the development of the aquaculture sector in the face of environmental crisis(14,9,15,16,17,18).The World Bank estimates annual global losses at around \$6 billion (19).Bacterial disease outbreaks represent a significant constraint on the production of cultured and wild fish species (20). The disease is a major cause of fish mortality. The bacteria most frequently associated with disease in farmed fish are species of the *Vibrionaceae* family (21).

Vibrio is a type of Gram-negative bacteria with short rod-shaped cells that are usually curved or comma-shaped (22, 23, 24, 25, 26, 27). The most significant pathogenic *Vibrio* is *Vibrio cholera* (28). However, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and other pathogenic species exist (24). The genus *Vibrio* contains highly contagious bacteria that can infect both humans and animals, as a result of its wide distribution and diverse reservoirs posing great danger to human health (29, 24). *Vibrio* survives better in salt water. Prevention of waterborne

Comment [r23]: suggested to please replace it by some better word

Comment [r24]: Is it unhealthy or unhygienic. Please clarify

Comment [r25]: Suggested to please remove this sentence from here. Or prefix this sentence before the preceding sentence ie Another major problem.....(9,10).

Comment [r26]: suggested to please delete this word

Comment [r27]: suggested to please replace advancement by development

Comment [r28]: suggested to please replace caused by "led to emergence of various..

Comment [r29]: suggested to please combine these two sentences agents which hindered the development.....

Comment [r30]: suggested to please rewrite asthe development of drug resistance by the microorganisms. Also suggested to insert References in this context

Comment [r31]: Suggested to please rewrite this sentence as " The infections of the fish especially caused by *Vibrio* spp., *Aeromonas* spp. etc have posed a significant challenge to aquaculture industry and disrupted the fish supply chain globally.

Comment [r32]: suggested to please delete from here and suffix/after ... "non spore forming..

Comment [r33]: insert space

Comment [r34]: suggested to write references numerics in chronological order

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Comment [r36]: suggested to please link these sentences with comment no 31. Reframing required to improve and avoid repetitive contexts

Comment [r37]: Description about morphological/biochemical characteristics have been presented in para 4 of introduction. suggested to please reframe the paragraphs to avoid repetitions.

contamination of *Vibrio cholerae* depends on appropriate hygiene measures, including wastewater treatment, maintenance of water quality, and purification of water supplies. *Vibrio* has been able to form cell clusters or aggregates, indicating the ability to form biofilms. Some researchers believe that the type of biofilm growth is important for the recovery and survival of *Vibrio* in water (30; 31)

These are fermentative, facultative anaerobic, monopolar, flagellated Gram-negative bacilli. Only *Vibrio cholerae*, *Vibrio cholerae* and *Vibrio* spp. In the case of *Vibrio mimicus*, all *Vibrio* species are halophilic (require salt) (32). A variety of virulence factors have been identified for *Vibrio* species, including cell-associated proteins, toxins, and various proteases. However, the exact role of these factors in the pathogenesis of this disease is unclear. Some non-O1, non-O139 *V. cholera* and *Vibrio* spp. (33). Mimic species can produce cholera toxin (32). (32) stated that the majority of *Vibrio* infections occur in summer and occur in warm water. Therefore, (34) reported that *Vibrio* bacteria include *Vibrio* and *Vibrio* bacteria that cause cholera, parahaemolyticus and *Vibrio vulnificus*.

Vibrio cholerae is often spread through contaminated water, and *Vibrio* bacteria can cause foodborne illness is often spread through contaminated water, and *Vibrio* bacteria is often spread through contaminated water can cause foodborne illness. It is associated with eating unhealthy seafood. Other species that have become more recognized as foodborne pathogens in recent years are *Vibrio mimicus* and *Vibrio alginolyticus*. *V. mimicus* shares genetic and biochemical similarities with *V. cholerae*, and its strains contain many toxins similar to *V. cholerae* (35, 36). However, this led to a massive decline in the aquaculture industry and was seen as a major impediment to economic growth, causing severe damage worldwide. According to (37) the frequent use of antibiotics has raised concerns about antibiotics in *Vibrio* species. Therefore, good and effective treatment must be provided.

Climate change, environmental constraints, globalization, and overpopulation pose many challenges, especially in developing countries such as Nigeria (38). Aquaculture in general and the food industry as a whole are faced with the problem of the rapid emergence of antibiotic resistance (ARB), which is gaining importance (39). This fight against infectious diseases has become a threat to global public health and environmental pollution (40). In addition, human health is at risk due to the presence of antimicrobial resistance agents (ARGs) in these

organisms, and this is considered a cause for concern. Both humans and the environment is under threat due to spread of antibiotic resistance (AMR) (41, 42, 43).

There is limited information on the isolation and identification of *Vibrio* Spp. from *Arius heudelotii* from Wild Environment, that is largely consumed by teaming population of Rivers State, Nigeria. Therefore, this study isolated and identified *Vibrio* species from gills, skin, and intestine of *Arius heudelotii* and water from New Calabar River in Choba, Rivers State, Nigeria.

Materials and Methods

Sample Collection

Live catfish (*Arius heudelotii*) freshly harvested from the fishermen at the river bank of the new Calabar River (Choba River) were purchased and collected in an ice cooler and transported to the laboratory for analysis. Fish die due to physical damage to the brain. The skin was removed before taking the gills and opening the ventral surface with a sterile blade to expose the cavity. Ten grams of skin, gills, and intestine were taken aseptically and homogenized in a stomacher bag containing 90ml sterile normal saline using a stomacher machine (44, 45).

The water samples from the New Calabar River were collected with sterile bottles 10-15cm below the water surface (46) and transported to the laboratory in an ice cooler, for physicochemical and microbiological analysis. The water samples were collected 100m away from the bank (47). The fish sample from *Arius heudelotii* from New Calabar River was identified by Department of Animal and Environmental Biology of Faculty of Sciences, Rivers State University, Npkolu-Oroworukwo, Port Harcourt, Rivers State.

A total of 24 *Arius heudelotii* from New Calabar River) from New Calabar River, were randomly collected over a 12-month period of study (every month, 2 *Arius heudelotii*, 2 water samples from New Calabar River).

Comment [r38]: Suggested to please include some justification on isolation and identification eg how isolation and identification would hel the aquaculture to grow? Or how isolation and identification is going to curtail drug resistance and so on..

Comment [r39]: All these lines indicate elements of repetitions and improper flow and linkages in different contexts. suggested to reframe all paragraphs in precise, non repetitive and proper continuity of sentences eg. cultural characteristics of vibrio are being repeated in different paragraphs vibrio: a waterborne pathogen. Its being directly/indirectly quoted many times. Aquaculture facing challenges is being said time and again

Comment [r40]: by

Comment [r41]: It is suggested to define the site of sample collection with longitude, latitude, ht cordinates and average temperature, RH etc.

Comment [r42]: homogenized all together or separately. Please specify

Comment [r43]: suggested to please rewrite aswere also collected from New Calabar River in sterile bottles. It would be fine to mention the volume of water sample collected.

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Pic 1: Catfish : *Arius heudelotii*
Source: New Calabar River

Total *Vibrio* counts

Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar was prepared according to the Manufacturer's specifications. The agar was allowed to gel; 0.1ml was spread-plated and incubated for 24 hours at 37°C (48). The total viable count was determined with the formula below:

$$\text{TVC(Cfu/ml)} = \frac{\text{Number of colonies} \times 1/\text{Dilution factor} \times 1/\text{Volume of inoculum}}$$

The identification of the *Vibrio* spp. from sampled catfish (*Arius heudelotii*) and water was based on the colonial morphology, Gram staining, and biochemical attributes (appendices 1). The *Vibrio* spp. were identified by ABIS online identification of bacterial. Appendix 1 shows the *Vibrio* spp. isolated.

Data analysis

Descriptive and inferential analysis was performed using Microsoft Excel Software (2010).

Results and Discussion

With regards to the intestine of *Arius heudelotii* associated with *Vibrio* disease, the total *Vibrio* counts was higher (7×10^4 cfu/g) in the month of September and nil in the months of June

Comment [r46]: suggested to please clarify whether serial dilutions were carried out. If yes, please incorporate here in the methods. Suggested to please add references for the method used for viable bacterial counts

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and July, respectively (Figure 1). The highest mean total *Vibrio* counts in the intestine was 5.12 ± 0.81 LogCFU/ml which occurred in October. Nil occurrence was observed in the months of June and July. The difference was significant for the various months under consideration ($p < 0.01$) (Table 1). The total *vibrio* counts in gills exhibited monthly variations throughout the study period, ranged from nil in the months of May and December to 8.4×10^4 cfu/g in the month of January (Figure 1). Figures 1, also indicates the total *Vibrio* count in the fish skin. The accumulation of *Vibrio* in the skin total *Vibrio* counts had a range of nil to 7×10^5 CFU/g, with the least in the months of February and April and the highest in October (Figure 1). The highest mean total vibrio count in the skin of *A. heudelotii* was 5.84 ± 0.00 which occurred in October. The difference was significant for the various months under investigation in this study ($p < 0.05$) (Table 1). The highest Total *Vibrio* density occurred in the water sample from New Calabar River was observed in the month of January (6×10^3 CFU/g) and nil in August, October, February, and April (Figure 1). The highest (5.22 ± 0.73 LogCFU/ml) and lowest (00 ± 0.00 LogCFU/ml) mean TVC density occurred in New Calabar River in the months of January for the highest August, October February, and April respectively. The difference in mean total vibrio count for the various months of observation in this study, was statistically significant ($p < 0.05$) (Table 1).

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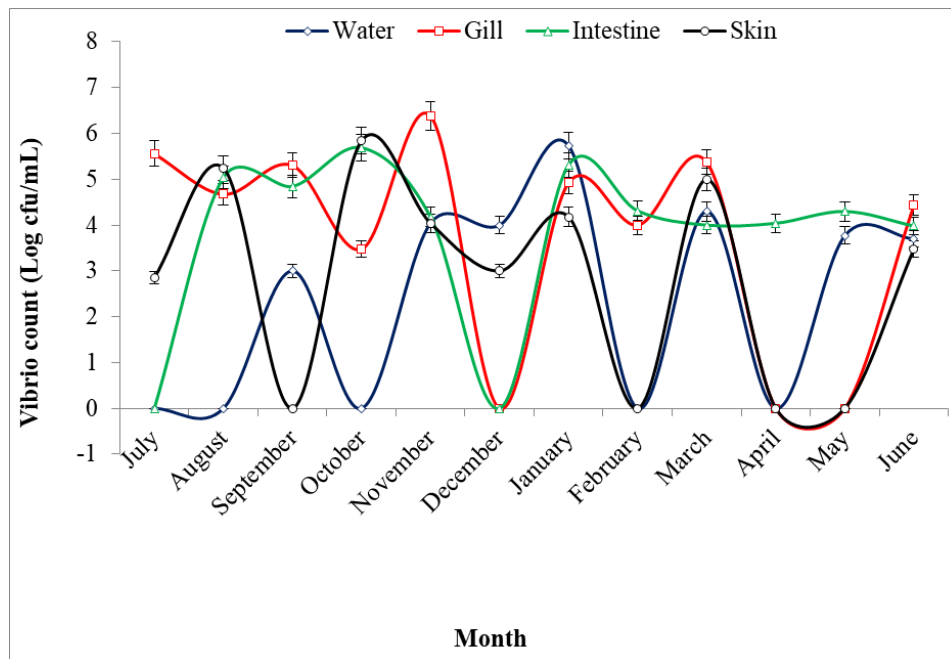


Figure 1: Total *Vibrio* Counts (TVC) from Water and Fish Parts Gills, Skin, and Intestine of Catfish (*Arius heudelotii*) Harvested from New Calabar River.

Table 1: Mean \pm Standard Deviation Total *Vibrio* counts (TVC) of the Water Samples and Fish Parts *Arius heudelotii* from New Calabar River for different months.

Parameters	<i>Arius heudelotii</i>			
	Gills	Intestine	Skin	Water
July	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^b	2.07 \pm 1.06 ^{cde}	1.00 \pm 1.41 ^{cd}
August	5.80 \pm 0.70 ^{ab}	4.67 \pm 0.52 ^a	5.36 \pm 0.16 ^{ab}	0.00 \pm 0.00 ^d
September	7.17 \pm 0.14 ^a	4.77 \pm 0.10 ^a	0.00 \pm 0.00 ^e	2.65 \pm 0.49 ^{bc}
October	4.73 \pm 0.83 ^{bc}	5.12 \pm 0.81 ^a	5.84 \pm 0.00 ^a	0.00 \pm 0.00 ^d
November	5.76 \pm 0.44 ^{ab}	3.98 \pm 0.27 ^a	3.67 \pm 0.52 ^{abcd}	3.67 \pm 0.52 ^{ab}
December	3.55 \pm 0.62 ^c	0.50 \pm 0.70 ^b	2.66 \pm 0.47 ^{bcd}	3.54 \pm 0.73 ^{ab}

January	4.25±0.06 ^{bc}	5.01±0.43 ^a	4.29±1.24 ^{abc}	5.22±0.73 ^a
February	5.38±0.12 ^b	3.90±0.59 ^a	0.00±0.00 ^e	0.00±0.00 ^d
March	5.76±0.05 ^{ab}	3.65±0.49 ^a	4.54±0.65 ^{abc}	4.00±0.42 ^{ab}
April	5.56±0.73 ^{ab}	4.18±0.19 ^a	0.00±0.00 ^e	0.00±0.00 ^d
May	0.00±0.00 ^d	3.65±0.92 ^a	1.00±1.41 ^{de}	3.73±0.05 ^{ab}
June	4.36±0.09 ^{bc}	0.00±0.00 ^b	2.88±0.83 ^{bcde}	3.58±0.15 ^{ab}
P-value	0.000	0.000	0.000	0.000

Legend: ^{ab} and ^c: mean values with the same superscript (letter) in each column indicate no significant difference ($p < 0.05$). Values are means of duplicates \pm standard deviation (SD) of the mean.

Prevalence of *Vibrio* spp. from *Arius heudelotii* and Water sample from New Calabar River

As shown in Table 2, results of *Arius heudelotii* from New Calabar River revealed 31, 11, 24, and 30 of *Vibrio* species, respectively were isolated from gills, skin, intestine, and water. Precisely, in Table 2 the result of *Arius heudelotii* revealed the incidence of *Vibrio* species detected n=31 (32.4%), n=11 (11.5%), n=24 (25%), n=30 (31.2%) for gills, skin, intestine and water, respectively.

Prevalence of *Vibrio* spp. from the gills of *Arius heudelotii*

Among the vibrio species isolated from the gills of *Arius heudelotii*, *V. Vuluificus* was the most predominant 7/31 (22.5%), this was followed by *V. Cholerae* and *V. Parahaemoliticus* had 5/31 (16.1%) each, *V. mimicus* 3/31 (9.6%), *V. alginolyticus*, *V. mytili*, *V. anguillarum*, *V.furnissi*, and *Alliivibrio fisheri* had 2/31(6.5%) each. *V.diazotrophic* was the least predominant 1/31 (3.2%) in the gills. However, *V. Xuii* and *V.guzogene* were not found in the gills of both catfish species used in this study (Table 2).

Prevalance of *Vibrio* spp. from the Skin of *Arius heudelotii*

The results of vibrio species isolated from the skin of *Arius heudelotii* are presented in the same Table 2, indicating that *V. parahaemoliticus* had the highest 3/11 (27.3%) (Figure 2). While *V. vuluificus* and *V. alginolyticus* had 2/11 (18.2%) each, and followed by *V. cholerae*, *V. mimicus*, *V. diazotrophic*, and *Alliivibrio fisheri* that were least predominant (1/11 (9.1%)). *V.*

Comment [r52]: small cap

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Comment [r55]: suggested to delete respectively from here.

Comment [r56]: Suggested to please insert of *Arius heudelotii*

Comment [r57]: Please insert from New Calabar River respectively.

Comment [r58]: Suggested to please delete the highlighted sentence and replace it with The study showed that the incidence of ...

Comment [r59]: suggested to delete "detected" and insert "was"

Comment [r60]: please check spell & follow scientific nomenclature norms

Comment [r61]: suggested to please check spelling and adhere to nomenclature norms

Comment [r62]: Suggested to please delete

Comment [r63]: suggested to replace comma by semicolon (;)

Comment [r64]: suggested to please delete "had"

Comment [r65]: Please clarify the use of "both catfish species". Previous text no where mentions about two species of catfish taken for study

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mytili, *V. anguillarum*, *V. furnissi*, *V. Xuui*, and *V.guzogene* were not recovered from the skin of *Arius heudelotii*.

Prevalance of *Vibrio* spp. from the intestine of *Arius heudelotii*

From the intestine of the *Arius heudolotti*, *V. vuluificus* had the highest 5/24 (20.8%) followed by *V. parahaemoliticus* and *V. alginolyticus* had the occurrence of 4/24 (16.7%) each. Other recovered *Vibrio* species are *V. Cholerae* 3/24 (12.5%) and *V.mimicus*, *V.furnissi*, and *V. Anguillarum* 2/24 (8.3%) each. While *Alliivibrio fisheri* and *V. mytili* had the lowest 1/24 (4.2%). Also, *V. Diazotrophic*, *V. Xuui* and *V.guzogene* were not found in the intestine of *Arius heudelotii* (Table 2)

Comment [r68]: suggested to please incorporate word "incidence"

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Prevalence of *Vibrio* spp. from Water sample of New Calabar River

Furthermore, Table 2 showed evidence of presence of *vibrio* in the water sample from the New Calabar River. The most predominant was *V. Parahaemoliticus* that had 6/30 (20.0%) followed by *V. Cholerae* 5/30 (16.7). More so, Table 4.16 revealed that *V. vuluificus*, *V. alginolyticus*, *V. mytili* and *V. furnissi* had 3/30 (10.0%) each. While *V. mimicus*, *V. anguillarum* and *Alliivibrio fisheri* had 2/30 (6.6%) each. The lowest 1/30 (3.3%) predominant was *V. diazotrophic*.

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Comment [r72]: suggested to please add "prevalance percentage of 10% each.

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Table 2: Incidence of *Vibrio* spp. isolated from Catfish (*Aruis heudelotii*) and Water from the New Calabar River

Fish Parts and water	<i>Vibrio</i> Positive samples	No. of isolates for		species
	No. (%)	No.	(%)	species
Gills	31(32.3)	7	22.5	<i>V.vuluificus</i>
		5	16.1	<i>V. cholerae</i>
		5	16.1	<i>V. parahaemoliticus</i>
		2	6.5	<i>V. alginolyticus</i>
		3	9.6	<i>V.mimicus</i>
		2	6.5	<i>V. mytili</i>
		2	6.5	<i>V. anguillarum</i>
		2	6.5	<i>V.furnissi</i>
		1	3.2	<i>V.diazotrophic</i>
		2	6.5	<i>Alliivibrio fisheri</i>

<i>Arius heudelotii</i>	Skin	11(11.5)	2	18.1	<i>V.vulnificus</i>
			1	9.1	<i>V. cholerae</i>
			3	27.3	<i>V. parahaemoliticus</i>
			2	18.2	<i>V. alginolyticus</i>
			1	9.1	<i>V.mimicus</i>
			1	9.1	<i>V.diazotrophic</i>
			1	9.1	<i>Allivibrio fisheri</i>
	Intestine	24(25)	5	20.8	<i>V.vulnificus</i>
			3	12.5	<i>V. cholerae</i>
			4	16.7	<i>V. parahaemoliticus</i>
4			16.7	<i>V. alginolyticus</i>	
2			8.3	<i>V.mimicus</i>	
1			4.2	<i>V. mytili</i>	
2			8.3	<i>V. angularum</i>	
2	8.3	<i>V.furnissi</i>			
Water	30(31.2)	1	4.2	<i>Allivibrio fisheri</i>	
		3	10.0	<i>V.vulnificus</i>	
		5	16.7	<i>V. cholerae</i>	
		6	20.0	<i>V. parahaemoliticus</i>	
		3	10.0	<i>V. alginolyticus</i>	
		2	6.7	<i>V.mimicus</i>	
		3	10.0	<i>V. mytili</i>	
		2	6.7	<i>V. angularum</i>	
		3	10.0	<i>V.furnissi</i>	
		1	3.3	<i>V.diazotrophic</i>	
2	6.6	<i>Allivibrio fisheri</i>			
96(100)					

In cognizance of distribution and frequency of occurrence of *Vibrio* species, the highest incidence in the *Arius heudelotii* and water samples from New Calabar River, the gills part of the fish was the most contaminated 31/96 (32.3%). This was followed by water and intestine with 30/96 (31.2%) and 24/96 (24%) respectively. The least contaminated sample was the skin 11/96 (11.5%) (Figure 1 and Table 2).

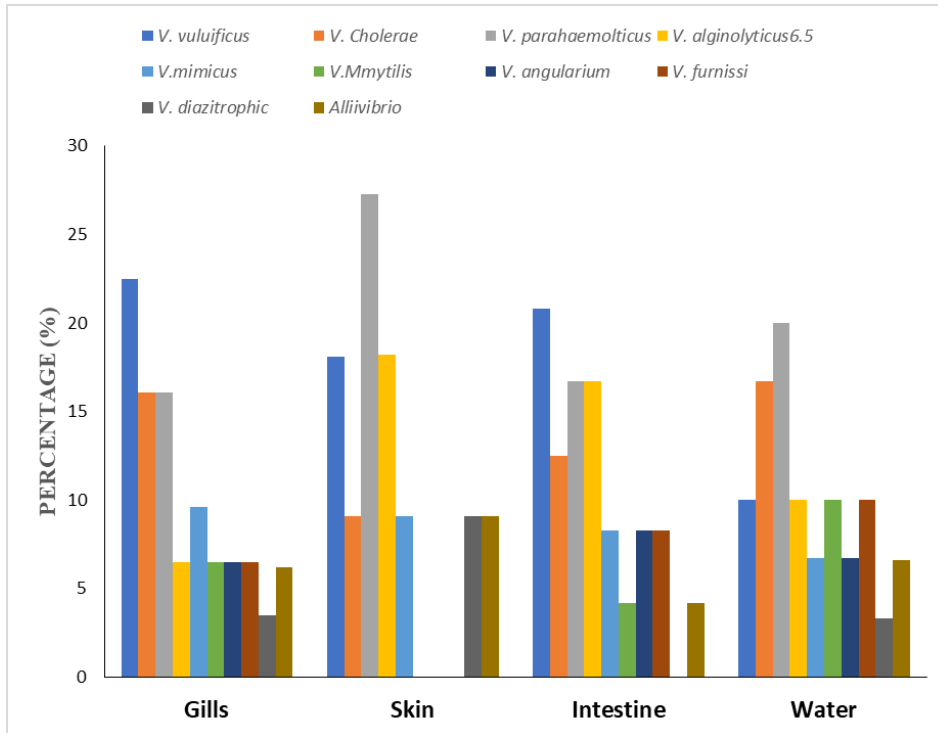


Figure 2: Prevalence of *Vibrio* species from *Arius heudelotii* (Gills, Skin, Intestine) and water from New Calabar River

Several members of the genus *Vibrio* are well-known pathogens of humans, fish, and some other animals (49). *V. parahaemolyticus* was found to be more prevalent. A similar result was reported by (16). *Vibrio parahaemolyticus* is one of the *Vibrio* spp. that has been claimed to pose a major concern in human and aquatic animals. For example, it can cause gastroenteritis (50, 51). *V. vulnificus* was reported as the third most predominant vibrio found in this study. *V. vulnificus* is one of the causative agents of the most severe diseases of marine and brackish aquaculture systems (52).

There were ten isolates of *Vibrio alginolyticus* that were identified in the examined catfish. According to (53), *V. alginolyticus* has the potential of increasing health risks for consumers. The presumptive biochemical tests indicated 10 (*V. parahaemolyticus*, *V. vulnificus*, *V. cholera*, *V. alginolyticus*, *V. furnissi*, *V. mimicus*, *V. anguillarum*, *V. mytili*, *Allivibrio fisheri*, *V. diazotrophic*, *V. xuii* and *V. guzogene*) *Vibrio* spp.

Comment [r75]: suggested to write this sentence as " A similar result was reported earlier also (16).

Comment [r76]: suggested to please rewrite this sentence as "*Vibrio parahaemolyticus* is known to cause gastroenteritis (50, 51).

Comment [r77]: suggested to please delete from here

Comment [r78]: please add reference (53) here

Comment [r79]: suggested to please insert ..the presence of ten different species (*V. parahaemolyticus*.....) of *Vibrio*.

Conclusion

In this study, ten *Vibrio* genera were isolated from the *Arius heudelotii* gills, skin, intestine and water samples, with *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* being the most common. These *Vibrio* species isolated has the ability to spread; cause high mortality and produce toxigenic strains that could contaminate the fish. It implies that *Vibrio* isolates can lead to serious pandemic causing serious health challenge to the final consumers of the fish. Fish farms especially those that harvest from the wild environments should equally embrace standard operating practices in cognizance of fish farming. They should be trained in hygienic practices.

Comment [r80]: italics and suffix/after word intestine of... it

Comment [r81]: Please clarify or replace

Comment [r82]: suggested to please use word "infect" in place of contaminate.

Comment [r83]: suggested to please replace final with "end "

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APPENDIX 1

ABIS IDENTIFICATION OF VIBRIO SPP ISOLATED FROM *ARIUS HEUDELOTII* AND WATER FROM NEW CALABAR RIVER

Comment [r84]: suggested to please expand ABIS in the text with appropriate name of the software maker etc.

SN	Cell	Gram	00% Naocl	10% Naocl	8% Naocl	Indole	Methv	Citrate	V	Motiliv	Catalase	Glucose	Lactose	Sucrose	Oxidase	Slant	Butt	Gase	H ₂ S	Suspecte d Isolates
1	Ro d	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
2	Ro d	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
3	Ro d	-	-	+	+	-	+	+	-	-	+	-	A G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
4	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
5	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
6	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
7	Ro d	-	-	+	+	+	+	-	+	+	+	A G	-	A G	+	B	A	-	-	<i>Vibrio alginolyticus</i>
8	Ro d	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
9	Ro d	-	-	+	+	+	-	-	+	-	+	A G	A G	A G	+	A	B	+	-	<i>Vibrio mytili</i>
10	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
11	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
12	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
13	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
14	Ro d	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
15	Ro d	-	-	+	+	-	+	+	-	-	+	-	A G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>
16	Ro d	-	-	+	+	+	+	+	+	-	+	-	A G	A G	+	A	A	-	-	<i>Vibrio diazotrophic</i>
17	Ro d	-	-	+	+	-	+	+	-	-	+	-	A	-	-	A	A	+	-	<i>Vibrio vulnificus</i>

3	d												G	G	G							
6	Ro	-	+	+	-	-	+	-	+	+	+		A	A	A	+	A	B	-	-		<i>Vibrio cholera</i>
4	d												G	G	G							
6	Ro	-	-	+	+	-	+	+	-	-	+		A	-	-	+	A	A	+	-		<i>Vibrio vulnificus</i>
5	d												G									
6	Ro	-	+	+	-	-	+	+	+	-	+		A	A-	-	+	B	A	-	-		<i>Vibrio mimicus</i>
6	d												-									
6	Ro	-	-	+	+	-	-	-	+	+	+		A	A	A	+	B	A	-	+		<i>Vibrio anguillarum</i>
7	d												G	G	G							
6	Ro	-	-	+	+	+	+	-	+	+	+		A	-	A	+	B	A	-	-		<i>Vibrio alginolyticus</i>
8	d												G		G							
6	Ro	-	+	+	-	-	+	-	+	+	+		A	A	A	+	A	B	-	-		<i>Vibrio cholera</i>
9	d												G	G	G							
7	Ro	-	-	+	+	-	-	-	-	-	+		-	-	A-	+	B	B	-	-		<i>Aliivibrio fischeri</i>
0	d																					
7	Ro	-	+	+	-	-	+	+	+	-	+		A	A-	-	+	B	A	-	-		<i>Vibrio mimicus</i>
7	d												-									
7	Ro	-	+	+	-	-	+	+	+	-	+		A	A-	-	+	B	A	-	-		<i>Vibrio mimicus</i>
7	d												-									
7	Ro	-	-	+	+	+	-	+	+	-	+		-	A	A	+	A	A	+	-		<i>Vibrio furnissii</i>
7	d														G							
7	Ro	-	-	+	+	+	+	-	-	+	+		A	-	A	+	B	A	-	-		<i>Vibrio Alginolyticus</i>
7	d												G		G							
7	Ro	-	-	+	+	-	+	+	-	-	+		-	A	-	-	A	A	+	-		<i>Vibrio vulnificus</i>
7	d												G									
7	Ro	-	-	+	+	+	-	+	+	-	+		-	A	A	+	A	A	+	-		<i>Vibrio furnissii</i>
7	d														G							
7	Ro	-	-	+	+	-	+	+	-	-	+		-	A	-	-	A	A	+	-		<i>Vibrio vulnificus</i>
8	d												G									
7	Ro	-	-	+	+	+	+	-	-	+	+		A	-	A	+	B	A	-	-		<i>Vibrio Alginolyticus</i>
9	d												G		G							
8	Ro	-	+	+	-	-	+	+	+	-	+		-	A-	-	+	B	A	-	-		<i>Vibrio mimicus</i>
0	d																					
8	Ro	-	-	+	+	+	-	+	+	-	+		-	A	A	+	A	A	+	-		<i>Vibrio furnissii</i>
8	d														G							
8	Ro	-	-	+	+	+	+	-	-	+	+		A	-	A	+	B	A	-	-		<i>Vibrio Alginolyticus</i>
8	d												G		G							
8	Ro	-	+	+	-	-	+	+	+	-	+		-	A-	-	+	B	A	-	-		<i>Vibrio mimicus</i>
8	d																					
8	Ro	-	+	+	+	-	+	-	+	+	+		A	-	A	+	B	A	-	-		<i>Vibrio cholera</i>
8	d												G		G							
8	Ro	-	-	+	+	-	+	+	-	-	+		-	A	-	+	A	A	+	-		<i>Vibrio vulnificus</i>
8	d												G									
8	Ro	-	-	+	+	-	-	-	+	+	+		A	A	A	+	B	A	-	+		<i>Vibrio anguillarum</i>

