Original Research Article

Isolation and Identification of *Vibrio* spp. from Catfish (Arius heudelotii) harvested from New Calabar River in Rivers State, Nigeria

ABSTRACT

This study was aimed at the isolation and identification of Vibrio spp. associated with *Arius heudelotii*, a catfish from a wild environment. Twenty-four *Arius heudelotii* (Catfish) were collected from the New Calabar River Choba, in Obio-Akpor LGA in Rivers State, Nigeria, and were processed to sample their skin, gills, and intestines. The Vibrio spp. associated with the gills, skins, and intestines of *Arius heudelotii* fish and water samples from the New Calabar River were isolated and identified. Ten *Vibrio* species *(V. vulnificus, V. cholerae, V. parahaemolyticus, V. alginolyticus, V. mimicus, V. mytili, V. anguilarum, V. furnissii, V. diazotrophic, Alliivibrio fisheri, V. xuii* and *V. gazogene*) were isolated and presumptively identified based on the morphological and biochemical test. A total of 96 *Vibrio* species were isolated 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* 18.6%, was the most predominant, this was followed by *V. Vulnificus* 17.7%, *V, Cholerae* 14.6%, *V. alginolyticus* 11.6%, *V. mimicus* 8.3%, *V. furnissii* 7.3%, *V. mytili* 6.3%, *V. anguilarum* 6.3%, and *Alliivibrio fisheri* 6.3% and *V. diazotrophic* was the least predominant 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 nutritious (4). It contains important vitamins that are essential for good health, such as vitamin K, which is responsible for anti-hemorrhage properties, and vitamin E known to reduce inflammation (5).

Various types of fish, *Arius heudelotti, Clarias gariepinus, Clarias batrachus, Tilapia, Heterobranchus nigrodi, Chrysichthys, Catlacatla* and others are readily available for human consumption. However, *Arius heudelotii*, an economic and nutritious food in Africa has been facing challenges for over a decade (8). *Arius heudelotii* is usually found in the brackish water, swampy, or paddy fields of wild environments and is consumed by individuals as food. *Arius* *heudelotti* has significant economic and nutritional values (6, 7). In recent times, the species have been declining in Africa, especially in Nigeria due to various unhygienic activities of man against the natural habitat. Over the years, the Nigerian aquaculture industry has suffered negligence and lack of adequate support to fish farmers until recently, due to incessant fall in oil production and prices. Another major problem affecting the growth of these species is pathogenic infections (9, 10).

Studies have shown that pathogenic infections are caused by the use of contaminated water from wild environments and artificial feeds which contributed to the high growth of several pathogens (11). In an attempt to curb the high rate of growth and development of infectious agents in cultured and wild environments, fish farmers used several antibiotics that have led to the emergence of various multidrug-resistant microorganisms in aquaculture (12). Aquaculture production has witnessed lower output as a result of contamination by these agents which hindered 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 the development of drug resistance by microorganisms (65). The aquaculture industry has suffered greatly due to the rapid spread of the disease. The infections of the fish especially caused by *Vibrio* spp., *Aeromonas* spp. e.tc has posed a significant challenge to the aquaculture industry and disrupted the fish supply chain globally. These microorganisms are opportunistic, gram-negative, rod-shaped, facultative anaerobic, oxidase-positive, motile or non-motile, and non-spore-forming bacteria (22,23,24,25,26,27). They are considered a major challenge for the development of the aquaculture sector in the face of environmental crisis (9,14,15,16,17,18). The World Bank estimates annual global losses at around $6 billion (19) due to bacterial infections.

Vibrio cholera is the most significant pathogenic *Vibrio* (28). Some non-O1 and non-O139 *V. cholera* exist and are often spread through contaminated water (33). 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). In the case of *Vibrio mimicus*, all *Vibrio* species are halophilic (require salt). Mimic species can produce cholera toxin (32). However, *Vibrio parahaemolyticus*, *Vibrio* *vulnificus,* and other pathogenic species exist (24). *Vibrio* bacteria that cause cholera include *Vibrio parahaemolyticus* and *Vibrio vulnificus* (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 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. 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, 30, 31). (32) stated that the majority of *Vibrio* infections occur in summer and occur in warm water.

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 the spread of antibiotic resistance (AMR) (41, 42, 43). 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.

Many researchers have noted that many species of *Vibrio* have been found in fish collected from both farmed and wild environments in Nigeria (54, 55, 56, 58). More importantly, most people in the Choba community and surrounding areas consume seafood from the New CalabarRiver. The risk of *Vibrio* infection and disease is high in Rivers, States and other parts of Nigeria, especially as consumption of *Arius heudelotii* is increasing in these areas. Hence, effective methods for isolation and identification of *Vibrio* spp. becomes paramount to enable first, health caregivers with understanding the types of Vibrio bacteria that make people sick. Second, it helps the aquaculture sector of the economy to adequately mitigate the high prevalence of the bacteria, thereby growing the aquaculture economy. Third, proper isolation and identification of these *Vibrio* species can improve antibiotic use and reduce resistance. Therefore, the study aimed at isolation and identification *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

"The New Calabar River and its tributaries are in Rivers State". It is a low deltaic river originating near Elele Alimini at about 5°10'N latitude. and 6°50'E. It flows south for about 150 km and ends at about 4°20'N. and 7°00 E. 0 to 50 m and 50 to 100 m Soils consist of clay, silt and sand and are rich in organic matter. A river flows or flows. Its peaks are freshwater, lowland, dense tropical rainforest and secondary forest/agricultural vegetation. However, the lower part is brackish and covered with mangrove swamps. The city's average annual temperature is usually between 25 and 28°C (46).

Live catfish (*Arius heudelott*i) freshly harvested by the fishermen at the river bank of the new Calabar River (Choba section) 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 separately in a stomacher bag containing 90ml sterile normal saline using a stomacher machine (44, 45).

The water samples were also collected from New Calabar River in 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 the New Calabar River was identified as *Arius heudelotii* by Department of Animal and Environmental Biology of Faculty of Sciences, Rivers State University, Npkolu-Oroworukwo, Port Harcourt, Rivers State.

The total number of Arius heudelotii randomly purchased from the New Calabar River over a period of 12 months were 24 and 24 water samples (every month, 2 Arius heudelotii, 2 water samples from Calabar River).

Pic 1: Catfish : *Arius heudolotti*
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. Tenfold serial dilution was carried out using normal saline as a diluent. Then 0.1ml of 10^{-2}, 10^{-3}, and 10^{-4} dilution levels were spread plated onto the medium in duplicate and incubated for 24 hours at $37^{o}C$. The bacterial colonies were counted after the incubation (48, 57, 58). The total viable count was determined with the formula below:

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

Identification of *Vibrio* spp.

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

Gram Staining

The Gram staining method was used as described in (59). During this process, the sample was smeared onto an oil-free microscope slide. The smear was heat-fixed by passing it through a Bunsen burner flame. The smear was flooded with crystal violet and allowed to stay for 60 seconds. The slide was washed off with water. The slide was flooded with Gram's iodine for 60 seconds and washed off with water, the smear was decolorized with 70% ethanol for 30 seconds and was washed off, and the smear was counterstained with safranin for 30 seconds and washed off. It was blotted dried and viewed at ×100 power (oil immersion) of the microscope (60).

Citrate Utilization Test

This test determines the ability of bacteria to use citrate as the sole source of carbon and energy and ammonium as the source of nitrogen for growth (61). The slant of Simmons citrate agar medium was inoculated with the 24 hours old culture of the test organism; the slant was streaked with a sterile wire loop while the butt of the medium was stabbed with a sterile inoculating needle. The tubes were incubated at 37°C for 48 hours. A colour change from green to blue indicates a positive effect, while maintaining the original green indicates a negative effect of citrate (59, 62).

Indole Production

The test determines the ability of certain bacteria to decompose the amino acid tryptophan to indole which accumulates in the medium. Sterile peptone water medium was inoculated with 24 hours old culture of the isolate and incubated at 37^{o} C for 24 to 48 hours. After incubation, 0.5ml of Kovac's reagent (P-dimethyl -amino benzaldehyde) was added and shaken gently. A red ring formation at the surface of the medium indicates a positive result (62, 63).

Methyl red-Voges Proskauer Test

The test determines the end products of glucose metabolism in bacteria. MR-positive organisms produce acid as their end products and VP-positive organisms produced 2, 3-butanediol, or acetyl methyl carbinol from the fermentation of pyruvic acid. The 24 hours old culture of the test organism was use to inoculate 10ml of sterile glucose phosphate medium using a sterile wire loop. The broth was incubated at 37^{o}C for 48 hours, after the incubation period the broth culture was divided into two parts, 5 drops of methyl red indicator were added to a part for methyl red test, and red colour formation indicated a positive result. For the VP test, 6 drops of 5% Alpha naphthol and 3 drops of 40% potassium hydroxide were added to the second half of the broth culture, it was gently shaken and left to stand for about 30mins. Positive organisms changed the colour of the medium to brick red (62).

Motility Test

This test was performed to determine the presence or absence of locomotive organelle characteristics such as flagella in bacteria. Tube method was used, with a sterile inoculation needle 18 hours old culture of the test organism was stabbed in the middle of the sterile semi-solid medium it was incubated at 37^{o}C for 24 -48 hours. After the incubation period, growth of organism was observed the tube was observed, diffusion of growth away from the stabbed line indicate a positive result (59, 63).

Triple Sugar Iron Agar

This test was used to indicate hydrogen sulphide (H_2S) production in addition to glucose and lactose fermentation with or without gas production. A sterile tube of triple sugar iron agar was inoculated with the test organism by streaking the slant and stabbing the butt and incubated at 37^{o}C for 48 hours. Hydrogen sulphide production was indicated by the blackening of the medium. Acid production was indicated by yellow colour development while gas production was shown by a crack or a split within the medium due to the tension production within the slope region signifies lactose utilization while at the butt signifies glucose (62).

Catalase Test

This test detects the presence of the enzyme catalase, which converts hydrogen peroxide to water and oxygen. There are two approaches to the catalase test. the slide approach and the test tube approach. For the slide approach (which was the one adopted). In line with (1), a drop of 3% hydrogen peroxide was placed on a glass slide. A sterile wire loop was used to pick up a colony from a culture plate and placed it in the drop. The presence of gas bubbles was indicative of a positive catalase test while a negative reaction was revealed by the absence of gas bubbles (1, 63).

Oxidase test

This test detects the presence of cytochrome c. oxidase that can reduce Oxygen. A filter paper was moistened with an oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride). With the aid of a sterile loop, a colony from an agar plate was smeared on the paper. The development of a purple colour within 10 seconds was indicative of a positive reaction (63).

Sugar Fermentation Test

A sugar fermentation test was performed to determine the ability of the isolates to metabolize some carbohydrates such as glucose, lactose, maltose, and sucrose with the production of either acid, gas, or both. one gram of each sugar was added with 1.5g of peptone dissolved in 100 ml distilled water. 0.3ml of bromocresol purple indicator was added to four (4) conical flasks containing the mixture and the flask was shaken properly. The mixture was dispensed into the test tubes and inverted Durham's tubes were added to the test tubes. The mixture was sterilized at 121^{o}C and 15psi for 15 minutes. The test tubes were inoculated with test isolates and incubated at 37^{o}C for 48 hours. Air spaces or vacuum in Durham's tube indicated gas production while a change in the colour of the indicator from purple-blue to yellow showed acid production (59, 63, 64).

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 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 (Table 1). 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* spp 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).

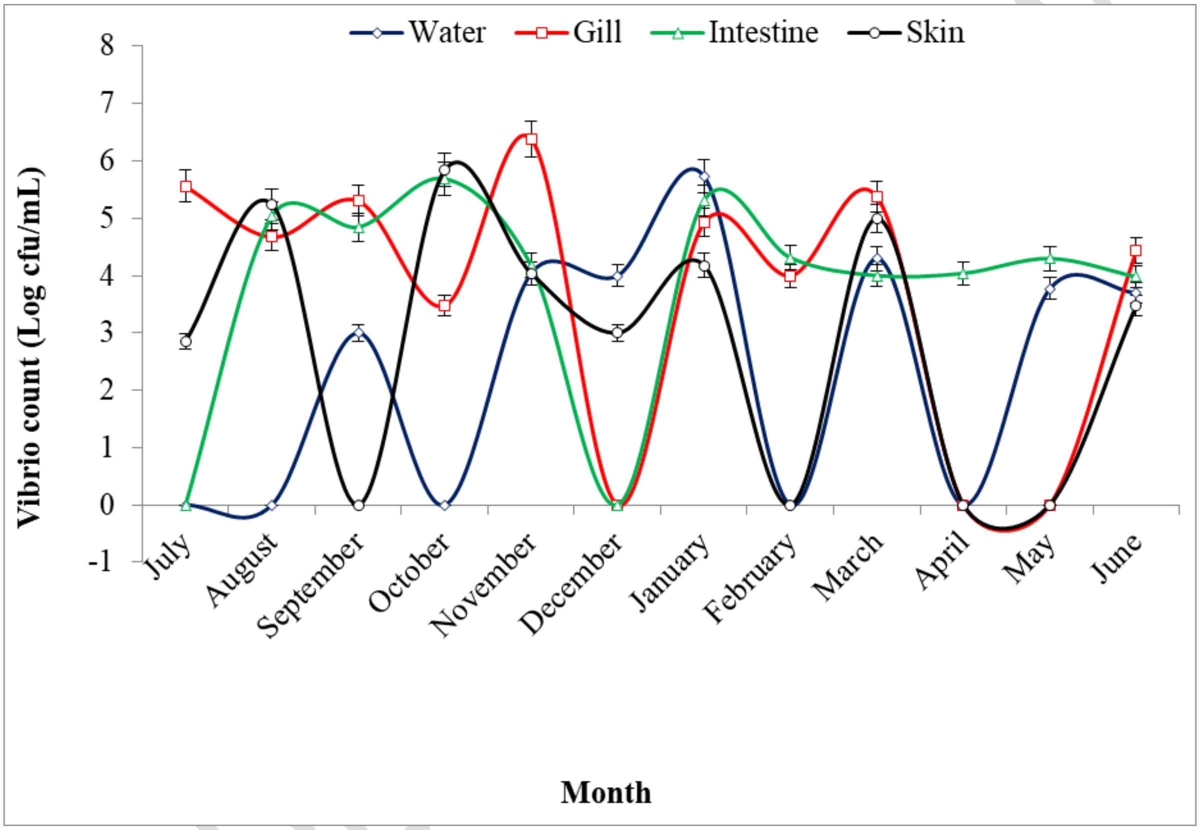

Figure 1: Total *Vibrio* Counts (TVC) from Water and Fish Parts Gills, Skin, and Intestine of Catfish (*Arius heudoletii*) Harvested from New Calabar River**.**

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

		Arius Heudelotii		
Parameters	Gills	Intestine	Skin	Water

July	0.00±0.00^{d}	0.00±0.00^{b}	2.07±1.06^{cde}	1.00±1.41^{cd}
August	5.80±0.70^{ab}	4.67±0.52^{a}	5.36±0.16^{ab}	0.00±0.00^{d}
September	7.17±0.14^{a}	4.77±0.10^{a}	0.00±0.00^{e}	2.65±0.49^{bc}
October	4.73±0.83^{bc}	5.12±0.81^{a}	5.84±0.00^{a}	0.00±0.00^{d}
November	5.76±0.44^{ab}	3.98±0.27^{a}	3.67±0.52^{abcd}	3.67±0.52^{ab}
December	3.55±0.62^{c}	0.50±0.70^{b}	2.66±0.47^{bcde}	3.54±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 ± 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, 31, 11, 24, and 30 *Vibrio* species were isolated from gills, skin, intestine *Arius heudelotii*, and water from New Calabar River, respectively. The study showed that the incidence of *Vibrio* species was 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 heudolotti*, *V. vulnificus* was the most predominant 7/31 (22.5%), this was followed by *V. Cholerae* and *V. parahaemolyticus* 5/31 (16.1%) each; *V. mimicus* 3/31 (9.6%), *V. alginolyticus*, *V. mytili*, *V. anguilarum*, *V.furnissi*, and *Alliivibrio fisheri* 2/31(6.5%) each. *V.diazotrophic* was the least predominant 1/31 (3.2%) in the gills. However, *V. Xuii* and *V.gazogene* were not found in the gills (Table 2).

Prelavance of *Vibrio* spp. from the skin of *Arius heudelotii*

The results of *Vibrio* species isolated from the skin of *Arius heudolotti* are presented in the same Table 2, indicating that *V. parahaemolyticus* had the highest 3/11 (27.3%) (Figure 2). While *V. vulnificus* and *V. alginolyticus* had 2/11 (18.2%) each, followed by *V. cholerae*, *V. mimcus*, *V. diazotrophic*, and *Alliivibrio fisheri* that were least predominant (1/11 (9.1%). *V. mytili*, *V. anguilarum*, *V. furnissi*, *V. Xuii*, and *V.gazogene* 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 incidence 5/24 (20.8%) followed by *V. parahaemolyticus* and *V. alginolyticus* 4/24 (16.7%) each. Other recovered *Vibrio* species are *V. Cholerae* 3/24 (12.5%) and *V.mimicus*, *V.furnissii*, and *V. Anguilarum* 2/24 (8.3%) each. While *Alliivibrio fisheri* and *V. mytili* had the lowest 1/24 (4.2%). Also, *V. Diazotrophic*, *V. Xuii* and *V.gazogene* were not found in the intestine of *Arius heudelotii* (Table 2)

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. Parahaemolyticus* 6/30 (20.0%) followed by *V. Cholerae* 5/30 (16.7). More so, Table 2 revealed that *V. vulnificus*, *V. alginolyticus*, *V. mytili* and *V. furnissi* had prevelance percentage of 10%. While *V. mimicus*, *V. anguilarium* and *Alliivibrio fisheri* had 2/30 (6.6%) each. The lowest 1/30 (3.3%) predominant of *Vibrio* species was *V. diazotrophic*. The 31.2% of Vibrio species isolated from water sample is similar to the reports by (66,67) in various wild environments. The result could be associated to high salanity and pollution of New Calabar River (46). Temperature and pH are also determinants of the occurrence of bacteria such as *Vibrio* in the marine environment (68).

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

	Vibrio Positive samples	No. of isolates for		species
Fish Parts and water	No. (%)	No.	(%)	species

Arius heudolotti	Gills	31(32.3)	7	22.5	*V.vulnificus*
			5	16.1	*V. cholerae*
			5	16.1	*V. parahaemolyticus*
			2	6.5	*V. alginolyticus*
			3	9.6	*V.mimicus*
			2	6.5	*V. mytili*
			2	6.5	*V. anguilarum*
			2	6.5	*V.furnissi*
			1	3.2	*V.diazotrophic*
			2	6.5	*Alliivibrio fisheri*
	Skin	11(11.5)	2	18.1	*V.vulnificus*
			1	9.1	*V. cholerae*
			3	27.3	*V. parahaemolyticus*
			2	18.2	*V. alginolyticus*
			1	9.1	*V.mimicus*
			1	9.1	*V.diazotrophic*
			1	9.1	*Alliivibrio fisheri*
	Intestine	24(25)	5	20.8	*V.vulnificus*
			3	12.5	*V. cholerae*
			4	16.7	*V. parahaemolyticus*
			4	16.7	*V. alginolyticus*
			2	8.3	*V.mimicus*
			1	4.2	*V. mytili*
			2	8.3	*V. anguilarum*
			2	8.3	*V.furnissi*
			1	4.2	*Alliivibrio fisheri*
	Water	30(31.2)	3	10.0	*V.vulnificus*
			5	16.7	*V. cholerae*
			6	20.0	*V. parahaemolyticus*
			3	10.0	*V. alginolyticus*
			2	6.7	*V.mimicus*
			3	10.0	*V. mytili*
			2	6.7	*V. anguilarum*
			3	10.0	*V.furnissi*
			1	3.3	*V.diazotrophic*
			2	6.6	*Alliivibrio fisheri*
	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).

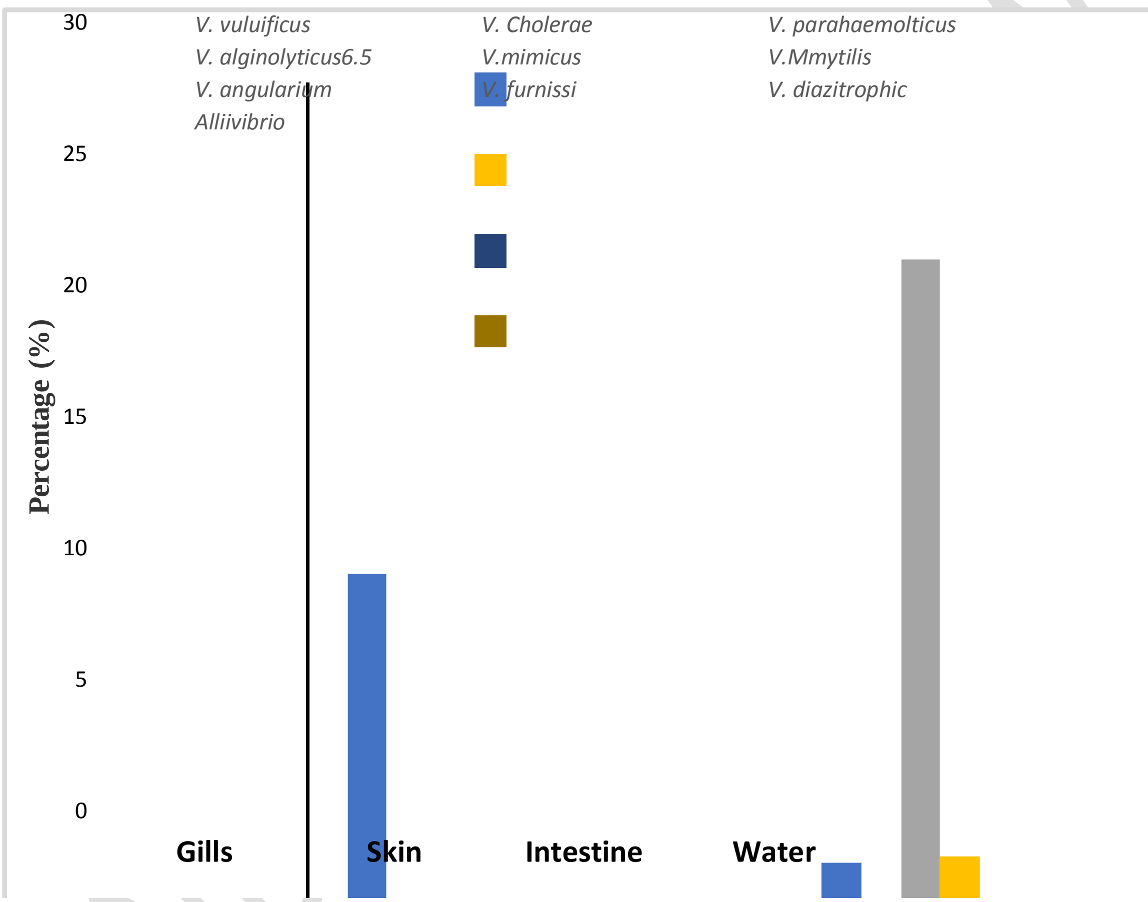

Figure 2: Prevalence of *Vibrio* species from *Arius heudelotii (Gills, Skin, Intestine)* and water from New Calabar River

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 earlier also (16). *Vibrio parahaemolyticus* is known to cause gastroenteritis human and other sicknesses in humans (50, 51, 52). Especially, when the seafood not well cooked before consumption (69). *V. vuluificus* was reported as the third most predominant vibrio found in this study. *V. vuluificus* 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. *V. alginolyticus* has the potential of increasing health risks for consumers (53). The presumptive biochemical tests indicated the presence of ten different species (*V. parahaemolyticus*, *V. vulnificus*, *V. cholera*, *V. alginolyticus*, *V. furnissii*, *V. mimicus*, *V. anguilarum*, *V. mytili*, *Alliivibrio fisheri*, *V. diazotraphic*, *V. xuii* and *V. gazogene) Vibrio* spp.

Conclusion

In this study, ten *Vibrio* genera were isolated from the gills, skin, intestine of *Arius heudelotii* 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 bacterial agents that could infect the fish. It implies that Vibrio isolates can lead to serious pandemic causing serious health challenge to the end 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.

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APPENDIX 1

ADVANCED BACTARIAL IDENTIFICATION SOFTWARE (ABIS) IDENTIFICATION OF VIBRIO SPP ISOLATED FROM *ARIUS HEUDELOTII* AND WATER FROM NEW CALABAR RIVER

SN	Cell M	Gram	0% Nacl	1% Nacl	8% Nacl	Indole	Methy	Citrate	V	Motility	Catalase	Glucose	Lactose	Sucrose	Oxidase	Slant	Butt	Gase	H_{2S}	*Suspected Isolates*
1	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
2	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
3	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
4	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
5	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
6	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
7	Rod	-	-	+	+	+	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio alginolyticus*
8	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
9	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	A	B	+	-	*Vibrio mytili*
10	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
11	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
12	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
13	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*

14	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
15	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
16	Rod	-	-	+	+	+	+	+	+	-	+	-	AG	AG	+	A	A	-	-	*Vibrio diazotrophic*
17	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
18	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
19	Rod	-	-	+	+	+	+	+	+	-	+	-	AG	AG	+	A	A	-	-	*Vibrio diazotrophic*
20	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio mytili*
21	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio parahaemolyticus*
22	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio mytili*
23	Rod	-	+	+	+	-	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio cholera*
24	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio mytili*
25	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
26	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
27	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
28	Rod	-	-	+	+	-	-	+	+	-	+	A-	A-	A-	+	A	A	-	-	*Vibrio vulnificus*
29	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
30	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
31	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
32	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
33	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
34	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
35	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
36	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*

37	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
38	Rod	-	-	+	+	+	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio alginolyticus*
39	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
40	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	A	B	+	-	*Vibrio mytili*
41	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
42	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
43	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
44	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
45	Rod	-	-	+	+	+	+	+	+	-	+	AG	-	-	+	B	A	-	-	*Vibrio parahaemolyticus*
46	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
47	Rod	-	-	+	+	+	+	+	+	-	+	-	AG	AG	+	A	A	-	-	*Vibrio diazotrophic*
48	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
49	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
50	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
51	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio mytili*
52	Rod	-	-	+	+	+	-	-	+	-	+	AG	AG	AG	+	B	A	+	-	*Vibrio parahaemolyticus*
53	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
54	Rod	-	+	+	+	-	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio cholera*
55	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
56	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
57	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
58	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
59	Rod	-	-	+	+	-	-	+	+	-	+	A-	A-	A-	+	A	A	-	-	*Vibrio vulnificus*

60	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
61	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
62	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
63	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
64	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
65	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
66	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
67	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
68	Rod	-	-	+	+	+	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio alginolyticus*
69	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
70	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	*Aliivibrio fischeri*
71	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
72	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
73	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
74	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
75	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
76	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
77	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
78	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	-	A	A	+	-	*Vibrio vulnificus*
79	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
80	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
81	Rod	-	-	+	+	+	-	+	+	-	+	-	A	AG	+	A	A	+	-	Vibrio furnissii
82	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*

83	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
84	Rod	-	+	+	+	-	+	-	+	+	+	AG	-	AG	+	B	A	-	-	*Vibrio cholera*
85	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
86	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
87	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
88	Rod	-	+	+	-	-	+	+	+	-	+	A-	A-	-	+	B	A	-	-	*Vibrio mimicus*
89	Rod	-	-	+	+	-	-	+	+	-	+	A-	A-	A-	+	A	A	-	-	*Vibrio vulnificus*
90	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
91	Rod	-	-	+	+	-	-	-	+	+	+	AG	AG	AG	+	B	A	-	+	*Vibrio anguilarum*
92	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
93	Rod	-	+	+	-	-	+	-	+	+	+	AG	AG	AG	+	A	B	-	-	*Vibrio cholera*
94	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*
95	Rod	-	-	+	+	-	+	+	-	-	+	-	AG	-	+	A	A	+	-	*Vibrio vulnificus*
96	Rod	-	-	+	+	+	+	-	-	+	+	AG	-	AG	+	B	A	-	-	*Vibrio Alginolyticus*