

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. parahaemoliticus*, *V. alginolyticus*, *V.mimicus*, *V. mytili*, *V. angular*, *V.furnissi*, *V.diazotrophic*, *Alliivibrio 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, respectively. Among the vibrio species isolated, *V. Parahaemoliticus* 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 *Alliivibrio 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 heudelotti*, *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 heudelotti* 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

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

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).



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:

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

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

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).

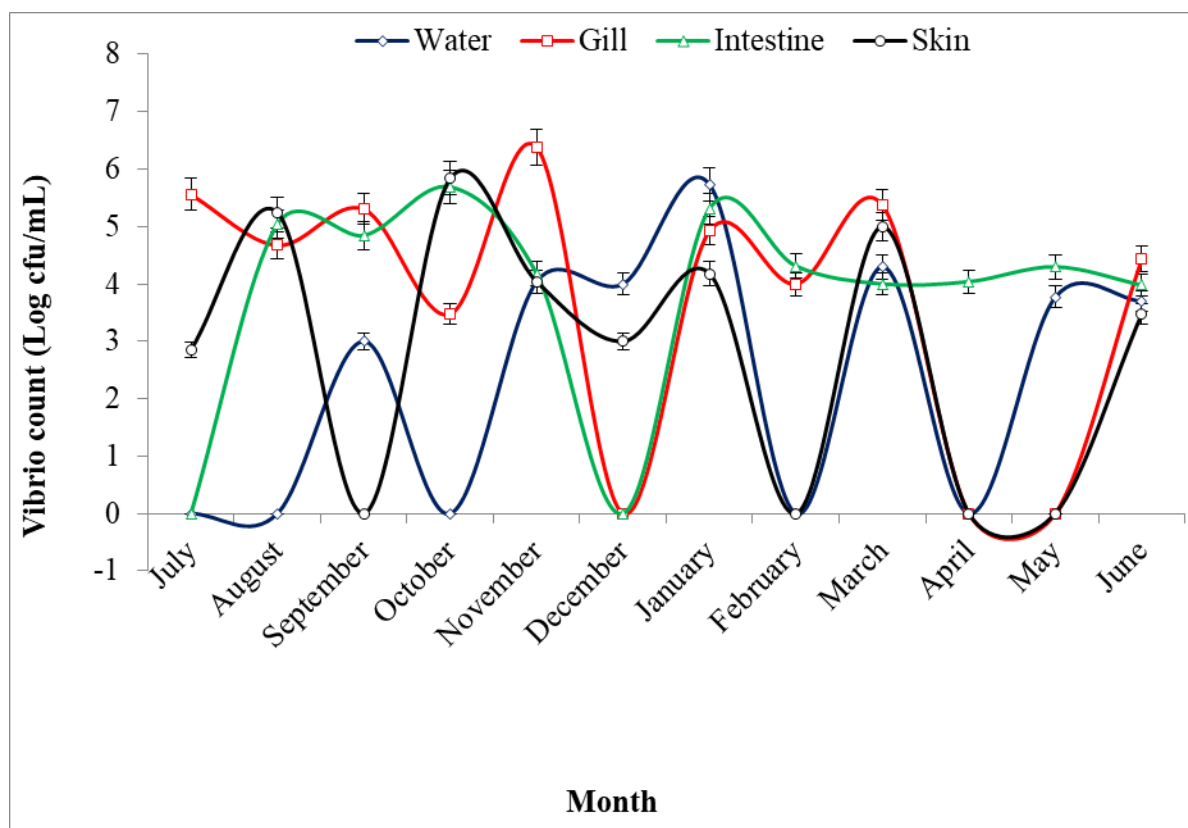


Figure 1: Total *Vibrio* Counts (TVC) from Water and Fish Parts Gills, Skin, and Intestine of Catfish (*Arius heudelotii*) Harvested from New Calabar River.

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 ^{bcde}	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 *Vibriosp.* 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.*

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 heudelotii*, *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)

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*.

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>Alliivibrio 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. anguillarum</i>	
2			8.3	<i>V.furnissi</i>	
1			4.2	<i>Alliivibrio fisheri</i>	
Water	30(31.2)	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. anguillarum</i>	
		3	10.0	<i>V.furnissi</i>	
		1	3.3	<i>V.diazotrophic</i>	
		2	6.6	<i>Alliivibrio 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).

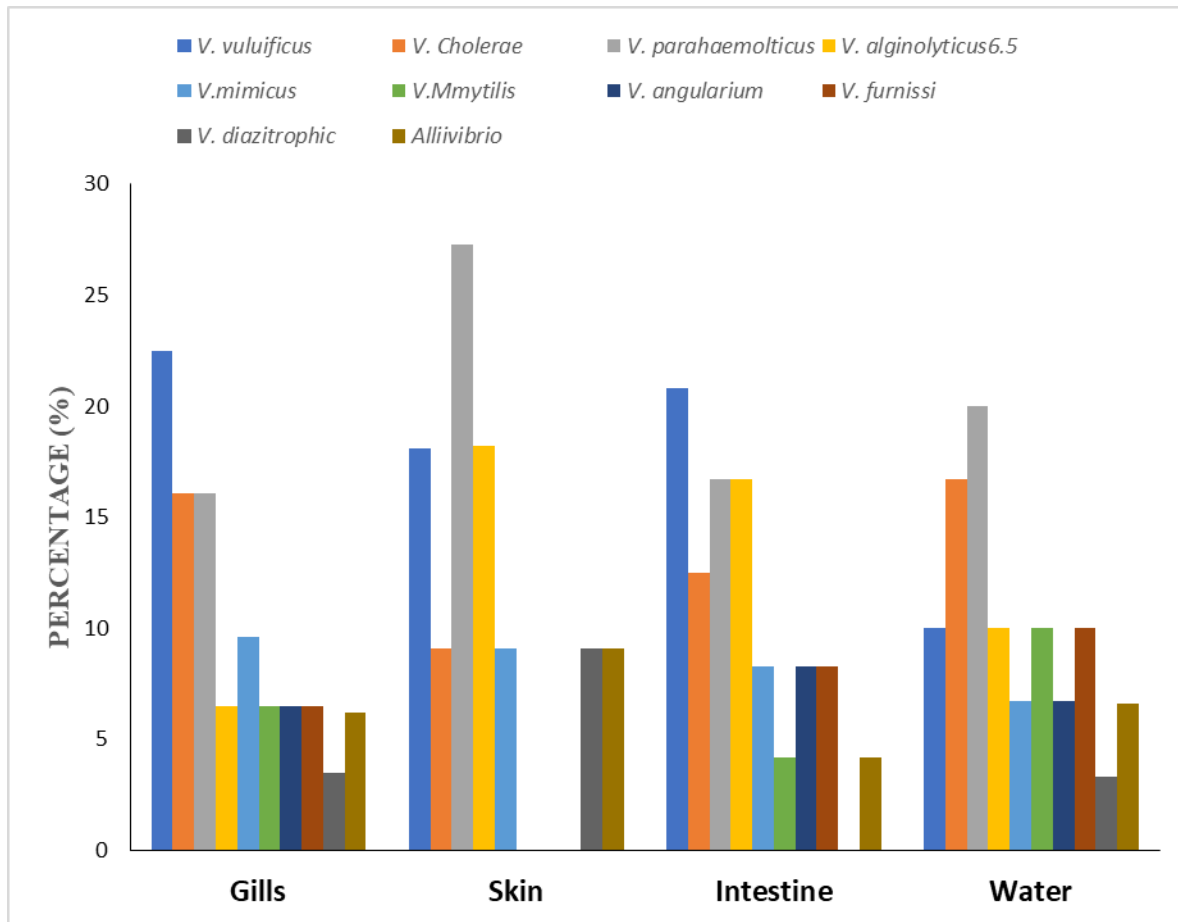


Figure 2: Prevalence of *Vibrio* species from *Arius heudelotii* (Gills, Skin, Intestine) and water from New Calabar River

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. 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. According to (53), *V. alginolyticus* has the potential of increasing health risks for consumers. The presumptive biochemical tests indicated 10 (*V. parahaemolyticus*, *V. vuluificus*, *V. cholera*, *V. alginolyticus*, *V. furnissi*, *V. mimicus*, *V. anguillarum*, *V. mytili*, *Alliivibrio fisheri*, *V. diazotrophic*, *V. xuii* and *V. guzogene*) *Vibrio spp.*

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.

REFERENCES

1. Olugbojo, J. A & Ayoola, S. O. (2015). Comparative studies of bacteria load in fish species of commercial importance at the aquaculture unit and lagoon front of the University of Lagos. *International Journal of Fisheries and Aquaculture*. 7(4): 37-46. Doi: 10.5897/IJFA
2. Rahman, A., Hussain, Md. A., Ahmmed, M. K., & Bekhit, A, E. A. (2022). Safety considerations in fish roe products, Elsevier BV, 2022
3. Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases microbe infection 2: 1651-1660
4. Soheir, S., Abd El-salam, F. M., Ghaly, Dina M., Baraka Shahira H.M., and Abeer A.E. (2016). Isolation and Identification of Bacterial Flora from /catfish (*Clarias gariepinus*) with Antimicrobial susceptibility and Herbal sensitivity. *Journal of pure and Applied Microbiology*. 10(3), 1835-1846.
5. Thomas, O. I. and Adenik, B. A. (2021). Seasonal variations in Microbial load and Heavy metal concentrations in fish from Yenagoa metropolis, Nigeria. *Journal of Applied Health Sciences and Medicine* 1(1)2021: 13-29.
6. Olajide, J. A., Oluwole, O. O., Fayokemi, C. & Olususi (2020). Assessment of bacterial loads of *Clarias gariepinus* (Bushell 1822) obtained from cultured and natural habitats. *The Journal of Basic and Applied Zoology*. 81:32. Doi.org/10.1186/s41936-020-00168-w
7. Food and Agriculture Organization (FAO) (2002). The State of World Fisheries and Aquaculture 2002. Rome: Food and Agriculture Organization of the United Nations, Fisheries Department. Available online at:

<http://www.fao.org/3/y7300e/y7300e00.pdf> (accessed November 6, 2022)

8. Food and Agriculture Organization of the United Nations, (2020). The state of world fisheries and aquaculture. Available at: <http://www.fao.org/state-of-fisheries-aquaculture> [Accessed on 18 December 2022].
9. Mulia DS, Isnansetyo A, Pratiwi R, Asmara W. (2021). Antibiotic resistance of *Aeromonas* spp. isolated from diseased walking catfish (*Clarias* sp.). *Biodiversitas* 22: 4839-4846.
10. Ahmed, R., Pandey, R. B., Arif, S. H., Nabi, N., Jabeen, M., & Hasnain, A. (2012). Polymorphic β and γ lens crystalline demonstrate latitudinal distribution of threatened walking catfish *Clarias batrachus* (Linn.) populations in north-western India. *Journal of Biological Sciences* 12: 98-104.
11. Food and Agriculture Organization of the United Nations (FAO). World aquaculture 2010: FAO fisheries and aquaculture technical paper. Rome: FAO 2011
12. Adebayo-Tayo, B. C., Okonko, I. O., John, M. O., Odu, N. N., Nwanze, J. C., & Ezediokpu, M. N. (2011). Occurrence of Potentially Pathogenic *Vibrio* Species in Sea Foods Obtained from Oron Creek. *Advances in Biological Research*, 5(6), 356–365. Retrieved from <https://pdfs.semanticscholar.org/fed8/b0005b892ffb72c48ab6139d0ec88778d629.pdf>.
13. Bondad-Reantaso, M. G., Subasinghe, R.P., Arthur, R. J., Ogawa, K., Chinabut, S., Adlard, R., Tan, Z. and Shariff, M. (2005). Disease and health management in Asian aquaculture. *Veterinar Parasitol.* 30:132(3-4):249-272.
14. Stratev, D. & Odeyemi, A. O. (2016). Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review *Journal of Infection and Public Health*, 9(5), 535—544. <https://doi.org/10.1016/j.jiph.2015.10.006>
15. Zehui, Y. U., Eriong, W., Yi, G., Kaiyu, W., Defang, C., Xiaoli, H., Ping, O., Zhicai, Z., Chao, H., Jing, F., Lizi, Y., Hongrui, G. & Zhijun, Z. (2020). Complete genome analysis of *Vibrio mimicus* strain SCCFO1, a highly virulent isolate from the freshwater catfish, *Virulence*, 11(1), 23-31. DOI: 10.1080/21505594.2019.1702797.
16. Suresh, Y., N. Subhashini, Ch. Bindu Kiranmayi, K. Srinivas, V. Prasastha Ram, G Chaitanya, B. Swathi Vimala and Srinivasa Rao, T. (2018). Isolation, Molecular Characterization and Antimicrobial Resistance Patterns of Four Different *Vibrio* Species

- Isolated from Fresh Water Fishes. *International Journal Current Microbiology Applied Science* 7(07): 3080-3088. doi: <https://doi.org/10.20546/ijcmas.2018.707.359>.
17. Abdelaziz, M., Ibrahim, M. D., Ibrahim, M. A., Abu-Elala, N. M., Abdel-moneam, D. A (2017). Monitoring of different *Vibrio* species affecting marine fishes in Lake Qarun and Gulf of Suez: Phenotypic and molecular characterization. *Egypt Journal Aquaculture Research*, 43 (2): 141-146
 18. Martnez-Murcia, A., Beaz-Hidalgo, R., Navarro, A., Carvalho, M. J., Aravena-Roman, M., Correia, A., Figueras, M. J., Saavedra, M. J. (2016) *Aeromonas lusitana* sp. nov., isolated from untreated water and vegetables. *Curr. Microbiology*, 72, 795-803.
 19. Debnath, S.C., McMurtrie, J., Temperton, B., Delamare-Deboutteville, J., Vishnumurthy, M., & Tyler, C. (2023). Tilapia aquaculture, emerging diseases, and the roles of the skin microbiomes in health and disease. *Aquaculture International*. <https://doi.org/10.1007/s10499-023-01117-4>
 20. Leung, T. L. F., & Bates, A. E. (2013). More rapid and severe disease outbreaks for aquaculture at the tropics: Implications for food security. *Journal of Applied Ecology*, 50(1), 215–222. <https://doi.org/10.1111/1365-2644.12017>
 21. Al-sunaiher, A. E., Ibrahim, A. S. S., & Al-salamah, A. A. (2010). Association of *Vibrio* Species with Disease Incidence in Some Cultured Fishes in the Kingdom of Saudi Arabia. *World Applied Sciences Journal*, 8(5), 653–660.
 22. Zeng, Q., Liao, C., Terhune, J., & Wang, L. (2019). Impacts of florfenicol on the microbiota landscape and resistome as revealed by metagenomic analysis. *Microbiome*, 7(1), 155. <https://doi.org/10.1186/s40158-019-0773-8>
 23. Zeng, Q., Liao, C., Terhune, J., & Wang, L. (2019). Impacts of florfenicol on the microbiota landscape and resistome as revealed by metagenomic analysis. *Microbiome*, 7(1), 155. <https://doi.org/10.1186/s40158-019-0773-8>
 24. Percival, S.L. and Williams, D.W. (2014) *Escherichia coli* (Chapter 6). In: Percival, S.L., Yates, M.V., Williams, D.W., et al., Eds., *Microbiology of Waterborne Diseases* (Second Edition), Elsevier Ltd., Amsterdam, 89-117
 25. Romalde, J. L., Dieguez, A. L., Lasa, A., & Balboa, S. (2014). New *Vibrio* species associated to molluscan microbiota: A review. *Frontiers in Microbiology*, 4(JAN), 1–11.

<https://doi.org/10.3389/fmicb.2013.00413>

26. Abdellrazeq, G. S., & Khaliel, S. A. (2014). Molecular characterization and antimicrobial susceptibility of *Vibrios* isolated from healthy and diseased aquacultured freshwater fishes. *Global Veterinaria*, 13(3), 397–407. 61 ref. <https://doi.org/10.5829/idosi.gv.2014.13.03.85107>
27. Rad, M., & Shahsavani, D. (2010). Isolation and characterization of *Vibrio* (*Listonella*) *anguillarum* from catfish. *Turkish Journal of Veterinary and Animal Sciences*, 34(4), 413–415. <https://doi.org/10.3906/vet-0812-5>
28. Okada, K., Iida, T., Kita-Tsukamoto, K., & Honda, T. (2005). *Vibrios* commonly possess two chromosomes. *Journal of Bacteriology*, 187(2), 752–757. <https://doi.org/10.1128/JB.187.2.752-757.2005>
29. You KG, Bong CW, Lee CW (2016). Antibiotic resistance and plasmid profiling of *Vibrio* spp. in tropical waters of Peninsular Malaysia. *Environmental Monitoring and Assessment*, 188(171).
30. Valente C. D & Wan A.H.L (2021). *Vibrio* and major commercially important vibriosis diseases in decapod crustaceans. *Journal of Invertebrate Pathology* 181 (2021) 107527 <https://doi.org/10.1016/j.jip.2020.107527>
31. Sultana, M., Nusrin, S., Hasan, N. A., Sadique, A., Ahmed, K. U., Islam, A., & Hossain, A. (2018). crossm Biofilms Comprise a Component of the Annual Cycle of *Vibrio cholerae* in the Bay of Bengal Estuary. *American Society for Microbiology*, 9(2), 1–13.
32. El-Bouhy, Z., El-Nobi, G., El-Murr, A., & Abd El-Hakim, S. (2016). Study on Vibriosis in *MugilCapito* in El-Dakahlia and Damitta Governorates, Egypt. *Abbassa International Journal Aquaculture*. 9(1), 19.
33. Dziejman, M., Serruto, D., Tam, V. C. & Mekalanos, J. J (2005). Genomic characterization of non-O1, non-O139 *Vibrio cholerae* reveals genes for a type III secretion system/ 102(9) 3465-3470. <https://doi.org/10.1073/pnas.0409918102>
34. Novotny, L.; Dvorska, L.; Lorencova, A.; Beran, V.; Pavlik, I. (2004). Fish: A potential source of bacterial pathogens for human beings. *Veterinární Medical*, 49, 343–358
35. Chonsin, K. S., Matsuda, C., Theethakaew, T., Kodama, J., Junjhon, Y., Suzuki, O., Suthienkul and Iida, T.(2016). Genetic diversity of *Vibrio parahaemolyticus* strains

- isolated from farmed Pacific White shrimp and ambient Pond water affected by acute hepatopancreatic necrosis disease outbreak in Thailand. *FEMS Microbiology Letters* 363: fnv 222.
36. Lloyd, N .A., Nazaret, S. & Barkay, T. (2019). Genome-facilitated discovery of RND efflux pump-mediated resistance to cephalosporins in *Vibrio* spp. isolated from the mummichog fish gut, *Journal of Global Antimicrobial Resistance*,19, 294-300
<https://doi.org/10.1016/j.jgar.2019.05.006>
 37. Plaza, N., Castillo, D., Pérez-reytor, D., Higuera, G., García, K., & Bastías, R. (2018). Bacteriophages in the control of pathogenic vibrios. *Electronic Journal of Biotechnology*, 31, 24–33. <https://doi.org/10.1016/j.ejbt.2017.10.012>
 38. Harrison, J., Nelson, K., Morcrette, H., Morcrette, C., Preston, J., Helmer, L., Titball, R. W., Butler, C. S. & Wagley, S. (2022). The increased prevalence of *Vibrio* species and the first reporting of *Vibrio jasicida* and *vibrio rotiferianus* at UK Shellfish sites. *Water Research*, 211, 117942
 39. Sheng, I and Wang, I. (2020). The microbial safety of fish and fish products: recent advances in understanding its significance, contamination sources, and control strategies. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 738–786.
<https://doi.org/10.1111/1541-4337.12671>
 40. Osunla, C. A., & Okoh, A. I. (2017). *Vibrio* pathogens: A public health concern in rural water resources in sub-Saharan Africa. *International Journal of Environmental Research and Public Health*, 14(10), 1–27. <https://doi.org/10.3390/ijerph14101188>
 41. Ram, Y. Dellus - Gut, E. Bibi, M and Hadany, L. (2019) Predicting Microbial growth in a mixed culture from growth curve data proceedings of the National Academy of Sciences 116(29): 14698-14707.
 42. Brunton, L. A., Desbois, A. P., Garza, M., Wieland, B., Mohan, C. V., Hasler, B.,...Guitian, J. (2019). Identifying hotspots for antibiotic resistance emergence and selection, and elucidating pathways to human exposure: Application of a systems-thinking approach to aquaculture systems. *Science of the Total Environment*. 687, 1344-1356.
 43. Preena, P. G., Swaminathan, T. R., Kumar, V. J. R., & Sing, I. S. B. (2020). Antimicrobial resistance in aquaculture: a crisis for concern. *Biologia*, 75, 1497-1517.
[Doi.org/10.2478/s11756-020-00456-4](https://doi.org/10.2478/s11756-020-00456-4)

44. Hoseinifar, S. H., Alireza M., & Merrifield D. L. (2011). The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*)", *Aquaculture*, 2011
45. Al-Harbi, A.H. (2005) "Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia", *Aquaculture*, 20051230.
46. Nwankwoala, H. O. An Evaluation of Heavy Metals Concentration in the Choba Section of the New Calabar River, Eastern Niger Delta, *Biodiversity International Journal*, 2017
47. Braide, W, Judith N, Samuel A. A, & Emmanuel O. E. (2016). Effects of Gas Flaring on the Physicochemical and Microbiological Quality of Water Sources in Egbema, Imo State, Nigeria, *International Letters of Natural Sciences*, 2016
48. Ong, H. M. G., Zhong, Y., Hu, C. C., Ong, K. H., Khor, W. C., Schlundt, J., & Aung, K. T. (2023). Quantitative Risk Evaluation of Antimicrobial-Resistant *Vibrio parahaemolyticus* Isolated from Farmed Grey Mullet in Singapore. *Pathogens*, 12(1), 93.
49. Mohamed Abdelaziz, Mai D. Ibrahim, Marwa A. Ibrahim, Nermeen M. Abu-Elala, Dalia A. Abdel-moneam. "Monitoring of different vibrio species affecting marine fishes in Lake Qarun and Gulf of Suez: Phenotypic and molecular characterization", *The Egyptian Journal of Aquatic Research*, 2017
50. He, M.; Lei, T.; Jiang, F.; Zhang, J.; Zeng, H.; Wang, J.; Chen, M.; Xue, L.; Wu, S.; Ye, Q.; et al. (2021). Genetic Diversity and Population Structure of *Vibrio parahaemolyticus* Isolated from Clinical and Food Sources. *Front. Microbiology*. 12, 708795.
51. Francisca, G. R., Menezes, D. E., Marina, T. T., Rodnguez Fatima, C. T., De Carvalho, Ford, C. I., Powell, A., Lau, D. Y.I., Turner, A. D., Dhanji-Rapkova, M., Martinez-Urtaza, J., & Baker-Austine, C. (2020). Isolation and characterisation of potentially pathogenic *Vibrio* species in a temperature, higher latitude hotspot. *Environmental Microbiology Reports* 12(4): 424-434.
52. Sony, M., Sumithra, T. G., V.N. Anusree, V. N., Amala, P. V., Reshma, K. J., Alex, S., Sanil, N. K. (2021) Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. *Aquaculture*. <https://doi.org/10.1016/j.aquaculture.2021.736608>

53. Baker - Austin C., Stockley, L., Rangdale, R., and Martinez- Urtraza, J (2010). Environmental occurrence and Clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. *Environmental Microbiology report*, 2, 7-18.

APPENDIX 1

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

SN	Cell	Gram	0% NaCl	1% NaCl	8% NaCl	Indole	Methyl	Citrate	V	Motility	Catalase	Glucose	Lactose	Sucrose	Oxidase	Slant	Butt	Gase	H ₂ S	Suspected Isolates
1	Rod	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
2	Rod	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
3	Rod	-	-	+	+	-	+	+	-	-	+	-	A G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
4	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
5	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
6	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
7	Rod	-	-	+	+	+	+	-	+	+	+	A G	-	A G	+	B	A	-	-	<i>Vibrio alginolyticus</i>
8	Rod	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
9	Rod	-	-	+	+	+	-	-	+	-	+	A G	A G	A G	+	A	B	+	-	<i>Vibrio mytili</i>
10	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
11	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
12	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
13	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
14	Rod	-	-	+	+	+	+	+	+	-	+	A G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>
15	Rod	-	-	+	+	-	+	+	-	-	+	-	A G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>
16	Rod	-	-	+	+	+	+	+	+	-	+	-	A G	A G	+	A	A	-	-	<i>Vibrio diazotrophic</i>
1	Ro	-	-	+	+	-	+	+	-	-	+	-	A	-	-	A	A	+	-	<i>Vibrio vulnificus</i>

7	d												G							
18	Rod	-	-	+	+	+	+	-	-	+	+	A	-	A	+	B	A	-	-	<i>Vibrio Alginolyticus</i>
19	Rod	-	-	+	+	+	+	+	+	-	+	-	A	A	+	A	A	-	-	<i>Vibrio diazotrophic</i>
20	Rod	-	-	+	+	+	-	-	+	-	+	A	A	A	+	B	A	+	-	<i>Vibrio mytili</i>
21	Rod	-	-	+	+	+	-	-	+	-	+	A	A	A	+	B	A	+	-	<i>Vibrio parahaemolyticus</i>
22	Rod	-	-	+	+	+	-	-	+	-	+	A	A	A	+	B	A	+	-	<i>Vibrio mytili</i>
23	Rod	-	+	+	+	-	+	-	+	+	+	A	-	A	+	B	A	-	-	<i>Vibrio cholera</i>
24	Rod	-	-	+	+	+	-	-	+	-	+	A	A	A	+	B	A	+	-	<i>Vibrio mytili</i>
25	Rod	-	-	+	+	-	-	-	+	+	+	A	A	A	+	B	A	-	+	<i>Vibrio anguillarum</i>
26	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	+	A	A	+	-	<i>Vibrio furnissii</i>
27	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	+	A	A	+	-	<i>Vibrio furnissii</i>
28	Rod	-	-	+	+	-	-	+	+	-	+	A	A-	A-	+	A	A	-	-	<i>Vibrio vulnificus</i>
29	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>
30	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>
31	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>
32	Rod	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>
33	Rod	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>
34	Rod	-	-	+	+	-	+	+	-	-	+	-	A	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
35	Rod	-	-	+	+	+	+	+	+	-	+	A	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
36	Rod	-	-	+	+	+	+	+	+	-	+	A	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
37	Rod	-	-	+	+	+	+	+	+	-	+	A	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticu</i>
38	Rod	-	-	+	+	+	+	-	+	+	+	A	-	A	+	B	A	-	-	<i>Vibrio alginolyticus</i>
39	Rod	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>
4	Ro	-	-	+	+	+	-	-	+	-	+	A	A	A	+	A	B	+	-	<i>Vibrio mytili</i>

0	d											G	G	G									
41	Rod	-	-	+	+	+	+	+	-	+	A	G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>			
42	Rod	-	-	+	+	+	+	+	-	+	A	G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>			
43	Rod	-	-	+	+	+	+	+	-	+	A	G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>			
44	Rod	-	-	+	+	+	+	+	-	+	A	G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>			
45	Rod	-	-	+	+	+	+	+	-	+	A	G	-	-	+	B	A	-	-	<i>Vibrio parahaemolyticus</i>			
46	Rod	-	-	+	+	-	+	+	-	+	-	A	G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>			
47	Rod	-	-	+	+	+	+	+	-	+	-	A	G	A	G	+	A	A	-	-	<i>Vibrio diazotrophic</i>		
48	Rod	-	-	+	+	-	+	+	-	+	-	A	G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>			
49	Rod	-	-	+	+	+	+	-	-	+	+	A	G	-	A	G	+	B	A	-	-	<i>Vibrio Alginolyticus</i>	
50	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	G	+	A	A	+	-	<i>Vibrio furnissii</i>		
51	Rod	-	-	+	+	+	-	-	+	-	+	A	G	A	G	A	G	+	B	A	+	-	<i>Vibrio mytili</i>
52	Rod	-	-	+	+	+	-	-	+	-	+	A	G	A	G	A	G	+	B	A	+	-	<i>Vibrio parahaemolyticus</i>
53	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	G	+	A	A	+	-	<i>Vibrio furnissii</i>		
54	Rod	-	+	+	+	-	+	-	+	+	+	A	G	-	A	G	+	B	A	-	-	<i>Vibrio cholera</i>	
55	Rod	-	-	+	+	-	-	-	+	+	+	A	G	A	G	A	G	+	B	A	-	+	<i>Vibrio anguillarum</i>
56	Rod	-	-	+	+	-	-	-	+	+	+	A	G	A	G	A	G	+	B	A	-	+	<i>Vibrio anguillarum</i>
57	Rod	-	-	+	+	-	+	+	-	-	+	-	A	G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>		
58	Rod	-	-	+	+	-	-	-	-	+	-	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>			
59	Rod	-	-	+	+	-	-	+	+	-	+	A	-	A-	A-	+	A	A	-	-	<i>Vibrio vulnificus</i>		
60	Rod	-	-	+	+	-	-	-	-	+	-	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>			
61	Rod	-	-	+	+	-	-	-	-	+	-	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>			
62	Rod	-	+	+	-	-	+	+	+	-	+	A	-	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>		
6	Ro	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>			

3	d											G	G	G							
64	Rod	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>	
65	Rod	-	-	+	+	-	+	+	-	-	+	-	A	G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
66	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>	
67	Rod	-	-	+	+	-	-	-	+	+	+	A	A	A	+	B	A	-	+	<i>Vibrio anguillarum</i>	
68	Rod	-	-	+	+	+	+	-	+	+	+	A	-	A	+	B	A	-	-	<i>Vibrio alginolyticus</i>	
69	Rod	-	+	+	-	-	+	-	+	+	+	A	A	A	+	A	B	-	-	<i>Vibrio cholera</i>	
70	Rod	-	-	+	+	-	-	-	-	-	+	-	-	A-	+	B	B	-	-	<i>Aliivibrio fischeri</i>	
71	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>	
72	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>	
73	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	+	A	A	+	-	<i>Vibrio furnissii</i>	
74	Rod	-	-	+	+	+	+	-	-	+	+	A	-	A	+	B	A	-	-	<i>Vibrio Alginolyticus</i>	
75	Rod	-	-	+	+	+	+	-	-	+	+	A	-	A	+	B	A	-	-	<i>Vibrio Alginolyticus</i>	
76	Rod	-	-	+	+	-	+	+	-	-	+	-	A	G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>
77	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	+	A	A	+	-	<i>Vibrio furnissii</i>	
78	Rod	-	-	+	+	-	+	+	-	-	+	-	A	G	-	-	A	A	+	-	<i>Vibrio vulnificus</i>
79	Rod	-	-	+	+	+	+	-	-	+	+	A	-	A	+	B	A	-	-	<i>Vibrio Alginolyticus</i>	
80	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>	
81	Rod	-	-	+	+	+	-	+	+	-	+	-	A	A	+	A	A	+	-	<i>Vibrio furnissii</i>	
82	Rod	-	-	+	+	+	+	-	-	+	+	A	-	A	+	B	A	-	-	<i>Vibrio Alginolyticus</i>	
83	Rod	-	+	+	-	-	+	+	+	-	+	A	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>	
84	Rod	-	+	+	+	-	+	-	+	+	+	A	-	A	+	B	A	-	-	<i>Vibrio cholera</i>	
85	Rod	-	-	+	+	-	+	+	-	-	+	-	A	G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
8	Ro	-	-	+	+	-	-	-	+	+	+	A	A	A	+	B	A	-	+	<i>Vibrio anguillarum</i>	

6	d											G	G	G						
87	Rod	-	-	+	+	-	+	+	-	-	+	-	A G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
88	Rod	-	+	+	-	-	+	+	+	-	+	A -	A-	-	+	B	A	-	-	<i>Vibrio mimicus</i>
89	Rod	-	-	+	+	-	-	+	+	-	+	A -	A-	A-	+	A	A	-	-	<i>Vibrio vulnificus</i>
90	Rod	-	-	+	+	-	+	+	-	-	+	-	A G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
91	Rod	-	-	+	+	-	-	-	+	+	+	A G	A G	A G	+	B	A	-	+	<i>Vibrio anguillarum</i>
92	Rod	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
93	Rod	-	+	+	-	-	+	-	+	+	+	A G	A G	A G	+	A	B	-	-	<i>Vibrio cholera</i>
94	Rod	-	-	+	+	+	+	-	-	+	+	A G	-	A G	+	B	A	-	-	<i>Vibrio Alginolyticus</i>
95	Rod	-	-	+	+	-	+	+	-	-	+	-	A G	-	+	A	A	+	-	<i>Vibrio vulnificus</i>
96	Rod	-	-	+	+	+	+	-	-	+	+	A G	-	A G	+	B	A	-	-	<i>Vibrio Alginolyticus</i>